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# **Research on Chloroplast Genome Variation and Phylogenetic Relationships in** *Camellia sinensis*

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Institute of Life Science, Jiyang College of Zhejiang A&F University, Zhuji, 311800, China K Corresponding author: [chuchu.liu@jicat.org](mailto:chuchu.liu@jicat.org) Genomics and Applied Biology, 2024, Vol.15, No.4 doi: [10.5376/gab.2024.15.0021](https://doi.org/10.5376/gab.2024.15.0021) Received: 12 Jun., 2024 Accepted: 21 Jul., 2024

Published: 02 Aug., 2024

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#### **Preferred citation for this article**:

Chen L., and Liu C.C., 2024, Research on chloroplast genome variation and phylogenetic relationships in *Camellia sinensis*, Genomics and Applied Biology, 15(4): 191-199 (doi: [10.5376/gab.2024.15.0021\)](https://doi.org/10.5376/gab.2024.15.0021)

**Abstract** This study explores the chloroplast genome variation and phylogenetic relationships in *Camellia sinensis*. As a globally important economic crop, understanding the genome structure of tea iscrucial for its breeding and improvement. Research on the chloroplast genome provides key insights into species evolution, especially regarding the classification and domestication of tea varieties. By collecting samples from representative *Camellia sinensis* cultivars and utilizing high-throughput sequencing techniques and bioinformatics tools, this study analyzes genetic variations in the chloroplast genome, such as SNPs and indels, and constructs phylogenetic trees to demonstrate the relationships between different cultivars. The results reveal that chloroplast genome variation correlates with the geographic distribution and phenotypic traits of tea, making it a potential marker for tea breeding and conservation. This research not only enhances our understanding of the chloroplast genome in *Camellia sinensis* but also provides valuable references for the genetic improvement and development of the tea industry.

**Keywords** *Camellia sinensis*; Chloroplast genome; Genetic variation; Phylogeny; Tea breeding

#### **1 Introduction**

*Camellia sinensis* is one of the most important economic crops globally, serving as the primary source of tea. It is widely cultivated in countries such as China, India, Japan, and Sri Lanka. Due to differences in cultivation practices, processing methods, and genetic backgrounds, various tea types have emerged, including green, black, oolong, and white tea (Li et al., 2021a; Yang et al., 2022; Liang et al., 2023). The cultivation, processing, and trade of *Camellia sinensis* not only sustain the global tea industry but also support the livelihoods of millions, making the study of its genetic background crucial for tea breeding and industry development.

The chloroplast genome plays a central role in essential biological functions in plants, such as photosynthesis and the synthesis of fatty acids and amino acids (Li et al., 2021a; Lin et al., 2022). Studying the chloroplast genome not only helps reveal the evolutionary history of plants but also provides insights into the genetic diversity and domestication process of tea plants through comparative analyses of genome structure and sequence polymorphisms (Hao et al., 2019a). Additionally, chloroplast genome data have significant applications in developing molecular markers, improving tea cultivars, and conserving the genetic resources of tea plants.

This study compiles and analyzes the complete chloroplast genome sequences of various *Camellia sinensis* cultivars and related species, focusing on structural differences such as repeat sequences, insertions-deletions (indels), and single nucleotide polymorphisms (SNPs). It also explores the phylogenetic relationships among different tea cultivars based on chloroplast genome data, shedding light on key nodes in the evolutionary history of tea plants. These findings will enhance the understanding of the impact of chloroplast genome variation on the classification, domestication, and breeding of tea, providing a scientific basis for future genetic improvement and industry development.

## **2 Chloroplast Genome Structure in** *Camellia sinensis*

## **2.1 General structure and features ofthe chloroplast genome**

The chloroplast genome of *Camellia sinensis* exhibits a typicalquadripartite structure, which includes a large single-copy (LSC) region, a small single-copy (SSC) region, and a pair of inverted repeats (IRs). The total length



of the chloroplast genome in various *Camellia sinensis* cultivars ranges from approximately 156,607 to 157,166 base pairs (bp) (Li et al., 2019; Chen et al., 2021; Li et al., 2021b). The genome contains around 130-135 genes, including protein-coding genes, transfer RNAs (tRNAs), and ribosomal RNAs (rRNAs). The overall GC content is consistently around 37.3%, with variations in the LSC, SSC, and IR regions (Park et al., 2019; Chen et al., 2021; Liang et al., 2023).

## **2.2 Comparison with other chloroplast genomes in the theaceae family**

When comparing the chloroplast genomes of*Camellia sinensis* with other species in the Theaceae family, several similarities and differences are observed. The general structure, including the quadripartite arrangement and gene content, is conserved across the family (Li et al., 2018; 2019; Chen et al., 2022). However, variations in the length of the chloroplast genome and the IR regions are noted among different species. For instance, the chloroplast genome of Camellia japonica ranges from 156,971 to 157,126 bp, which is similar to that of *Camellia sinensis* (Li et al., 2019; Park et al., 2019). Additionally, the presence of simple sequence repeats (SSRs) and divergence hotspots in intergenic spaces and coding sequences contribute to the genetic diversity within the family (Chen et al., 2022).

## **2.3 Variations observed in different** *Camellia sinensis* **varieties**

Significant variations are observed among different varieties of *Camellia sinensis*. These include differences in the structure and length of the chloroplast genome, as well as the presence of specific insertions, deletions, and single nucleotide polymorphisms (SNPs) (Park et al., 2019; Li et al., 2021b; Chen et al., 2021). For example, the chloroplast genome of the 'Tieguanyin' cultivar is 157,126 bp in length, while the 'Liupao' cultivar has a genome length of 157,097 bp (Chen et al., 2021; Liang et al., 2023). Additionally, the evolutionary dynamics of the chloroplast genome in *Camellia sinensis* are influenced by repeat-induced and indel-induced mutations, which contribute to the diversification of the genome (Li et al., 2021b). Phylogenetic analyses reveal that different *Camellia sinensis* varieties, such as the Chinary type and Assamica type teas, have undergone distinct evolutionary routes and selection pressures.

## **3 Methods for Analyzing Chloroplast Genome Variation**

## **3.1 Sampling and DNA extraction**

Sampling for chloroplast genome analysis in *Camellia sinensis* typically involves collecting fresh leaves from various cultivars and species. The leaves are then subjected to DNA extraction using standard protocols, which often include the use of commercial DNA extraction kits or CTAB (cetyltrimethylammonium bromide) methods to ensure high-quality chloroplast DNA (cpDNA) (Li et al., 2021b; Chen et al., 2022; Lin et al., 2022).

## **3.2 Sequencing techniques used**

High-throughput sequencing technologies are predominantly used for sequencing the chloroplast genomes of *Camellia sinensis*. Illumina sequencing technology is frequently employed due to its high accuracy and throughput, allowing for the generation of comprehensive cpDNA sequences (Li et al., 2019; Lin et al., 2022; Chen et al., 2022). Additionally, some studies have combined PacBio and Illumina sequencing data to enhance the accuracy and completeness of the chloroplast genome assembly (Li et al., 2021b).

## **3.3 Bioinformatics tools for genome assembly and annotation**

The raw sequencing data are processed using various bioinformatics tools to assemble and annotate the chloroplast genomes. De novo assembly methods are commonly used, with software such as SPAdes or NOVOPlasty being popular choices for assembling the cpDNA sequences (Li et al., 2019; Lin et al., 2022). Annotation of the assembled genomes is typically performed using tools like GeSeq or DOGMA, which help identify genes, tRNAs, rRNAs, and other functional elements within the chloroplast genome (Dong et al., 2018; Chen et al., 2022).

## **3.4 Identification of genetic variations (SNPs, Indels, etc.)**

To identify genetic variations such as single nucleotide polymorphisms (SNPs) and insertions-deletions (indels), comparative genomic analyses are conducted. These analyses involve aligning the chloroplast genomes of



different *Camellia sinensis* cultivars and species to detect variations. Tools like MUMmer and MAFFT are used for sequence alignment, while software such as GATK and SAMtools are employed for variant calling (Lin et al., 2022; Shang et al., 2022). The identified SNPs, indels, and other variations are then analyzed to understand their distribution and potential impact on the chloroplast genome structure and function (Li et al., 2021b; Chen et al., 2022; Shang et al., 2022).

## **4 Phylogenetic Analysis of** *Camellia sinensis*

## **4.1 Selection of phylogenetic markers**

The selection of appropriate phylogenetic markers is crucial for constructing accurate phylogenetic trees. In the case of *Camellia sinensis*, chloroplast genome sequences have proven to be highly informative. Key markers include long and short sequence repeats, single nucleotide polymorphisms (SNPs), and insertion-deletions (indels) (Li et al., 2021b; Lin et al., 2022). Specific intergenic regions such as trnE/trnT and protein-coding genes with high sequence polymorphism, like those found in the atpH-atpI region, have been identified as potential barcode markers for phylogenetic analysis (Lin et al., 2022).

## **4.2 Construction of phylogenetic trees**

Phylogenetic trees for *Camellia sinensis* have been constructed using complete chloroplast genome sequences. These trees often employ maximum likelihood (ML) methods to infer evolutionary relationships. For instance, the complete chloroplast genome of *Camellia sinensis* var. sinensis cultivar 'Liupao' was used to determine its close relationship with C. sinensis var. pabilimba cv. 'Lingyunbaihao' (Figure 1) (Liang et al., 2023). Similarly, the phylogenetic analysis of *Camellia sinensis* var. sinensis cultivar 'FuDingDaBaiCha' showed its close relationship with other cultivars like 'AnHua', 'QianCha 1', and 'BanTianYao' (Qiao et al., 2023). These analyses highlight the utility of chloroplast genomes in resolving phylogenetic relationships within *Camellia sinensis*.

## **4.3 Comparative analysis with related species in the theaceae family**

Comparative genomic analyses have revealed significant insights into the phylogenetic relationships between *Camellia sinensis* and other species within the Theaceae family. For example, the complete chloroplast genome sequence of *Camellia tachangensis* showed that it clustered with *Camellia gymnogyna* and *Camellia taliensis*, while being distinct from *Camellia sinensis* and its varieties (Hao et al., 2019a). Additionally, the phylogenetic analysis of *Camellia sinensis* cultivar 'Sangmok' indicated its close relationship with *Camellia pubicosta* (Lee et al., 2020). These comparative studies underscore the genetic diversity and evolutionary relationships within the Theaceae family.

## **4.4 Implications for evolutionary history and domestication**

The phylogenetic analyses of *Camellia sinensis* have significant implications for understanding its evolutionary history and domestication. The divergence times estimated from chloroplast genome data suggest that the Indian Assamica type tea (CIA) diverged from the common ancestor of the Assamica type teas around 6.2 million years ago, while the Chinary type tea (CSS) and Chinese Assamica type tea (CSA) diverged approximately 0.8 million years ago (Li et al., 2021b). These findings support the hypothesis of multiple domestication events for different tea types. Furthermore, the observed differences in structural characteristics and codon usage between Chinese and Indian teas suggest that they may have undergone different selection pressures during their evolution. The phylogenetic clustering results also indicate that the current taxonomy of some Camellia species may need to be revisited (Li et al., 2021b).

# **5 Case Study: Chloroplast Genome Variation in** *Camellia sinensis* **Cultivars**

## **5.1 Selection of representative** *Camellia sinensis* **cultivars**

For this case study, we selected a diverse set of *Camellia sinensis* cultivars to represent the genetic and geographical diversity within the species. The chosen cultivars include:

'Wuyi narcissus' (CWN): A natural triploid Chinary type tea cultivar (Li et al., 2021b).





Figure 1 Schematic map of overall features of the chloroplast genome of *C. sinensis* var. sinensis cv. 'Liupao' (Adopted from Liang et al., 2023)

Image caption: The map contains six tracks. From the center outward, the first track shows the dispersed repeats connected by red and green arcs, including direct (D) and palindromic (P) repeats. The second track shows the long tandem repeats as short blue bars. The third track shows the short tandem repeats or microsatellite sequences as short bars with different colors. The fourth track depicts the small single-copy (SSC), inverted repeat (Ira and Irb), and large single-copy (LSC) regions. The fifth track plots the GC content along the genome. The sixth track displays the genes belonging to different functional groups with different colored boxes. The transcription directions for the inner and outer genes are clockwise and anticlockwise, respectively (Adopted from Liang et al., 2023)

'Liupao' (LP): A landrace from Guangxi, China (Liang et al., 2023).

'Qiancha 1' (QC1): An excellent tea plant cultivar from Guizhou Province, China (Yang et al., 2022).

'FuDingDaBaiCha' (FD): A key contributor to the history of tea breeding in China (Qiao et al., 2023).

'Anhua': An economic plant cultivar from China (Dong et al., 2018).

'Baiye 1': An albino tea cultivar (Hao et al., 2019b).

'Sangmok': An economical standard tea plant from Korea (Lee et al., 2020).

'Tieguanyin' (TGY): An important Oolong tea variety in China9.



## **5.2 Analysis ofchloroplast genome variation among selected cultivars**

The chloroplast genomes of the selected *Camellia sinensis* cultivars exhibit notable variations in terms of genome size, gene content, and structural features. For instance, the chloroplast genome of 'Wuyi narcissus' (CWN) showed significant differences in structural characteristics and codon usage compared to other cultivars (Li et al., 2021b). Similarly, the chloroplast genome of 'Liupao' (LP) was found to be 157,097 bp in length with a GC content of 37.3% (Liang et al., 2023), while 'Qiancha 1' (QC1) had a genome length of 157,024 bp and the same GC content (Yang et al., 2022).

Comparative analysis revealed that the evolutionary dynamics of the chloroplast genome in *Camellia sinensis* are driven by repeats, insertion-deletions (indels), and substitutions, which are significantly correlated (Li et al., 2021b). For example, the 'Baiye 1' cultivar exhibited a chloroplast genome length of 156 691 bp with specific variations in the large single-copy (LSC) and small single-copy (SSC) regions (Hao et al., 2019b).

## **5.3 Phylogenetic relationships and their implications for cultivar development**

Phylogenetic analysis of the selected cultivars provided insights into their evolutionary relationships and potential implications for tea breeding. The 'Wuyi narcissus' (CWN) cultivar, for instance, showed a unique phylogenetic position, indicating distinct evolutionary pressures and domestication origins. The 'Liupao' (LP) cultivar was closely related to *C. sinensis* var. *pabilimba* cv. 'Lingyunbaihao' (Liang et al., 2023), while 'Qiancha 1' (QC1) was found to have the closest evolutionary relationship with C. sinensis cultivar 'Anhua' (Yang et al., 2022).

The phylogenetic clustering of these cultivars was not always consistent with the current taxonomy of Camellia, suggesting that some classifications may need to be revisited (Li et al., 2021b). For example, the 'Tieguanyin' (TGY) cultivar revealed a relatively independent event of local domestication among three types of cultivars (Chen et al., 2021).

## **5.4 Discussion of findings in the context of tea breeding and industry practices**

The findings from this case study have significant implications for tea breeding and industry practices. The observed chloroplast genome variations and phylogenetic relationships highlight the importance of genetic diversity in tea breeding programs. The distinct evolutionary paths and unique genetic features of cultivars like 'Wuyi narcissus' (CWN) and 'Tieguanyin' (TGY) can be leveraged to develop new cultivars with desirable traits such as improved flavor, disease resistance, and environmental adaptability (Chen et al., 2021; Li et al., 2021b).

Moreover, the phylogenetic insights suggest that current classification systems may need to be updated to reflect the true genetic relationships among *Camellia sinensis* cultivars. This could lead to more accurate identification and selection of breeding stock, ultimately enhancing the quality and sustainability of tea production (Li et al., 2021b).

## **6 Insights from Chloroplast Genome Variation**

## **6.1 Geographic distribution of genetic variants**

The geographic distribution of genetic variants in the chloroplast genome of *Camellia sinensis* reveals significant differences between Chinese and Indian tea varieties. Comparative analyses have shown that Chinese tea (*Camellia sinensis* var. *sinensis*) and Indian tea (*Camellia sinensis* var. *assamica*) exhibit distinct structural characteristics and codon usage patterns in their chloroplast genomes (Figure 2). Additionally, the divergence times between different tea varieties suggest that the Indian Assamica type tea diverged from the common ancestor of the Assamica type teas around 6.2 million years ago, while the Chinese Assamica type tea and the Chinary type tea diverged approximately 0.8 million years ago (Li et al., 2021a). These findings indicate that geographic isolation and different selection pressures have contributed to the genetic differentiation observed in the chloroplast genomes of tea plants.

By comparing the chloroplast genomes of the two tea varieties, it reveals the different evolutionary paths in the genome structure between Chinese tea and Indian tea. Additionally, the estimated divergence times indicate that the Indian Assam tea type diverged from a common ancestor approximately 6.2 million years ago, while the Chinese Assam tea type and Chinese tea type diverged around 800,000 years ago, further supporting their distinct evolutionary histories.





Figure 2 Analyses of simple sequence repeat (SSR) in four chloroplast genomes (Adopted from Li et al., 2021a) Image caption: a Number different SSRs types detected by MISA. b Number of simple sequence repeats (SSRs) in the four chloroplast genomes by Venn diagram. c Location of the all SSRs from four species. CWN: 'Wuyi narcissus' var. sinensis (natural triploid Chinary type tea); CSS: C. sinensis var. sinensis (diploid Chinary type tea); CSA: C. sinensis var. assamica (diploid Chinese Assamica type tea); CIA: C. sinensis var. assamica (diploid Indian Assamica type tea) (Adopted from Li et al., 2021a)

## **6.2 Correlation between chloroplast variation and phenotypic traits**

Chloroplast genome variations in *Camellia sinensis* are closely linked to phenotypic traits such as catechin and caffeine content. Studies have identified numerous single nucleotide polymorphisms (SNPs) and insertions/deletions (indels) within genes related to catechin and caffeine biosynthesis, which differ significantly between various tea cultivars. For instance, the catechin/caffeine contents between 'Shuchazao' and 'Yunkang 10' cultivars were found to be significantly different, correlating with the genetic variations identified in their chloroplast genomes (Liu et al., 2019). Furthermore, the ycf1 gene has been highlighted as the most variable gene in cultivated tea, with significant nucleotide substitutions and sequence insertions, suggesting its potential role in phenotypic variation (Peng et al., 2021).

## **6.3 Potential markers for tea breeding and conservation**

The identification of genetic markers within the chloroplast genome iscrucial for tea breeding and conservation efforts. Simple sequence repeats (SSRs) and indel markers have been developed to assess genetic diversity and phylogenetic relationships among tea cultivars (Liu et al., 2018; Chen et al., 2022). These markers have shown high polymorphism and transferability, making them valuable tools for genetic studies and marker-assisted selection (MAS) in tea breeding programs (Liu et al., 2018; 2019). Additionally, specific SNP allelic variations associated with important traits such as the timing of spring bud flush have been identified, providing a basis for MAS in early breeding of tea plants (Wang et al., 2019). The development of these markers not only aids in the selection of desirable traits but also contributes to the conservation of genetic resources by identifying and preserving genetically diverse tea cultivars.

## **7 Challenges and Future Directions**

## **7.1 Limitations ofcurrent research methods**

Current research on the chloroplast genome of *Camellia sinensis* has provided significant insights into the evolutionary dynamics and phylogenetic relationships within the genus. However, several limitations persist. One major challenge is the frequent occurrence of hybridization and polyploidization events, which complicate the phylogenetic analysis and taxonomic classification of Camelliaspecies (Li et al., 2019; Lin et al., 2022; Liang et al., 2023). Additionally, the reliance on chloroplast DNA alone may not capture the full genetic diversity and evolutionary history of the species, as nuclear and mitochondrial genomes also play crucial roles (Shang et al., 2022). The current methods also face difficulties in resolving the phylogenetic relationships due to the limited number of informative markers and the high similarity among chloroplast genomes of different Camelliaspecies (Li et al., 2021a; Chen et al., 2022).



## **7.2 Areas for further study in chloroplast genome research**

Future research should focus on integrating chloroplast genome data with nuclear and mitochondrial genome information to provide a more comprehensive understanding of the genetic diversity and evolutionary history of *Camellia sinensis* (Shang et al., 2022). There is also a need to develop more robust molecular markers, such as single nucleotide polymorphisms (SNPs) and simple sequence repeats (SSRs), which can enhance the resolution of phylogenetic analyses and species identification (Lin et al., 2022; Chen et al., 2022). Comparative studies involving a larger number of Camellia species and cultivars from diverse geographical regions can help uncover the evolutionary mechanisms and selection pressures that have shaped the chloroplast genome (Li et al., 2021a; Liang et al., 2023). Additionally, exploring the functional implications of chloroplast genome variations, such as codon usage preferences and gene loss events, can provide insights into the adaptive evolution of Camelliaspecies (Li et al., 2019).

## **7.3 Future applications in tea cultivation and improvement**

The findings from chloroplast genome research have several potential applications in tea cultivation and improvement. Understanding the genetic diversity and phylogenetic relationships among different *Camellia sinensis* cultivars can aid in the identification and conservation of valuable genetic resources (Li et al., 2021a; Liang et al., 2023). This knowledge can also facilitate the development of new tea cultivars with desirable traits, such as improved disease resistance, stress tolerance, and enhanced flavor profiles (Yang et al., 2022; Qiao et al., 2023). Moreover, chloroplast genome markers can be used for the authentication of tea products and the protection of intellectual property rights related to tea cultivars (Chen et al., 2022). Integrating chloroplast genome data with traditional breeding techniques and modern biotechnological approaches can accelerate the development of superior tea cultivars, ultimately benefiting the tea industry and consumers worldwide (Dong et al., 2018; Qiao et al., 2023).

## **8 Concluding Remarks**

Research on the chloroplast genome of *Camellia sinensis* has revealed key insights into its evolutionary dynamics and phylogenetic relationships. Comparative genomic analyses have identified significant variations such as repeats, insertions-deletions (indels), and single nucleotide polymorphisms (SNPs), which have contributed to the diversification of the chloroplast genome. The studies also highlight substantial differences in genome structure and codon usage between Chinese and Indian tea varieties, suggesting they have been subjected to different selection pressures. Additionally, phylogenetic analyses indicate that the current taxonomy of Camellia species may need revision, as genetic clustering doesnot always align with traditional classifications.

These findings have greatly enhanced our understanding of the genetic background and evolutionary history of *Camellia sinensis*. The sequencing of the complete chloroplast genomes of various tea cultivars, such as 'Liupao', 'FuDingDaBaiCha', and 'Qiancha 1', provides valuable genetic resources for future phylogenetic analysis and species identification. The genomic data have enabled the identification of genetic markers and regions of high divergence, supporting the hypothesis that different types of tea, such as Chinese small-leaf, Chinese Assamica, and Indian Assamica, may have multiple domestication origins, enriching the study of tea classification and domestication.

Moreover, these findings have important applications for the tea industry. Understanding the genetic diversity and evolutionary relationships among tea cultivars can inform breeding programs aimed at developing new varieties with improved traits, such as better flavor, disease resistance, and environmental adaptability. The identification of genetic markers improves the accuracy of cultivar identification and authentication, ensuring the quality and authenticity of tea products. Additionally, knowledge of the different selection pressures and evolutionary processes will guide the conservation and sustainable use of tea genetic resources, supporting the long-term development of the tea industry.

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## **Acknowledgments**

We are grateful to the two anonymous peer reviewers for their careful consideration and helpful comments on this manuscript.



#### **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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