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Application of Chloroplast Genomes in Phylogenetic and Taxonomic Studies of the *Cucumis* Genus

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Abstract This study explores the application of chloroplast genomes in addressing phylogenetic and taxonomic challenges within the *Cucumis* genus. Chloroplast genomes, due to their conserved nature and maternal inheritance, provide valuable genetic markers for elucidating evolutionary relationships and resolving taxonomic ambiguities. The complete sequencing of chloroplast genomes from various *Cucumis* species, including synthetic allotetraploids like *Cucumis*×*hytivus*, has revealed significant genetic variations such as indels and SNPs that clarify species' evolutionary positions. Comparative genomic studies have also identified hypervariable regions and mutational hotspots within chloroplast DNA, which are essential for species identification and understanding adaptive evolution. Integrating chloroplast genomic data with nuclear and mitochondrial genomes has proven effective in constructing robust phylogenetic frameworks and resolving deep phylogenetic nodes. Furthermore, these genomic insights have practical applications in breeding programs, where they aid in developing new cultivars with desirable traits. Future research should focus on expanding chloroplast genome sequencing across a broader range of *Cucumis* species and employing advanced genomic technologies to enhance phylogenetic resolution and understand the genus's evolutionary history comprehensively.

Keywords *Cucumis*; Chloroplast genomes; Phylogenetics; Taxonomy; Genetic markers

1 Introduction

The *Cucumis* genus, belonging to the Cucurbitaceae family, encompasses a variety of species that are of significant economic and botanical importance. This genus includes well-known crops such as cucumbers (*Cucumis sativus*) and melons (*Cucumis melo*), which are cultivated worldwide for their nutritional and economic value. *Cucumis* species are not only vital for human consumption but also play a crucial role in agricultural systems due to their diverse uses and adaptability to different climatic conditions. The genus is characterized by a wide range of morphological diversity, which has led to challenges in its taxonomic classification and phylogenetic relationships (Zhai et al., 2021; Xia et al., 2023).

Chloroplast genomes (cp genomes) have emerged as powerful tools in phylogenetic and taxonomic studies due to their highly conserved nature and maternal inheritance. The cp genome provides valuable molecular markers that can be used to resolve phylogenetic relationships and clarify taxonomic ambiguities within and among plant species (Fučíková et al., 2016; Somaratne et al., 2019). The comparative analysis of cp genomes allows for the identification of mutational hotspots, simple sequence repeats (SSRs), and other genetic variations that are crucial for species identification and evolutionary studies (Li et al., 2020; Alzahrani et al., 2021). In the context of the *Cucumis* genus, cp genomes have been instrumental in understanding the genetic and evolutionary dynamics, especially in complex scenarios such as allopolyploidization (Zhai et al., 2021; Xia et al., 2023).

The aim of this study is to explore the application of chloroplast genomes in addressing phylogenetic and taxonomic challenges within the *Cucumis* genus. By leveraging the complete chloroplast genome sequences, this study aims to clarify the phylogenetic relationships among *Cucumis* species, including those with complex genetic backgrounds, by constructing robust phylogenetic trees based on chloroplast genome data. Additionally, it seeks to resolve taxonomic ambiguities by identifying and analyzing genetic markers within the chloroplast genomes that can aid in the accurate classification and differentiation of *Cucumis* species, thereby addressing existing taxonomic discrepancies. Furthermore, the study aims to enhance our understanding of the evolutionary dynamics within the *Cucumis* genus by investigating genetic variations and evolutionary patterns in their chloroplast genomes, with a particular focus on hybridization and polyploidization events.

2 Chloroplast Genome Structure and Features

2.1 General structure and function of chloroplast genomes in plants

Chloroplast genomes in plants are typically circular DNA molecules that are highly conserved across different species. They generally exhibit a quadripartite structure, which includes a large single-copy (LSC) region, a small single-copy (SSC) region, and two inverted repeat (IR) regions (Niu et al., 2023; Xia et al., 2023; Xin et al., 2023). The size of chloroplast genomes can vary, but they usually range from approximately 150 000 to 170 000 base pairs (bp) (Liang et al., 2020). These genomes encode essential genes for photosynthesis and other metabolic processes, including protein-coding genes, transfer RNA (tRNA) genes, and ribosomal RNA (rRNA) genes (Abdullah et al., 2020). The conserved nature of chloroplast genomes makes them valuable tools for studying plant phylogeny, species identification, and evolutionary relationships (Wang et al., 2023).

2.2 Specific characteristics of chloroplast genomes in *Cucumis* species

The chloroplast genomes of *Cucumis* species, such as cucumber (*Cucumis sativus*), exhibit typical quadripartite structures with sizes ranging from approximately 154 673 to 157 641 bp. These genomes consist of a large single-copy (LSC) region, a small single-copy (SSC) region, and two inverted repeats (IRs) (Zhai et al., 2021). Comparative genomic analyses have revealed significant genetic variation within the chloroplast genomes of different *Cucumis* species, particularly between Indian ecotype cucumbers and other cultivars. This genetic diversity is crucial for understanding the evolutionary history and adaptation mechanisms of *Cucumis* species. Additionally, studies have shown that chloroplast genes in *Cucumis* species respond to environmental stresses, such as temperature changes, by regulating lipid metabolism and ribosome metabolism (Xia et al., 2023).

2.3 Advances in sequencing technologies for chloroplast genomes

Advances in sequencing technologies, particularly next-generation sequencing (NGS), have revolutionized the study of chloroplast genomes. NGS allows for the rapid and accurate assembly of complete chloroplast genomes, facilitating comparative genomic analyses and phylogenetic studies (Liang et al., 2020; Song et al., 2022). The use of NGS has enabled researchers to sequence and characterize the chloroplast genomes of numerous plant species, including those within the *Cucumis* genus. These technologies have also made it possible to identify hypervariable regions and simple sequence repeats (SSRs) within chloroplast genomes, which can serve as molecular markers for species identification and genetic diversity studies. The increasing availability of complete chloroplast genome sequences in public databases has further enhanced our understanding of chloroplast genome evolution and its implications for plant taxonomy and phylogeny (Zhou et al., 2021).

3 Phylogenetic Analysis Using Chloroplast Genomes

3.1 Techniques for chloroplast genome-based phylogenetic studies

Chloroplast genome-based phylogenetic studies leverage the highly conserved nature of chloroplast DNA (cpDNA) to infer evolutionary relationships among plant species. Techniques commonly used include whole chloroplast genome sequencing, comparative genomic analysis, and the identification of hypervariable regions that serve as molecular markers. For instance, sequencing and comparative analysis of chloroplast genomes have been employed to study genetic variations and evolutionary relationships in various plant species, such as *Cucumis*, Cleomaceae, and *Mangifera* (Alzahrani et al., 2021; Zhai et al., 2021). These studies often involve the assembly of complete chloroplast genomes, annotation of genes, and the use of phylogenetic trees constructed through methods like maximum likelihood (ML) and Bayesian Inference (BI) (Liu et al., 2021; Xin et al., 2023). Additionally, the identification of simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) within the chloroplast genome can provide further insights into genetic diversity and phylogenetic relationships (Li et al., 2020).

3.2 Phylogenetic relationships within the *Cucumis* genus based on chloroplast DNA

Phylogenetic analysis within the *Cucumis* genus using chloroplast DNA has revealed significant insights into the evolutionary relationships and taxonomic positions of various species. For example, the chloroplast genome of the synthetic allotetraploid *Cucumis*×*hytivus* and its diploid parents (*C. hystrix* and *C. sativus*) was sequenced and compared, revealing genetic variations such as indels and SNPs (Zhai et al., 2021). This analysis clarified the evolutionary and taxonomic position of *C.*×*hytivus* within the *Cucumis* subgenus. Similarly, the construction of a cucumber chloroplast pan-genome based on 121 cucumber germplasms identified three distinct phylogenetic groups: East Asian, Eurasian + Indian, and Xishuangbanna + Indian (Xia et al., 2023). Figure 1 presents a genetic variation and structure analysis of 50 cucumber chloroplast genomes, illustrating the nucleotide diversity and SNP-based phylogenetic relationships, which are crucial for understanding the genetic basis of species differentiation and breeding strategies within the *Cucumis* genus.

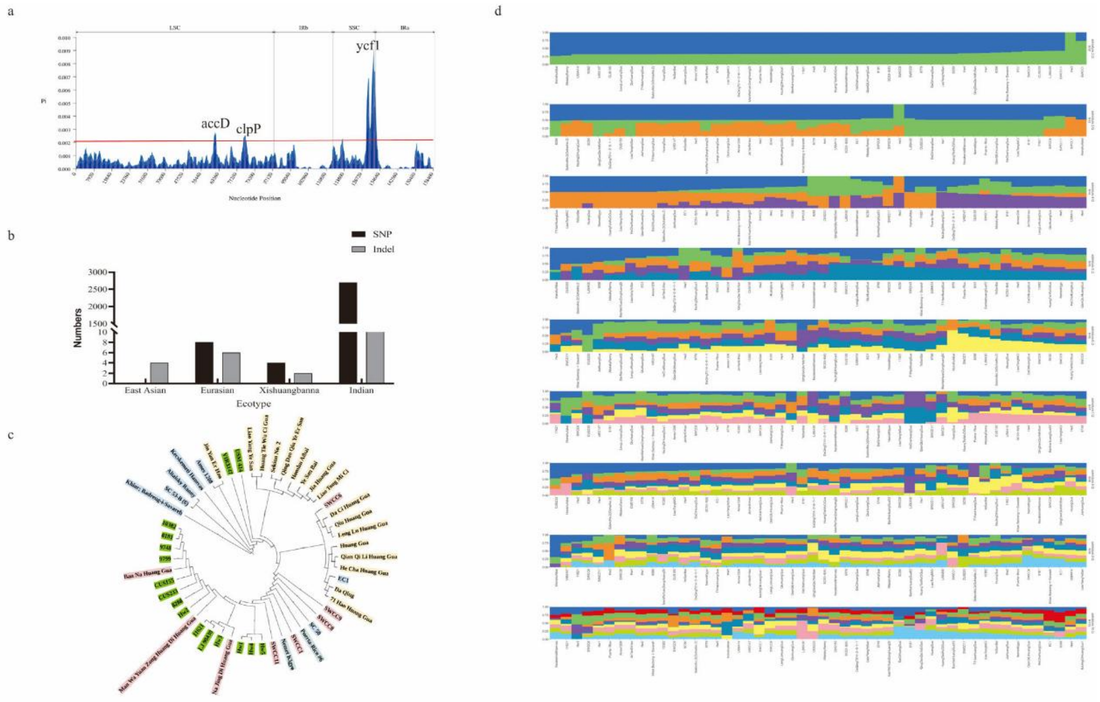

Figure 1 Genetic variation and structure analysis of 50 cucumber chloroplast genomes (Adopted from Xia et al., 2023)

Image caption: (a) Statistics of nucleotide diversity (Pi) in 50 cucumber chloroplast genomes with parameters of a window length of 1 000 bp and a step size of 100 bp. (b) The variations in 50 cucumber chloroplast genomes. The phylogenetic tree (c) and population structure analyses (d) are based on SNP (Adopted from Xia et al., 2023)

3.3 Comparison of chloroplast-derived phylogenetic trees with nuclear genomic data

Comparing phylogenetic trees derived from chloroplast genomes with those based on nuclear genomic data can provide a more comprehensive understanding of plant evolution and taxonomy. Chloroplast genomes, being maternally inherited and highly conserved, often reveal different evolutionary patterns compared to nuclear genomes, which undergo biparental inheritance and recombination. For instance, in the study of the synthetic allotetraploid *Cucumis*×*hytivus*, chloroplast genome analysis supported maternal inheritance and provided insights into cytonuclear evolution (Zhai et al., 2021). In contrast, nuclear genomic studies might focus on different aspects of genetic variation and evolutionary history. The integration of both chloroplast and nuclear genomic data can help resolve discrepancies and provide a more robust phylogenetic framework. For example, in the case of the cucumber, chloroplast genome analysis revealed distinct phylogenetic groups, while nuclear genomic studies might further elucidate the genetic basis of these groupings and their evolutionary significance (Xia et al., 2023).

4 Taxonomic Implications of Chloroplast Genomic Studies

4.1 Resolving taxonomic discrepancies in the *Cucumis* genus using chloroplast DNA

Chloroplast DNA (cpDNA) has proven to be a valuable tool in resolving taxonomic discrepancies within various plant genera, including *Cucumis*. The comparative analysis of chloroplast genomes can reveal significant genetic variations that are not apparent through morphological studies alone. For instance, the study on the synthetic allotetraploid *Cucumis*×*hytivus* and its diploid parents demonstrated that chloroplast genomes can clarify evolutionary and taxonomic positions by identifying specific genetic variations such as Indels and SNPs (Zhai et al., 2021). Similarly, the identification of mutational hotspots and highly polymorphic regions in other genera, such as *Artemisia* and *Abelmoschus*, has been instrumental in resolving taxonomic ambiguities and inferring phylogenetic relationships (Li et al., 2020; Shahzadi et al., 2020). These findings underscore the potential of cpDNA in addressing taxonomic challenges within the *Cucumis* genus.

4.2 Chloroplast genome markers for species identification and classification

Chloroplast genome markers are essential for species identification and classification due to their high resolution and specificity. In the genus *Chenopodium*, for example, the development of species-specific molecular markers based on InDels and SNPs has enabled the precise identification of closely related species (Wei et al., 2023). Similarly, in the genus Abelmoschus, mutational hotspots such as start-psbA and atpB-rbcL have been identified as potential markers for resolving taxonomic discrepancies (Li et al., 2020). Figure 2 illustrates the chloroplast genome map of three *Abelmoschus* species, highlighting the organization of genes within the chloroplast genome and their transcriptional orientation, which provides a comparative framework for understanding the chloroplast genome structures across different plant genera. These markers are particularly useful in distinguishing species with high morphological similarity, as demonstrated in the study of *Lespedeza* species, where complete chloroplast genomes provided unique molecular features for accurate species identification (Somaratne et al., 2019). The application of such markers in the *Cucumis* genus could significantly enhance the accuracy of species classification and identification.

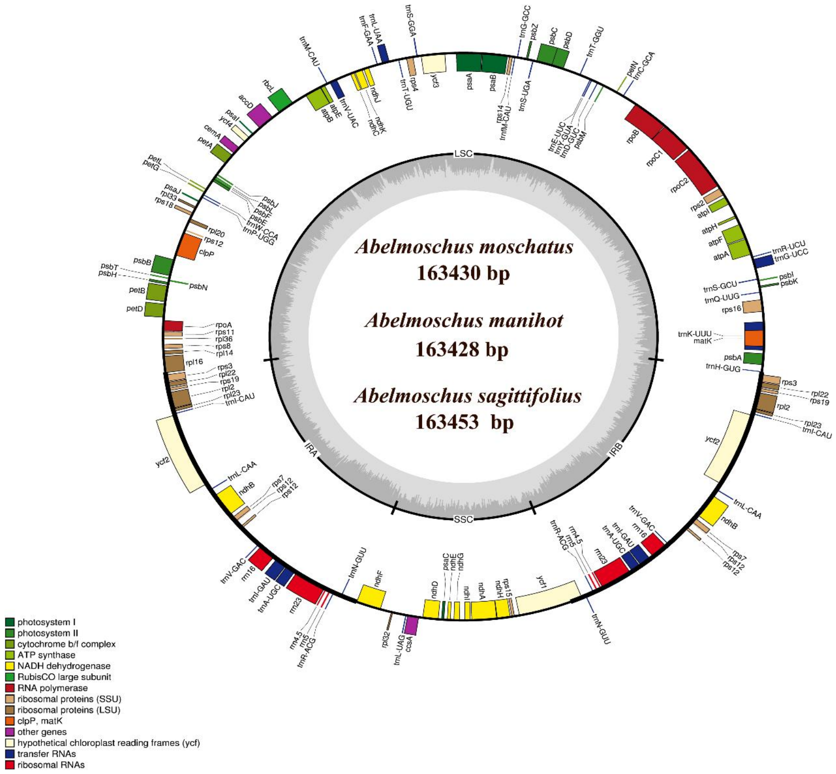

Figure 2 Chloroplast genome map of three *Abelmoschus* species (Adopted from Li et al., 2020)

Image caption: Genes shown outside the circle are transcribed clockwise and those inside counterclockwise. Genes belonging to different functional groups are color-coded (Adopted from Li et al., 2020)

4.3 Implications of chloroplast genomics for understanding speciation and divergence

Chloroplast genomics offers profound insights into the processes of speciation and divergence. The study of chloroplast genomes in the Zingiberaceae family revealed that hybridization events within related groups could explain the complex phylogeny observed in these species (Liang et al., 2020). This highlights the role of chloroplast DNA in tracing evolutionary histories and understanding the mechanisms of speciation. In the case of *Cucumis*, the comparative analysis of chloroplast genomes between allotetraploid and diploid species provided evidence of cytonuclear evolution and the genetic changes associated with allopolyploidization (Zhai et al., 2021). Furthermore, the identification of hypervariable regions in the chloroplast genomes of Eragrostideae species has facilitated the understanding of interspecies relationships and divergence within the tribe (Liu et al., 2021). These studies collectively demonstrate that chloroplast genomics is a powerful tool for elucidating the evolutionary dynamics and speciation processes in the *Cucumis* genus and beyond.

5 Case Studies: Chloroplast Genomes in Cucumis Taxonomy

5.1 Resolving intraspecific taxonomic conflicts in the *Cucumis* genus using chloroplast genomics

Chloroplast genomics has proven to be a powerful tool in resolving intraspecific taxonomic conflicts within the *Cucumis* genus. The comparative analysis of chloroplast genomes from different *Cucumis* species has revealed significant genetic variations that can clarify taxonomic ambiguities. For instance, the study on the synthetic allotetraploid *Cucumis*×*hytivus*, derived from hybridization between *C. hystrix* and *C. sativus*, demonstrated that chloroplast genomes can provide clear insights into the evolutionary and taxonomic positions of hybrid species. The identification of 51 Indels and 292 SNPs in the chloroplast genome of *C.*×*hytivus* relative to its female parent C. hystrix highlights the potential of chloroplast genomics in resolving taxonomic conflicts by providing detailed genetic information (Zhai et al., 2021).

5.2 Application of chloroplast genome sequencing to uncover cryptic species within *Cucumis*

Chloroplast genome sequencing has been instrumental in uncovering cryptic species within the *Cucumis* genus. Cryptic species, which are morphologically similar but genetically distinct, can be difficult to identify using traditional taxonomic methods. The comprehensive sequencing and analysis of chloroplast genomes allow for the detection of subtle genetic differences that may not be apparent through morphological studies alone. For example, the construction of a cucumber chloroplast pan-genome based on 121 cucumber germplasms revealed significant genetic variation among different ecotypes, particularly in Indian cucumbers. This genetic diversity suggests the presence of cryptic species that were previously unrecognized, thereby enhancing our understanding of the genetic landscape within the *Cucumis* genus (Xia et al., 2023).

5.3 Use of chloroplast DNA barcoding in re-evaluating phylogenetic relationships among *Cucumis* species

Chloroplast DNA barcoding has emerged as a valuable method for re-evaluating phylogenetic relationships among *Cucumis* species. By analyzing specific regions of the chloroplast genome, researchers can construct detailed phylogenetic trees that provide insights into the evolutionary relationships between species. The study of the chloroplast genomes of various *Cucumis* species has identified several hypervariable regions that serve as effective DNA barcodes. These regions, such as those identified in the comparative analysis of the chloroplast genomes of *Dipterygium glaucum* and *Cleome chrysantha*, can be used to infer phylogenetic relationships with high confidence. The identification of hotspot genes like *ycf1* and *rpoC2* further supports the utility of chloroplast DNA barcoding in resolving phylogenetic relationships within the *Cucumis* genus (Alzahrani et al., 2021).

6 Functional Applications of Chloroplast Genomic Data

6.1 Practical applications of chloroplast genomic data in cultivar development and breeding programs

Chloroplast genomic data have proven to be invaluable in the development of new cultivars and the enhancement of breeding programs. The highly conserved nature of chloroplast genomes allows for the identification of genetic variations that can be exploited for cultivar improvement. For instance, the study on cucumber chloroplast genomes revealed significant genetic variations among different ecotypes, particularly in Indian cucumbers, suggesting untapped genetic resources that could be harnessed for breeding programs (Xia et al., 2023). Additionally, the inheritance patterns of chloroplast genomes, as demonstrated in reciprocal F_1 hybrids of cucumber, confirm maternal inheritance, which is crucial for maintaining desired traits in breeding lines (Park et al., 2021). These insights facilitate the development of molecular markers that can be used for purity testing of hybrid seeds, ensuring the consistency and quality of new cultivars.

6.2 Role of chloroplast genomes in understanding adaptive evolution

Chloroplast genomes play a critical role in understanding how plants adapt to environmental stresses. The comparative analysis of cucumber chloroplast genomes under temperature stress conditions highlighted the up-regulation of specific genes such as *matK* and *accD*, which are involved in lipid and ribosome metabolism, respectively. These findings suggest that chloroplasts contribute to temperature tolerance by modulating metabolic pathways (Xia et al., 2023). Similarly, the study on the chloroplast genomes of *Populus* species revealed dynamic patterns of evolution, including variations in genome size, gene content, and repeat sequences, which are essential for understanding the adaptive mechanisms of this genus (Zhou et al., 2021). These evolutionary insights can inform breeding strategies aimed at enhancing stress tolerance in crops.

6.3 Breeding programs benefiting from chloroplast genomic insights

Several breeding programs have benefited from the insights provided by chloroplast genomic data. For example, the synthetic allotetraploid *Cucumis*×*hytivus*, derived from hybridization between *C. hystrix* and *C. sativus*, exhibited significant genetic variations in its chloroplast genome compared to its diploid parents. These variations have clarified the evolutionary and taxonomic position of the synthetic allotetraploid, providing a foundation for future breeding efforts (Zhai et al., 2021). Additionally, the complete chloroplast genome sequences of Cleomaceae species have identified variable regions that can serve as molecular markers for species authentication and phylogenetic studies, aiding in the selection of desirable traits for breeding (Alzahrani et al., 2021). These case studies underscore the practical applications of chloroplast genomic data in enhancing the efficiency and effectiveness of breeding programs.

7 Challenges and Limitations of Chloroplast Genomics in Phylogenetics

7.1 Technical challenges in sequencing and assembling chloroplast genomes

Sequencing and assembling chloroplast genomes present several technical challenges. One significant issue is the presence of highly conserved regions and repetitive sequences, which can complicate the assembly process. For instance, the study on the chloroplast genomes of *Coelogyne* spp. highlighted the strong structural and gene content similarities, which can make distinguishing between closely related species difficult (Jiang et al., 2020). Additionally, the presence of inverted repeats (IRs) and simple sequence repeats (SSRs) can lead to assembly errors and misalignments, as seen in the comparative analysis of *Euonymus* chloroplast genomes (Li et al., 2021). These technical difficulties necessitate the use of advanced sequencing technologies and bioinformatics tools to ensure accurate assembly and annotation.

7.2 Limitations of chloroplast genomic data in resolving deep phylogenetic nodes

While chloroplast genomes are valuable for phylogenetic studies, they have limitations in resolving deep phylogenetic nodes. The highly conserved nature of chloroplast genomes can result in insufficient genetic variation to distinguish between distantly related taxa. For example, the study on the Ranunculaceae family found that many traditional taxonomic characters were unreliable due to parallel, convergent, or reversal evolution, which complicates the resolution of deep phylogenetic relationships (Zhai et al., 2019). Similarly, the analysis of the *Ficus sarmentosa* complex revealed that despite the promise of chloroplast genomes for studying evolutionary relationships, cyto-nuclear discordance can still pose challenges (Zhang et al., 2022). These limitations highlight the need for integrating chloroplast genomic data with nuclear and mitochondrial genomes to achieve a more comprehensive phylogenetic resolution.

7.3 Future directions to overcome current limitations in chloroplast genome research

To overcome the current limitations in chloroplast genome research, several future directions can be pursued. First, the development and application of more sophisticated sequencing technologies, such as long-read sequencing, can help resolve repetitive regions and improve genome assembly accuracy (Zhai et al., 2021). Second, integrating chloroplast genomic data with nuclear and mitochondrial genomes can provide a more holistic view of phylogenetic relationships and help resolve deep nodes (Zhai et al., 2019). Third, expanding the taxonomic sampling and constructing pan-genomes, as demonstrated in the cucumber chloroplast pan-genome study, can uncover more genetic variations and provide insights into species adaptation and evolution (Xia et al., 2023). Finally, the identification and utilization of hypervariable regions and positive selection genes, as seen in the studies on *Amphilophium* and Eragrostideae, can enhance the resolution of phylogenetic analyses at both shallow and deep levels (Thode and Lohmann, 2019; Liu et al., 2021).

8 Integrative Approaches in Genomic Studies

8.1 Combining chloroplast genomic data with nuclear and mitochondrial genomes

Integrating chloroplast genomic data with nuclear and mitochondrial genomes provides a comprehensive view of plant evolution and taxonomy. For instance, the study on the synthetic allotetraploid *Cucumis*×*hytivus* demonstrated how chloroplast genomes can be used alongside nuclear data to understand cytonuclear variation and evolutionary processes post-allopolyploidization (Zhai et al., 2021). Similarly, the research on the *Amelanchier-Malacomeles-Peraphyllum* clade utilized both chloroplast and nuclear ribosomal DNA to resolve phylogenetic relationships, highlighting the importance of combining different genomic data sources to address complex evolutionary questions (Liu et al., 2020). These integrative approaches allow for a more robust reconstruction of phylogenetic trees and can clarify taxonomic ambiguities that single-genome studies might miss.

8.2 Role of multi-locus phylogeny in enhancing resolution of evolutionary relationships

Multi-locus phylogeny, which involves the use of multiple genetic loci from different genomes, significantly enhances the resolution of evolutionary relationships. For example, the study on the *Ficus sarmentosa* complex used multiple chloroplast genomes to identify hypervariable regions and phylogenetic performance, which provided a clearer picture of the evolutionary dynamics within this taxonomically complex group (Zhang et al., 2022). Additionally, the research on *Euonymus* species combined data from various chloroplast regions to construct a detailed evolutionary tree, demonstrating the effectiveness of multi-locus approaches in resolving phylogenetic relationships (Li et al., 2021). These studies underscore the value of using multiple loci to achieve higher resolution and more accurate phylogenetic inferences.

8.3 Potential of integrative genomics in comprehensive taxonomic studies

Integrative genomics, which combines data from chloroplast, nuclear, and mitochondrial genomes, holds great potential for comprehensive taxonomic studies. The comparative analysis of chloroplast genomes in the genus *Populus* revealed dynamic patterns of evolution and provided corrected annotations, showcasing how integrative approaches can refine taxonomic classifications and improve evolutionary inferences (Zhou et al., 2021). Similarly, the study on *Bupleurum* species used chloroplast genome data to explore evolutionary characteristics and phylogenetic relationships, demonstrating the utility of integrative genomics in understanding the taxonomy and evolution of complex plant groups (Huang et al., 2021). By leveraging the strengths of different genomic data sources, integrative genomics can offer a holistic view of plant taxonomy and evolution, leading to more accurate and comprehensive classifications.

9 Future Research Directions

9.1 Emerging technologies and their potential impact on chloroplast genome research

The advent of next-generation sequencing (NGS) technologies has revolutionized the field of chloroplast genomics, enabling the rapid and cost-effective sequencing of complete chloroplast genomes. These technologies have facilitated the assembly and comparative analysis of chloroplast genomes across various species, providing insights into genetic variation, phylogenetic relationships, and evolutionary processes (Zhai et al., 2021). For instance, the use of NGS allowed for the construction of the first cucumber chloroplast pan-genome, revealing significant genetic variations and adaptations to temperature stress (Xia et al., 2023). Additionally, the application of NGS in the study of *Curcuma* species has provided valuable data on genome evolution and phylogenetic relationships within the Zingiberaceae family (Liang et al., 2020). Future research should focus on integrating NGS with other emerging technologies such as CRISPR-Cas9 for targeted genome editing, which could further elucidate the functional roles of specific chloroplast genes and their contributions to plant adaptation and evolution.

9.2 Collaborative efforts and data sharing in *Cucumis* genomic studies

Collaborative efforts and data sharing are crucial for advancing our understanding of chloroplast genomics in the *Cucumis* genus. The establishment of comprehensive databases that include chloroplast genome sequences, genetic variations, and associated phenotypic data can facilitate comparative studies and meta-analyses. For example, the comparative analysis of chloroplast genomes from different *Cucumis* species has already provided insights into cytonuclear evolution and the taxonomic position of synthetic allotetraploids (Zhai et al., 2021). Collaborative projects that pool resources and expertise from multiple research groups can accelerate the discovery of novel genetic markers and the development of robust phylogenetic frameworks. Additionally, open-access repositories and bioinformatics tools should be promoted to ensure that data generated from chloroplast genome studies are readily available to the scientific community, fostering a collaborative environment for ongoing research (Li et al., 2021; Park et al., 2021).

9.3 Priorities for future research in chloroplast genomics and their applications

Future research in chloroplast genomics should prioritize the following areas to enhance our understanding and application of chloroplast genome data. Investigating the genetic diversity within and between *Cucumis* species using chloroplast genome data can uncover valuable genetic resources for breeding programs. Studies have shown that certain ecotypes, such as Indian cucumbers, harbor significant genetic variation that remains underexplored (Xia et al., 2023). Functional characterization of chloroplast genes, particularly those involved in stress responses and adaptation, can provide insights into the mechanisms underlying plant resilience. For example, the upregulation of *matK* under temperature stress in cucumbers highlights the importance of chloroplast genes in environmental adaptation (Xia et al., 2023). Continued efforts to sequence and analyze chloroplast genomes from a broader range of *Cucumis* species and related taxa will refine our understanding of phylogenetic relationships and evolutionary history. Comparative studies across different genera, such as those conducted in the Zingiberaceae and Cleomaceae families, have demonstrated the utility of chloroplast genomes in resolving taxonomic ambiguities and inferring evolutionary relationships (Liang et al., 2020; Alzahrani et al., 2021). Identifying and validating molecular markers from chloroplast genomes can aid in species identification, genetic diversity studies, and breeding programs. Hotspot regions and simple sequence repeats (SSRs) identified in chloroplast genomes have proven useful for these purposes (Alzahrani et al., 2021; Li et al., 2021).

10 Concluding Remarks

The application of chloroplast genomes in the study of the *Cucumis* genus has yielded significant insights into the genetic and evolutionary dynamics of these species. The complete chloroplast genome sequencing of *Cucumis*×*hytivus* and its diploid parents revealed substantial genetic variations, including 51 Indels and 292 SNPs, which are crucial for understanding the inheritance and changes in organelle genomes post-allopolyploidization. These findings underscore the utility of chloroplast genomes in elucidating the evolutionary relationships and taxonomic positions within the *Cucumis* genus.

Chloroplast genome studies have significantly contributed to the phylogenetic and taxonomic understanding of various plant genera, including *Cucumis*. The comparative analysis of chloroplast genomes has provided robust phylogenetic frameworks and clarified taxonomic ambiguities. For instance, the phylogenetic network based on chloroplast genome sequences has been instrumental in clarifying the evolution and taxonomic position of synthetic allotetraploid *Cucumis*×*hytivus*. Similar studies in other genera, such as *Abelmoschus* and *Lespedeza*, have demonstrated the effectiveness of chloroplast genomes in resolving taxonomic discrepancies and providing insights into species divergence and evolutionary relationships.

Future research should focus on expanding the chloroplast genome sequencing to include a broader range of *Cucumis* species and related genera. This will enhance the resolution of phylogenetic relationships and provide a more comprehensive understanding of the evolutionary history of the *Cucumis* genus. Additionally, the identification of hypervariable regions and mutational hotspots within chloroplast genomes can be leveraged to develop molecular markers for species authentication, genetic diversity studies, and conservation efforts. Practical applications of these findings include the development of DNA barcodes for accurate species identification and the use of chloroplast genome data in breeding programs to improve crop varieties within the *Cucumis* genus.

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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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