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Microstructural Changes in the Plastid Genome of *Zea*: Evolutionary Insights

Liting Wang
Hainan Institute of Biotechnology, Haikou, 570206, Hainan, China
Corresponding email: liting.wang@hibio.org

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Abstract This study explores the evolutionary dynamics within the genus *Zea* by analyzing complete plastid genomes. The research sequenced plastomes from five *Zea* species, identifying 193 indels and 15 inversions, with tandem repeat indels being the most prevalent microstructural changes. Divergence times were estimated, revealing that the stem lineage of all *Zea* species diverged approximately 176 000 years before present (YBP), with mutation rates ranging from 1.7E-8 to 3.5E-8 changes per site per year. Additionally, the study examined plastome diversity within *Zea mays*, identifying 124 polymorphic loci and 27 distinct haplotypes, which reflect the geographic structuring of nuclear gene pools in South American maize landraces. The findings underscore the non-uniform rates of microstructural changes despite close taxonomic relationships and provide a comprehensive framework for understanding evolutionary processes at low taxonomic levels. This research contributes significantly to the phylogenomic knowledge of *Zea*, confirming previous mitochondrial and nuclear data analyses and highlighting the importance of plastid genome studies in elucidating evolutionary histories.

Keywords Plastid genome; *Zea* evolution; Microstructural changes; Phylogenomics; Divergence times

1 Introduction

Plastids are essential organelles in plant cells, originating from a photosynthetic bacterial endosymbiont. They possess their own genome, which has undergone significant reduction and rearrangement over evolutionary time (Barbrook et al., 2006; Rogalski et al., 2015). The plastid genome, or plastome, typically contains around 130 genes in higher plants, organized in a circular DNA molecule ranging from 100 to 220 kb in size (Rogalski et al., 2015). These genes are crucial for various cellular functions, including photosynthesis, transcription, and translation (Liebers et al., 2017). Plastids exhibit remarkable diversity, with different types such as chloroplasts, chromoplasts, and amyloplasts, each playing specific roles in plant development and metabolism (Liebers et al., 2017; Sierra et al., 2023).

The evolution of plastid genomes provides critical insights into plant phylogeny and the adaptation of plants to different environments. The retention of plastid genomes in both photosynthetic and non-photosynthetic organisms highlights their essential roles beyond photosynthesis, such as in the synthesis of metabolic compounds and stress responses (Barbrook et al., 2006; Rogalski et al., 2015). Comparative analyses of plastid genomes across various plant species have revealed patterns of gene loss, retention, and rearrangement, which are key to understanding the evolutionary dynamics of these organelles (Huang et al., 2013; Yang et al., 2022). For instance, the study of plastid genomes in parasitic plants has shown how the loss of photosynthetic genes can lead to the retention of other essential genes, providing a model for genome degradation and adaptation (Krause, 2008; Graham et al., 2017).

This study aims to explore the microstructural changes in the plastid genome of the *genus Zea*, focusing on evolutionary insights gained from these changes. By examining complete plastid genomes of various *Zea* species, including *Zea mays* and its congeneric species, this study seeks to identify and analyze the frequencies and types of microstructural changes such as inversions and indels. Additionally, the study will estimate divergence times and mutation rates within the genus, providing a comprehensive understanding of the evolutionary history and phylogenetic relationships among *Zea* species. This knowledge will contribute to broader discussions on plastid genome evolution and its implications for plant biology and biotechnology.

2 Evolutionary Background of *Zea* Plastids

2.1 Historical evolution of *Zea*

The genus *Zea*, which includes the well-known species *Zea mays* (maize), has a rich evolutionary history that is intricately linked to its plastid genome. The plastid genome, or plastome, of *Zea* species has been a focal point of evolutionary studies due to its relatively stable inheritance and the critical role it plays in photosynthesis and other cellular functions. The historical evolution of *Zea* can be traced back to its divergence from a common ancestor with other grasses. The stem lineage of all *Zea* species was calculated to have diverged approximately 176 000 years before present (YBP) (Orton et al., 2017). This divergence set the stage for the evolution of various *Zea* species, each adapting to different ecological niches and undergoing unique microstructural changes in their plastomes.

2.2 Phylogenetic context

Phylogenetic analyses have been instrumental in understanding the evolutionary relationships within the genus *Zea*. Complete plastome sequences from multiple *Zea* species, including *Zea diploperennis*, *Zea perennis*, *Zea luxurians*, *Zea nicaraguensis*, and *Zea mays* subsp. *huehuetenangensis*, have been used to construct phylogenetic trees that reveal the rates and patterns of microstructural changes such as inversions and insertion or deletion mutations (indels) (Orton et al., 2017). These analyses have confirmed the close relationships among *Zea* species and have provided insights into the divergence times of specific nodes within the genus. For instance, divergence dates for specific nodes relative to *Zea* were estimated to fall between 38 000 YBP for certain subspecies and 23 000 YBP for the section Luxuriantes (Orton et al., 2017).

The phylogenetic context of *Zea plastids* is further enriched by studies on nuclear ribosomal internal transcribed spacer (ITS) sequences, which have been used to evaluate patterns of concerted evolution and substitution rates among *Zea* taxa (Buckler and Holtsford, 1996). These studies have identified significant differences in ITS substitution rates among *Zea* species, highlighting the role of selection in shaping the observed polymorphisms and substitutions.

2.3 Genetic divergence

Genetic divergence within the genus *Zea* is marked by a variety of microstructural changes in the plastid genome. A comprehensive study identified 193 indels and 15 inversions across the examined plastomes of *Zea* species, with tandem repeat indels being the most common type of microstructural change observed (Orton et al., 2017). These changes are not uniformly distributed, indicating that different *Zea* species have undergone unique evolutionary paths despite their close relationships.

The genetic divergence of *Zea plastids* is also reflected in the response to polyploidization, a process that has played a significant role in the evolutionary history of many eukaryotic species, including *Zea mays*. Polyploidization often leads to large-scale genomic reorganizations and phenotypic alterations. Studies have shown that different *Zea mays* inbred lines exhibit varying morphological responses to changes in ploidy, demonstrating the existence of genetic variation for the response to ploidy change within the species (Riddle et al., 2006).

Moreover, the plastome diversity within *Zea mays* has been explored through the sequencing of complete plastomes from various South American maize landraces and teosintes. These analyses have identified a total of 124 polymorphic plastome loci, which have been used to infer evolutionary relationships among haplotypes and to understand the phylogeographic structuring of maize landraces . The structuring of haplotype diversity in these landraces reflects the distinction between Andean and South American lowland gene pools, providing further insights into the genetic divergence within *Zea mays*.

The evolutionary background of *Zea plastids* is characterized by a complex interplay of historical divergence, phylogenetic relationships, and genetic divergence. The plastid genome of *Zea* species has undergone significant microstructural changes, which have been shaped by various evolutionary forces, including selection, polyploidization, and geographic structuring. These insights not only enhance our understanding of the

evolutionary history of *Zea* but also provide a framework for future studies on the genetic and functional diversity of plastid genomes in this important genus.

3 Methods and Techniques for Studying Changes in the *Zea* Plasmid Genome

3.1 Molecular biology techniques

Molecular biology techniques are fundamental in studying the microstructural changes in the plastid genome of *Zea* species. One of the primary methods used is sequencing, which allows for the detailed examination of plastid genomes. In the study by Orton et al. (2017), both Sanger and next-generation sequencing methods were employed to sequence the complete plastomes of five *Zea* species. This comprehensive sequencing enabled the identification of 193 indels and 15 inversions, providing insights into the mutation rates and divergence times within the genus.

Another critical molecular technique is the use of restriction endonucleases, which can compare the digestion patterns of affected and normal plastids. This method was utilized in the study of the iojap gene in *Zea mays*, which conditions a permanent deficiency in plastid differentiation. The restriction endonuclease digestion patterns confirmed that iojap-affected plastids contain a normal genome despite their inability to differentiate properly (Walbot and Coe, 1979).

Additionally, the analysis of ribosomal internal transcribed spacer (ITS) sequences is a valuable molecular technique. ITS sequences were used to evaluate patterns of concerted evolution, rates of substitutions, and structural constraints in *Zea* and Tripsacum. This analysis revealed significant differences in ITS substitution rates among *Zea taxa* and identified methylation-induced deamination as a potent mutation source (Buckler and Holtsford, 1996).

3.2 Bioinformatics analysis

Bioinformatics plays a crucial role in analyzing the vast amounts of data generated by sequencing technologies. Comparative genomic analysis is one such bioinformatics approach, which was used to study the plastomes of seven Lonicera species. This analysis identified various repeat sequence variations and protein sequence evolution, highlighting divergence hotspot regions and genes under positive selection (Liu et al., 2018).

Phylogenomic analyses are another essential bioinformatics tool. In the study of *Zea* species, full plastome alignments were used to compare tree topologies from different types of mutations. This approach confirmed previous work examining *Zea mitochondrial* and nuclear data, providing a comprehensive view of the evolutionary relationships within the genus (Orton et al., 2017).

Furthermore, the selection of appropriate evolutionary models and character partition strategies is critical for accurate phylogenetic analyses. The study on Amphilophium species demonstrated the importance of these models in recovering phylogenetic relationships and addressing sources of systematic error, such as compositional heterogeneity and codon usage bias (Thode et al., 2020).

3.3 Experimental and observational methods

Experimental and observational methods are indispensable for understanding the functional implications of microstructural changes in the plastid genome. For instance, the effect of adenosine 5'-triphosphate (ATP) on the Shibata shift and associated structural changes in maize etioplasts was investigated through controlled experiments. The study found that ATP inhibits the Shibata shift and prolamellar body transformation, suggesting a regulatory role for ATP in early plastid development (Horton and Leech, 1975).

Another experimental approach involves the reconstruction of gene transfer events from plastids to the nucleus. This method allows researchers to observe genome evolution in real-time and study the molecular mechanisms by which plastid genes are converted into functional nuclear genes. Such experiments have provided valuable insights into the ongoing process of DNA transfer from organelles to the nucleus (Bock and Timmis, 2008).

Observational methods, such as the analysis of plastome diversity within *Zea mays*, are also crucial. By examining the complete plastomes of South American maize landraces and teosintes, researchers identified polymorphic plastome loci and inferred evolutionary relationships among haplotypes. This study highlighted the importance of intraspecific plastome variation in understanding evolutionary processes at low taxonomic levels (López et al., 2021).

A combination of molecular biology techniques, bioinformatics analysis, and experimental and observational methods provides a comprehensive toolkit for studying microstructural changes in the plastid genome of *Zea*. These approaches offer valuable insights into the evolutionary dynamics and functional implications of these changes, contributing to researchers understanding of plant genome evolution.

4 Microstructural Changes in the *Zea* Plasmid Genome

4.1 Changes in genome size and content

The plastid genomes (plastomes) of *Zea* species exhibit notable variations in size and content. A comprehensive study on the genus *Zea*, which includes species such as Zea mays, Zea diploperennis, Zea perennis, Zea luxurians, and *Zea nicaraguensis*, revealed significant differences in genome size among these species. The study sequenced four complete plastomes and a nearly complete plastome, identifying 193 insertion or deletion mutations (indels) and 15 inversions across the examined plastomes (Orton et al., 2017). These microstructural changes contribute to the overall variation in genome size and content within the genus.

Additionally, the plastid genome of *Zea mays* has been shown to undergo changes during different developmental stages. For instance, the plastid DNA (ptDNA) in maize seedlings transitions from large, intact molecules in proplastids to fragmented forms in photosynthetically active chloroplasts. This fragmentation process does not alter the overall genome sequence but results in variations in the physical state of the ptDNA (Figure 1) (Tripathi et al., 2022). These findings highlight the dynamic nature of plastid genome size and content in *Zea* species.

4.2 Changes in gene arrangement and repeat sequences

The arrangement of genes and the presence of repeat sequences in the plastid genome of *Zea* species are subject to significant alterations. In the genus *Zea*, tandem repeat indels were identified as the most common type of microstructural change, indicating a high frequency of repeat sequence variations (Orton et al., 2017). These changes in repeat sequences can impact the stability and function of the plastid genome.

Moreover, the loss of inverted repeat (IR) regions, which are crucial for genome stability, has been observed in other plant lineages and can provide insights into the evolutionary dynamics of *Zea* plastomes. For example, the loss of IR regions in conifer plastomes has been associated with changes in the selection pressure and substitution rates of protein-coding genes (Ping et al., 2022). Although this specific phenomenon has not been documented in *Zea*, it underscores the potential impact of IR region variations on plastid genome evolution.

4.3 Gene loss and acquisition in the plastid genome

Gene loss and acquisition are critical aspects of plastid genome evolution in *Zea* species. The study of plastid genomes in the genus *Zea* revealed that certain genes are retained or lost at different rates across species. For instance, the nuclear gene iojap in *Zea mays* conditions a permanent deficiency in plastid differentiation, leading to the loss of ribosomes and high molecular weight RNA in affected plastids (Walbot and Coe, 1979). This gene loss can have profound effects on plastid function and overall plant development.

In addition, the evolutionary history of plastid genomes in other plant lineages provides valuable insights into gene loss and acquisition in *Zea*. For example, the plastid genomes of parasitic plants in the Scrophulariaceae and Orobanchaceae families have experienced extreme reductions in gene content, with the loss of photosynthetic genes and the retention of translational genes like *rps2* (dePamphilis et al., 1997). These patterns of gene loss and retention can inform our understanding of similar processes in *Zea plastomes*.

Furthermore, the complete plastome sequences of seven species in the Gentiana sect. Kudoa revealed significant gene loss, particularly in the ndh gene family, which is involved in photosynthesis (Sun et al., 2018). This gene

loss pattern is comparable to the observed variations in *Zea plastomes* and highlights the evolutionary pressures that shape plastid genome content.

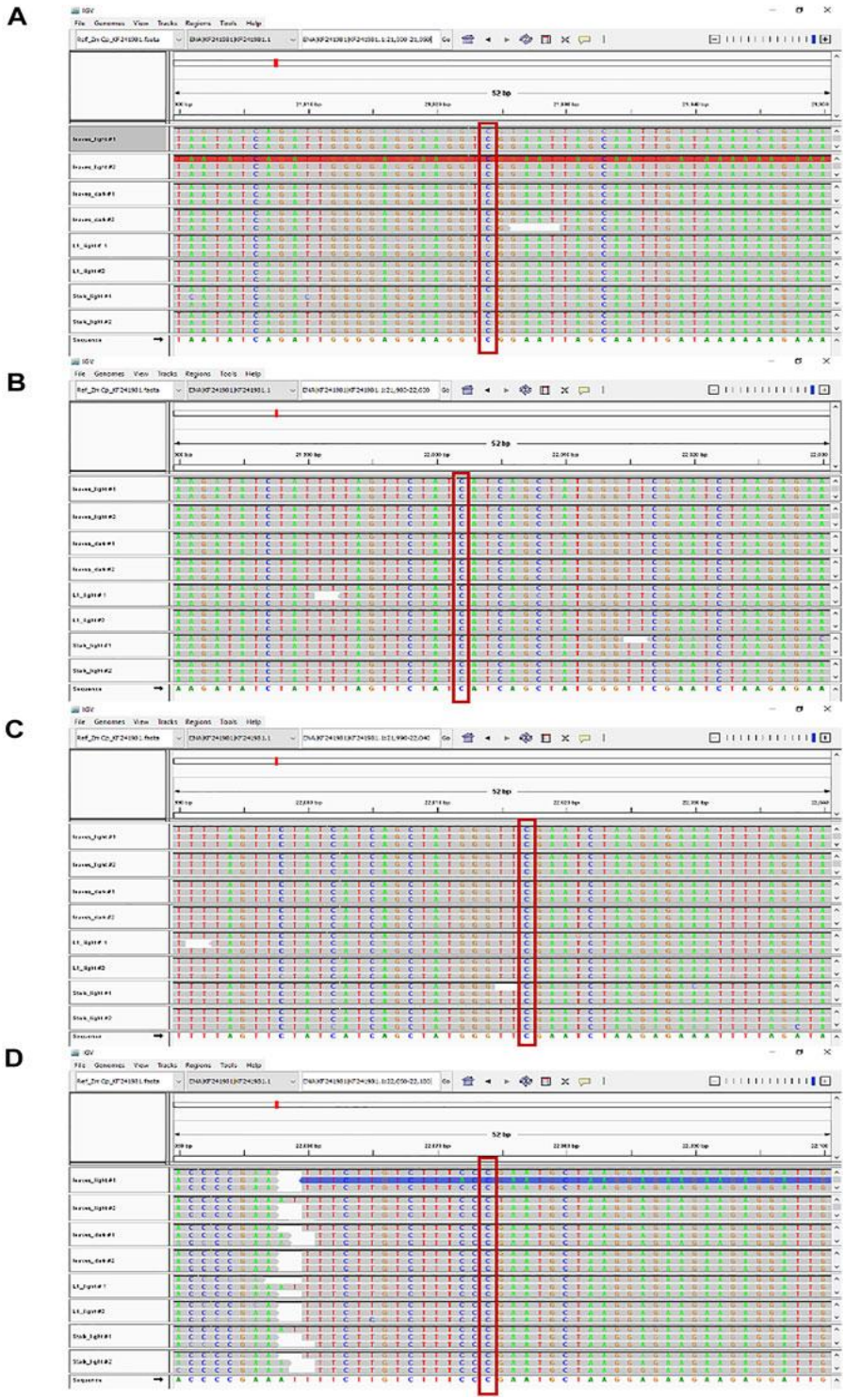

Figure 1 RNA editing sites within the rpoB gene (Adopted from Tripathi et al., 2022)

Image caption: Maize ptDNA sequences from four tissues were aligned against the rpoB gene of the B73 plastid reference genome (NCBI # KF241981.1) using the Integrative genome browser (IGV). Comparison of sequences around four RNA editing sites are shown and located at nts (A) 21924, (B) 22002, (C) 22017, and (D) 22074, respectively. The conserved RNA editing sites are shown in red boxes (Adopted from Tripathi et al., 2022)

The plastid genomes of *Zea* species exhibit dynamic changes in size, content, gene arrangement, repeat sequences, and gene loss/acquisition. These microstructural changes provide valuable insights into the evolutionary processes

that govern plastid genome evolution in the genus *Zea*. The studies cited here contribute to a comprehensive understanding of these changes and their implications for plant biology and evolution (Walbot and Coe, 1979; Orton et al., 2017; Sun et al., 2018; Ping et al., 2022; Tripathi et al., 2022).

5 Case Study: Comparative Analysis of the Plastid Genome in Maize and Its Wild Relatives

5.1 Comparative analysis of the plastid genomes of maize (*Zea mays*) and teosinte

The comparative analysis of the plastid genomes of maize (*Zea mays*) and its wild progenitor, teosinte (*Zea mays* ssp. *parviglumis*), reveals significant insights into the evolutionary processes that have shaped modern maize. Teosinte, which grows naturally in Southern Mexico, maintains a high level of genetic diversity that is crucial for the diversification and domestication of maize (Adhikari et al., 2021). Single-molecule long-read sequencing has been employed to analyze the teosinte genome, identifying 70 044 nonredundant transcript isoforms and constructing a draft genome with 16 633 high-quality contigs (Li et al., 2021). This analysis showed that genes from families that expanded from teosinte to maize were significantly enriched in the RNA modification pathway and had more transcript isoforms in teosinte than in maize (Li et al., 2021).

Furthermore, cellular studies have shown that many cellular traits observed in developing maize caryopses are also present in teosinte, suggesting that these traits evolved independently of domestication and predate human selection pressure (Dermastia et al., 2009). This includes early programmed cell death in the maternal placento-chalazal layer, accumulation of phenolics and flavonoids, and the formation of wall ingrowths in the basal endosperm transfer layer (Dermastia et al., 2009). These findings indicate a high degree of conservation in the cellular processes between maize and teosinte, providing a deeper understanding of the evolutionary history of maize.

5.2 Impact of microstructural changes on maize domestication and cultivation

The domestication of maize from teosinte involved significant microstructural changes in the genome, driven by artificial selection. Analysis of single-nucleotide polymorphisms (SNPs) in 774 genes indicates that 2 to 4% of these genes experienced artificial selection, while the remaining genes show evidence of a population bottleneck associated with domestication (Wright et al., 2005). This artificial selection has led to the clustering of candidate selected genes near quantitative trait loci (QTL) that contribute to phenotypic differences between maize and teosinte (Wright et al., 2005).

One notable example is the *ZEA CENTRORADIALIS 8* (*ZCN8*) gene, which plays a central role in mediating flowering time in maize. A SNP in the ZCN8 promoter was identified as being strongly associated with flowering time and was a target of selection during early domestication. This SNP co-segregated with a major QTL for flowering time in maize-teosinte mapping populations, highlighting the impact of microstructural changes on the adaptation of maize to different environments (Figure 2) (Guo et al., 2018).

Additionally, the introgression of chromosome segments from teosinte into maize has been shown to enhance traits such as flooding tolerance. For instance, a flooding-tolerant genotype containing a chromosome segment from *Zea nicaraguensis* was identified, suggesting the presence of a major QTL for flooding tolerance in that region (Mano and Omori, 2013). This demonstrates how microstructural changes and introgression from wild relatives can be utilized to improve maize cultivation under various environmental conditions.

5.3 Application of genome comparison in maize improvement

The comparative analysis of the plastid genomes of maize and its wild relatives has significant implications for maize improvement. The genetic diversity present in teosinte can be harnessed to develop maize lines with enhanced traits such as stress tolerance and yield. For example, the introgression of alleles from teosinte into maize has been shown to produce lines with unique features such as protogynous behavior, short anthesis-silking intervals, and multiple ears per plant (Adhikari et al., 2021). These traits are valuable for breeding programs aimed at improving maize resilience and productivity.

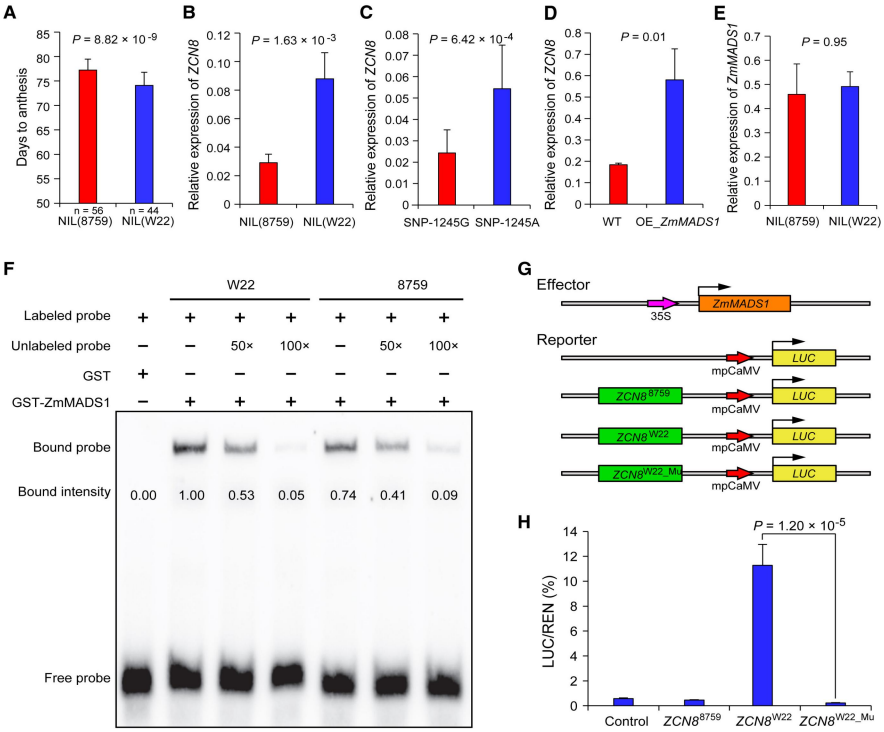

Figure 2 SNP-1245 Affects ZCN8 Expression and Is Associated with Differential Regulation by ZmMADS1 (Adopted from Guo et al., 2018)

Image caption: (A) The difference in days to anthesis between NIL(W22) and NIL(8759); Data represent the mean±SD; p values were determined by Student's t test., B) Relative expression levels of ZCN8 in NIL(W22) and NIL(8759); Data represent the mean±SD (n=3 biological replicates); p values were determined by Student's t test (see also Figure S1)., C) Relative expression levels of ZCN8 in maize inbred lines carrying different alleles of SNP-1245; For each allelic group, 10 maize inbred lines were randomly selected from the maize association panel; Data represent the mean±SD; p values were determined by Student's t test., D) Relative expression levels of ZCN8 in ZmMADS1-overexpressing plants and wild-type plants; Data represent the mean±SD (n=3 biological replicates); p values were determined by Student's t test., E) ZmMADS1 exhibited similar expression levels in the two NILs for qDTA8; Values are presented as the means±SD (n=3 biological replicates); *p* values were determined by Student's t test., F) Electrophoretic mobility shift assay (EMSA) showing that SNP-1245 is associated with differential binding by ZmMADS1; Each biotin-labeled DNA fragment was incubated with GST-ZmMADS1 or GST proteins; Competition assays for the labeled probes were performed by adding an excess of unlabeled probes; The relative bound intensity was quantified with ImageJ software (http://imagej.nih.gov/ij/) (see also Table S4); G and H) Dual-luciferase transient expression assay in maize protoplasts; G) Constructs used in the transient expression assay; The coding sequence of ZmMADS1 driven by the 35S promoter was used as the effector; The luciferase (LUC) gene driven by an~2-kb promoter sequence from NIL(W22) or NIL(8759) was used as the reporter; To examine the effect of SNP-1245, a mutant reporter containing the G nucleotide of SNP-1245 in the NIL(W22) promoter was constructed., H) The effect of overexpression of ZmMADS1 on the activity of the luciferase (LUC) gene driven by the promoter from NIL(W22) or NIL(8759); Values are presented as the means±SD (n=5 biological replicates); *p* values were determined by Student's t test.

Moreover, the identification of genes and QTLs associated with important traits through genome comparison can inform targeted breeding strategies. The *ZCN8* gene, for instance, provides a target for manipulating flowering time to adapt maize to different latitudes and growing seasons (Guo et al., 2018). Similarly, the flooding-tolerant genotype identified through the introgression of chromosome segments from *Zea nicaraguensis* can be used to develop maize lines that are better suited to flood-prone areas (Mano and Omori, 2013).

The comparative analysis of the plastid genomes of maize and its wild relatives offers valuable insights into the evolutionary processes that have shaped modern maize. The microstructural changes identified through this analysis have significant implications for maize domestication and cultivation, providing a foundation for the development of improved maize varieties through targeted breeding and introgression of beneficial alleles from wild relatives.

6 Effects of Plastid Genome Changes on the Evolution of *Zea* Plants

6.1 Relationship with plant diversity

The plastid genome, or plastome, plays a crucial role in the diversification of plant species, including those within the genus *Zea*. The study of plastid genomes across different *Zea* species has revealed significant microstructural changes, such as inversions and insertion or deletion mutations (indels), which contribute to genetic diversity. For instance, a comprehensive analysis of five *Zea* species identified 193 indels and 15 inversions, with tandem repeat indels being the most common type of microstructural change observed (Orton et al., 2017). These genetic variations are essential for understanding the evolutionary relationships and divergence times within the genus. The divergence dates for specific nodes relative to *Zea* were calculated to fall between 38 000 years before present (YBP) for the subspecies and 23 000 YBP for section Luxuriantes, with the stem lineage of all *Zea* species diverging at 176 000 YBP (Orton et al., 2017).

The plastid genome's relatively low number of genes and its uniparental inheritance make it a valuable tool for phylogenetic studies. The gene content and genome rearrangements in plastids are efficient markers for capturing and understanding evolutionary events between different plant species (Rogalski et al., 2015). This genetic diversity within the plastid genome contributes to the overall diversity of the *Zea genus*, influencing traits such as morphology, physiology, and ecological adaptation.

6.2 Impact on plant adaptability

Plastid genome changes significantly impact the adaptability of *Zea* plants to various environmental conditions. The plastid genome's role in photosynthesis and other metabolic processes is crucial for plant survival and adaptation. Structural rearrangements in the plastid genome, such as inversions and deletions, can lead to changes in gene expression and function, thereby affecting the plant's ability to adapt to different environments (Sugimoto et al., 2020). For example, the introduction of plastid-targeted restriction endonucleases in Arabidopsis resulted in various types of rearrangements in the plastid genome, leading to phenotypic changes such as leaf variegation and impaired chloroplast development (Sugimoto et al., 2020). These findings suggest that similar mechanisms could be at play in *Zea* plants, where plastid genome changes could enhance or impair adaptability.

Moreover, the co-evolution of plastid and nuclear genomes plays a significant role in plant adaptability. The complex interactions between these genomes can result in plastome-genome incompatibilities, which can manifest as hybrid bleaching, hybrid variegation, or disturbances in the sexual phase (Greiner et al., 2011). These incompatibilities can act as barriers to hybridization, thereby influencing the adaptability and evolutionary trajectory of *Zea* plants. The ability of *Zea* plants to adapt to different environmental conditions is also influenced by the presence of stress-induced changes in chromosome and ploidy integrity, which can boost adaptive genome evolution in hostile environments (Storme and Mason, 2014).

6.3 Contribution to plant hybridization and speciation

Plastid genome changes are instrumental in the processes of hybridization and speciation in *Zea* plants. The co-evolution of plastid and nuclear genomes can lead to cytonuclear genetic incompatibilities, which are crucial for reproductive isolation and speciation (Postel and Touzet, 2014). These incompatibilities can establish hybridization barriers, similar to the Dobzhansky-Muller model of speciation processes, thereby contributing to the formation of new species (Greiner et al., 2011). For instance, the study of plastid genomes in the genus *Zea* has shown that microstructural changes, such as indels and inversions, can influence the genetic compatibility between different species, affecting their ability to hybridize and form viable offspring (Orton et al., 2017).

Hybridization and polyploidization are major evolutionary forces in plant evolution, including in *Zea* plants. The integration of nuclear and plastid genomic data has revealed that hybridization and polyploidization events are common in plant speciation (Karbstein et al., 2022). In the case of *Zea*, the presence of multiple hybrid origins involving different progenitor species and substantial post-origin evolution suggests that plastid genome changes play a significant role in the speciation process (Karbstein et al., 2022). Additionally, the study of plastid genomes in other plant groups, such as the inverted-repeat-lacking clade (IRLC) of legumes, has shown that plastid genome variation can provide insights into the mechanisms of genomic evolution and the potential for genetic

improvement via plastid transformation (Sabir et al., 2014). These findings highlight the importance of plastid genome changes in the hybridization and speciation of *Zea* plants.

The plastid genome changes in *Zea* plants have profound effects on their evolution, influencing plant diversity, adaptability, and the processes of hybridization and speciation. The study of plastid genomes provides valuable insights into the genetic and evolutionary mechanisms that shape the diversity and adaptability of *Zea* plants, contributing to our understanding of their evolutionary history and potential for future genetic improvement.

7 Future Directions for Research on the *Zea* Plasmid Genome

7.1 Application of new technologies in plastid genome research

The advent of next-generation sequencing (NGS) technologies has revolutionized the field of genomics, including plastid genome research. These technologies allow for the rapid and cost-effective sequencing of entire plastid genomes, providing detailed insights into their structure, function, and evolution. For instance, the study of the plastid genomes of five *Zea* species using both Sanger and NGS methods revealed significant microstructural changes, such as inversions and indels, which are crucial for understanding the evolutionary dynamics within the genus (Orton et al., 2017). Future research should focus on leveraging these advanced sequencing technologies to explore the plastid genomes of lesser-studied *Zea* species and other related genera. This could help in identifying novel genetic variations and evolutionary patterns that are not apparent in well-studied species like *Zea mays*.

Moreover, the integration of high-throughput sequencing with bioinformatics tools can facilitate the identification of functional elements within the plastid genome, such as regulatory sequences and non-coding RNAs. This approach can also be used to study the plastid transcriptome, providing insights into gene expression patterns and their regulation under different environmental conditions. For example, the analysis of plastid genome sequences during maize seedling development has shown that sequence variants are more prevalent in dark-grown leaves compared to light-grown ones, suggesting a role for light in plastid genome stability (Fu et al., 2021). Future studies should aim to elucidate the molecular mechanisms underlying these observations, potentially uncovering new aspects of plastid biology.

7.2 Comparative studies of *Zea* plastid genomes with other plant genera

Comparative genomics is a powerful approach for understanding the evolutionary history and functional diversity of plastid genomes. By comparing the plastid genomes of *Zea* species with those of other plant genera, researchers can identify conserved and divergent features that shed light on the evolutionary processes shaping these genomes. For instance, the study of plastid genomes in the legume family (Fabaceae) has revealed significant structural changes, such as gene losses and rearrangements, which provide a model for understanding genomic evolution in seed plants (Moghaddam and Kazempour-Osaloo, 2020). Similar comparative studies involving *Zea* and other grass species could reveal unique evolutionary adaptations in the plastid genomes of these plants.

Additionally, comparative studies can help identify genes that are under positive selection or have undergone pseudogenization in specific lineages. For example, the ycf4 gene in the tribe Fabeae has been shown to exhibit lineage-specific accelerated evolution and pseudogenization, highlighting the dynamic nature of plastid genomes (Moghaddam and Kazempour-Osaloo, 2020). Investigating whether similar patterns exist in *Zea* and related genera could provide insights into the selective pressures acting on plastid genes and their functional implications.

7.3 Potential applications of plastid genome research in agriculture and ecology

Plastid genome research has significant implications for agriculture and ecology, particularly in the context of crop improvement and conservation. The plastid genome harbors genes that are essential for photosynthesis and other metabolic processes, making it a valuable target for genetic engineering. For example, plastid transformation techniques can be used to introduce traits such as enhanced photosynthetic efficiency, resistance to biotic and abiotic stresses, and the production of valuable metabolites (Rogalski et al., 2015). In *Zea*, such genetic modifications could lead to the development of maize varieties with improved yield, stress tolerance, and nutritional quality.

Furthermore, the uniparental inheritance of plastid genomes makes them ideal markers for phylogenetic and population genetic studies. This feature has been exploited to study the evolutionary relationships and population structure of various plant species, including horticultural crops (Rogalski et al., 2015). In *Zea*, plastid genome markers can be used to trace the domestication history of maize and its wild relatives, providing insights into the genetic diversity and evolutionary dynamics of these species. Such information is crucial for the conservation and sustainable use of genetic resources in breeding programs.

In addition to their agricultural applications, plastid genomes can also provide valuable information for ecological studies. For instance, the study of plastid genome degradation in the subtribe Gentianinae has revealed patterns of gene loss and substitution rate shifts that are associated with different habitats and ecological niches (Fu et al., 2021). Similar studies in *Zea* could help elucidate the ecological adaptations of maize and its wild relatives, contributing to our understanding of plant-environment interactions and the impact of environmental changes on plant genomes.

In conclusion, future research on the *Zea* plastid genome should focus on the application of new sequencing technologies, comparative genomics, and the exploration of potential agricultural and ecological applications. By addressing these areas, researchers can gain a deeper understanding of the evolutionary dynamics and functional diversity of plastid genomes, ultimately contributing to the improvement and conservation of maize and other related species.

8 Concluding Remarks

The research on the plastid genome of *Zea* species has revealed significant microstructural changes that provide insights into the evolutionary dynamics within this genus. The study of complete plastomes from five *Zea* species identified 193 indels and 15 inversions, with tandem repeat indels being the most common type of microstructural change. Additionally, the divergence times for various nodes within *Zea* were estimated, highlighting the evolutionary timeline of these species. The mutation rates were found to vary, despite the close relationships among the taxa studied. Furthermore, the analysis of plastome diversity within *Zea mays* landraces indicated significant haplotype differentiation between Andean and lowland South American gene pools, although overall patterns were not informative for subspecies diversification.

The findings from these studies have broader implications for understanding the evolutionary processes at play within the genus *Zea*. The identification of specific microstructural changes and their frequencies can help in reconstructing the phylogenetic relationships and evolutionary history of these species. The observed variation in mutation rates suggests that evolutionary pressures and environmental factors may differentially influence the plastid genomes of closely related species. Additionally, the structuring of haplotype diversity in maize landraces underscores the importance of considering both nuclear and plastid markers in phylogeographic studies, which can provide a more comprehensive understanding of genetic diversity and evolutionary trajectories.

Future research on plastid genomes should continue to explore the microstructural changes and their evolutionary significance across a broader range of species within the genus *Zea* and other related taxa. Advances in sequencing technologies and bioinformatics tools will facilitate more detailed and comprehensive analyses of plastid genomes, enabling researchers to uncover finer-scale evolutionary patterns and processes. Additionally, integrating plastid genome data with nuclear and mitochondrial genome information will provide a holistic view of plant evolution and adaptation. Understanding the functional implications of specific microstructural changes and their role in plant physiology and development will also be a critical area of future research, potentially leading to applications in crop improvement and conservation biology.

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Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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