

The complete chloroplast genome sequence of *Camellia sinensis* var. *sinensis* cultivar “Yuexiangzao”

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ABSTRACT

Camellia sinensis var. *sinensis* cultivar “Yuexiangzao” (YXZ) was a new tea cultivar in China. In this study, we reported a complete chloroplast (CP) genome based on the DNGSEQ sequencing technology. The CP genome sequence of YXZ was 157,041 bp in length and contained a large single copy (LSC, 86,594 bp), a small single copy (SSC, 18,291 bp), and two inverted repeats (IRs, 26,078 bp). The GC contents of LSC, SSC, and two IRs were 35.31%, 30.53%, and 42.94%, respectively. The phylogenetic analysis showed that YXZ was closely related to *C. sinensis* cv. “Tieluohan” and *C. sinensis* cv. “Xinyang 10.”

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Introduction


The tea plant (*Camellia sinensis* (L.) O. Kuntze 1881) is an important economic plant in the world, native to China (Wang et al. 2020). Its leaves can be processed into tea, rich in various antioxidant ingredients, such as catechins, amino acids, and caffeine, which exhibits anticancer, anti-inflammatory, hepatoprotective benefits and so on (Aadrika et al. 2022; Evren et al. 2024). *C. sinensis* var. *sinensis* cv. “Yuexiangzao” (YXZ) was a new tea cultivar breeding from “Jiukengzhong” by the Shaoxing Agricultural Science Research Institute, Zhejiang province, China, good characteristics in shoots and leaves, high yield, fine adaptability for making green tea, strong resistance and so on (Fu et al. 2012). YXZ showed good adaptability in the tea region of southern Henan, China, which was suitable for promotion and application here (Li, Jiang, et al. 2023). In early work, we found that YXZ had comparatively stronger fecundity by regular field investigation, and seeds of YXZ not only owned a high kernel rate but also shared a great oil content (Li, Zhu, et al. 2023). So, we considered that YXZ could be used as a fine candidate cultivar for harvesting both tea leaves and seeds in the tea area of Southern Henan, China. And also, YXZ may be selected as a parent for constructing the mapping population in our following study. The complete chloroplast genome sequence of YXZ not only provides a precious resource for further research on the phylogenetic analysis of tea plants, but also useful for identifying important genes and screening molecular markers. The chloroplast (CP) genome, which mostly belongs to maternally inherited, is more easily to

provide species evolution information compared with the nuclear genome (Kaundun and Matsumoto 2011). For all we know, a total of 93 tea plant verified and complete cp genomes were reported and released in Genbank (Table S1). Plenty of scholars have applied the complete cp genome to explore the phylogenetic relationship of tea plants in recent years (Yang et al. 2024). For example, Qiao et al. (2023) found the famous fine cultivar of *C. sinensis* cv. “FuDingDaBaiCha” was closely related to *C. sinensis* cv. “Anhua,” *C. sinensis* cv. “Qiancha 1,” *C. sinensis* cv. “BanTianYao.” In this study, we sequenced and analyzed the complete cp genome sequence of YXZ, and constructed its phylogenetic relationship with other different tea cultivars as well.

Materials and methods

The first and second leaves under apical bud of YXZ were collected from an eleven-year-old plant (Figure 1) in the experimental field of the Tea Research Institute, Xinyang Academy of Agricultural Science (114°18'N, 32°9'E) in August 2022. Materials were collected following the rules and regulations of Tea Research Institute, Xinyang Academy of Agricultural Sciences, with no special permission statement being required for the collection. The leaves were immediately preserved in liquid nitrogen before DNA extraction. The total genomic DNA was extracted with the CTAB method and purified with Qiagen Genomic-tip kit 13343 (Qiagen, Germany). A specimen was

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Figure 1. The species image of *Camellia sinensis* cv. “Yuexiangzao.” (A) Whole plant morphology in autumn. (B) Tea shoot in autumn. All photos were taken by suangfeng Jiang in the Tea Research Institute, Xinyang Academy of Agricultural Science, Xinyang, Henan, China.

deposited in the Tea Germplasm Resource Nursery of Tea Research Institute, Xinyang Academy of Agricultural Science (<http://www.xynk.cn/>, associate researcher Shuangfeng Jiang, shuangfeng130@sina.com) under the voucher number XYCS0021. The library construction and sequencing were performed by the Benagen Technology Corporation Limited (Wuhan, China) on the DNBSEQ-T7 platform, following the manufacturer protocol (DNBSEQ, Shenzhen, China). The CP genome was de novo assembled using GetOrganelle v1.7.5 (Jin et al. 2020) and annotated using CPGAVAS2 (Shi et al. 2019). All annotation errors were manually corrected by Apollo (Lewis et al. 2002). The circular structure of the CP genome was drawn using OGDRAW (Stephan et al. 2019). The complete CP genomes of 29 representative *Camellia* cultivars and YXZ were aligned using MAFFT v.7.505 software (Katoh and Standley 2013). A Maximum Likelihood (ML) tree was constructed using IQ-TREE v1.6.12 (Nguyen et al. 2015) under the maximum composite likelihood model with 1000 bootstraps replicates and was visualized using ITOL v6 software (Letunic & Bork, 2019).

Results

The complete CP genome of YXZ was 157,041 bp in length with a typical quadripartite structure (Figure 2). All position of the CP genome of YXZ had high depth coverage (Figure S1). It contained a pair of inverted repeats (IRa and IRb) of 26,078 bp each, which were separated by a large single-copy (LSC, 86,594 bp) and a small single-copy (SSC, 18,291 bp). GC contents of the two IRs, LSC, and SSC were 42.94%, 35.31%, and 30.55%, respectively. A total of 112 genes were predicted, including 80 unique protein-coding genes, 28 tRNA genes, and 4 rRNA genes. Among them, all protein-coding genes contained 11 NADH dehydrogenase subunit genes, 5

photosystem I subunit genes, 16 photosystem II subunit genes, 6 cytochrome b/f complex subunit genes, 6 ATP synthase subunit genes, a single 1,5-diphosphate ribose carbonylase/oxygenase large subunit gene, 4 DNA-dependent RNA polymerase genes, 9 ribosomal large subunit genes, 12 ribosomal small subunit genes, 1 mature enzyme gene, 1 C-type cytochrome synthase gene, 1 membrane protein gene, 1 protease gene, 1 acetyl-CoA gene, 1 translation initiation factor gene, and 4 conserved open reading frame genes (Figure S2 and S3). The complete CP genome sequences and annotations of YXZ were submitted to the NCBI GenBank under the accession number PP933804.

To explore the phylogenetic position of YXZ, phylogenetic analysis was performed on the complete CP genomes of 29 cultivars with *Camellia oleifera* as outgroup species. As shown in the ML tree (Figure 3), first, *C. sinensis* distinctly separated from wild relative species *C. taliensis*, *C. tachangensis* and *C. tachangensis*; then, all cultivars of *C. sinensis* clustered three different branches, *C. sinensis* var. *sinensis*, *C. sinensis* var. *assamica* and *C. sinensis* var. *Pubilimba*, respectively. YXZ was closely related to *C. sinensis* cv. “Tieluohan” and *C. sinensis* cv. “Xinyang 10,” where the sequence of YXZ was completely consistent with the latter two.

The CP genome structures of 24 representative tea cultivars were compared and visualized by the IRscope software (Amiryousefi et al. 2018). The results showed that CP genome structures of different tea cultivars were highly conserved (Figure S4). For example, the LSC/IRb boundaries of 19 tea cultivars were located within *rps19* genes and 46 bp away from the last bases of these genes, and their boundaries of IRa/LSC were all located at 1 base before *trnH* genes. The CP genome length and gene locations of YXZ were completely consistent with *C. sinensis* cv. “Tieluohan” and *C. sinensis* cv.

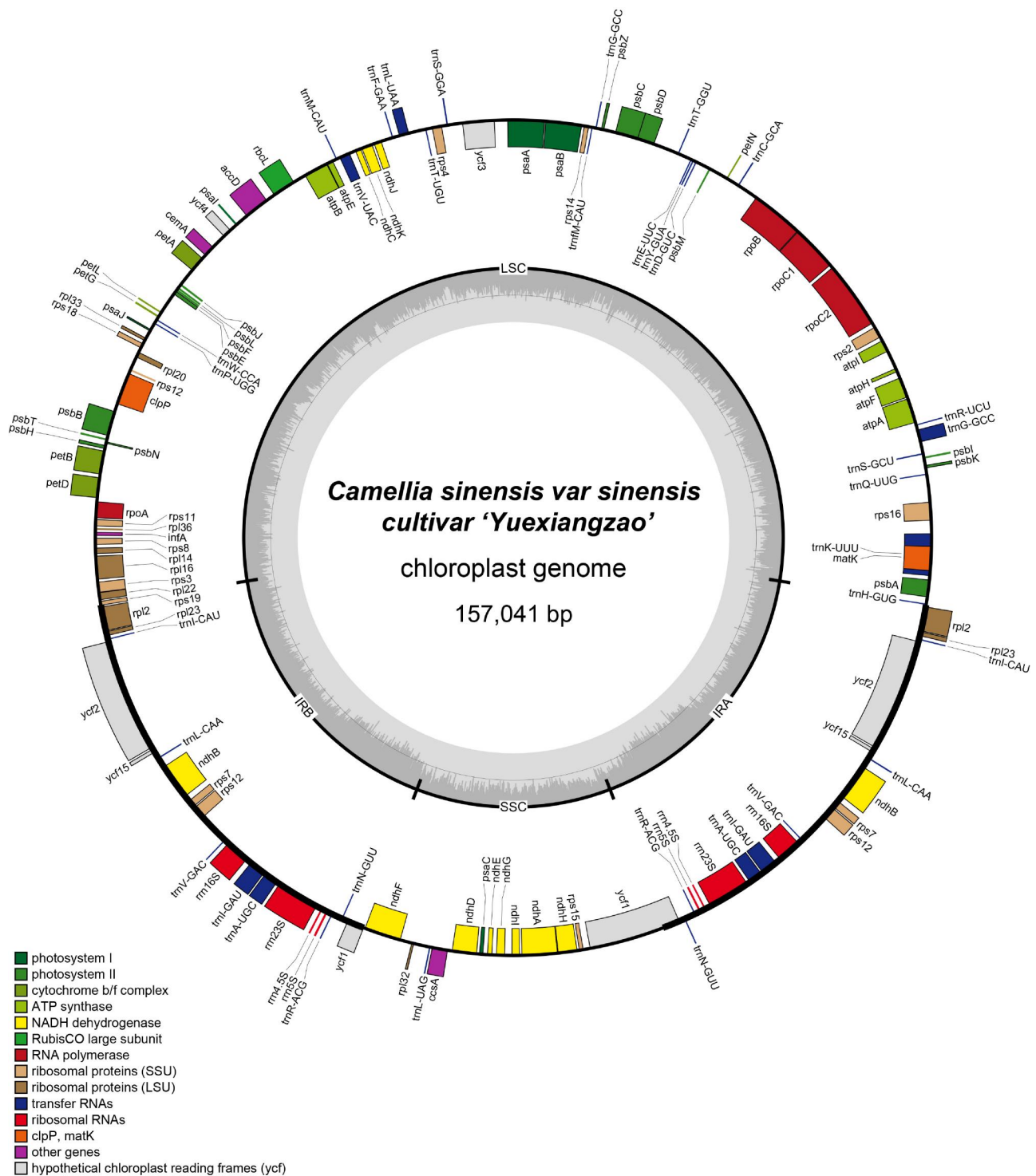


Figure 2. Chloroplast genome map of *Camellia sinensis* cv. “Yuexiangzao.” Genes outside the main circle was transcribed clockwise, while genes on the inside were transcribed counterclockwise. The organellar genome DRAW (OGDraw) online software (v1.3.1) was used to draw this map. Different colors represent genes with other functions. The inner circle's gray portion indicates the chloroplast genome's GC content.

“Xinyang 10.” In addition, the nucleotide diversity (Pi values) of the CP genomes of these cultivars were further calculated by the DnaSP 6.0 software (Rozas et al. 2017). There were 8 regions with relatively high Pi values (> 0.007), which were located into *ycf2*, *trnL-ndhB*, *rps7-rps12*, *rps12*, *ndhB-trnL*, and *trnL* genes, respectively (Figure S5). The Pi values of other regions were less than 0.004, indicating that the CP genomes of different tea cultivars were highly conserved as well.

Discussion and conclusions

To meet the changing demands of the market, new tea cultivars have been bred continuously (Wang et al. 2019). The phylogenetic position of a new tea cultivar could be preliminarily realized by phylogenetic analysis based on the CP genome sequence. In this study, we reported a complete CP genome sequence of YXZ, which was completely consistent

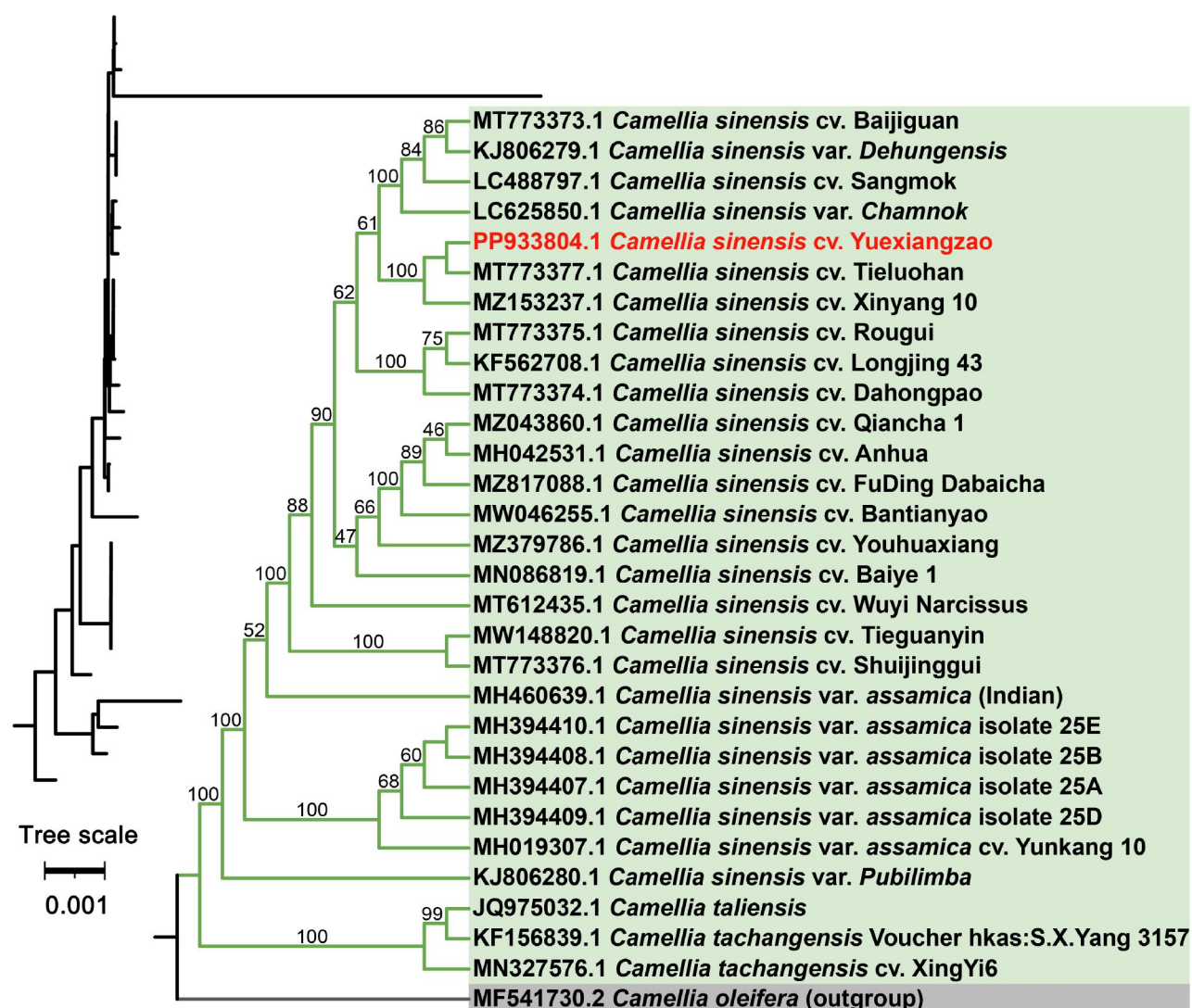


Figure 3. Phylogenetic relationships of 30 *Camellia sinensis* cultivars based on their complete chloroplast genome sequences. The phylogenetic tree was constructed using Neighbor-Joining (NJ) method with 1000 bootstrap replicates. The bootstrap values were labeled at each branch node. The following published sequences were applied to construct the NJ-tree: MT773373.1, (Fan et al. 2022), KJ806279.1 (Huang et al. 2014), LC488797.1 (Lee et al. 2020), MT773377.1 (Fan et al. 2022), MZ153237.1 (Yan et al. 2021), MT773375.1 (Fan et al. 2022), KF562708.1 (Ye et al. 2014), MT773374 (Li et al. 2021), MZ043860.1 (Yang et al. 2022), MH042531.1 (Dong et al. 2018), MZ817088.1 (Qiao et al. 2023), MW046255.1, MN086819.1 (Hao et al. 2019), MT773376.1 (Fan et al. 2022), MH460639.1 (Rawal et al. 2020), MH394410.1 (Zeng et al. 2018), MH394408.1 (Zeng et al. 2018), MH394407.1 (Zeng et al. 2018), MH394409.1 (Zeng et al. 2018), MH019307.1 (Zhang et al. 2019), KJ806280.1 (Huang et al. 2014), JQ975032.1 (Shi et al. 2013), KF156839.1 (Yang et al. 2013), MN327576.1 (Hao et al. 2019). All sequences can be downloaded from NCBI GenBank.

with *C. sinensis* cv. "Tieluohan" and *C. sinensis* cv. "Xinyang 10," suggesting that these cultivars may have been derived from the same maternal parent and with a short divergence time. However, the former was from Henan Province, China, and the latter two were from Fujian Province and Zhejiang Province, China, respectively (Zhang et al. 2004; Huang et al. 1994; Fu et al. 2012). And there was no any breeding record to show that three cultivars were related with each other. We speculate that this may be closely related to the spread of tea seeds due to the migration and flow of human population.

Author contributions

Yali Chang completed this manuscript. Lingzhi Chen and Jinlei Luo contacted and collected the experiment materials. Shuangfeng Jiang

provided the experimental information and guided the sampling work. Yi Chen and Jinlei Luo performed the data analysis. Mufang Sun conceived this work and roundly reviewed this manuscript. All authors have read and agree to the published version of this manuscript, and agreed to be accountable for all aspects of the work.

Ethics statement

The collection of specimens conformed to the requirement of international ethics, which did not incur any damage to the environment and the species itself. The process and purpose of this experimental research were in line with the rules and regulations of our college. There are no ethical issues or specific permissions are required.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The data that support the findings of this study are openly available in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov/>, under the accession no. PP933804. The associated Bioproject, SRA, and Bio-Sample numbers are PRJNA1164410, SRR30835843, and SAMN43901166.

References

- Aadrika B, Punita A, Amita R, Nitesh K. 2022. Pharmacological actions and underlying mechanisms of catechin: a review. *Mini Rev Med Chem*. 22:821–833. doi:10.2174/1389557521666210902162120
- Amiryousefi A, Hyvönen J, Pocai P. 2018. IRscope: an online program to visualize the junction sites of chloroplast genomes. *Bioinformatics*. 34(17):3030–3031. doi:10.1093/bioinformatics/bty220
- Dong M, Liu S, Xu Z, Hu Z, Ku W, Wu L. 2018. The complete chloroplast genome of an economic plant, *Camellia sinensis* cultivar Anhua, China. *Mitochondrial DNA B Resour*. 3(2):558–559. doi:10.1080/23802359.2018.1462124
- Evren DK, Guldu OK, Tut E, Medine EI. 2024. Nanoencapsulation of green tea catechin (-)-Epigallocatechin-3-Gallate (EGCG) in niosomes and assessment of its anticancer activity against lung cancer. *J Drug Deliv Sci Tec*. 93:105412. doi:10.1016/j.jddst.2024.105412
- Fan L, Li L, Hu Y, Huang Y, Hong Y, Zhang B. 2022. Complete chloroplast genomes of five classical Wuyi tea varieties (*Camellia sinensis*, Synonym: *thea bohea* L.), the most famous Oolong tea in China. *Mitochondrial DNA B Resour*. 7(4):655–657. doi:10.1080/23802359.2022.2062263
- Fu J, Chen XD, Xia XH. 2012. Preliminary breeding report of new tea cultivar *Camellia sinensis* var. *sinensis* cv. *Yuexiangzao*. *J China Tea*. 34(01):16–17.
- Hao WJ, Ma JQ, Ma C, Jin J, Chen L. 2019. The complete chloroplast genome sequence of *Camellia tachangensis* F. C. Zhang (Theaceae). *Mitochondrial DNA B Resour*. 4(2):3344–3345. doi:10.1080/23802359.2019.1673247
- Huang DP, Lv LZ, Wei H. 1994. Breeding report of new tea cultivar *Camellia sinensis* var. *sinensis* cv. 'Xinyang'. *J Tea*. 20(10):8–11.
- Huang H, Shi C, Liu Y, Mao SY, Gao LZ. 2014. Thirteen *Camellia* chloroplast genome sequences determined by high-throughput sequencing: genome structure and phylogenetic relationships. *BMC Evol Biol*. 14(1):151. doi:10.1186/1471-2148-14-151
- Jin JJ, Yu WB, Yang JB, Song Y, dePamphilis CW, Yi TS, Li DZ. 2020. GetOrganelle: a fast and versatile toolkit for accurate de novo assembly of organelle genomes. *Genome Biol*. 21(1):241. doi:10.1186/s13059-020-02154-5
- Kaundun SS, Matsumoto S. 2011. Molecular evidence for maternal inheritance of the chloroplast genome in tea, *Camellia sinensis* (L.) O. Kuntze. *J Sci Food Agric*. 91(14):2660–2663. doi:10.1002/jsfa.4508
- Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol*. 30(4):772–780. doi:10.1093/molbev/mst010
- Lee DJ, Kim CK, Lee TH, Lee SJ, Moon DG, Kwon YH, Cho JY. 2020. The complete chloroplast genome sequence of economical standard tea plant, *Camellia sinensis* L. cultivar Sangmok, in Korea. *Mitochondrial DNA B Resour*. 5(3):2835–2836. doi:10.1080/23802359.2020.1790311
- Letunic I, Bork P. 2019. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Res*. 47(W1):W256–W259. doi:10.1093/nar/gkz239
- Lewis SE, Searle SMJ, Harris N, Gibson M, Lyer V, Richter J, Wiel C, Bayraktaroglu L, Birney E, Crosby MA, et al. 2002. Apollo: a sequence annotation editor. *Genome Biol*. 3(12):RESEARCH0082. doi:10.1186/gb-2002-3-12-research0082
- Li JL, Jiang SF, Cai YM, Chen ZY, Feng Y, Ren TY, Li J. 2023. Study on the adaptability of the new tea cultivar “Yuexiangzao” in the Southern Henan Tea Region. *J Tea Commun*. 50:312–316.
- Li L, Hu Y, Wu L, Chen R, Luo S. 2021. The complete chloroplast genome sequence of *Camellia sinensis* cv. Dahongpao: a most famous variety of Wuyi tea (Synonym: *Thea bohea* L.). *Mitochondrial DNA B Resour*. 6(1):3–5. doi:10.1080/23802359.2020.1844093
- Li YW, Zhu Y, Huang SJ, Luo JL, Sun MF, Chen Y, Jiang SF, Guo GY, Chang YL. 2023. Comparison of fatty acid composition in seed kernels of tea plant (*Camellia sinensis*), between conventional cultivars and albino cultivars. *Cogent Food Agr*. 9:2272464.
- Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol*. 32(1):268–274. doi:10.1093/molbev/msu300
- Qiao DH, Yang C, Guo Y. 2023. The complete chloroplast genome sequence of *Camellia sinensis* var. *sinensis* cultivar ‘FuDingDaBaiCha’. *Mitochondrial DNA B Resour*. 8(1):100–104. doi:10.1080/23802359.2022.2161327
- Rawal HC, Kumar PM, Bera B, Singh NK, Mondal TK. 2020. Decoding and analysis of organelle genomes of Indian tea (*Camellia assamica*) for phylogenetic confirmation. *Genomics*. 112(1):659–668. doi:10.1016/j.ygeno.2019.04.018
- Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A. 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Mol Biol Evol*. 34(12):3299–3302. doi:10.1093/molbev/msx248
- Shi L, Chen H, Jiang M, Wang L, Wu X, Huang L, Liu C. 2019. CPGAVAS2, an integrated plastome sequence annotator and analyzer. *Nucleic Acids Res*. 47(W1):W65–W73. doi:10.1093/nar/gkz345
- Shi C, Liu Y, Huang H, Xia E-H, Zhang H-B, Gao L-Z. 2013. Contradiction between plastid gene transcription and function due to complex posttranscriptional splicing: an exemplary study of *ycf15* function and evolution in angiosperms. *PLoS One*. 8(3):e59620. doi:10.1371/journal.pone.0059620
- Stephan G, Pascal L, Ralph B. 2019. OrganellarGenomeDRAW (OGDRAW) version 1.3.1: expanded toolkit for the graphical visualization of organellar genomes. *Nucleic Acids Res*. 47(W1):W59–W64.
- Wang XC, Wang L, Hao XY, Zeng JM, Yang YJ. 2019. Progress of tea genetic breeding study in 40 years of China. *Chinese Tea*. 41:1–6.
- Wang XC, Feng H, Chang YX, Ma CL, Wang LY, Hao XY, Li AL, Cheng H, Wang L, Cui P, et al. 2020. Population sequencing enhances understanding of tea plant evolution. *Nature Commun*. 11:4447.
- Yan MH, Liu K, Wang M, Lu Y, Zhang Q. 2021. Complete chloroplast genome of *Camellia sinensis* cv. Xinyang 10 and its phylogenetic evolution. *J Tea Sci*. 41:777–788.
- Yang C, Qiao D, Guo Y, Chen J, Chen Z. 2022. The complete chloroplast genome sequence of *Camellia sinensis* cultivar ‘Qiancha1’ from Guizhou Province, China. *Mitochondrial DNA B Resour*. 7(2):404–405. doi:10.1080/23802359.2021.2005490
- Yang YQ, Tan J, Wang F, Peng SL, Deng J, Tan MY, Lv MY, Zhou FY, Liu SC. 2024. Research and application progress in chloroplast genome of tea plant (*Camellia sinensis*). *Biotech Bull*. 40:20–30.
- Yang JB, Yang SX, Li HT, Yang J, Li DZ. 2013. Comparative chloroplast genomes of *Camellia* species. *PLoS One*. 8(8):e73053. doi:10.1371/journal.pone.0073053
- Ye XQ, Zhao ZH, Zhu QW, Wang YY, Lin ZX, Ye CY, Fan LJ, Xu HR. 2014. Entire chloroplast genome sequence of tea (*Camellia sinensis* cv. Longjing 43): a molecular phylogenetic analysis. *J Zhejiang Univ*. 40(4):404–412.
- Zhang F, Li W, Gao CW, Zhang D, Gao LZ. 2019. Deciphering tea tree chloroplast and mitochondrial genomes of *Camellia sinensis* var. *assamica*. *Sci Data*. 6(1):209. doi:10.1038/s41597-019-0201-8
- Zhang FZ, Zheng NH, Chen L. 2004. Varieties of oolong tea (continued). *J China Tea*. (03):8–9.
- Zeng CX, Hollingsworth PM, Yang J, He ZS, Zhang ZR, Li DZ, Yang JB. 2018. Genome skimming herbarium specimens for DNA barcoding and phylogenomics. *Plant Methods*. 14(1):43. doi:10.1186/s13007-018-0300-0