

# CANCER AND CANCER THERAPY

December 07, 2022 | Dubai, UAE

Received date-22-07-2022 | Accepted date-25-08-2022 | Published date- 12-12-2022



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### Complete chloroplast genomes of two medicinal *Swertia* species: The comparative evolutionary analysis of *Swertia* genus in the Gentianaceae family

**Background/Aims:** We developed the comparative research of *Swertia kouitchensis* chloroplast genome with that of 22 *Swertia* species in the *Gentianaceae* family from the aspects of phenotype characteristics, structural and genetic features, and evolutionary relationship. Thus, we could exploit the distinctive differentia and identified traits to fully illustrate the relevance and distinction among these species on account of the complete database statement for these chloroplast genomes.

**Methods:** Total genomic DNA was extracted using the plant genomic DNA kit (Tiangen Biotech, Beijing, China) (Vieira *et al.* 2014). The DNA purity was detected with 1.0% agarose gel and the concentration of cpDNA was determined using a Nanodrop spectrophotometer 2000 (Rowan *et al.* 2011). DNA extracts were fragmented for 300 bp short-insert library construction. The library was sequenced in pair-end mode with the read length of 150 bp on an Illumina HiSeq 2500 platform (Cronn *et al.* 2008). The raw reads were filtered using Trimmomatic 0.35 with default parameters to remove adapters and low-quality bases (Bolger 2014). Then, the chloroplast genome of *S. kouitchensis* was assembled using the NOVOPlasty (v 4.2) software (Dierckxsens *et al.* 2017) with default parameters and the *rbcl* sequences as the seed. We used Gepard software to draw dot plots between the assembled genome and reference genomes to identify the structure of the chloroplast genome (Krumšek *et al.* 2007). The chloroplast genome can be annotated using the CPGAVAS2 web service (<http://www.herba.lgenomics.org/cpgavas2>) (Shi *et al.* 2019). The annotation was manually corrected using the Apollo software (Firtina *et al.* 2020). We calculated the GC content using BioXM software (Losko and Heumann 2009). Lastly, the assembly and annotation results of the *S. kouitchensis* chloroplast genome was submitted to GenBank and the accession number

OM617848 was acquired. Then, the comparative data of *S. bimaculata* chloroplast genome was downloaded from the NCBI database (MW344296.1), which was developed by the team of professor Weizhen from Zhengzhou University.

**Results:** The significant distinction in the specification between *Strigoptera bimaculata* and *Swertia kouitchensis* lies in the shape of the stem (1), pedicel (2), and leaf (3); The color of corolla (4) and seeds, spots (5), and the shape of the seed surface. In the species of *S. bimaculata*, it has a round stem (1), thick pedicel (2), erect or oblique extension, unequal length, oval to ovate-lanceolate leaf (3), mostly small purple spots (5) in the upper part of the yellow corolla (4), two semicircular yellow-green large gland spots (5) in the middle, and the brown round seeds with protruding on the surface. Meanwhile, the species of *S. kouitchensis* has four-angular erect stems (1); narrow pedicel wings (2), lanceolate leaf (3), yellow, white, and green corolla (4), apex and long pointed fruit that slightly increases with two glands (5) at the base; and yellow-brown seeds with a nearly smooth surface.

The chloroplast genome of *S. kouitchensis* encodes a total of 131 genes (110 unique genes), which include 88 PCGs (80 unique genes), 37 tRNAs (29 unique genes), and 8 genes encoding ribosome RNA (4 unique genes). The 88 PCGs encoded a total of 26,243 and 25,961 codons in the *S. kouitchensis* and *S. bimaculata* chloroplast genome. The most abundant codon of the two species was for isoleucine, and the least abundant codon was for cysteine.

The number of microsatellites repeat sequences was twenty-eight and thirty-two identified in the chloroplast genomes of *S. kouitchensis* and *S. bimaculata*, respectively. A total of 1127 repeat sequences were identified in all the 23 *Swertia* chloroplast genomes, and they fell into

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four categories. Furthermore, five divergence hotspot regions can be applied to discriminate these 23 *Swertia* species through genomes comparison. One pair of genus-specific DNA barcodes primer has been accurately identified. Therefore, the diverse regions cloned by a specific primer may become an effective and powerful molecular marker for the identification of *Swertia* genus. Moreover, four genes (*ccsA*, *ndhK*, *rpoC1*, and *rps12*) were positive selective pressure. The phylogenetic tree showed that the 23 *Swertia* species were clustered into a large clade including four evident subbranches, whereas the two species of *S. kouitchensis* and *S. bimaculata* were separately clustered into the diverse but correlated species group.

**Conclusion:** The complete chloroplast genome of *Swertia kouitchensis* has been sequenced and assembled, compared with that of *S. bimaculata* to determine the evolutionary relationships among species of the *Swertia* in the *Gentianaceae* family.

## Recent Publications

1. Du Q, ZENG J, Wang L, *et al.* Comparative genomics and phylogenetic analysis of the chloroplast genomes in three medicinal *Salvia* species for bioexploration. Research Square; 2022. DOI: 10.21203/rs.3.rs-1582501/v1
2. Du Q, Li J, Wang L, *et al.* Complete chloroplast genomes of two medicinal *Swertia* species: the comparative evolutionary analysis of *Swertia* genus in the *Gentianaceae* family. *Planta*. 2022 Sep;256(4):73. DOI: 10.1007/s00425-022-03987-z. PMID: 36083348.
3. Du Q, Jiang M, Sun S, *et al.* The complete chloroplast genome sequence of *Clerodendranthus spicatus*, a medicinal plant for preventing and treating kidney diseases from Lamiaceae family. *Molecular Biology Reports*. 2022 Apr;49(4):3073-3083. DOI: 10.1007/s11033-022-07135-4. PMID: 35059973.

## Biography

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