

THE COMPLETE MITOCHONDRIAL GENOMES OF TWO APPLE CULTIVARS REVEAL THE HIGHLY CONSERVED STRUCTURE IN CULTIVATED APPLES

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*Apples are as one of the most popular and economically important fruits worldwide, have the important nutritional and ornamental value. However, because of their complexity, studies on apple mitochondrial (mt) genomes have been limited. In this study, the mt genomes of Aksu Fuji and Ralls Janet cultivars were assembled, annotated, and analyzed based on a hybrid strategy using Illumina, and comprehensive comparisons of their structure, gene content, intercellular gene transfer, phylogeny, and RNA editing sites were performed. The mt genome length and gene structure of the two apple cultivars were identical (396,592 bp) and included 63 protein-coding genes (PCGs), 20 transfer RNA (tRNA) genes, and 4 ribosomal RNA (rRNA) genes. There were 44 and 31 mitochondrial plastid fragments (MTPTs) identified between the mt and plastid genomes of the Ralls Janet and Aksu Fuji cultivars, accounting for 1.98 and 2.19 % of their mt genomes, respectively. Furthermore, there were 419 and 421 RNA editing sites detected in Aksu Fuji and Ralls Janet, respectively. Analyses of coding usage bias, nucleotide diversity, selection pressure, and genetic distance revealed that the mt genomes of the two cultivars were highly conserved. Phylogenetic analysis of 29 Rosaceae species showed that Aksu Fuji and Ralls Janet clustered with *Malus baccata* and *M. kansuensis*. This study provides new insights into the genetics, systematics, and evolution of apple mt genomes*

Key words: cultivated apple, mitochondrial genome, mitochondrial plastid sequences, RNA editing sites, phylogenetic analysis.

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ПОВНІ МИТОХОНДРІАЛЬНІ ГЕНОМИ ДВОХ СОРТІВ ЯБЛУК ДЕМОНСТРУЮТЬ ВИСОКОКОНСЕРВАТИВНУ СТРУКТУРУ

Яблука є одними з найпопулярніших та економічно важливих фруктів у всьому світі і мають як харчову, так і декоративну цінність. Однак, дослідження мітохондріальних (mt) геномів яблук проводяться рідко через їхню складність. У цьому дослідженні було зібрано, анатовано та проаналізовано mt-геноми сортів Аксу Фуджі та Роллс Дженет на основі гібридної стратегії з використанням Illumina, а також проведено всебічне порівняння їхньої структури, вмісту генів, міжклітинного переносу генів, філогенезу та сайтів редактування РНК. Довжина геному mt і структура генів двох сортів яблук були ідентичними (396, 592 п.н.) і включали 63 гени, що кодують білки (PCG), 20 генів транспортних РНК (тРНК) і 4 гени рибосомних РНК (рРНК). Між mt і пластидними геномами сортів Роллс Дженет і Аксу Фуджі було виявлено 44 і 31 мітохондріально-пластидних фрагментів (МТРТ), що становить 1,98 і 2,19 % від їхніх mt геномів, відповідно. Крім того, було виявлено 419 та 421 сайт редактування РНК у Аксу Фуджі та Роллс Дженет, відповідно. Аналіз зсуву використання кодування, нуклеотидного різноманіття, селекційного тиску та генетичної дистанції показав, що mt-геноми обох сортів є висококонсервативними. Філогенетичний аналіз 29 видів розоцвітих показав, що Аксу Фуджі та Роллс Дженет класифікуються з *Malus baccata* та *M. kansuensis*. Це дослідження дає нові уявлення про генетику, систематику та еволюцію mt-геномів яблук.

Ключові слова: вирощувані яблука, мітохондріальний геном, послідовність мітохондріальних пластид, сайти редактування РНК, філогенетичний аналіз.

REFERENCE

- Beier S, Thiel T, Münch T, Scholz U et al (2017) MISA-web: a web server for microsatellite prediction. Bioinformatics 33:2583–2585. <https://doi.org/10.1093/bioinformatics/btx198>
- Benson G (1999) Tandem repeats finder: a program to analyze DNA sequences. Nucl Acids Res 27:573–580. <https://doi.org/10.1093/nar/27.2.573>
- Brand MD, Orr AL, Perevoshchikova IV, Quinlan CL (2013) The role of mitochondrial function and cellular bioenergetics in ageing and disease. Br J Dermatol 169:1–8. <https://doi.org/10.1111/bjd.12208>
- Bu YF, Wu XY, Sun N, Man Y et al (2021) Codon usage bias predicts the functional *MYB10* gene in *Populus*. J. Plant Physiol 265:153491. <https://doi.org/10.1016/j.jplph.2021.153491>

- Chan PP, Lowe TM (2019) tRNAscan-SE: searching for tRNA genes in genomic sequences. *Methods Mol Biol* 1962:1–14. https://doi.org/10.1007/978-1-4939-9173-0_1

Chen CJ, Chen H, Zhang Y, Thomas HR et al (2020) TB-tools: an integrative toolkit developed for interactive analyses of big biological data. *Mol Plant* 13:1194–1202. <https://doi.org/10.1016/j.molp.2020.06.009>

Chen PX, Li ZX, Zhang DH, Shen WY et al (2021) Insights into the effect of human civilization on *Malus* evolution and domestication. *Plant Biotechnol J* 19:2206–2220. <https://doi.org/10.1111/pbi.13648>

Chen J, Zang Y, Liang S, Xue S et al (2022) Comparative analysis of mitochondrial genomes reveals marine adaptation in seagrasses. *BMC Genom* 23:800. <https://doi.org/10.1186/s12864-022-09046-x>

Cornille A, Giraud T, Smulders MJ, Roldán-Ruiz I et al (2014) The domestication and evolutionary ecology of apples. *Trends Genet* 30:57–65. <https://doi.org/10.1016/j.tig.2013.10.002>

Deng YL, Wang S, Zheng Y, Yao YC (2023) Sequence and comparative analysis of chloroplast genome of apple rootstock ‘SH6’. *J Beijing Univ Agric* 38:23–27. <https://doi.org/10.13473/j.cnki.issn.1002-3186.2023.0305>

Edera AA, Small I, Milone DH, Sanchez-Puerta MV (2021) Deepred-Mt: deep representation learning for predicting C-to-U RNA editing in plant mitochondria. *Comput Biol Med* 136:104682. <https://doi.org/10.1016/j.combiomed.2021.104682>

Fang J, Jiang XH, Wang TF, Zhang XJ et al (2021) Tissue-specificity of RNA editing in plant: analysis of transcripts from three tobacco (*Nicotiana Tabacum*) varieties. *Plant Biotechnol Rep* 15:471–482. <https://doi.org/10.1007/s11816-021-00692-3>

Gao FL, Chen CJ, Arab DA, Du ZG et al (2019) Easy-CodeML: a visual tool for analysis of selection using CodeML. *Ecol Evol* 9:3891–3898. <https://doi.org/10.1002/ece3.5015>

Ge DP, Dong JM, Guo LH, Yan M et al (2020) The complete mitochondrial genome sequence of cultivated apple (*Malus domestica* cv. ‘Yantai Fuji 8’), Mitochondrial DNA Part B 5:1317–1318. <https://doi.org/10.1080/23802359.2020.1733447>

Goremykin VV, Lockhart PJ, Viola R, Velasco R (2012) The mitochondrial genome of *Malus domestica* and the import-driven hypothesis of mitochondrial genome expansion in seed plants. *Plant J* 71:615–626. <https://doi.org/10.1111/j.1365-313X.2012.05014.x>

Goryunov DV, Goryunova SV, Kuznetsova OI, Logacheva MD et al (2018) Complete mitochondrial genome sequence of the “copper moss” *Mielichhoferia elongata* reveals independent nad7 gene functionality loss, *Peer J* 6:e4350. <https://doi.org/10.7717/peerj.4350>

Greiner S, Lehwerk P, Bock R (2019) OrganelleGenomeDRAW (OGDRAW) version 1.3.1: expanded toolkit for the graphical visualization of organellar genomes. *Nucl Acids Res* 47:W59–W64. <https://doi.org/10.1093/nar/gkz238>

Gualberto JM, Newton KJ (2017) Plant mitochondrial genomes: dynamics and mechanisms of mutation, *Ann Rev Plant Biol* 68:225–252. <https://doi.org/10.1146/annurev-arplant-043015-112232>

Gupta PK, Varshney RK (2000) The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica* 113:163–185. <https://doi.org/10.1023/A:1003910819967>

Hahn C, Bachmann L, Chevreux B (2013) Reconstructing mitochondrial genomes directly from genomic next-generation sequencing reads – a baiting and iterative mapping approach. *Nucl Acids Res* 41:e129. <https://doi.org/10.1093/nar/gkt371>

Harris SA, Robinson JP, Juniper BE (2002) Genetic clues to the origin of the apple. *Trends Genet* 18:426–430. [https://doi.org/10.1016/s0168-9525\(02\)02689-6](https://doi.org/10.1016/s0168-9525(02)02689-6)

Janouškovec J, Liu SL, Martone PT, Carré W et al (2013) Evolution of red algal plastid genomes: ancient architectures, introns, horizontal gene transfer, and taxonomic utility of plastid markers. *PLoS One* 8:e59001. <https://doi.org/10.1371/journal.pone.0059001>

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Hae-seler A et al (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat Methods* 14:587–589. <https://doi.org/10.1038/nmeth.4285>

Kan SL, Shen TT, Ran JH, Wang XQ (2021) Both Conifer II and Gnetales are characterized by a high frequency of ancient mitochondrial gene transfer to the nuclear genome. *BMC Biol* 19:146. <https://doi.org/10.1186/s12915-021-01096-z>

Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Brief Bioinformatics* 20:1160–1166. <https://doi.org/10.1093/bib/bbx108>

Kleine T, Maier UG, Leister D (2009) DNA transfer from organelles to the nucleus: the idiosyncratic genetics of endosymbiosis. *Ann Rev Plant Biol* 60: 115–138. <https://doi.org/10.1146/annurev.arplant.043008.092119>

Kubo N, Kadokawa KI (2001) The gene encoding mitochondrial succinate dehydrogenase subunit 4 has been successfully transferred to the nuclear genome in pea, while leaving an original sequence as a pseudogene in the mitochondrial genome. *Plant Biotechnol* 18:283–287. <https://doi.org/10.5511/plantbiotechnology.18.283>

- Kurtz S, Choudhuri JV, Ohlebusch E, Schleiermacher C et al (2001) REPuter: the manifold applications of repeat analysis on a genomic scale. *Nucl Acids Res* 29:4633–4642. <https://doi.org/10.1093/nar/29.22.4633>
- Lai CJ, Wang J, Kan SL, Zhang S et al (2022) Comparative analysis of mitochondrial genomes of *Broussonetia* spp. (Moraceae) reveals heterogeneity in structure, synteny, intercellular gene transfer, and RNA editing. *Front Plant Sci* 13:1052151. <https://doi.org/10.3389/fpls.2022.1052151>
- Lai R, Feng X, Chen J, Zhong C et al (2019) Codon usage bias of *Canarium album* (Lour.) R. transcriptome and its influence factors. *J Nucl Agric Sci* 33:31–38. <https://doi.org/10.11869/j.issn.100-8551.2019.01.0031>
- Lee J, Kang Y, Shin SC, Park H et al (2014) Combined analysis of the chloroplast genome and transcriptome of the antarctic vascular plant *Deschampsia antarctica* Desv. *PLoS One* 9:e92501. <https://doi.org/10.1371/journal.pone.0092501>
- Liang XJ, Zhu CS, Li KX, An JC et al (2021) Codon bias of transcriptomic genes in *Cinnamomum camphora*. *Guizhaia* 41:2077–2083. <https://doi.org/10.11931/guizhaia.gxzw202003074>
- Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25:1451–1452. <https://doi.org/10.1093/bioinformatics/btp187>
- Liu H (2023) Assembly and evolution analysis of the soybean mitochondrial and chloroplast genomes. Ph. D. Thesis (University of Northwestern, Xi'an)
- Liu G, Xie YJ, Zhang DQ, Chen HP (2018) Analysis of SSR loci and development of SSR primers in *Eucalyptus*. *J For Res* 29:273–282. <https://doi.org/10.1007/s11676-017-0434-3>
- Lonsdale DM (1984) A review of the structure and organization of the mitochondrial genome of higher plants. *Plant Mol Biol* 3:201–206. <https://doi.org/10.1007/BF00029655>
- Ma K (1993) Discuss the concept of biodiversity. *Biodivers Sci* 1:20–22.
- Mower JP (2020) Variation in protein gene and intron content among land plant mitogenomes. *Mitochondrion* 53:203–213. <https://doi.org/10.1016/j.mito.2020.06.002>
- 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* 32:268–274. <https://doi.org/10.1093/molbev/msu300>
- Olson MS, McCauley DE (2000) Linkage disequilibrium and phylogenetic congruence between chloroplast and mitochondrial haplotypes in silene vulgaris. *Proc Royal Soc B* 267:1801–1808. <https://doi.org/10.1098/rspb.2000.1213>
- Park J, Xi H, Kim Y, Nam S et al (2020) The complete mitochondrial genome of new species candidate of *Rosa Rugosa* (Rosaceae). *Mitochondrial DNA Part B* 5:3435–3437. <https://doi.org/10.1080/23802359.2020.1821820>
- Pereira F, Carneiro J, Amorim A (2008) Identification of species with DNA-based technology: current progress and challenges. *Recent Pat DNA Gene Seq* 2:187–199. <https://doi.org/10.2174/187221508786241738>
- Prjibelski A, Antipov D, Meleshko D, Lapidus A et al (2020) Using SPAdes De Novo assembler. *Curr Protoc Bioinformatics* 70. <https://doi.org/10.1002/cpb.102>
- Ratajczak E, Małecka A, Ciereszko I, Staszak AM (2019) Mitochondria are important determinants of the aging of seeds. *Int J Mol Sci* 20:1568. <https://doi.org/10.3390/ijms20071568>
- Rieseberg LH, Soltis DE (1991) Phylogenetic consequences of cytoplasmic gene flow in plants. *Am J Bot* 5:65–84. <https://doi.org/10.1007/BF00021248>
- Ruhsam M, Bell D, Hart M, Hollingsworth P (2022) The genome sequence of the European crab apple, *Malus sylvestris* (L.) Mill., 1768. *Wellcome Open Res* 7:296. <https://doi.org/10.12688/wellcomeopenres.18645.1>
- Sarkissian CD, Vilstrup JT, Schubert M, Seguin-Orlando A et al (2015) Mitochondrial genomes reveal the extinct *Hippidion* as an outgroup to all living equids. *Biol Lett* 11:20141058. <https://doi.org/10.1098/rsbl.2014.1058>
- Shan YY, Li JL, Zhang X, Yu J (2023) The complete mitochondrial genome of *Amorphophallus albus* and development of molecular markers for five *Amorphophallus* species based on mitochondrial DNA. *Front Plant Sci* 14:1180417. <https://doi.org/10.3389/fpls.2023.1180417>
- Sloan DB, Wu ZQ (2016) Molecular Evolution: The perplexing diversity of mitochondrial rna editing systems. *Curr. Biol* 26:R22–R24. <https://doi.org/10.1016/j.cub.2015.11.009>
- Straub SCK, Cronn RC, Edwards C, Fishbein M et al (2013) Horizontal transfer of DNA from the mitochondrial to the plastid genome and its subsequent evolution in Milkweeds (Apocynaceae). *Genome Biol Evol* 5:1872–1885. <https://doi.org/10.1093/gbe/evt140>
- Su ZL, Wilson B, Kumar P, Dutta A (2020) Noncanonical roles of tRNAs: tRNA fragments and beyond. *Ann Rev Genet* 54:47–69. <https://doi.org/10.1146/annurev-genet-022620-101840>
- Sun XP, Jiao C, Schwaninger H, Chao CT et al (2020) Phased diploid genome assemblies and pan-genomes provide insights into the genetic history of apple do-

- mestication. *Nat Genet* 52:1423–1432. <https://doi.org/10.1038/s41588-020-00723-9>

Tamura K, Stecher G, Kumar S (2021) MEGA11: molecular evolutionary genetics analysis version 11. *Mol Biol Evol* 38:3022–3027. <https://doi.org/10.1093/molbev/msab120>

Tillich M, Lehark P, Pellizzer T, Ulbricht-Jones ES et al (2017) GeSeq – versatile and accurate annotation of organelle genomes. *Nucl Acids Res* 45:W6–W11. <https://doi.org/10.1093/nar/gkx391>

Velasco R, Zharkikh A, Affourtit J, Dhingra A et al (2010) The genome of the domesticated apple (*Malus × domestica* Borkh.). *Nat Genet* 42:833–839. <https://doi.org/10.1038/ng.654>

Wanga VO, Dong X, Oulo MA, Mkala EM et al (2021) Complete chloroplast genomes of *Acanthochlamys bracteata* (China) and *Xerophyta* (Africa) (Velloziaceae): comparative genomics and phylogenomic placement. *Front Plant Sci* 12:691833. <https://doi.org/10.3389/fpls.2021.691833>

Wu ZQ, Liao XZ, Zhang XN, Tembrock LR et al (2022) Genomic architectural variation of plant mitochondria – a review of multichromosomal structuring. *J Syst Evol* 60:160–168. <https://doi.org/10.1111/jse.12655>

Wynn EL, Christensen AC (2019) Repeats of unusual size in plant mitochondrial genomes: identification, incidence and evolution. *G3-Genes Genom. Genet* 9:549–559. <https://doi.org/10.1534/g3.118.200948>

Xu SJ, Teng K, Zhang H, Wu JY et al (2023) The first complete mitochondrial genome of *Carex (C. breviculmis)*: a significantly expanded genome with highly structural variations. *Planta* 258:43. <https://doi.org/10.1007/s00425-023-04169-1>

Zhai XY, Wang S, Zheng Y, Yao YC (2023) Assembly and comparative analysis of four mitochondrial genomes of *Malus*. *J Beijing Univ Agric* 38:28–33. <https://doi.org/10.13473/j.cnki.issn.1002-3186.2023.0306>

Zhang D, Gao FL, Jaković I, Zou H et al (2020) PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Mol Ecol Resour* 20:348–355. <https://doi.org/10.1111/1755-0998.13096>

Zhao N, Grover CE, Chen ZW, Wendel JF et al (2019) Intergenomic gene transfer in diploid and allopolyploid *Gossypium*. *BMC Plant Biol* 19:492. <https://doi.org/10.1186/s12870-019-2041-2>

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