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PLASTOME REPORT

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The complete chloroplast genome sequence of *Prunus salicina* cultivar 'Zuili' (Rosaceae)

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ABSTRACT

'Zuili' is a distinguished plum (*Prunus salicina* Lindley 1830) originating from Tongxiang City, Zhejiang Province, China, and a nationally recognized geographical indication product. In this study, we reported the complete chloroplast genome of *P. salicina* cultivar 'Zuili'. The genome has a circular structure of 157,935 bp containing a large single-copy (LSC) region of 86,133 bp, a small single-copy (SSC) region of 19,028 bp, and two inverted repeats (IRs) of 26,387 by each. It harbors 130 genes (111 unique genes), including 85 protein-coding genes (78 are unique), eight ribosomal RNA genes (four are unique), and 37 transfer genes (29 are unique). The phylogenetic analysis based on whole chloroplast genomes showed 'Zuili' was clustered with *Prunus salicina* cultivar 'Wuyuecui' (MW406461.1) and 'No. 2 Guofeng' (MW406472.1). This study provides valuable information that can contribute to the identification and further evolutionary analysis of *Prunus salicina* cultivar 'Zuili'.

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Introduction

Prunus salicina Lindley 1830, commonly known as the Japanese plum or Chinese plum, is a species of fruit tree in the Rosaceae family. It is native to China and is widely cultivated throughout the world, particularly in East Asia, where it is known for its delicious and nutritious fruits (Jia et al. 2013). This species boasts a diverse range of cultivars, varying greatly in size, color, and cultivation requirements. For instance, the 'Santa Rosa' cultivar is known for its large size and red to purple hue, and is popularly consumed fresh, canned, or in cooked forms. Conversely, the 'Shiro' cultivar, recognized by its medium-sized, yellow-skinned fruits, thrives only in warm, sheltered environments.

Prunus salicina cultivar 'Zuili' is a characteristic fruit that originated from Zuili City of ancient China, and a nationally recognized geographical indication product with very high economic value. The cultivation of this famous plum has spanned across centuries. 'Zuili' plum is renowned for its specific characteristics: it weighs around 50 g per fruit and has a dark purple-red skin adorned with a patchwork of varying yellow-brown spots and a layer of white fruit powder (Figure 1). The flesh is light orange, dense, sweet, and aromatic. When ripe, it turns into a juicy pulp that emits a strong wine-like aroma and creates a unique flavor (Jia et al. 2013). In the present study, the whole chloroplast genome of 'Zuili' was characterized and assembled for the first time, which provided data resources for its phylogenetic study.

Materials and methods

The fresh leaves of *P. salicina* cultivar 'Zuili' were sampled at Tongxiang, Jiaxing city, Zhejiang Province, China (30°42′38″N and 120°31′38″E). The voucher specimen (TXZL001) was deposited at Institute of Eco-Environmental Sciences, Jiaxing Academy of Agricultural Sciences (https://www.jxnky.com, Zhangliang Yao is the contact person, zlyao513@126.com). Total genomic DNA was extracted using the CTAB method (Doyle and Doyle 1987). The extracted whole genomic DNA

Figure 1. Appearance of *Prunus salicina* cultivar 'Zuili'. The photo was taken by Liangling Cao at Tongxiang, Jiaxing city, Zhejiang Province, China (30°42′38″N and 120°31′38″E). The fruit weighs around 50 g and has a dark purple-red skin adorned with a patchwork of varying yellow-brown spots. The leaves are simple, ovate to oblong in shape, with a finely serrated margin.

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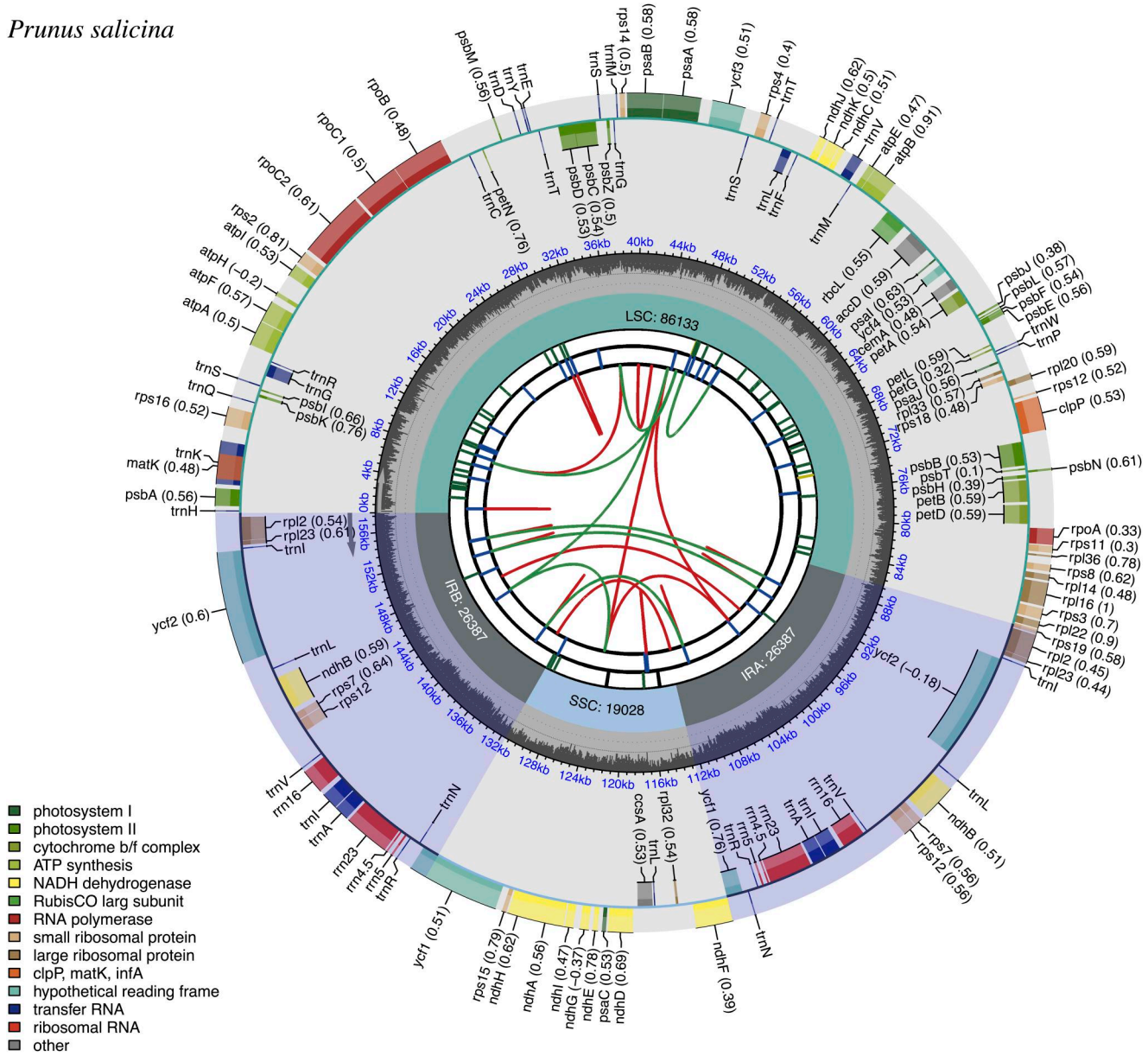

Figure 2. Chloroplast genome map of *Prunus salicina* cultivar 'Zuili' by CPGview. The map contains six tracks in default. From the center outward, the first track shows the dispersed repeats. The dispersed repeats consist of direct (D) and Palindromic (P) repeats, connected with red and green arcs. 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 colors, the type of repeat they represent, and the description of the repeat types are as follows: black: c (complex repeat); green: p1 (repeat unit size = 1); yellow: p2 (repeat unit size = 2); purple: p3 (repeat unit size = 3); blue: p4 (repeat unit size = 4); orange: p5 (repeat unit size = 5); red: p6 (repeat unit size = 6). The quadripartite structure (LSC, SSC, IRA, and IRB) is illustrated on the fourth track. The GC content is plotted on the fifth track. The genes are shown on the sixth track. The optional codon usage bias is displayed in the parenthesis after the gene name. Genes are color-coded by their functional classification. The transcription directions for the inner and outer genes are clockwise and anticlockwise, respectively. The functional classification of the genes is shown in the bottom left corner.

was used to construct sequencing library and sequenced using Illumina NovaSeq 6000 sequencing platform (Illumina Inc., San Diego, CA) with a paired-end sequencing length of 150 bp. The raw data were filtered, and adapter trimmed using fastp (version 0.23.2) (Chen et al. 2018) with default parameters. The complete chloroplast genome was then assembled using the clean data by GetOrganelle (version 1.7.7.0) (Jin et al. 2020). The complete chloroplast genome of 'Zuili' was annotated using CpGAVAS2 (Shi et al. 2019). The Geneious Prime (2023.2) (Kearse et al. 2012) was employed to correct these annotations with problem. The OrganellarGenomeDRAW (OGDRAW) (version 1.3.1) (Greiner et al. 2019) and CPGView (www.1kmpg.cn/cpgview/; Liu et al. 2023) were used to draw the chloroplast genome map of 'Zuili'. The sequencing depth and coverage plot of the assembled chloroplast genome were generated by mapping the raw reads onto the assembled chloroplast genome using BWA-mem (version v0.7.17) (Li and Durbin 2009). The alignment file was then used to calculate the depth using SAMtools depth (version 1.16.1) (Li et al. 2009). The complete chloroplast genome sequences of 'Zuili' assembled in current study and other species/varieties downloaded from NCBI were aligned using MAFFT (version 7) (Katoh and Standley 2013) with auto strategy. These aligned sequences were then used to construct the maximum-likelihood (ML) tree by IQ-TREE (version 2.0.3) (Nguyen et al. 2015) with 1000 bootstrap replicates. The phylogeny tree was

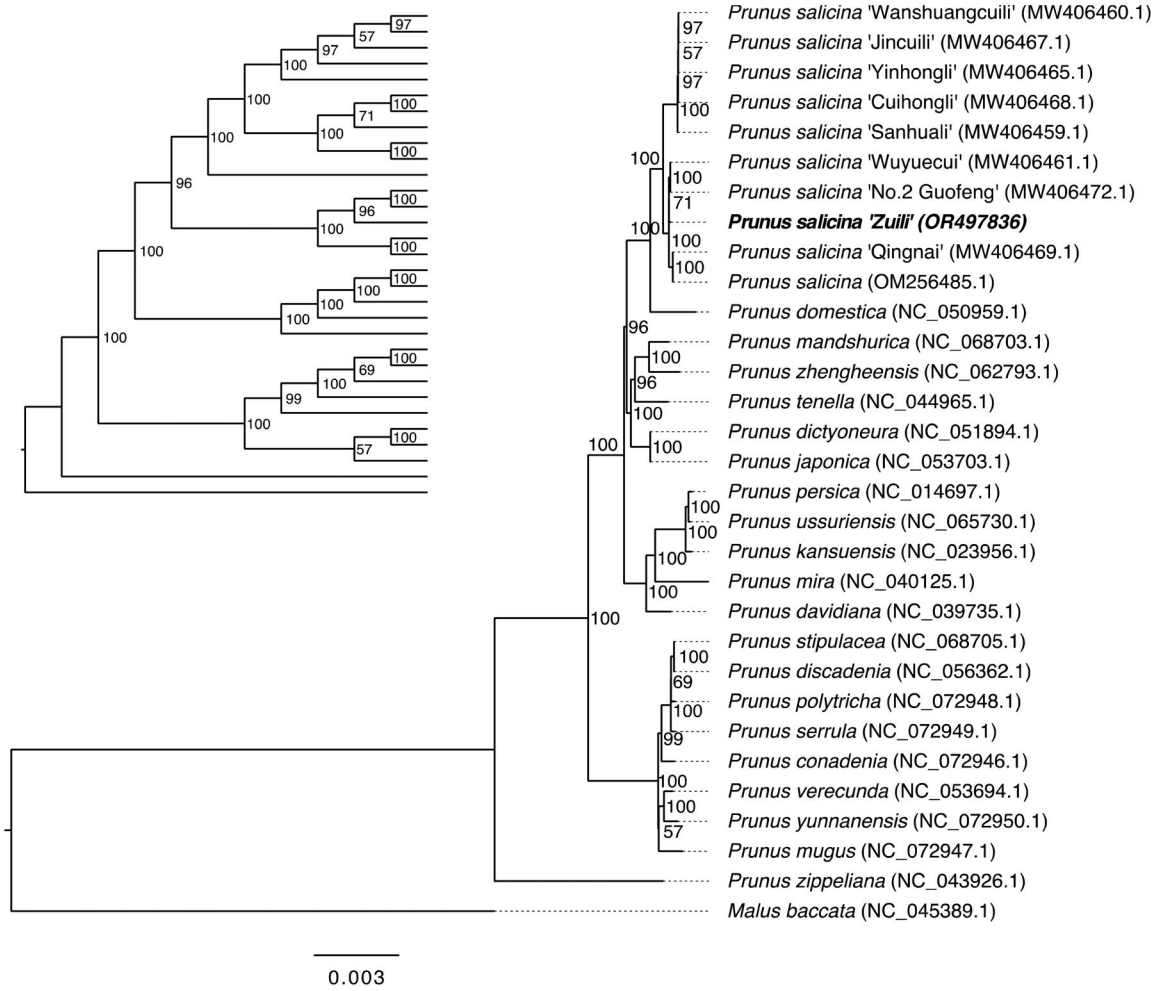

Figure 3. Phylogenetic tree inferred by maximum-likelihood (ML) method based on the complete chloroplast genomes of 30 *Prunus* species and one outlier (*Malus baccata*). For clearly displaying the phylogenetic relationship, a cladogram without species name is placed in the top left. A total of 1000 bootstrap replicates were performed, and the bootstrap support values (percentage) are indicated behind nodes. The chloroplast genome of *Prunus* salicina cultivar Zuili is shown in bold. The NCBI GenBank accession numbers are shown in parentheses. The gray lines were used to supplement the branch length, and the branch length of the phylogram was black. The following sequences were used: *Prunus salicina* 'Wanshuangcuili' MW406460.1, *Prunus salicina* 'Jincuili' MW406467.1, *Prunus salicina* 'Yinhongli' MW406465.1, *Prunus salicina* 'Cuihongli' MW406468.1, *Prunus salicina* 'Sanhuali' MW406459.1, *Prunus salicina* 'Wuyuecui' MW406461.1, *Prunus salicina* 'No.2 Guofeng' MW406472.1, *Prunus salicina* 'Qingnai MW406469.1, *Prunus salicina* OM256485.1 (Su et al. 2023), *Prunus domestica* NC_050959.1 (Geng et al. 2020), *Prunus mandshurica* NC_068703.1, *Prunus zhengheensis* NC_062793.1, *Prunus tenella* NC_044965.1, *Prunus dictyoneura* NC_051894.1 (Liu et al. 2019), *Prunus japonica* NC_053703.1 (Mu et al. 2021), *Prunus persica* NC_014697.1 (Jansen et al. 2011), *Prunus ussuriensis* NC_065730.1, *Prunus kansuensis* NC_023956.1, *Prunus mira* NC_040125.1 (Bao et al. 2019), *Prunus davidiana* NC_039735.1 (Zhang et al. 2018), *Prunus stipulacea* NC_068705.1, *Prunus discadenia* NC_056362.1, *Prunus polytricha* NC_072948.1, *Prunus serrula* NC_072949.1, *Prunus conadenia* NC_072946.1, *Prunus verecunda* NC_053694.1 (Jiang et al. 2019), *Prunus yunnanensis* NC_072950.1, *Prunus mugus* NC_072947.1, *Prunus zippeliana* NC_043926.1, and *Malus baccata* NC_045389.1.

visualized using FigTree (version v1.4.4) (https://github.com/rambaut/figtree/).

Results

The complete circular chloroplast genome of *P. salicina* cultivar 'Zuili' is 157,935 bp in size with a large single-copy (LSC) region of 86,133, small single-copy (SSC) region of 19,028 bp, and two inverted repeats (IRs) of 26,387 bp by each (Figure 2). The genome assembly quality was assessed. The sequencing depth of the bases in the assembled genome was between 154× and 1587×, with a mean of 1110.3× (Figure S1). The chloroplast genome of *P. salicina* cultivar 'Zuili' comprises 130 genes (111 are unique genes), including 85 protein-coding genes (78 are unique), eight ribosomal RNA (rRNA) genes (four are unique), and 37 transfer RNA (tRNA) genes (28 are unique). Nineteen genes were single-intron genes, and four genes had two introns (Tables S1 and S2). The cis-splicing genes and the trans-splicing gene rps12 are shown in Figures S2 and S3. The length of the protein-coding sequence (CDS) in the chloroplast genome of 'Zuili' is 79,545 bp. The overall GC content is 36.76%, while the GC contents in IR regions (42.62%) are significantly higher than that in LSC (39.35%) and SSC regions (30.40%). The complete chloroplast genome of *P. salicina* cultivar 'Zuili' with annotations was submitted to GenBank (accession no. OR497836). The raw reads were deposited in the NCBI Sequence Read Archive (accession no. SRR26329588).

To figure out the phylogenetic position of *P. salicina* cultivar 'Zuili', a ML tree was constructed by using the complete chloroplast genome of 'Zuili' assembled in current study and other 29 *Prunus* species and one outgroup (*Malus baccata*) downloaded from NCBI database. The phylogenetic analysis

based on chloroplast genomes fully resolved *P. salicina* cultivar 'Zuili' in a clade with *Prunus salicina* cultivar 'Wuyuecui' (MW406461.1) and 'No. 2 Guofeng' (MW406472.1) (Figure 3).

Discussion and conclusions

In this study, we successfully sequenced and characterized the complete chloroplast genome of *Prunus salicina* cultivar 'Zuili'. Phylogenetic analysis revealed that 'Zuili' clustered closely with the cultivars 'Wuyuecui' (MW406461.1) and 'No. 2 Guofeng' (MW406472.1) within a distinct clade. Notably, 'No. 2 Guofeng', 'Wuyuecui', and 'Zuili' share similar fruit skin colors, ranging from red to purple. However, the average fruit weight of 'No. 2 Guofeng' is approximately 125 g, double that of 'Zuili'. Chloroplast genome analysis suggests the closest relationship between 'No. 2 Guofeng' and 'Wuyuecui', both of which feature yellow flesh. Despite the genetic similarity of chloroplast genome, these two cultivars still exhibit great differences in other morphological characteristics. Additionally, 'Wanshuangcuili' and 'Jincuili' were grouped together in the phylogenetic tree, both characterized by their crisp and tender fruit flesh, a trait that is even reflected in their names. While we have very limited knowledge about the breeding history, phenotype, and physiology of most cultivars, a more comprehensive study still needs to be conducted in future. Also, in order to further explore the historical origin of 'Zuili', more ancient local varieties and cultivars are needed to be included. Nevertheless, this study provided valuable information that can contribute to the identification and further evolutionary analysis of *Prunus salicina* cultivar 'Zuili'.

Author contributions

The research was designed by ZLY, JSM, and QL. The experiment was conducted by ZLY, QL, WDX, and LLC. The data analysis was conducted by JSM, ZLY, and QL. The manuscript draft was prepared by JSM, ZLY, QL, and WDX. All authors participated in the discussion and editing of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The genome sequence 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. OR497836.1. The associated 'BioProject', 'SRA', and 'Bio-Sample' numbers are PRJNA1026316, SRR26329588, and SAMN37737007, respectively.

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