





The complete chloroplast genome of a new candidate cultivar, Sang Jae, of *Abeliophyllum distichum* Nakai (Oleaceae): initial step of *A. distichum* intraspecies variations atlas

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ABSTRACT

Chloroplast genome of Sang Jae, new candidate cultivar of *Abeliophyllum distichum* Nakai, has been sequenced to reveal infraspecific relationship of wildtypes and cultivars. Its length is 156,008 bp long (GC ratio is 37.8%) and has four subregions: 86,773 bp of large single copy (35.8%) and 17,827 bp of small single copy (31.9%) regions are separated by 25,704 bp of inverted repeat (43.2%) regions including 133 genes (87 protein-coding genes, eight rRNAs, and 37 tRNAs). Phylogenetic trees show that *A. distichum* is clustered with twelve *Forsythia* species and intraspecies variations level is similar to *Olea europaea*.

ARTICLE HISTORY

Received 17 July 2019
Accepted 3 August 2019

KEYWORDS

Abeliophyllum distichum;
chloroplast genome;
cultivar-specific variation;
Sang Jae; Korea endemic



Abeliophyllum distichum Nakai, is endemic to Korea and monotypic species of genus *Abeliophyllum* (Nakai 1919; Melchior 1964). A novel candidate cultivar of *A. distichum*, Sang Jae, has been discovered, which has remarkable phenotypic differences from wildtype: flower is yellow and curved petal has wavy margin (Figure 1(A)) and fruit shape is obovate, different from that of wild type (Figure 1(B)). *A. distichum* is widely used as landscaping tree even though its natural habitats are limited and threatening (Kim and Maunder 1998; Lim et al. 2009; Lee et al. 2014). Four chloroplast genomes of two *A. distichum* are available presenting intraspecies relationship between cultivars and natural isolates (Kim et al. 2016; Ha et al. 2018; Park, Kim et al. 2019; Park, Min et al. 2019). Here, we completed third cultivar chloroplast genome of *A. distichum* for understanding intraspecies variations.

Total DNA of Sang Jae collected in GoesanBunjae-Nongwon (Goesan-gun, Chungbuk Province, Republic of Korea, Voucher in InfoBoss Cyber Herbarium (IN); Y. Kim, IB-01026) was extracted from fresh leaves by using a DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). Genome was sequenced using HiSeqX at Macrogen Inc., Korea, and *de novo* assembly and sequence confirmation were done by 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 chloroplast genome annotation based on *A. distichum* chloroplast (MK616470; Park et al. 2019).

Chloroplast genome of *A. distichum*, Sang Jae, (Genbank accession is MN127986) is 156,008 bp (GC ratio is 37.8%) and has four subregions: 86,773 bp of large single copy (35.8%) and 17,827 bp of small single copy (31.9%) regions are separated by 25,704 bp of inverted repeat (IR; 43.2%). It contains 133 genes (87 protein-coding genes, eight rRNAs, and 37 tRNAs); 19 genes (7 protein-coding genes, 4 rRNAs, and 7 tRNAs) are duplicated in IR regions. It is identical to that of Ok-Hwang-1-ho because this cultivar was rescued from the same region.

Based on alignments with four *A. distichum* chloroplast genome, 9 single nucleotide polymorphisms (SNPs) and 11 insertions and deletions (INDELs) were found between Sang Jae and Dae Ryun (MN116559). While there are 93 SNPs and 56 INDELs between Sang Jae and natural isolate (NC_031445) caused by exceptional sequence variations (56 SNPs and 15 INDELs) in *rpoC2*. All INDELs do not affect frame of *rpoC2*. Only one INDEL was found in the comparison of another natural isolate (MF407183).

Forty-eight Oleaceae chloroplast genomes including five *A. distichum* chloroplast genomes were used for constructing bootstrapped neighbor joining and maximum likelihood phylogenetic trees using MEGA X (Kumar et al. 2018) via modifying directions of subregions and aligning whole chloroplast genomes by MAFFT 7.388 (Kato and Standley 2013). Phylogenetic trees show that five *A. distichum* form an independent clade from *Forsythia* species, supporting that genus *Abeliophyllum* has evolved separated from *Forsythia* clade. In

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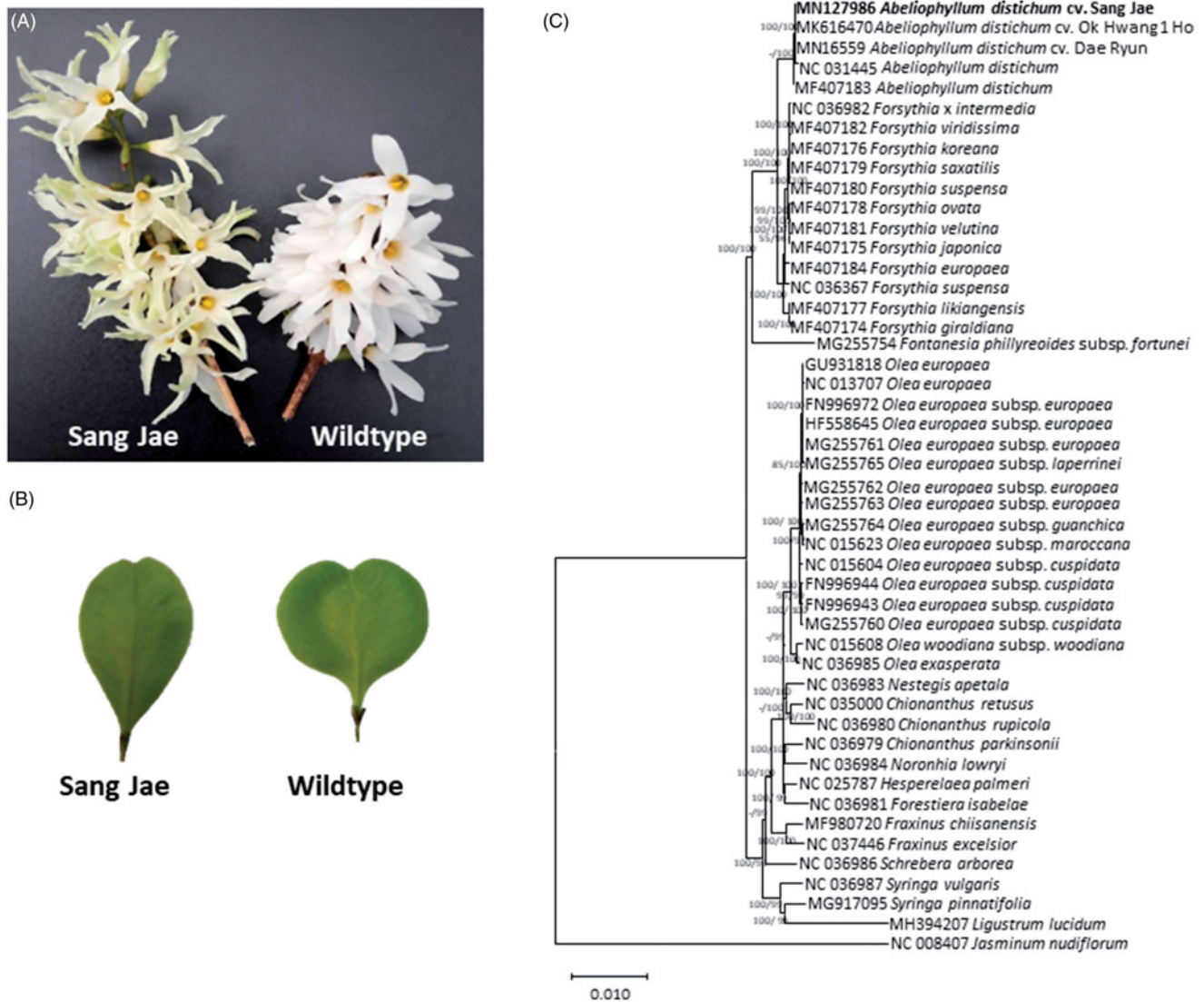


Figure 1. (A) Pictures of flowers of Sang Jae and wildtype of *A. distichum*. (B) Pictures of fruits of Sang Jae and wildtype of *A. distichum*. (C) Maximum likelihood (bootstrap repeat is 1,000) and neighbor joining (bootstrap repeat is 10,000) phylogenetic trees of forty-eight Oleaceae chloroplast genomes: *Abeliophyllum distichum* (MN127986 in this study, MN116559, MK616470, MF407183, and NC_031445), *Forsythia x intermedia* (NC_036982), *Forsythia giraldiana* (MF407174), *Forsythia likiangensis* (MF407177), *Forsythia suspensa* (NC_036367, MF407180), *Forsythia europaea* (MF407184), *Forsythia japonica* (MF407175), *Forsythia velutina* (MF407181), *Forsythia ovata* (MF407178), *Forsythia saxatilis* (MF407179), *Forsythia koreana* (MF407176), *Forsythia viridissima* (MF407182), *Fontanesia phillyreoides* subsp. *fortunei* (MG255754), *Olea woodiana* subsp. *woodiana* (NC_015608), *Olea exasperata* voucher A. Costa 1 (NC_036985), *Olea europaea* subsp. *maroccana* (NC_015623), *Olea europaea* subsp. *guanchica* isolate La Gomera 10 (MG255764), *Olea europaea* cultivar Frantoio (GU931818), *Olea europaea* cultivar Bianchera (NC_013707), *Olea europaea* subsp. *europaea* cultivar Manzanilla (FN996972), *Olea europaea* subsp. *europaea* isolate Stavrovouni 11 (HF558645), *Olea europaea* subsp. *laperrinei* isolate Adjelella 10 (MG255765), *Olea europaea* subsp. *europaea* isolate Vallee du Fango 5 (MG255762), *Olea europaea* subsp. *cuspidata* (NC_015604), *Hesperelaea palmeri* (NC_025787), *Chionanthus retusus* (NC_035000), *Olea europaea* subsp. *europaea* isolate Oeiras 1 (MG255763), *Schrebera arborea* (NC_036986), *Fraxinus excelsior* (NC_037446), *Syringa vulgaris* (NC_036987), *Noronhia lowryi* (NC_036984), *Fraxinus chiisanensis* (MF980720), *Chionanthus rupicola* (NC_036980), *Olea europaea* subsp. *cuspidata* (FN996944), *Olea europaea* subsp. *cuspidata* isolate Menagesha Forest 14 (MG255760), *Olea europaea* subsp. *cuspidata* isolate Almihwit 5.1 (FN996943), *Chionanthus parkinsonii* (NC_036979), *Syringa pinnatifolia* (MG917095), *Forestiera isabelae* (NC_036981), *Olea europaea* subsp. *europaea* isolate Stavrovouni Monastery 11 (MG255761), *Nestegis apetala* (NC_036983), *Ligustrum lucidum* (MH394207), and *Jasminum nudiflorum* (NC_008407). Phylogenetic tree was drawn based on neighbor joining tree. The numbers above branches indicate bootstrap support values of maximum likelihood and neighbor joining phylogenetic trees, respectively.

addition, intraspecies variations among five *A. distichum* is similar to those of *Olea europaea*.

Disclosure statement

The authors declare that they have no competing interests.

Funding

This research was supported by Basic Science Research Programme through the National Research Foundation of Korea (NRF) funded by the Ministry of Education [NRF-2016R1D1A1B03934869].

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