Maize Genomics and Genetics 2024, Vol.15, No.5, 228-238
http://cropscipublisher.com/index.php/mgg

Systematic Review Open Access

# Genetic Diversity in the Genus *Zea*: Insights from Chloroplast Genome Variability

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Maize Genomics and Genetics, 2024, Vol.15, No.5 doi: 10.5376/mgg.2024.15.0022
Received: 22 Jul., 2024
Accepted: 03 Sep., 2024
Published: 23 Sep., 2024

**Preferred citation for this article**:
Chen B., Hou J.F., Cai Y.F., Wang G.Y., Cai R.X., and Zhao F.C., 2024, Genetic diversity in the genus *zea*: insights from chloroplast genome variability, Maize Genomics and Genetics, 15(5): 228-238 (doi: 10.5376/mgg.2024.15.0022)

**Abstract** The genus *Zea*, which includes maize and its wild relatives, exhibits significant genetic diversity, particularly within the chloroplast genome. This study investigates the variability in chloroplast genomes across different *Zea* species to understand their evolutionary relationships and potential for genetic improvement. By analyzing whole chloroplast genomes, researchers identified substantial nucleotide sequence variations and evolutionary rates among different *Zea* species. Our findings reveal that photosynthetic genes are under strong purifying selection, while other genes exhibit heterogeneous substitution rates, indicating diverse evolutionary pressures. This research highlights the importance of chloroplast genome diversity in the adaptation and evolution of *Zea* species, providing valuable insights for breeding programs aimed at enhancing stress tolerance and other agronomic traits.

**Keywords** Genetic diversity; Chloroplast genome; *Zea* species; Evolutionary rates; Plant breeding

## 1 Introduction

The genus *Zea*, particularly *Zea mays* (maize), holds a pivotal role in global agriculture and nutrition. Maize is one of the world's most important cereal crops, extensively cultivated for its versatility in human food, animal feed, and industrial applications (Nuss and Tanumihardjo, 2010; Lee et al., 2019; Revilla et al., 2022). Originating from Central America, maize has adapted to diverse environments, resulting in a wide range of genetic resources with significant variability (Revilla et al., 2022). This adaptability has made maize a staple food for a substantial portion of the global population, particularly in regions such as sub-Saharan Africa, Southeast Asia, and Latin America, where it is a primary source of nutrition (Nuss and Tanumihardjo, 2010). The genetic diversity within maize is not only crucial for its adaptability and yield improvement but also for its nutritional enhancement through biofortification strategies (Nuss and Tanumihardjo, 2010; Dong et al., 2023).

The chloroplast genome plays a critical role in plant phylogeny and evolutionary studies. Chloroplast DNA (cpDNA) is maternally inherited in most plant species and exhibits a relatively slow mutation rate compared to nuclear DNA, making it a valuable tool for studying evolutionary relationships and genetic diversity among plant species (Vivodík et al., 2017). In maize, the chloroplast genome has been utilized to explore genetic diversity and phylogenetic relationships, providing insights into the evolutionary history and domestication of this important crop (Strable and Scanlon, 2009; Vivodík et al., 2017). The cpDNA markers are particularly useful in assessing genetic variation and identifying distinct genetic lineages within the genus *Zea*, which can inform breeding programs and conservation efforts (Vivodík et al., 2017).

This research is to investigate the genetic diversity within the genus *Zea* by analyzing chloroplast genome variability. This study aims to elucidate the phylogenetic relationships among different *Zea* species and subspecies, with a particular focus on *Zea mays*. By leveraging cpDNA markers, we seek to uncover the extent of genetic variation and identify distinct genetic lineages within the genus. This research will contribute to a deeper understanding of the evolutionary history and domestication processes of maize, providing valuable information for breeding programs aimed at improving crop yield, adaptability, and nutritional quality. Additionally, the findings will have implications for the conservation of genetic resources within the genus *Zea*, ensuring the sustainability and resilience of this vital crop in the face of environmental challenges.

## 2 Methods for Comparing Chloroplast Genes

### 2.1 Collection and analysis of chloroplast genome data

To investigate the genetic diversity within the genus *Zea*, we collected chloroplast genome data from various sources. The primary data sources included newly sequenced genomes and publicly available sequences from databases such as GenBank. Sequencing technologies employed in these studies ranged from traditional Sanger sequencing to next-generation sequencing platforms like Illumina, which provide high-throughput and accurate genome sequences (Bayly et al., 2013; Li et al., 2020; Loeuille et al., 2021).

The analysis of chloroplast genome variability involved several bioinformatics tools and methods. For instance, tools like GYDLE Inc. pipelines were used for direct chloroplast genome assembly, ensuring high-quality finished genomes without the need for PCR gap-filling or contig order resolution (Bayly et al., 2013). Comparative genomics tools such as CGView Comparison Tool (CCT) were utilized to compare chloroplast genomes across different species, identifying structural variations and sequence similarities (Gao et al., 2019). Additionally, software like MEGA and PAUP* were employed for phylogenetic analyses, while custom scripts in languages like Python and R were used for detailed sequence analysis and visualization (Doebley et al., 1987; Dong et al., 2012).

### 2.2 Genome alignment and variation detection

Aligning chloroplast genome sequences is a critical step in identifying genetic variations. Multiple sequence alignment tools such as MAFFT and ClustalW were used to align the chloroplast genomes of *Zea* species and their relatives. These tools help in aligning sequences accurately, allowing for the detection of conserved and variable regions (Saski et al., 2007; Li et al., 2020; Loeuille et al., 2021).

To identify and classify variations, several strategies were employed. Single nucleotide polymorphisms (SNPs) and insertions/deletions (indels) were detected using tools like GATK and SAMtools, which provide robust methods for variant calling from aligned sequence data (Dong et al., 2012; Li et al., 2020). Additionally, regions with high nucleotide diversity were identified using sliding window analysis, which helps in pinpointing hotspots of genetic variation (Shaw et al., 2007; Xie et al., 2018). The identified variations were then annotated using databases like dbSNP and tools such as SnpEff, which provide functional insights into the detected variants (Doebley et al., 1987; Soltis et al., 1991).

### 2.3 Phylogenetic and population genetics analysis

Phylogenetic tree construction is essential for understanding the evolutionary relationships among species. In this study, phylogenetic trees were constructed using maximum parsimony and Bayesian inference methods. Software like MrBayes and RAxML were employed to generate phylogenetic trees based on chloroplast genome sequences, providing insights into the interspecies relationships within the genus *Zea* (Saski et al., 2007; Bayly et al., 2013). These analyses revealed that chloroplast DNA data could produce trees consistent with other measures of species affinity, such as isoenzymatic and morphological data (Doebley et al., 1987).

Population genetics analysis was conducted to study gene flow and population structure within Zea species. Tools like STRUCTURE and Arlequin were used to analyze genetic diversity and population structure, providing insights into the genetic differentiation and admixture among populations (Xie et al., 2018). These analyses were complemented by the use of highly variable chloroplast markers, which are particularly useful for evaluating phylogeny at low taxonomic levels and for DNA barcoding (Soltis et al., 1991; Dong et al., 2012). The combination of phylogenetic and population genetics analyses allowed for a comprehensive understanding of the genetic diversity and evolutionary history of the genus *Zea*.

## 3 Genetic Diversity in *Zea* Revealed by Chloroplast Genome Variability

### 3.1 Structural variation in chloroplast genomes

The chloroplast genomes of *Zea* species exhibit notable structural variations that contribute to our understanding of genetic diversity within this genus. Structural rearrangements, such as inversions and transpositions, have been observed in the chloroplast genomes of various plant species, including *Zea*. For instance, in the genus Anemoneae, multiple inversions and transpositions were detected, which provided significant phylogenetic information (Liu et al., 2018). Similarly, in *Zea*, the distribution of restriction site mutations throughout the chloroplast genome indicates that different regions of the genome evolve at different rates, with the inverted repeat regions evolving more slowly than the unique sequence regions (Doebley et al., 1987).

These structural variations can have profound impacts on species adaptation and evolution. For example, the presence of large and small inversions in the chloroplast genomes of Aldama species suggests that such structural changes may play a role in the evolutionary processes of these plants (Loeuille et al., 2021). In *Zea*, the structural variations in the chloroplast genome could influence the plant's ability to adapt to different environmental conditions, thereby contributing to the evolutionary success of the genus.

### 3.2 Single nucleotide polymorphisms and insertions/deletions

Single nucleotide polymorphisms (SNPs) and insertions/deletions (indels) are critical markers of genetic differentiation among *Zea* species. In the chloroplast genomes of Zingiber species, a significant number of SNPs and indels were identified, highlighting the genetic diversity within the genus (Li et al., 2020). Similarly, in *Zea*, the analysis of 580 restriction sites in the chloroplast DNA revealed 24 variable sites, indicating the presence of nucleotide substitutions and other genetic variations (Doebley et al., 1987).

These variations reflect the genetic differentiation among *Zea* species. For instance, the comparative analysis of chloroplast genomes in the genus Urophysa identified numerous variable repeats and SSR markers, which are indicative of genetic diversity and differentiation (Xie et al., 2018). In *Zea*, the presence of SNPs and indels in the chloroplast genome can provide insights into the genetic relationships and evolutionary history of different species within the genus.

### 3.3 Phylogenetic relationships and species divergence

Phylogenetic relationships constructed based on chloroplast genome data are essential for understanding species divergence within *Zea*. Chloroplast DNA analysis has been used to produce phylogenetic trees that are consistent with other measures of species affinity, such as isoenzymatic and morphological data (Doebley et al., 1987). For example, the phylogenetic analysis of chloroplast genomes in the Heuchera group revealed complex relationships and instances of chloroplast capture, which have implications for understanding species divergence and hybridization events (Soltis et al., 1991).

The variability in chloroplast genomes plays a crucial role in elucidating the phylogenetic relationships and species divergence within *Zea*. In the case of Bretschneidera sinensis, the comparative analysis of chloroplast genomes from different geographical locations revealed significant genetic divergence, which was consistent with the species' geographical distribution (Shang et al., 2022). Similarly, in *Zea*, the analysis of chloroplast genome variability can help to identify distinct clades and understand the evolutionary processes that have shaped the diversity within the genus.

## 4 Gene Flow and Population Structure in *Zea*

### 4.1 Patterns of gene flow

The study of gene flow patterns in the genus *Zea*, as revealed by chloroplast genome variability, provides significant insights into the genetic connectivity among populations. Chloroplast DNA (cpDNA) is maternally inherited in most angiosperms, including *Zea*, and thus primarily reflects seed-mediated gene flow. Research has shown that cpDNA variation can be a powerful tool for understanding gene flow and population structure. For instance, a study on the genus *Zea* using restriction site variation in the chloroplast genome revealed that the cpDNA of maize (*Zea mays* subsp. *mays*) and some teosintes (*Z. mays* subsps. *mexicana* and *parviglumis*) are indistinguishable, supporting the hypothesis that maize is a domesticated form of teosinte (Doebley et al., 1987) This indicates a high level of gene flow between these subspecies, which has implications for the genetic diversity and adaptability of maize.

Gene flow influences population structure by facilitating the exchange of genetic material between populations, thereby reducing genetic differentiation. In *Zea*, the movement of seeds, as indicated by cpDNA, plays a crucial role in shaping the genetic landscape. The study of cpDNA haplotype variation within and among populations of Coreopsis grandiflora, another plant species, demonstrated that cpDNA polymorphisms can transcend species boundaries, suggesting significant interpopulational gene flow (Mason-Gamer et al., 1995). This pattern is likely similar in *Zea*, where gene flow via seeds can lead to the sharing of cpDNA haplotypes among geographically separated populations, thereby influencing population structure and genetic diversity.

### 4.2 Population structure and diversity

The analysis of population structure in *Zea*, as revealed by chloroplast genome data, provides a detailed understanding of genetic diversity among populations. Chloroplast genomes are highly conserved, yet they contain enough variation to distinguish between different populations and species. For example, a study on the chloroplast genomes of the genus Populus revealed dynamic patterns of evolution and significant variation in genome features such as size, gene content, and repeat sequences (Zhou et al., 2021). Similar analyses in *Zea* can uncover the extent of genetic diversity and the relationships between different populations.

Chloroplast genome data can help identify distinct genetic clusters within *Zea* populations. For instance, research on the chloroplast genomes of Urophysa species identified regions with high nucleotide diversity and positive selection, which are valuable for phylogenetic and population genetics studies (Xie et al., 2018). In *Zea*, such data can reveal the genetic structure of populations, highlighting areas of high diversity and potential adaptive significance. This information is crucial for understanding the evolutionary history and adaptive potential of *Zea* populations, as well as for conservation and breeding programs.

### 4.3 Environmental factors and genome variability

Environmental factors play a significant role in influencing chloroplast genome variability in *Zea* species. Different habitat conditions can impact gene flow and genetic diversity by creating barriers or facilitating the movement of seeds. For example, a study on the genetic diversity of Erysimum teretifolium, a plant species endemic to island-like habitats, found that environmental barriers led to significant genetic differentiation among populations (Valle et al., 2020). In *Zea*, similar environmental factors, such as geographical barriers and habitat fragmentation, can influence the distribution of cpDNA haplotypes and overall genetic diversity.

The impact of different habitat conditions on gene flow and genetic diversity in *Zea* can be profound. For instance, research on the chloroplast genomes of Aldama species revealed extremely low nucleotide diversity, suggesting that environmental stability and limited gene flow contribute to genetic conservation (Figure 1) (Loeuille et al., 2021). In contrast, dynamic environments with frequent disturbances may promote higher genetic diversity by facilitating gene flow and creating new opportunities for adaptation. Understanding these environmental influences is essential for managing *Zea* populations and ensuring their long-term survival and adaptability.

## 5 Conservation and Breeding Applications in *Zea*

### 5.1 Using chloroplast genome data for species conservation

Chloroplast genome variability offers a powerful tool for developing conservation strategies for *Zea* species. By analyzing the chloroplast DNA (cpDNA) of various *Zea* species, researchers can identify regions and populations with high genetic diversity, which are crucial for conservation efforts. For instance, the study on the genus *Zea* revealed that the nucleotide substitutions per site among *Zea* species are relatively low, indicating a conserved genetic structure (Doebley et al., 1987). This information can help prioritize conservation efforts towards populations that maintain unique genetic variations (Hu et al., 2024).

Moreover, comparative analyses of chloroplast genomes, as demonstrated in studies on other plant species, can reveal polymorphic simple sequence repeats (SSRs), single nucleotide polymorphisms (SNPs), and indels that are essential for assessing genetic variation and diversity (Figure 2) (Shang et al., 2022). These markers can be used to identify and protect genetically diverse populations of *Zea*, ensuring the preservation of their evolutionary potential.

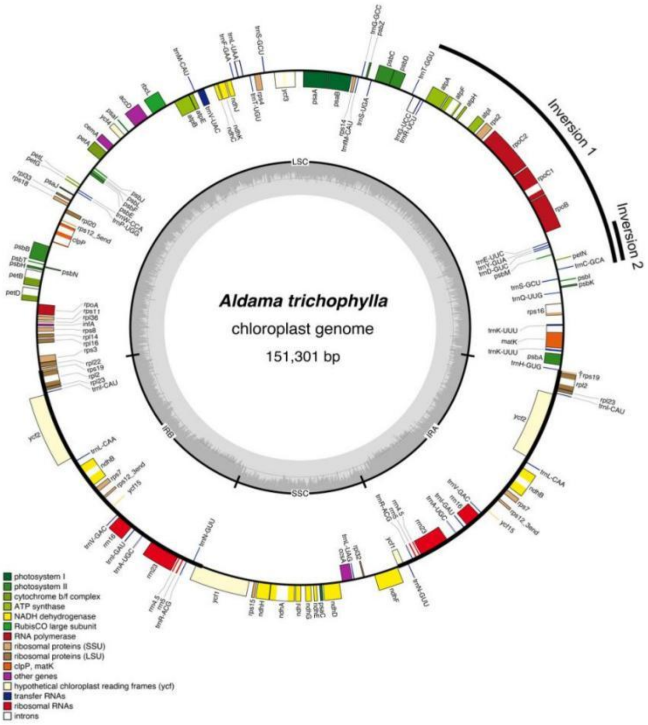

Figure 1 Gene map of the Aldama trichophylla plastome representing the genus Aldama and the plastomes of other five Heliantheae genera, which present the same general structure and gene content (Adopted from Loeuille et al., 2021)

Image caption: Genes drawn inside the circle are transcribed clockwise, and those outside are transcribed counterclockwise. Genes belonging to different functional groups are colored following the legend. The darker and lighter gray in the inner circle correspond to GC content and AT content, respectively (Adopted from Loeuille et al., 2021)

Phylogenetic analyses based on chloroplast genome data can also inform conservation strategies by identifying distinct genetic lineages and their geographical distributions. For example, the phylogenetic study of Zelkova schneideriana populations highlighted the importance of protecting populations with high nucleotide and haplotype diversity (Liu et al., 2016). Similar approaches can be applied to *Zea* species to identify and conserve genetically significant populations.

### 5.2 Breeding strategies based on genetic diversity

Chloroplast genome variability can significantly enhance breeding programs for *Zea* by providing insights into the genetic diversity and evolutionary history of different species and subspecies. The identification of beneficial gene variations through chloroplast genome analysis can guide the selection of parent lines for breeding, ultimately improving crop adaptability and yield.

For instance, the study on Zingiber species identified several highly divergent regions in the chloroplast genome that could be useful for species identification and phylogeny (Li et al., 2020). These regions can serve as markers for selecting *Zea* varieties with desirable traits. Additionally, the detection of positive selection in specific genes, such as those involved in ATP synthesis and photosynthesis, can inform breeding strategies aimed at enhancing these functions in *Zea* crops (Li et al., 2020; Fan et al., 2018).

Furthermore, the use of PCR-restriction fragment length polymorphism (RFLP) analysis to assess genetic variation in chloroplast DNA has proven effective in other plant families, such as Zingiberaceae (Ahmad et al., 2009). This technique can be adapted for *Zea* breeding programs to identify and select varieties with optimal genetic profiles for specific environmental conditions.

### 5.3 Long-term conservation and genetic resource management

The role of chloroplast genome data in the long-term management of genetic resources is multifaceted. It provides a comprehensive understanding of the genetic structure and diversity within and between *Zea* species, which is essential for developing effective conservation and management plans.

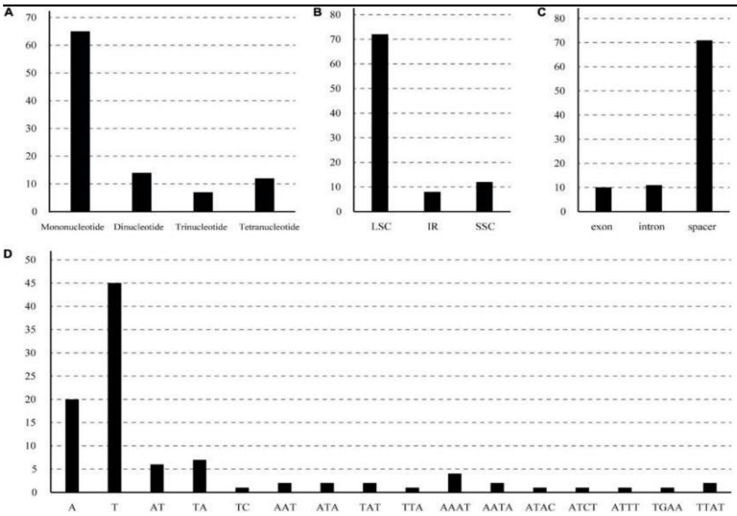

Figure 2 Type and distribution of simple sequence repeats (SSRs) in B. sinensis chloroplast genomes (Adopted from Shang et al., 2022)

Image caption: (A) Number of different SSR types detected by MISA; (B) Number of SSRs in the LSC, SSC, and IR regions; (C) Number of SSRs in spacers, exons, and introns; (D) Frequencies of identified SSR motifs in the different repeat classes (Adopted from Shang et al., 2022)

Long-term conservation efforts should focus on maintaining the genetic diversity of *Zea* species by protecting both in situ and ex situ populations. In situ conservation involves preserving natural habitats and ecosystems that support diverse *Zea* populations, while ex situ conservation includes seed banks and botanical gardens that safeguard genetic material for future use. The study on Bretschneidera sinensis emphasized the importance of ex situ conservation for endangered species, highlighting the need to preserve genetic variation through seed collections and other methods (Shang et al., 2022).

Future conservation efforts in *Zea* should also consider the integration of chloroplast genome data with other genomic resources, such as mitochondrial and nuclear genomes, to provide a holistic view of genetic diversity and evolutionary dynamics (Xie et al., 2018; Shang et al., 2022). This integrated approach can enhance the effectiveness of conservation strategies and ensure the long-term sustainability of *Zea* genetic resources.

## 6 Future Research Directions

### 6.1 Advances in chloroplast genomics technology

Emerging sequencing technologies, particularly long-read sequencing, are revolutionizing chloroplast genome research. Long-read sequencing technologies, such as those provided by Pacific Biosciences and Oxford Nanopore, allow for the generation of highly contiguous and accurate chloroplast genome assemblies. These technologies overcome the limitations of short-read sequencing by resolving complex regions, such as repetitive sequences and structural variations, which are often challenging to assemble accurately with short reads alone For instance, the hybrid assembly approach combining second- and third-generation sequencing has been successfully applied to analyze complex chloroplast genomes, revealing significant structural variations and expansions caused by long repeats (Figure 3) (Xu et al., 2023).

The future prospects of chloroplast genomics in *Zea* research are promising. With the continuous improvement in sequencing technologies, it is expected that more complete and accurate chloroplast genome sequences will be obtained, facilitating detailed comparative analyses and evolutionary studies. Additionally, the application of long-read sequencing can uncover previously undetected structural variations and rearrangements in the chloroplast genomes of *Zea* species, providing new insights into their evolutionary history and genetic diversity (Li et al., 2020; Xu et al., 2023). Furthermore, the integration of high-throughput sequencing data with advanced bioinformatics tools will enable the identification of novel genetic markers and the development of more robust phylogenetic frameworks for *Zea* species (Gallaher et al., 2018; Thode and Lohmann, 2019).

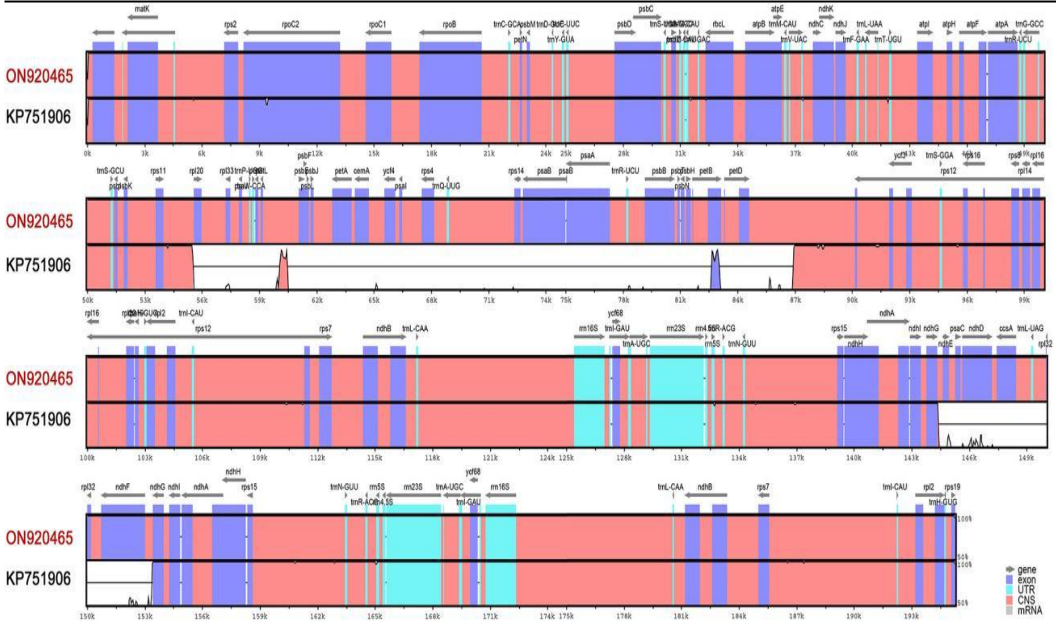

Figure 3 mVISTA identity diagram based on LAGAN alignment for Carex siderosticta assembled by two different methods and data sources (Adopted from Xu et al., 2023)

Image caption: ON920465 was used as the reference sequence, which was derived from a hybrid assembly of second and third generation data. A cut-off value of 70% similarity was used for the plot, and the Y scale indicates the percent similarity between 50 and 100%. Blue represents coding regions and pink represents non-coding regions (Adopted from Xu et al., 2023)

### 6.2 Integration of multi-omics data

Integrating chloroplast genome data with nuclear genome, transcriptome, and metabolome data offers a comprehensive understanding of evolution and adaptation in *Zea*. The chloroplast genome, being maternally inherited and relatively conserved, provides valuable information on the evolutionary history and phylogenetic relationships among species. However, to gain a holistic view of the genetic and functional diversity in *Zea*, it is essential to combine chloroplast genome data with other omics datasets.

The integration of multi-omics data allows for the identification of correlations between genetic variations in the chloroplast genome and phenotypic traits, such as stress tolerance, photosynthetic efficiency, and metabolic pathways. For example, RNA-Seq data can be used to quantify the expression levels of chloroplast genes under different environmental conditions, providing insights into the regulatory mechanisms underlying adaptation (Gallaher et al., 2018). Additionally, metabolomic analyses can reveal the impact of chloroplast genome variations on the production of key metabolites, contributing to our understanding of the metabolic networks in *Zea* (Gallaher et al., 2018; Li et al., 2020).

By combining chloroplast genome data with nuclear genome sequences, researchers can investigate the co-evolution of nuclear and chloroplast genomes and identify potential nuclear-encoded factors that influence chloroplast function. This integrative approach can also help in identifying candidate genes for crop improvement and breeding programs aimed at enhancing desirable traits in *Zea* species (Gallaher et al., 2018; Thode and Lohmann, 2019).

### 6.3 Unresolved questions and research outlook

Despite significant advancements in chloroplast genomics, several unresolved questions remain regarding chloroplast genome variability in *Zea*. One of the primary challenges is understanding the extent and impact of structural variations, such as inversions, duplications, and deletions, on the function and evolution of chloroplast genomes. While some studies have reported structural variations in chloroplast genomes of other plant species, the specific patterns and consequences of these variations in *Zea* are not well understood (Loeuille et al., 2021; Liu and Melton, 2021; Xu et al., 2023).

Another unresolved question is the role of positive selection in shaping the chloroplast genomes of *Zea* species. Although some genes have been identified as undergoing positive selection in other plant genera, the specific genes and selective pressures driving chloroplast genome evolution in *Zea* remain to be elucidated (Fan et al., 2018; Loeuille et al., 2021). Future research should focus on identifying and characterizing positively selected genes in *Zea* chloroplast genomes and understanding their functional significance in adaptation to different environmental conditions.

Additionally, the genetic diversity and phylogenetic relationships among *Zea* species based on chloroplast genome data need further investigation. While some studies have provided insights into the phylogenetic relationships within closely related genera, comprehensive phylogenetic analyses of *Zea* species using complete chloroplast genome sequences are still lacking (Shaw et al., 2007; Dong et al., 2012; Loeuille et al., 2021). Such analyses can help resolve taxonomic ambiguities and provide a clearer picture of the evolutionary history of *Zea*.

To address these unresolved questions, future research should employ advanced sequencing technologies and integrative approaches combining chloroplast genome data with other omics datasets. Comparative genomics studies involving multiple *Zea* species and closely related genera can reveal patterns of genome evolution and identify key genetic markers for phylogenetic and evolutionary studies (Shaw et al., 2007; Dong et al., 2012; Loeuille et al., 2021). Furthermore, functional studies using gene editing and transcriptomic analyses can elucidate the roles of specific genes and regulatory elements in chloroplast function and adaptation.

## 7 Concluding Remarks

This study has provided significant insights into the genetic diversity within the genus *Zea* by analyzing chloroplast genome variability. The comparative analysis of chloroplast genomes across different species and subspecies of *Zea* revealed a relatively low nucleotide diversity, with only 24 out of 580 restriction sites being variable. This low level of genetic variation is consistent with findings in other angiosperm genera and supports the hypothesis that maize (*Z. mays* subsp. *mays*) is a domesticated form of teosinte (Z. mays subsps. mexicana and parviglumis). The study also highlighted the conserved nature of chloroplast genomes within *Zea*, which aligns with observations in other plant genera where chloroplast genomes tend to be highly conserved.

Understanding chloroplast genome variability has profound implications for both conservation and agricultural applications in *Zea*. The identification of specific genetic markers and regions of variability can aid in the conservation of genetic diversity within wild and cultivated populations. For instance, the detection of polymorphic simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) in chloroplast genomes can be used to monitor genetic diversity and manage conservation efforts effectively. Additionally, the insights gained from chloroplast genome studies can inform breeding programs aimed at improving crop resilience and productivity. By identifying genes under positive selection, such as those involved in photosynthesis and stress responses, breeders can develop maize varieties with enhanced adaptability to changing environmental conditions.

The findings from this study have significant implications for future plant genomics research and agricultural improvement. The comprehensive analysis of chloroplast genomes provides a valuable genetic resource for further studies on the evolutionary history and phylogenetic relationships within *Zea* and related genera. The identification of highly divergent regions and genes under positive selection offers potential targets for genetic engineering and marker-assisted selection in crop improvement programs. Moreover, the methodologies and insights from this study can be applied to other plant species, enhancing our understanding of chloroplast genome evolution and its role in plant adaptation and diversity. This knowledge will be crucial in developing strategies to improve crop resilience, productivity, and sustainability in the face of global challenges such as climate change and food security.

In conclusion, this study underscores the importance of chloroplast genome research in revealing genetic diversity, informing conservation efforts, and driving agricultural innovation in the genus *Zea*. The integration of chloroplast genomic data with other molecular markers will continue to advance our understanding of plant genetics and contribute to the development of improved crop varieties.

## Acknowledgements

The authors would like to thank Mr. Zheng for his invaluable guidance, insightful suggestions, and continuous support throughout the development of this study

## Conflict of Interest Disclosure

The authors affirm 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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