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MITOGENOME ANNOUNCEMENT

The complete chloroplast genome sequence of rose-gold pussy willow, *Salix gracilistyla* Miq. (Salicaceae)

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ABSTRACT

To understand genetic background of *Salix gracilistyla* Miq., we presented its complete chloroplast genome which is 155,557 bp and has four sub regions: 84,530 bp of large single copy (LSC) and 16,218 bp of small single copy (SSC) regions are separated by 27,405 bp of inverted repeat (IR) regions including 130 genes (84 protein-coding gene, eight rRNAs, and 38 tRNAs). The overall GC content of the chloroplast genome is 36.7% and those in the LSC, SSC, and IR regions are 34.5%, 31.0%, and 41.9%, respectively. Phylogenetic trees show phylogenetic position of *S. gracilistyla* with low level of inter-species sequence variations.

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Salix gracilistyla; chloroplast genome; *Salix*; SSC inversion; Salix phylogeny

Salix gracilistyla Miq., belonging to *Salix* L., the largest genus in Salicaceae (Argus 1997), lives nearby river or streams (Lee 2003). *Salix gracilistyla* is dioecious species, separating male and female individuals (Argus 1997). *S. gracilistyla* has been studied about ability to endure flooding and draught, affecting its distribution on riverbank (Nakai et al. 2009, 2010); however, there is no molecular study of this species conducted till now.

Total DNA was extracted from fresh leaves of *S. gracilistyla* male individual isolated from Hwacheon-gun, Gangwon-do, Korea (Voucher deposited in InfoBoss Cyber Herbarium (IN): Y. Kim IB-01020) using a DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). Genome sequencing was performed using HiSeqX at Macrogen Inc., Korea. Raw reads were trimmed by Trimmomatic (Bolger et al. 2014) and *de novo* assembly and confirmation were carried out using Velvet 1.2.10 (Zerbino and Birney 2008), SOAPGapCloser 1.12 (Zhao et al. 2011), BWA 0.7.17 (Li 2013), and SAMtools 1.9 (Li et al. 2009). Geneious R11 11.0.5 (Biomatters Ltd, Auckland, New Zealand) was used for annotation based on *S. koryanagi* chloroplast complete genome (MK120982; Kim, Kim, et al. 2019).

The chloroplast genome of *S. gracilistyla* (Genbank accession is MK814774; GC ratio is 36.7%) is 155,557 bp and has four subregions: 84,530 bp of large single copy (LSC; 34.5%) and 16,218 bp of small single copy (SSC; 31.0%) regions are separated by 27,405 bp of inverted repeat (IR; 41.9%). It contains 130 genes (84 protein-coding genes, eight rRNAs, and 38 tRNAs); 19 genes (eight protein-coding genes, four rRNAs, and seven tRNAs) are duplicated in IR regions. Both LSC and SSC of *S. gracilistyla* are inverted in comparison to that of *S. koryianagi* female individual, which is similar but different from those of *S. koryianagi* male individual (Park, Kim, and Xi 2019), *Hibiscus syriacus* (Kim, Oh et al. 2019), and *Pseudostellaria heterophylla* (Kim et al. under review).

Sequence alignment of 21 *Salix* and one *Populus* chloroplast genomes (Kim, Kim, et al. 2019) with correcting directions of LSC and SSC of five *Salix* chloroplast genomes (*Salix suchowensis*, *Salix purpurea*, *Salix arbutifolia*, *Salix babylonica*, and *S. gracilistyla*) was conducted using MAFFT (Katoh and Standley 2013). Maximum likelihood (bootstrap repeat is 1,000) and neighbor joining (bootstrap repeat is 10,000) phylogenetic trees were constructed using MEGA X (Kumar et al. 2018), presenting congruent with previous *Salix* (Lauron-Moreau et al. 2015; Wu et al. 2015; Figure 1). Phylogenetic trees show phylogenetic position of *S. gracilistyla* clustered with two chloroplast genomes of *S. koryianagi* and separated from those of three *Salix* species originated from China (Figure 1). In addition, numbers of single nucleotide polymorphisms and insertions and deletions between *S. gracilistyla* and *S. koryianagi* are 40 and 139, respectively, which is lower than intraspecies variations of *Marchantia polymorpha* (Kwon et al. 2019), *Camellia japonica* (Park, Kim, Xi, et al. 2019), *Pseudostellaria palibiniana* (Kim, Heo, et al. 2019), *Pyrus ussuriensis* (Cho et al. under review), *Rehmannia glutinosa* (Jeon et al. 2019), and *Eucommia ulmoides* (Wang et al. 2016).

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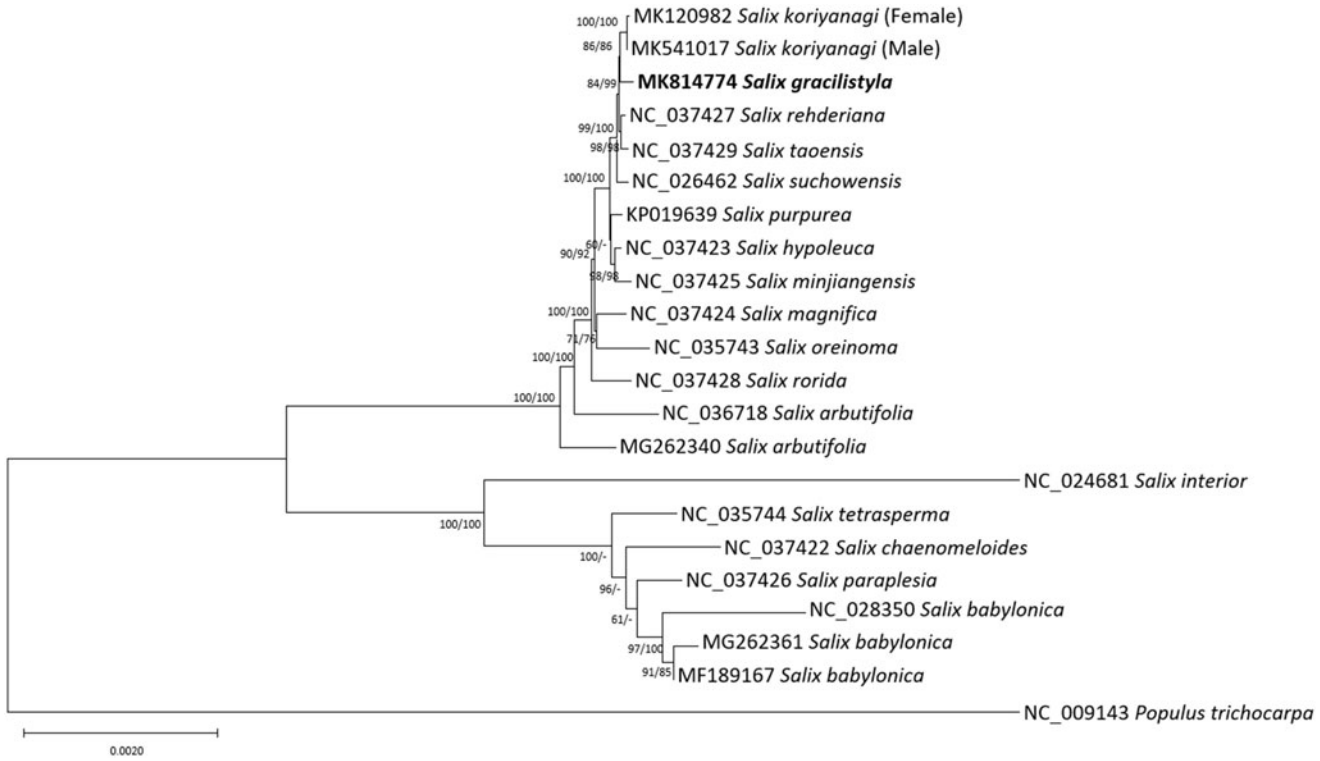

Figure 1. Neighbor joining (bootstrap repeat is 10,000) and maximum likelihood (bootstrap repeat is 1,000) phylogenetic trees of 21 *Salix* and one *Populus* complete chloroplast genomes from Salicaceae: *Salix gracilistyla* (MK814774 in this study), *Salix koriyanagi* (MK541017 and MK120982), Salix suchowensis (NC_026462), *Salix purpurea* (KP019639), *Salix rehderiana* (NC_037427), *Salix rorida* (NC_037428), *Salix taoensis* (NC_037429), *Salix tetrasperma* (NC_035744), *Salix paraplesia* (NC_037426), *Salix oreinoma* (NC_035743), *Salix minjiangensis* (NC_037425), *Salix magnifica* (NC_037424), *Salix interior* (NC 024681), *Salix interior* (NC_024681), *Salix hypoleuca* (NC_037423), *Salix chaenomeloides* (NC_037422), *Salix babylonica* (NC_028350, MG262361, and MF189167), *Salix arbutifolia* (NC 036718 and MG262340), and *Populus trichocarpa* (NC 009143). Neighbor joining tree was used for displaying phylogenetic tree. The numbers above branches indicate bootstrap support values of neighbor joining and maximum likelihood trees, respectively.

More *Salix* chloroplast genomes will present landscape of sequence variations on chloroplast genomes.

Disclosure statement

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