Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Comparative Morphological and SSR-Based Molecular Characterization of Zea nicaraguensis and the Maize Inbred Line

Senthilkumar Velmurugan
Govind Ballabh Pant University of Agriculture and Technology
Priya Garkoti
Govind Ballabh Pant University of Agriculture and Technology
Sharat Prabhakaran
Govind Ballabh Pant University of Agriculture and Technology
Anu Singh
Govind Ballabh Pant University of Agriculture and Technology
Dinesh Pandey
Govind Ballabh Pant University of Agriculture and Technology
Usha Pant
Govind Ballabh Pant University of Agriculture and Technology
Jai Prakash Jaiswal
Govind Ballabh Pant University of Agriculture and Technology
Narendra Kumar Singh
narendraksingh2@gmail.com

Govind Ballabh Pant University of Agriculture and Technology

Research Article

Keywords: Zea nicaraguensis, Maize, Genetic diversity, SSR markers, Morphological traits

Posted Date: July 31st, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10144969/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Understanding the divergence between cultivated maize and its wild relatives is important for exploiting novel alleles in crop improvement. In the present investigation, Zea *nicaraguensis* was compared with LM13, a cultivated maize inbred line selected as the agronomic comparator because of its adaptation and suitability as a potential recurrent parent in introgression breeding. Field evaluation at Pantnagar and Kyrdemkulai for 25 agro-morphological traits revealed clear phenotypic differences between the two genotypes. LM13 flowered earlier, exhibited a positive anthesis-silking interval, and had longer ears and higher 100-seed weight, whereas *Z. nicaraguensis* showed delayed flowering, protogyny, stronger anthocyanin pigmentation, and taller plant stature. SSR marker analysis identified 95 polymorphic markers, indicating clear molecular differentiation between the two genotypes. These findings demonstrate the potential of *Z. nicaraguensis*as a valuable donor of novel alleles for pre-breeding and introgression programmes aimed at broadening the maize gene pool and improving stress adaptation.

1. Introduction

Maize (*Zea mays* L. subsp. *mays*), is a cornerstone of global agriculture, serving as a fundamental source of food, livestock feed, and raw materials for various industrial applications, sustaining millions of lives around the world (Senthilkumar et al., 2024). Due to its high genetic yield potential, it has been referred to as the “Queen of Cereals” (Dass et al., 2012). Despite its importance on a global scale, growing maize faces many difficulties as a result of climate change, including biotic stresses like pests and diseases and abiotic stresses like salinity, drought, and waterlogging (Sahoo et al., 2021). The genetic complexity of the traits, phenotypic challenges, limited germplasm, and resource constraints make it difficult to breed climate resilient maize for combined tolerance and yield traits (Prasanna et al., 2021). Because traditional maize varieties lack the genetic traits necessary to thrive under future conditions, it is imperative that breeders develop new lines with enhanced tolerance and improved kernel traits (Hossain et al., 2022).

Addressing the challenges faced by maize under environmental stresses necessitates a multifaceted strategy. Teosinte species, particularly *Zea nicaraguensis*, provide a valuable reservoir of genetic diversity that can be utilized to enhance maize's stress resilience (Garkoti et al., 2025). *Z. nicaraguensis*, first identified along the Pacific coast of Nicaragua, was classified as a novel species within the genus *Zea* and is part of the teosinte group (Iltis and Benz, 2000). This species occurs in geographically isolated regions of the Nicaraguan coastal lowlands and embayments, situated at elevations between 9 to 75 meters above sea level in the Gulf of Fonseca (Iltis and Benz, 2000). It typically grows in partially submerged conditions, with shoots and prop roots in stagnant water 10–40 cm deep, flourishing both under canopy shade and at sunlit woodland edges. Its ability to thrive in flood-prone habitats and demonstrate rapid growth suggests the presence of unique physiological adaptations for waterlogging tolerance (Voesenek and Bailey-Serres, 2015). Incorporating genetic material from teosinte into cultivated maize offers promising opportunities to enhance stress tolerance and productivity. This potential could be realized through comprehensive studies utilizing interspecific introgression lines to

explore the effects of *Z. nicaraguensis* gene integration into maize, particularly for traits such as flood tolerance **(Mano et al., 2007).**

Its unique morphological traits—such as prolific ear production, narrow leaf architecture, and pigmentation—combined with its adaptation to flooded and saline conditions, make it a potential genetic donor for enhancing resilience and diversity in maize breeding programs **(**Garkoti et al., 2025). However, its genetic distance from cultivated maize and reproductive barriers necessitate a comprehensive understanding of its compatibility and diversity **(**Sahoo et al., 2021). To achieve this, it is essential to first determine the genetic relationship between *Zea nicaraguensis* and the LM13 maize inbred line. These insights will guide the development of maize lines with enhanced tolerance and improved kernel traits, ensuring better adaptation to climate stress **(Mano et al., 2008 and** Sahoo et al., 2021). Molecular markers, particularly Simple Sequence Repeats (SSRs), have been extensively used to evaluate genetic diversity due to their high polymorphism, co-dominant inheritance, and genome-wide distribution **(Idrees et al., 2014).** When combined with phenotypic characterization, SSR markers help identify rare or underutilized alleles and provide strong insights into the relationships between wild and cultivated taxa (Kumar et al., 2019).

Wild introgression offers a valuable strategy to broaden the narrow genetic base of modern maize and to recover adaptive alleles that may have been lost during domestication and subsequent selection **(**Garkoti et al., 2025). Wild relatives such as teosintes represent important reservoirs of novel genetic variation for stress tolerance, yield-related traits, and environmental adaptation. In particular, teosinte-derived chromosomal segments have been useful for identifying genomic regions associated with adaptive traits, including flooding tolerance, thereby demonstrating their significance in pre-breeding and climate-resilient maize improvement **(Mano et al., 2015).** However, the utilization of wild germplasm is constrained by several factors, including reproductive barriers, cross-incompatibility within the genus *Zea*, linkage drag, and the poor agronomic performance of wild donor backgrounds. These limitations often require repeated backcrossing, rigorous phenotypic selection, and marker-assisted recovery of elite maize genomic regions **(**Sahoo et al., 2021). Therefore, a clear comparative characterization of the wild donor and cultivated parent is an essential prerequisite for effective introgression breeding.

The goal of the current study was to use SSR markers and agro-morphological descriptors to compare a maize inbred line LM13 with *Zea nicaraguensis.* The objectives were to assess interspecific divergence using morphological traits, SSR polymorphism, allele size variation, and pairwise genetic relationship, and to evaluate the potential of *Z. nicaraguensis* as a pre-breeding donor **(**Mano et al., 2012). This study provides an integrated assessment of morphological and molecular divergence between LM13 and *Zea nicaraguensis*, establishing a baseline for the use of teosinte alleles in maize improvement **(**Senthilkumar et al., 2024). The findings will support future introgression breeding and the identification of useful genomic regions associated with adaptive traits.

2. Materials and Methods

2.1. Plant Materials and Experimental Sites

The study was conducted to compare *Zea nicaraguensis* (Accession Number: EC938022) and the maize inbred line LM13 for morphological and molecular divergence. *Zea nicaraguensis* accession EC938022 was obtained from ICAR–National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India, and is maintained at the Department of Genetics and Plant Breeding, College of Agriculture, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India. The maize inbred line LM13 was used as the cultivated comparator in the present study. LM13 is a normal yellow, flint-type, late-maturity maize inbred line with high combining ability and was obtained from ICAR–Indian Institute of Maize Research (IIMR), Ludhiana, India. LM13 was chosen for comparison because it provides a clear contrast to *Z. nicaraguensis* with respect to flowering behaviour, plant architecture, and kernel characteristics, thereby making it suitable for estimating morpho-molecular divergence between the wild and cultivated genotypes **(**Senthilkumar et al., 2024; Garkoti et al., 2025; Bhatla et al., 2025). In addition, its cultivated background makes it a relevant reference parent for assessing the potential use of *Z. nicaraguensis* in future introgression breeding.

Field experiments were carried out at the N.E.B. Crop Research Centre, Pantnagar, and the College of Agriculture (COA) Farm, Kyrdemkulai. These locations represented diverse agro-climatic environments for evaluating the two genotypes. Pantnagar, located at 243.8 m above mean sea level (28.97°N, 79.41°E), lies in the subtropical Tarai region and receives an average annual rainfall of 1443.9 mm. Kyrdemkulai, situated at approximately 914 m above mean sea level (25.7°N, 91.9°E) in Meghalaya, receives 2771 mm of annual rainfall and represents a high-rainfall environment. The inclusion of Kyrdemkulai was particularly important because *Zea nicaraguensis* is adapted to waterlogged and flood-prone habitats, making this location relevant for evaluating its field performance under high-moisture conditions **(**El-Shamarka et al., 2024). The weather during the crop seasons was generally favourable for screening and growth studies. Both genotypes were evaluated using standardized maize descriptors recommended by Bioversity International (2009**).** Molecular characterization was performed in the Plant Biotechnology and Molecular Breeding Laboratory, Department of Genetics and Plant Breeding, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. The following sections describe the methods used for data recording, experimental protocols, materials used in each experiment, and the statistical analyses employed. No plant material was collected from the wild specifically for the present study. The experimental cultivation, maintenance, and use of *Zea nicaraguensis* accession EC938022 and LM13 were carried out with institutional permission and complied with relevant institutional, local, national, and international guidelines for plant material use.

2.2. Experimental Design and Field Layout

Both LM13 and *Zea nicaraguensis* were sown in five rows of 4 meters each, with inter-row spacing of 60 cm and intra-row spacing of 20 cm, maintaining a population of 10–15 plants per row. Five plants per row were randomly tagged for trait observation.

2.3. Morphological Trait Evaluation

A total of 25 agro-morphological traits were recorded to compare phenotypic diversity between *Zea nicaraguensis* and the maize inbred line LM13. The observations were made on five randomly tagged plants per row at both locations. The traits were selected based on CIMMYT maize descriptors **(**Bioversity International, 2009**)** and included a comprehensive representation of phenological, vegetative, and reproductive characteristics. The recorded traits represented phenological, vegetative, reproductive, and yield-related characteristics. Phenological observations included days to 50% anthesis, days to 50% silking, and anthesis-silking interval (ASI) to assess flowering behaviour and reproductive synchrony. Prolificacy was evaluated based on the number of ears per plant and ear clusters. Vegetative traits comprised plant height, height of the first cob, number of leaves, leaf length and width, and flag leaf length, width, and angle, measured using a meter scale and protractor at the vegetative to tasseling stages. Tassel traits, including tassel length and spikelet density, were recorded to characterize male floral architecture and potential pollen production. Anthocyanin pigmentation was qualitatively scored as present or absent in the leaf sheath, tassel base, glumes, anthers, and cobs. Yield-related traits included ear length and 100-seed weight, with ear length measured after harvest and seed weight determined by shelling and weighing kernels from each tagged plant using a precision balance. Trait measurements were recorded using standardized field phenotyping protocols, and data from both environments were used to assess environmental interactions and genotype stability **(**Bioversity International, 2009**)**.

2.4. DNA Extraction and SSR Genotyping

Genomic DNA was extracted from leaf samples collected at the knee-high stage using the CTAB method of Doyle and Doyle (1990**)**, with modifications according to Aboul-Maaty and Oraby (2019**).** For molecular characterization, a total of 197 SSR markers were selected to ensure genome-wide coverage across all 10 maize chromosomes, with 15–24 markers per chromosome. Parental screening between LM13 and *Zea nicaraguensis* identified 95 polymorphic markers, which were used for further analysis. The chromosome-wise distribution of total markers screened, polymorphic markers obtained, and percent polymorphism is presented in Table 1. This summary was included to show the genomic coverage of the marker set and to highlight chromosome-wise variation in polymorphism between LM13 and *Zea nicaraguensis*. PCR amplification was carried out following standard protocols, and the amplified products were resolved on 3% agarose gel stained with ethidium bromide. Banding profiles were visualized using a gel documentation system, and allele sizes were scored manually.

Table 1
Chromosome-wise distribution of SSR markers and percent polymorphism between LM13 and *Zea nicaraguensis*

Chromosome	Total markers screened	Polymorphic markers	Percent polymorphism
1	24	14	58.33%
2	18	7	38.89%
3	20	8	40.00%
4	19	9	47.37%
5	19	5	26.32%
6	17	11	64.71%
7	21	10	47.62%
8	21	14	66.67%
9	15	6	40.00%
10	23	11	47.83%
Total	**197**	**95**	**48.22%**

2.5. Genetic Diversity Analysis

Molecular comparison between LM13 and *Zea nicaraguensis* was primarily based on SSR polymorphism, allele size variation, and pairwise genetic relationship. The total number of markers screened, number of polymorphic markers, and percent polymorphism were used to assess the extent of molecular differentiation between the two genotypes. Allele size range for each polymorphic marker was determined from the minimum and maximum amplified fragment sizes. Pairwise genetic relationship was further evaluated using similarity and dissimilarity matrices based on the Jaccard similarity coefficient **(**Jaccard, 1908**)**, where similarity = a / (a + b + c) and dissimilarity = 1 − similarity, with *a* representing the number of shared bands, *b* the number of bands unique to genotype 1, and *c* the number of bands unique to genotype 2. Polymorphic information content (PIC) was calculated according to Botstein et al. (1980) as PIC = 1 − Σ(Pi)², where *Pi* is the frequency of the i^{th} allele at a locus, and was used here only as an indicator of relative marker informativeness in this pairwise parental comparison. Nei's genetic distance was estimated following Nei (1973) to describe overall molecular divergence between the two genotypes. Because the analysis involved only two genotypes, statistics such as PIC and genetic distance were interpreted cautiously as measures of pairwise marker-based differentiation rather than population-level allelic diversity. All analyses were performed in R software using the **poppr** package (Kamvar et al., 2014) and **adegenet** package **(**Jombart, 2008**).**

3. Results and Discussion

3.1. Morphological characterization of LM13 inbred line and *Zea nicaraguensis*

Phenotypic differences between the maize inbred line LM13 and Zea nicaraguensis were comprehensively assessed using 25 agro-morphological traits across two geographically distinct locations, Pantnagar, Uttarakhand, and Kyrdemkulai, Meghalaya. The evaluation revealed marked divergence between the two genotypes in vegetative growth, flowering behaviour, plant architecture, and reproductive traits (Bioversity International, 2009). Representative differences at the seedling stage, in plant structure, cob morphology, and kernel characteristics are illustrated in Figs. 1 and 2. These morphological observations provided valuable insights into the adaptive divergence and contrasting phenotype of LM13 and Zea nicaraguensis and also highlighted the potential breeding value of the wild relative for maize improvement (Garkoti et al., 2025). The quantitative performance of the evaluated traits is summarized in Table 2.

The comparative agro-morphological evaluation revealed a clear and consistent distinction between the cultivated maize inbred LM13 and the wild relative *Zea nicaraguensis*. LM13 flowered early, attaining 50% tasselling in 54.5–58.0 days and 50% silking in 57.0–62.0 days, with a positive anthesis–silking interval of 2–3 days, thereby confirming its protandrous cultivated habit. In contrast, *Zea nicaraguensis* exhibited distinctly delayed flowering, requiring 106–110 days for tasselling and 100–106 days for silking, and maintained a negative anthesis–silking interval of about − 4.0 to − 5.5 days, indicating protogyny. Reproductive organisation differed just as sharply: LM13 produced no ear clusters and only one ear per plant, whereas *Zea nicaraguensis* formed 20–30 ear clusters and 110–120 total ears per plant. Similar divergence was evident in sink traits, where LM13 produced longer ears (12.5–14.0 cm) and heavier dent-type seeds (20.5–23.0 g per 100 seeds), while *Zea nicaraguensis* had shorter ears (10–12 cm) and lighter trapezoidal compressed seeds (7.8–8.85 g per 100 seeds). These contrasts agree well with earlier reports describing the marked phenotypic separation between cultivated maize and teosinte-like material, particularly in relation to flowering behaviour and reproductive form, as noted by Singh et al. (2017), Rivera-Rodríguez et al. (2019), Sahoo et al. (2021), and Garkoti et al. (2025).

These differences are best interpreted in the light of domestication and ecological adaptation. LM13 expressed the classical cultivated maize ideotype, characterized by early flowering, positive ASI, single-ear habit, and larger reproductive structures, all of which contribute to synchronized reproduction and improved grain productivity under field cultivation. By contrast, *Zea nicaraguensis* retained the characteristic wild syndrome of delayed flowering, protogyny, prolific ear formation, and vigorous vegetative growth. The architectural data further strengthened this interpretation: *Zea nicaraguensis* was substantially taller (263.5–275.5 cm) than LM13 (118–135 cm), displayed high ear placement rather than the medium ear placement of LM13, and produced 60–65 leaves compared with only 13.5–16.0 leaves in the maize inbred. In addition, LM13 had broader leaves and a wider flag leaf angle, whereas *Zea nicaraguensis* maintained a narrower and relatively upright canopy. Such cultivated-versus-wild differentiation has long been regarded as a hallmark of maize domestication by Iltis and Benz (2000),

and later studies by Singh et al. (2017) and Sahoo et al. (2021) similarly emphasized that teosintes preserve adaptive traits that were modified or reduced during crop improvement. The vigorous phenotype of *Zea nicaraguensis* under field conditions, as documented by Garkoti et al. (2025), further supports this ecological interpretation.

Table 2
Morphological Comparison of LM13 inbred line and *Zea nicaraguensis*

S. No.	Characters	LM13 maize inbred line		*Zea nicaraguensis*	
	Location	**Pantnagar**	**Kyrdemkulai**	**Pantnagar**	**Kyrdemkulai**
1	Days to 50% tasseling	56.5 to 58.0	54.5 to 57.0	108 to 110	106 to 108
2	Days to 50% silking	61.5 to 62.0	57.0 to 58.0	100 to 106	100 to 104
3	Flowering behavior	Protandry	Protandry	Protogyny	Protogyny
4	Anthesis-silking interval (ASI)	2 to 3	2 to 3	-5 to -5.5	−4.0 to -5
5	Ear clusters per plant	Absent / not clustered	Absent / not clustered	20 to 30	20 to 28
6	Total ears per plant	1	1	110 to 120	110 to 117
7	Plant height (cm)	118 to 135	111 to 131	265 to 275	263.5 to 275.5
8	Ear placement	Medium	Medium	High	High
9	Angle between leaf and stem	< 45°	< 45°	< 45°	< 45°
10	Total number of leaves	13.5 to 15.0	15.0 to 16.0	60 to 65	62 to 65
11	Leaf length (cm)	56 to 57	52 to 55	36 to 38	39 to 41
12	Leaf width (cm)	6.0 to 6.5	6.5 to 7.2	5.2 to 5.5	5.5 to 5.6
13	Flag leaf angle	> 45°	> 45°	< 45°	< 45°
14	Flag leaf length (cm)	22 to 26	20.5 to 23.0	18.5 to 20.5	18.0 to 22.0
15	Flag leaf width (cm)	5.40 to 6.10	4.75 to 5.50	2.5 to 3.5	3.0 to 3.2
16	Anthocyanin Colouration of leaf sheath	Present	Present	Present	Present
17	Tassel: Anthocyanin Colouration at base of glume (in middle third of main axis)	Absent	Absent	Present	Present
18	Tassel: Anthocyanin Colouration of anthers (in middle third of main axis on fresh anthers)	Present	Present	Present	Present

S. No.	Characters	LM13 maize inbred line		*Zea nicaraguensis*	
	Location	**Pantnagar**	**Kyrdemkulai**	**Pantnagar**	**Kyrdemkulai**
19	Tassel: Anthocyanin Colouration of glume excluding base (in middle third of main axis)	Absent	Absent	Present	Present
20	Tassel density of spikelets	Dense	Dense	Sparse	Sparse
21	Tassel length (cm)	> 30	> 30	25–35	25–35
22	Anthocyanin in cobs	Absent	Absent	Absent	Absent
23	Ear length (cm)	13.5 to 14.0	12.5 to 13.8	10 to 12	10 to 12
24	100-seed weight (g)	22.0 to 23.0	20.5 to 22.5	8.25 to 8.85	7.8 to 8.25
25	Kernel type / seed shape	Dent	Dent	Trapezoidal / compressed	Trapezoidal / compressed

From a breeding perspective, these results highlight *Zea nicaraguensis* not as a directly usable agronomic type, but as a strategically valuable pre-breeding resource. Although it carries several undesirable wild attributes, including late flowering, extreme prolificacy, smaller ears, and lighter seeds, it simultaneously contributes novel variation for flowering behaviour, reproductive biology, plant architecture, pigmentation, and adaptation-related morphology. In the present study, *Zea nicaraguensis* also differed from LM13 in anthocyanin-related traits, showing pigmentation in the leaf sheath, tassel base, anthers, and glumes, whereas LM13 displayed a more restricted pattern, thereby underlining the distinct adaptive and morphological variability available in the wild relative. The usefulness of teosinte for broadening maize diversity has been repeatedly stressed by Singh et al. (2017) and Sahoo et al. (2021), while more recent work such as Senthilkumar et al. (2024) and Garkoti et al. (2025) has further reinforced the value of *Zea nicaraguensis* for introgression-based diversification and resilience-oriented breeding. Thus, the corrected LM13–*Zea nicaraguensis* comparison not only demonstrates a strong domestication-related divergence, but also confirms the practical significance of this wild relative as a donor of novel alleles for long-term maize genetic enhancement.

3.2. Molecular characterization of LM13 inbred line and *Zea nicaraguensis*

A total of 197 SSR markers were screened across the 10 maize chromosomes to assess molecular differentiation between LM13 and *Zea nicaraguensis*, of which 95 markers were found to be polymorphic, resulting in an overall polymorphism of 48.22% (Abdul Hussein et al., 2014). The chromosome-wise distribution of total markers screened, polymorphic markers, and percent polymorphism is presented in Table 1. The level of polymorphism varied among chromosomes, with the highest percent polymorphism

recorded on chromosome 8 (66.67%), followed by chromosome 6 (64.71%), whereas the lowest was observed on chromosome 5 (26.32%). The distribution of polymorphic SSR markers across all chromosomes indicated that molecular differentiation between LM13 and *Zea nicaraguensis* was widespread throughout the maize genome, although the extent of polymorphism differed among chromosomal regions (Loaisiga et al., 2011). The relatively higher polymorphism observed on chromosomes 8 and 6 suggests greater marker-based differentiation in these regions, whereas the lower polymorphism on chromosome 5 may indicate comparatively conserved genomic segments between the two genotypes. Similar chromosome-wise variation in SSR polymorphism has been reported in maize and teosinte-related germplasm and has been used to identify informative genomic regions for comparative analysis (Terra et al., 2011). Pairwise comparison of the two genotypes further revealed differences in allele size range. In LM13, amplified alleles ranged from 100 to 300 bp, whereas in *Zea nicaraguensis* the observed range was 120 to 320 bp, indicating broader allele size variation in the wild genotype (**Loaisiga et al., 2012**). The average PIC value across polymorphic markers was approximately 0.768, suggesting that the selected SSR markers were informative for detecting polymorphism between the two parental genotypes (Eisele et al., 2021). Nei's genetic distance between LM13 and *Zea nicaraguensis* was 0.185, indicating measurable molecular divergence between the two genotypes (Terra et al., 2011).

The pairwise genetic relationship was further supported by similarity and dissimilarity analysis. The genetic similarity value of 0.831 indicated that LM13 and *Zea nicaraguensis* still shared a substantial proportion of marker bands, whereas the genetic dissimilarity value of 0.169 reflected clear molecular differentiation between them (Vathana et al., 2019; Xia et al., 2005). Because the present analysis involved only two genotypes, these molecular parameters are interpreted here as indicators of pairwise marker-based differentiation rather than population-level diversity. Overall, the SSR analysis demonstrated clear molecular differentiation between LM13 and *Zea nicaraguensis* and supports the potential usefulness of *Zea nicaraguensis* as a donor source for broadening the genetic base of cultivated maize (**Mano et al., 2007;** Garkoti et al., 2025). The detailed molecular results and representative SSR amplification profiles are presented in **Table 3 and** Fig. 3.

Table 3. Molecular metrics of Zea nicaraguensis and the maize inbred line LM13

Metrics	LM13 and Zea *nicaraguensis*
Total markers screened	197
Polymorphic markers	95
Overall polymorphism (%)	48.22%
Combined allele size range (bp)	100 to 320
Average PIC of polymorphic markers	~ 0.768
Nei genetic distance	0.185
Genetic similarity	0.831
Genetic dissimilarity	0.169

3.3 Integrated Interpretation of Morphological and Molecular Divergence

The observed morphological and molecular contrasts between LM13 and *Zea nicaraguensis* underscore the genetic divergence between cultivated and wild maize. LM13 demonstrated traits favourable for high-yielding environments, such as early maturity, compact plant architecture, and bold seeds, whereas *Zea nicaraguensis* exhibited delayed flowering, high prolificacy, anthocyanin pigmentation, and taller growth, reflecting adaptation to stress-prone and waterlogged habitats. Molecular analysis revealed broader allele size variation and clear SSR polymorphism between LM13 and *Zea nicaraguensis*, indicating measurable marker-based differentiation between the two genotypes. These results are consistent with previous reports by Mano et al. (2016) on the role of *Zea nicaraguensis* in improving waterlogging tolerance in maize. The moderate genetic distance and high similarity index observed between the two genotypes suggest that introgression of beneficial alleles from *Zea nicaraguensis* into maize is feasible through pre-breeding. Notably, traits reported in earlier studies, such as stem elongation and adaptive responses under flooding, may contribute to improved performance under waterlogged conditions **(**Mano and Omori, 2013**).** These findings establish *Zea nicaraguensis* as a promising genetic resource for improving stress resilience, adaptability, and genetic base expansion in maize, particularly under challenging agro-ecological conditions.

4. Conclusion and Future Prospects

The present study revealed substantial morphological and molecular divergence between *Zea nicaraguensis* and the maize inbred line LM13. The wild relative showed distinct adaptive features and clear molecular differentiation from LM13, highlighting its potential as a valuable donor for broadening the genetic base of cultivated maize and improving stress adaptation. The identified polymorphic SSR markers provide useful tools for future introgression breeding and marker-assisted selection. Further studies on trait-linked genomic regions will support the effective utilization of *Zea nicaraguensis* in maize improvement programmes.

Declarations

Conflict of Interest:

The authors declare that they have no conflict of interest.

Ethics, Consent to Participate, and Consent to Publish declarations

The plant materials used in the present study were obtained from recognized institutional germplasm sources with appropriate permission. *Zea nicaraguensis* accession EC938022 was obtained from ICAR–National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India, and LM13 was obtained from ICAR–Indian Institute of Maize Research (IIMR), Ludhiana, India. The materials were maintained and used at the Department of Genetics and Plant Breeding, College of Agriculture, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India. No endangered or protected plant species were collected from the wild for this study. The collection and use of plant materials complied with all relevant institutional, national, and international guidelines, including the IUCN Policy Statement on Research Involving Species at Risk of Extinction and CITES regulations.

Consent to publish:

Not applicable.

Plant reproducibility

Seeds of Zea nicaraguensis accession EC938022 and LM13 used in this study are maintained at the Department of Genetics and Plant Breeding, College of Agriculture, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India. Detailed information on the plant materials and their sources has been provided in the Materials and Methods section.

Funding:

No funding was received for conducting this study.

Author Contribution

V.S., P.G., S.P., and N.K.S. conceptualized the study and conducted the field and laboratory experiments. V.S., P.G., S.P., and A.S. drafted the manuscript. N.K.S., D.P., U.P., and J.P.J. provided materials and technical support. N.K.S. supervised the study and critically reviewed the manuscript. All authors read and approved the final manuscript.

Acknowledgement

The authors acknowledge the Department of Genetics and Plant Breeding and the Directorate of Research, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India, for providing the facilities and support necessary for this study.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Abdul Hussein N, Al-Saadi AH, Merza TK. Analysis of genetic diversity in maize (*Zea mays L.*) varieties using simple sequence repeat (SSR) markers. J Univ Babylon. 2014;22(6):1768–74.
2. Aboul-Maaty NAF, Oraby HAS. Extraction of high-quality genomic DNA from different plant orders applying a modified CTAB-based method. Bull Natl Res Centre. 2019;43:25.
3. Adhikari S, Joshi A, Kumar A, Singh NK. Diversification of maize (*Zea mays L.*) through teosinte (*Zea mays subsp. parviglumis* Iltis and Doebley) allelic influx. Genet Resour Crop Evol. 2021;68(7):2983–95.
4. Adhikari S, Joshi A, Sahoo S, Singh NK. Teosinte (*Zea mays subsp. parviglumis*) allelic influx as a measure to enhance and strengthen diversity in maize. Maize J. 2020;9(1):1–9.
5. Bhatla A, Angidi S, Thomas N, Madankar K, Shahi JP. Classification of maize inbred lines into heterotic groups based on yield and yield attributing traits. Plant Genetic Resources: Charact Utilization. 2025;23:27–39.
6. Bioversity International. Key access and utilization descriptors for maize genetic resources. Mexico: CIMMYT; 2009. p. 6.
7. Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet. 1980;32(3):314–31.
8. Chen L, Luo J, Jin M, Yang N, Liu X, Peng Y, Li W, Phillips A, Cameron B, Bernal JS, Rellán-Álvarez R. Genome sequencing reveals evidence of adaptive variation in the genus *Zea*. Nat Genet. 2022;54(11):1736–45.
9. Dass S, Kumar A, Jat SL, Parihar CM, Singh AK, Chikkappa GK, Jat ML. Maize holds potential for diversification and livelihood security. Ind J Agron. 2012;57(3):32–7.
10. Doyle JJ, Doyle JL. Isolation of plant DNA from fresh tissue. Focus. 1990;12:13–5.
11. Eisele TG, Lazzari D, Silva TAD, Pinto RJB, Matsuzaki RA, Maioli MF, Alves AV, Amaral ATD. Combining ability and genetic divergence among tropical maize inbred lines using SSR markers. Acta Scientiarum Agron. 2021;43:53317.

12. El-Shamarka S, Ali OA, El-Nahas M. Evaluation of some maize-teosinte hybrids and their parents under water stress conditions. Menoufia J Plant Prod. 2024;9(3):109–41.
13. Garkoti P, Senthilkumar V, Naresh T, Prakash S, Anirudh TV, Tiwari M, Kumar K, Singh NK. 2025. Deciphering the potential *Zea nicaraguensis* for addressing yield plateau in maize. *Genetic Resources and Crop Evolution*. Advance online publication. 10.1007/s10722-025-02412-0
14. Hossain F, Muthusamy V, Bhat JS, Zunjare RU, Kumar S, Prakash NR, Mehta BK. 2022. Maize breeding. Fundamentals Field Crop Breed: 221–58.
15. Idrees M, Irshad M. Molecular markers in plants for analysis of genetic diversity: a review. Eur Acad Res. 2014;2(1):1513–40.
16. Iltis HH, Benz BF. *Zea nicaraguensis* (Poaceae), a new teosinte from Pacific coastal Nicaragua. Novon. 2000;10:382–90.
17. Jaccard P. Nouvelles recherches sur la distribution florale. Bull de la Société Vaudoise des Sci Nat. 1908;44:223–70.
18. Jombart T. adegenet: A R package for the multivariate analysis of genetic markers. Bioinformatics. 2008;24(11):1403–5.
19. Joshi A, Adhikari S, Singh NK. Genetic diversity in interspecific hybridization derived advanced maize inbred lines. Maydica. 2021;66(1):15.
20. Kamvar ZN, Tabima JF, Grünwald NJ. poppr: An R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ. 2014;2:e281.
21. Kumar A, Singh NK, Adhikari S, Joshi A. Morphological and molecular characterization of teosinte derived maize population. Indian J Genet Plant Breed. 2019;79(4):670–7.
22. Laurentin H. Data analysis for molecular characterization of plant genetic resources. Genet Resour Crop Evol. 2009;56:277–92.
23. Loaisiga CH, Brantestam AK, Diaz O, Salomon B, Merker A. Genetic diversity in seven populations of Nicaraguan teosinte (*Zea nicaraguensis* Iltis et Benz) as estimated by microsatellite variation. Genet Resour Crop Evol. 2011;58:1021–8.
24. Loáisiga CH, Rocha O, Brantestam AK, Salomon B, Merker A. Genetic diversity and gene flow in six accessions of Meso-American teosintes. Genet Resour Crop Evol. 2012;59:95–111.
25. Mano Y, Omori F. Breeding for flooding tolerant maize using teosinte as a germplasm resource. Plant Root. 2007;1:17–21.
26. Mano Y, Omori F. Verification of QTL controlling root aerenchyma formation in a maize × teosinte *Zea nicaraguensis* advanced backcross population. Breed Sci. 2008;58(3):217–23.
27. Mano Y, Omori F. Flooding tolerance in interspecific introgression lines containing chromosome segments from teosinte (*Zea nicaraguensis*) in maize (*Zea mays subsp. mays*). Ann Botany. 2013;112(6):1125–39.
28. Mano Y, Omori F. Flooding tolerance in maize (*Zea mays subsp. mays*) F1 hybrids containing a QTL introgressed from teosinte (*Zea nicaraguensis*). Euphytica. 2015;205:255–67.

29. Mano Y, Omori F, Takeda K. Construction of intraspecific linkage maps, detection of a chromosome inversion, and mapping of QTL for constitutive root aerenchyma formation in the teosinte *Zea nicaraguensis*. Mol Breeding. 2012;29:137–46.
30. Mano Y, Omori F, Loaisiga CH, Bird RM. QTL mapping of above-ground adventitious roots during flooding in maize × teosinte *Zea nicaraguensis* backcross population. Plant Root. 2009;3:3–9.
31. Mano Y, Omori F, Tamaki H, Mitsuhashi S, Takahashi W. DNA marker-assisted selection approach for developing flooding-tolerant maize. Japan Agricultural Res Q. 2016;50(3):175–82.
32. Nei M. Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA. 1973;70(12):3321–3.
33. Pineda-Hidalgo KV, Méndez-Marroquín KP, Alvarez EV, Chávez-Ontiveros J, Sánchez-Peña P, Garzón-Tiznado JA, Vega-García MO, López-Valenzuela JA. Microsatellite-based genetic diversity among accessions of maize landraces from Sinaloa in México. Hereditas. 2013;150(4):53–9.
34. Prasanna BM, Cairns JE, Zaidi PH, Beyene Y, Makumbi D, Gowda M, Magorokosho C, Zaman-Allah M, Olsen M, Das A, Worku M. Beat the stress: breeding for climate resilience in maize for the tropical rainfed environments. Theor Appl Genet. 2021;134(6):1729–52.
35. Rivera-Rodríguez DM, de Jesús Sánchez González J, De la Cruz Larios L, Santacruz-Ruvalcaba F, Corral R, J.A. Morphological and climatic variability of teosinte (*Zea spp.*) and relationships among taxa. Syst Bot. 2019;44(1):41–51.
36. Sahoo S, Adhikari S, Joshi A, Singh NK. Use of wild progenitor teosinte in maize (*Zea mays subsp. mays*) improvement: present status and future prospects. Trop Plant Biology. 2021;14(2):156–79.
37. Senthilkumar V, Garkoti P, Naresh T, Tiwari M, Anirudh TV, Singh NK. Impact of *Zea nicaraguensis* introgression on kernel trait variability in maize lines. Pantnagar J Res. 2024;22(2):241–53.
38. Shannon CE, Weaver W. The mathematical theory of communication. University of Illinois Press; 1949.
39. Singh NK, Garkoti P, Senthilkumar V, Thotla N, Kashyap S, Varalakshmi S. 2023. Wild relatives: potential and prospects in development and diversification of maize germplasm. Technical bulletin released at the Maize Annual Workshop, Pantnagar, 2023.
40. Singh NK, Kumar A, Chandra H, Pal K, Verma SS. Enhancement of maize allelic diversity using wild relative teosinte (*Zea mays ssp. parviglumis*). Indian J Plant Genetic Resour. 2017;30(3):253–7.
41. Terra TDF, Wiethölter P, Almeida CCDS, Silva SDDA, Bered F, Sereno MJCDM, Neto B, J.F. Genetic variability in maize and teosinte populations estimated by microsatellite markers. Ciência Rural. 2011;41:205–11.
42. Varalakshmi S, Singh NK, Senthilkumar V, Sahoo S, Singh P, Garkoti P. Morphological characterization for leaf architecture in teosinte (*Zea mays subsp. parviglumis*) derived BC1F2 population of maize. Pantnagar J Res. 2022;20:3.
43. Vathana Y, Sa KJ, Lim SE, Lee JK. Genetic diversity and association analyses of Chinese maize inbred lines using SSR markers. Plant Breed Biotechnol. 2019;7(3):186–99.

44. Voesenek LACJ, Bailey-Serres J. Flood adaptive traits and processes: an overview. New Phytol. 2015;206(1):57–73.
45. Xia XC, Reif JC, Melchinger AE, Frisch M, Hoisington DA, Beck D, Pixley K, Warburton ML. Genetic diversity among CIMMYT maize inbred lines investigated with SSR markers: II. Subtropical, tropical midaltitude, and highland maize inbred lines and their relationships with elite US and European maize. Crop Sci. 2005;45(6):2573–82.

Figures



Figure 1

Comparative morphology of LM13 and *Zea nicaraguensis* at seedling and vegetative stages



Figure 2

Comparative cob and kernel morphology of LM13 and *Zea nicaraguensis*.

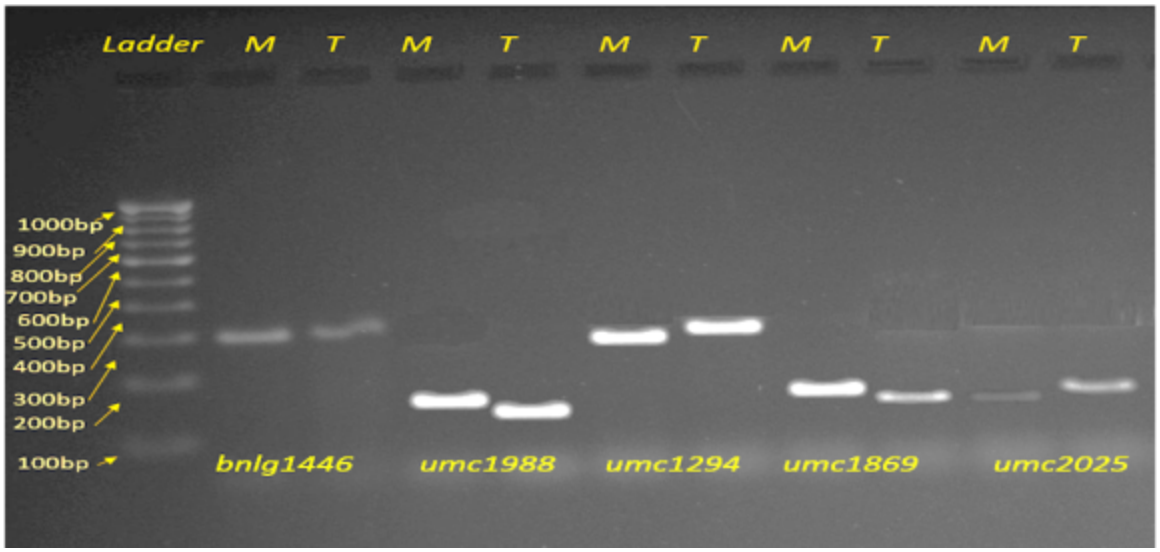


Figure 3

SSR amplification profiles of LM13 and *Zea nicaraguensis* generated using the polymorphic primers bnlg1446, umc1988, umc1294, umc1869, and umc2025. Lane assignments: 100 bp DNA ladder; M, LM13; T, *Zea nicaraguensis*.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- RevisedSupplementaryTableSenthilkumarV.docx