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MITOGENOME ANNOUNCEMENT

The complete chloroplast genome sequence of a hybrid blackberry (*Rubus* spp.) cultivar

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ABSTRACT

Blackberry (*Rubus* spp.) is an important hybrid fruit crop popular in the US Pacific Northwest and the European region with complex origins. In this study, we report the complete chloroplast genome sequence of a hybrid blackberry cultivar 'Arapohol' using next-generation sequencing technology. The complete chloroplast genome size is 156,621 bp. The genome contains 134 genes, including 40 tRNA genes, 86 protein-coding genes, and 8 rRNA genes. Phylogenetic analysis based on 11 complete chloroplast genomes revealed that taxa is closely related to *Rubus niveus*. The complete chloroplast genome of this *Rubus* sp. provides valuable information for understanding the origination of this crop species.

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Blackberry (*Rubus* spp.) belongs to the genus *Rubus* of the Rosaceae family (Ding et al. 2006). *Rubus* spp. has been introduced and cultivated in many countries or regions (Esselen et al. 2011). Most of the cultivated blackberry cultivars were selected by several runs of inter- and intra-species hybridization (Moore and Clark 1993). Seldom full records can be traced to resolve the origin of these materials. The characteristics of highly conserved genes of the chloroplast genome provide favorable tools for studying the phylogenetic and origination of different plants (Kaila et al. 2017). Reports on phylogenetic and species identification using information from the chloroplast genome are widely reported (Song et al. 2016; Lin et al. 2018).

We used high-throughput sequencing approach to assemble and identify the complete chloroplast genome sequence of a blackberry cultivar for the first time. The complete chloroplast genome sequence has been deposited in GenBank (Accession number: MH992399). Fresh leaves of blackberry cv 'Arapahol' were collected from the Teaching and Research Base of Sichuan Agricultural University (29°59′10.56″ N, 102°58′51.54″). Voucher specimens were deposited in the herbarium of the College of Horticulture of the University with sample No. R09-16. The leaves were used to extract DNA by a modified CTAB method. Fifteen universal primer pairs were used to amplify the complete chloroplast genome referring to Zhang et al. (2016).

Equal amount of the purified PCR products from all reactions were pooled. Ten microgram of DNA was used as a template for constructing a sequencing library by using the Illumina Hiseq 2000 platform. A stringent filtering process was used to remove adapter sequences, low-quality reads, and duplicated reads by Trimmomatic v0.22. In total, 6.8 million of the high-quality 150 bp short reads were assembled to develop the preliminary chloroplast genome sequence by using Geneious v11. The protein-coding genes, transfer RNA genes, and ribosomal RNA genes were annotated by the software DOGMA. A physical map of the chloroplast genome was generated using OGDRAW (Li et al. 2017; Guo et al. 2018).

The complete chloroplast genome of the *Rubus* sp. is 156,621 bp. It has a pair of inverted repeats (IR) of 25,957 bp that separate a large single-copy (LSC) region of 85,967 bp and a small single-copy (SSC) region of 18,740 bp. It contains 40 tRNA genes, 86 protein-coding genes, and 8 rRNA genes. 18 genes are duplicated in the IRs (7 tRNA genes, 7 protein-coding genes, and 4 rRNA genes). A total of 11 chloroplast genomes were aligned using the HomBlock (Bi et al. 2018). Maximum likelihood analysis was performed in the IQTREE package (Trifinopoulos et al. 2016). It showed that the 6 species from *Rubus* formed a well-resolved single clade, and the hybrid cultivar was mostly related to *R. niveus*, with strong bootstrap support (Figure 1). *Malus prunifolia*, *Prunus padus*, and *Pyrus pyrifolia* formed a single cluster with 100% bootstrap support. We provide this entire chloroplast genome of *Rubus* spp, which may be used in the phylogenetic and taxonomic studies for the genus *Rubus*.

Disclosure statement

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

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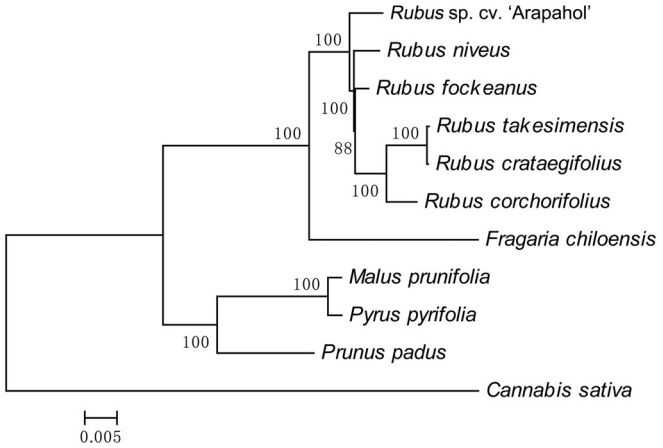

Figure 1. Maximum likelihood (ML) tree based on conserved collinear blocks of the complete chloroplast genome of *Rubus* species, using *Cannabis sativa*as an outgroup. The numbers on the node are the fast bootstrap value based on 10,000 replicates. The bar indicates 0.005 mutations per site.Accession numbers are listed as below: *Rubus* spp, MH992399, *Rubus niveus*, KY419961, *Rubus fockeanus*, KY420018, *Rubus takesimensis*, NC_037991, *Rubus crataegifolius* MG189543, *Rubus corchorifolius*, KY419958, *Fragaria chiloensis*, NC_019601, *Malus prunifolia*, NC_031163, *Pyrus pyrifolia*, NC_015996, *Prunus padus*, NC_026982, *Cannabis sativa*, NC_026562.

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Data availability statement

The data that support the findings of this study are openly available in the Genbank database at https://www.ncbi.nlm.nih.gov/, under accession number [MH992399].

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