






## The complete chloroplast genome of a new candidate cultivar, Dae Ryun, of *Abeliophyllum distichum* Nakai (Oleaceae)

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### ABSTRACT

To understand genetic features of new candidate cultivar, Dae Ryun of *A. distichum*, chloroplast genome was sequenced. Its length is 156,019 bp and has four subregions: 86,783 bp of large single-copy (LSC) and 17,828 bp of small single-copy (SSC) regions are separated by 25,704 bp of inverted repeat (IR) regions including 133 genes (87 protein-coding genes, 8 rRNAs, and 37 tRNAs). Overall GC content is 35.8%. Intraspecies sequence variations among four *A. distichum* chloroplast genomes present various numbers. Phylogenetic trees show that *A. distichum* is clustered with 12 *Forsythia* species.

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*Abeliophyllum distichum* Nakai (Oleaceae) is a monotypic genus endemic to Korea (Chung 1999). Nine natural habitats have been found (Lee et al. 2014). *Abeliophyllum distichum* displays various phenotypes of colours and size of flowers (Lee 1976) and also shows continuous phenotypic variations inside habitat (Kim 2001). Ok-Hwang-1-ho became first official cultivar of *A. distichum* and its chloroplast genome was completed presenting enough number of variations (Park, Kim, Xi, Jang, et al. 2019). To unravel relationship between phenotypic and genetic variations of *A. distichum* with three available chloroplasts (Kim et al. 2016; Ha et al. 2018; Park, Kim, Xi, Jang, et al. 2019), chloroplast of new candidate cultivar, named as Dae Ryun, of which flower is larger than wildtype (Figure 1(A)) was sequenced.





Total DNA of Dae-Ryun collected in GoesanBunjae-Nongwon (Goesan-gun, Chungbuk Province, Korea, Voucher in InfoBoss Cyber Herbarium (IN); IB-01025) 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 from *A. distichum* (MK616470; Park, Kim, Xi, Jang, et al. 2019).

Chloroplast genome of *A. distichum* (Genbank accession is MN116559) is 156,019 bp (GC ratio is 37.8%) and has four subregions: 86,783 bp of large single-copy (35.8%) and

17,828 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, 8 rRNAs, and 37 tRNAs); 18 genes (7 protein-coding genes, 4 rRNAs, and 7 tRNAs) are duplicated in IR regions.

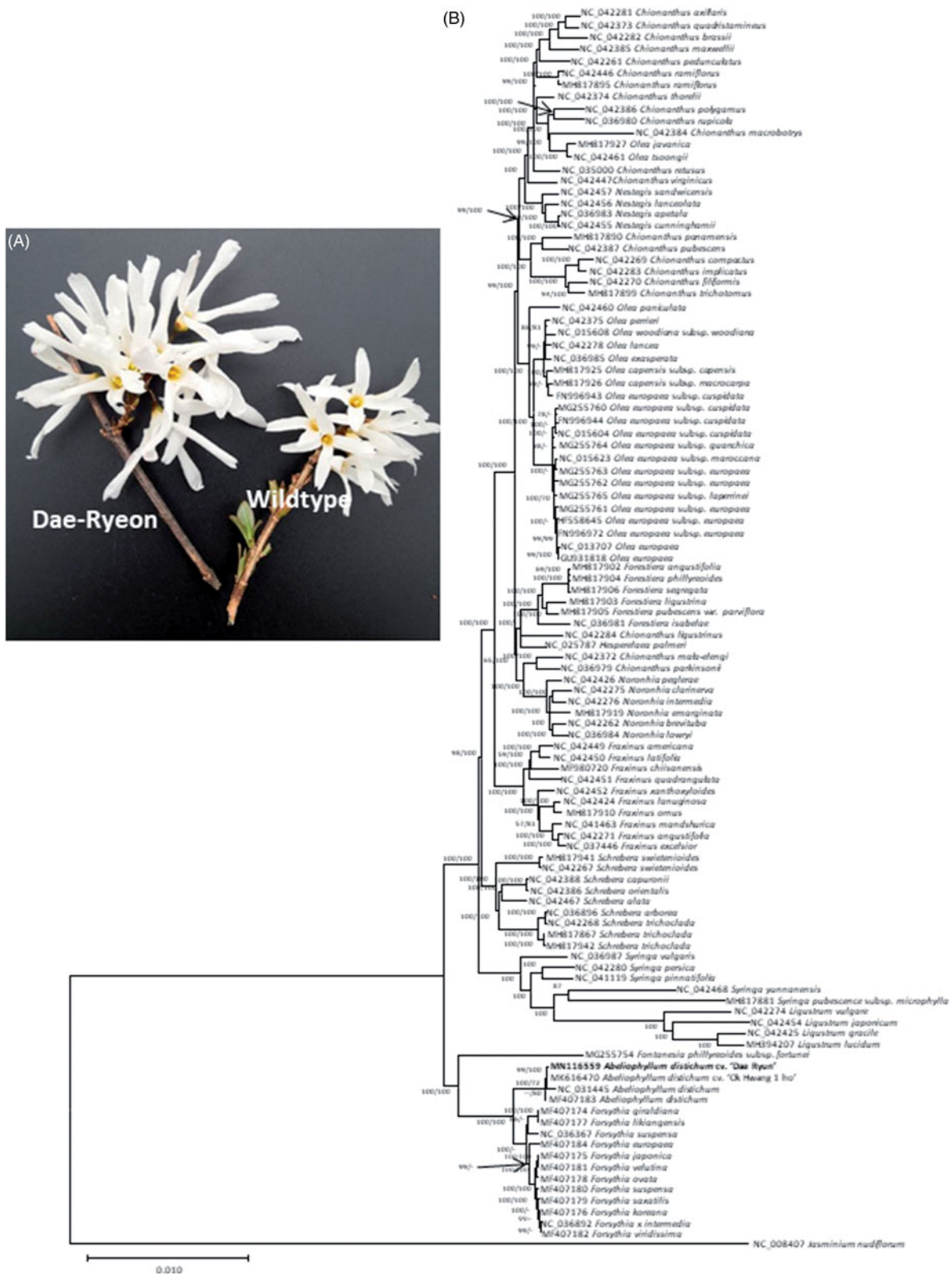
Based on alignments with three *A. distichum* chloroplast genomes, 9 single nucleotide polymorphisms (SNPs) and 11 insertions and deletions (INDELs) were found between DaeRyun and Ok-Hwang-1-ho or isolate (MF407183), which is higher than those of *Coffea arabica* (Park, Kim, Xi, Heo 2019; Park, Kim, Xi, Nho, et al. 2019; Park, Xi, et al. 2019), *Marchantia polymorpha* (Kwon et al. 2019), *Camellia japonica* (Park, Kim, Xi, Oh, et al. 2019), *Dysphania pumilio* (Park and Kim 2019), and *Cucumis melo* (Zhu et al. 2016). While, there are 102 SNPs and 63 INDELs between Dae Ryun and another isolate (NC\_031445), similar to those of *Pseudostellaria palibiniana* (Kim et al. 2019), *Illicium anisatum* (Park, Kim, Xi 2019), *Duchesnea chrysantha* (Park, Kim, Lee 2019), and *Dioscorea polystachya* (Cao et al. 2018).

One hundred and eight Oleaceae chloroplast genomes including four *A. distichum* chloroplasts were used for constructing bootstrapped neighbor joining and maximum likelihood trees using MEGA X (Kumar et al. 2018) and IQ-TREE 1.6.6 (Nguyen et al. 2015), respectively, after aligning whole chloroplast genomes with modifying directions of subregions by MAFFT 7.388 (Katoh and Standley 2013). Trees show that four *A. distichum* formed independent clade from twelve *Forsythia* species (Figure 1(B)).

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**Figure 1.** (A) Picture of flowers of Dae Ryeon and wildtype of *A. distichum*. (B) Neighbor joining (bootstrap repeat is 10,000) and maximum likelihood (bootstrap repeat is 1,000) phylogenetic trees of 108 Oleaceae chloroplast genomes: *Abeliophyllum distichum* (MN116559, MN116559, NC\_031445, and MF407183), *Chionanthus axillaris* (NC\_042281), *Chionanthus quadristamineus* (NC\_042373), *Chionanthus brassii* (NC\_042282), *Chionanthus maxwellii* (NC\_042385), *Chionanthus pedunculatus* (NC\_042261), *Chionanthus ramiflorus* (NC\_042446 and MH817895), *Chionanthus thorelii* (NC\_042374), *Chionanthus polygamus* (NC\_042386), *Chionanthus rupicola* (NC\_036980), *Chionanthus macrobotrys* (NC\_042384), *Olea javanica* (MH817927), *Olea tsoongii* (NC\_042461), *Chionanthus retusus* (NC\_035000), *Chionanthus virginicus* (NC\_042447), *Nestegis sandwicensis* (NC\_042457), *Nestegis lanceolata* (NC\_042456), *Nestegis apetala* (NC\_036983), *Nestegis cunninghamii* (NC\_042455), *Chionanthus panamensis* (MH817890), *Chionanthus pubescens* (NC\_042387), *Chionanthus compactus* (NC\_042269), *Chionanthus implicatus* (NC\_042283), *Chionanthus filiformis* (NC\_042270), *Chionanthus trichotomus* (MH817899), *Olea paniculata* (NC\_042460), *Olea perrieri* (NC\_042375), *Olea woodiana* subsp. *woodiana* (NC\_015608), *Olea lancea* (NC\_042278), *Olea exasperata* (NC\_036985), *Olea capensis* subsp. *capensis* (MH817925), *Olea capensis* subsp. *macrocarpa* (MH817926), *Olea europaea* subsp. *cuspidata* (FN996943, MG255760, FN996944, and NC\_015604), *Olea europaea* subsp. *guanchica* (MG255764), *Olea europaea* subsp. *maroccana* (NC\_015623), *Olea europaea* subsp. *europaea* (MG255763, MG255762, MG255761, HF558645 and FN996972), *Olea europaea* subsp. *laperrinei* (MG255765), *Olea europaea* (NC\_013707),

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## Figure 1. (Continued)

GU931818), *Forestiera angustifolia* (MH817902), *Forestiera phillyreoides* (MH817904), *Forestiera segregata* (MH817906), *Forestiera ligustrina* (MH817903), *Forestiera pubescens* var. *parviflora* (MH817905), *Forestiera isabellae* (NC\_036981), *Chionanthus ligustrinus* (NC\_042284), *Hesperelaea palmeri* (NC\_025787), *Chionanthus mala-elengi* (NC\_042372), *Chionanthus parkinsonii* (NC\_036979), *Noronhia peglerae* (NC\_042426), *Noronhia clarinerva* (NC\_042275), *Noronhia intermedia* (NC\_042276), *Noronhia emarginata* (MH817919), *Noronhia breviflora* (NC\_042262), *Noronhia lowryi* (NC\_036984), *Fraxinus americana* (NC\_042449), *Fraxinus latifolia* (NC\_042450), *Fraxinus chiisanensis* (MF980720), *Fraxinus quadrangulata* (NC\_042451), *Fraxinus xanthoxyloides* (NC\_042452), *Fraxinus lanuginosa* (NC\_042424), *Fraxinus ornus* (MH817910), *Fraxinus mandshurica* (NC\_041463), *Fraxinus angustifolia* (NC\_042271), *Fraxinus excelsior* (NC\_037446), *Schrebera swietenoides* (MH817941 and NC\_042267), *Schrebera capuronii* (NC\_042388), *Schrebera orientalis* (NC\_042266), *Schrebera alata* (NC\_042467), *Schrebera arborea* (NC\_036986), *Schrebera trichoclada* (NC\_042268), *MH817867* and *MH817942*), *Syringa vulgaris* (NC\_036987), *Syringa persica* (NC\_042280), *Syringa pinnatifolia* (NC\_041119), *Syringa yunnanensis* (NC\_042468), *Syringa pubescens* subsp. *microphylla* (MH817881), *Ligustrum vulgare* (NC\_042274), *Ligustrum japonicum* (NC\_042454), *Ligustrum gracile* (NC\_042425), *Ligustrum lucidum* (MH394207), *Fontanesia phillyreoides* subsp. *fortunei* (MG255754), *Forsythia gairdiana* (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 x intermedia* (NC\_036982), *Forsythia viridissima* (MF407182), and *Jasminum nudiflorum* (NC\_008407) as an outgroup. Phylogenetic tree was drawn based on neighbor joining tree. The numbers above branches indicate bootstrap support values of neighbor joining and maximum likelihood phylogenetic trees, respectively.