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Characterization of the complete chloroplast genome of *Hordeum vulgare* L. var. *trifurcatum* with phylogenetic analysis

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ABSTRACT

In the present study, the complete chloroplast genome of *Hordeum vulgare* L. var. *trifurcatum* was sequenced, assembled and compared with closely related species. The chloroplast genome of *Hordeum vulgare* L. var. *trifurcatum* was composed of 84 protein-coding genes (PCG), 8 ribosomal RNA (rRNA) genes, and 38 transfer RNA (tRNA) genes. The *Hordeum vulgare L. var. trifurcatum* chloroplast genome is 136,485 bp in size, with the GC content of 38.32%. Phylogenetic analysis based on the combined chloroplast gene dataset indicated that the *Hordeum vulgare* L. var. *trifurcatum* exhibited a close relationship with *Hordeum vulgare* subsp. *spontaneum* and *Hordeum vulgare* subsp. *vulgare*.

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Hordeum vulgare L. var. *trifurcatum*, belonging to the Poaceae family (Soreng et al. 2015; Saarela et al. 2018), is one of the staple foods for Tibetans and an important livestock feed in the Tibetan Plateau (Zeng et al. 2015; Huang et al. 2020). The highland barley (*Hordeum vulgare* L. var. *trifurcatum*) shows good environmental tolerance, which can be successfully planted in high altitude, low temperature, and high salinity places (Walia et al. 2006; El-Esawi et al. 2018; Elsawy et al. 2018). In addition, the highland barley also showed good antioxidant activity (Asif et al. 2020). Thus, *Hordeum vulgare* L. var. *trifurcatum* is a promising nutritious food source and traditional Chinese medicine grows widely in plateau, which concerning by more and more researchers, just like the *Fagopyrum tataricum* (L.) Gaertn (Song et al. 2016; Xiang et al. 2016; Xiang, Ma, et al. 2019; Xiang, Song, et al. 2019) and *Stellera chamaejasme* L. (Ren et al. 2019, 2021a, 2021b). The genus *Hordeum* comprises more than 30 species. Some varieties are also found in this genus (Malysheva-Otto et al. 2006; Forsberg et al. 2019; Hagenblad and Morales 2020; Kumar et al. 2020). Organelle genomes have been widely used in study of taxonomy, evolution and genetics (Wang et al. 2016; Yang et al. 2019; Wang et al. 2020; Li, He, et al. 2020). However, no complete chloroplast genome of *Hordeum vulgare* L. var. *trifurcatum* was reported to date, which limits its breeding and application (Su et al. 2020).

The specimen (*Hordeum vulgare* L. var. *trifurcatum*) used for chloroplast genome assembly was collected from Qinghai, China (101.97 E; 53.70 N). A specimen was deposited at Collection Center of Chengdu University (Y. Ren, renyuanhang@cdu.edu.cn) under the voucher number ZQK_R1. The chloroplast genome of *Hordeum vulgare* L. var. *trifurcatum* was sequenced and assembled according to methods previously described (Li, Ren, et al. 2020). First, we extracted the total genomic DNA of *Hordeum vulgare* L. var. *trifurcatum* using a Plant DNA Kit (D3485-00, Omega Bio-Tek, Norcross, GA, USA). Then we purified the genomic DNA using a Gel Extraction Kit (Omega Bio-Tek, Norcross, GA, USA). The purified DNA was stored in Chengdu University (No. DNA_ZQK_R1). Sequencing libraries of *Hordeum vulgare* L. var. *trifurcatum* was constructed using a NEBNext® Ultra™ II DNA Library Prep Kit (NEB, Beijing, China). We conducted the whole genomic sequencing (WGS) of *Hordeum vulgare* L. var. *trifurcatum* using the Illumina HiSeq 2500 Platform (Illumina, SanDiego, CA). The chloroplast genome of *Hordeum vulgare* L. var. *trifurcatum* was initially assembled using SPAdes v3.11.0 (Bankevich et al. 2012). The chloroplast sequences obtained in the above steps were used as seed sequences to assemble the complete chloroplast genome of *Hordeum vulgare* L. var. *trifurcatum* using NOVOPlasty v4.3.1 with a k-mer size of 35 (Dierckxsens et al. 2017). Approximately 1.10 million reads were assembled into a complete circular chloroplast genome. The average chloroplast sequence coverage was 2,168 ×. The complete chloroplast genome of *Hordeum vulgare* L. var. *trifurcatum* was annotated by GeSeq (Tillich et al. 2017) using the chloroplast genome of *Hordeum vulgare* subsp. *spontaneum* as the reference (Bdolach et al. 2019).

The complete chloroplast genome of *Hordeum vulgare* L. var. *trifurcatum* is 136,485 bp in length, which was larger than *Hordeum vulgare* subsp. *vulgare* (136,462 bp) (Zeng et al. 2017) and smaller than *Hordeum vulgare* subsp. *spontaneum* (136,536 bp) (Bdolach et al. 2019). The GC content of the *Hordeum vulgare* L. var. *trifurcatum* chloroplast genome is

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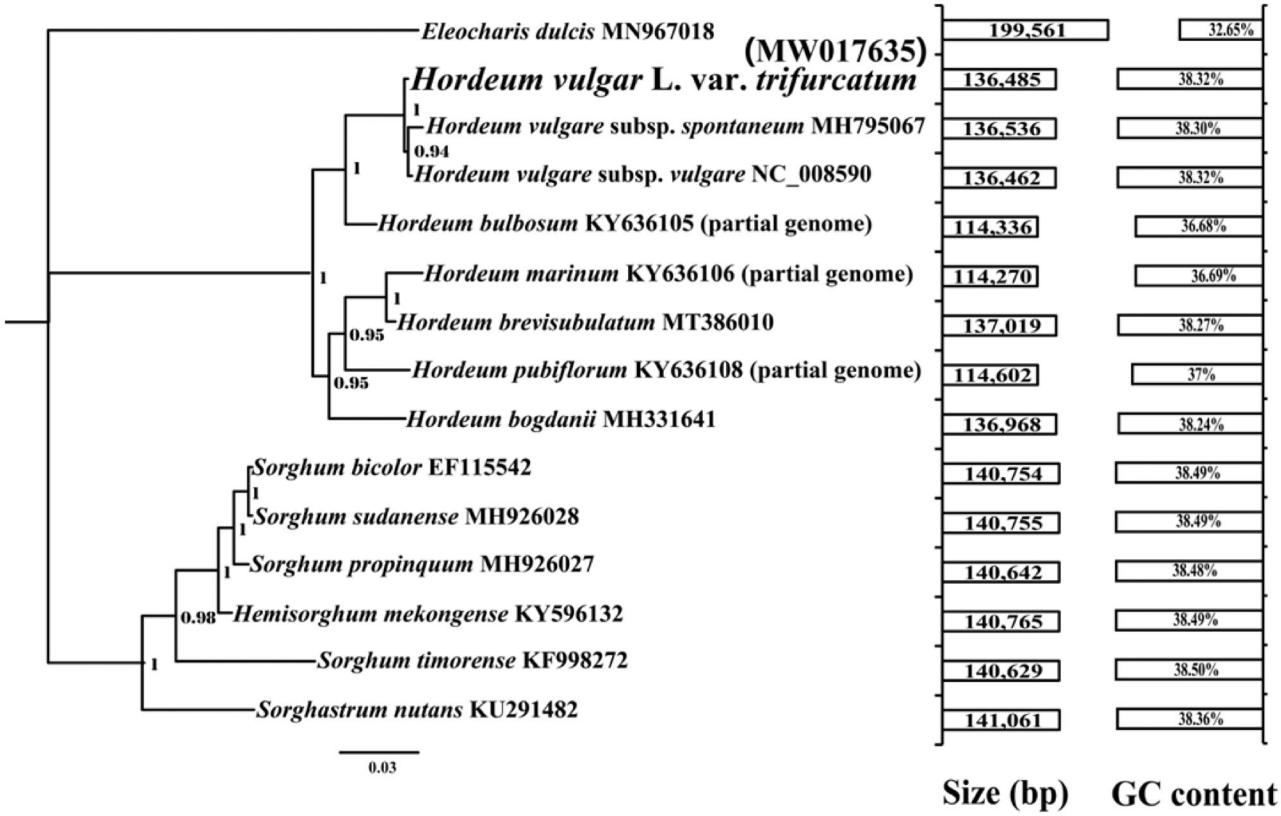

Figure 1. Bayesian phylogenetic analysis and comparative chloroplast genomic analysis of 15 species based on the combined protein-coding gene sets. Support values are Bayesian posterior probabilities (BPP). Accession numbers of chloroplast sequences used in the phylogenetic analysis are listed after the species names.

38.32%, which is larger than that of *Hordeum vulgare* subsp. *spontaneum* (38.30%). The base compositions of the *Hordeum vulgare* L. var. *trifurcatum* chloroplast genome were as follows: A (30.93%), T (30.76%), G (19.22%) and C (19.10%). The complete chloroplast genome of *Hordeum vulgare* L. var. *trifurcatum* contains 84 protein-coding genes, 8 ribosomal RNA genes, and 38 transfer RNA (tRNA) genes. The number of protein-coding genes in *Hordeum vulgare* L. var. *trifurcatum* chloroplast genome was more than that in two subspecies (*Hordeum vulgare* L. var. *trifurcatum* and *Hordeum vulgare* subsp. *vulgare*), while the number of tRNA was less than that in the two subspecies. To investigate the phylogenetic status of *Hordeum vulgare* L. var. *trifurcatum*, we constructed a phylogenetic tree for 15 species. The protein-coding region of 13 genes conserved in the 15 species was used to construct combined a chloroplast gene set according to previous methods (Li, Xiang, et al. 2019; Wu et al. 2021). Bayesian (BI) analysis method (Li, Wu, et al. 2021) was used to construct the phylogenetic tree based on combined protein-coding genes of chloroplast genome as described by previous methods (Li, Yang, et al. 2020; Cheng et al. 2021; Li, Li, et al. 2021). MrBayes v3.2.6 (Ronquist et al. 2012) was used to construct the phylogenetic tree using Bayesian inference (BI) method. Two independent runs with four chains (three heated and one cold) each were conducted simultaneously for 2×10^6 generations. Each run was sampled every 100 generations. We assumed that stationarity had been reached when estimated sample size (ESS) was greater than 100, and the potential scale reduction factor (PSRF) approached 1.0. The first 25% samples were discarded as burn-in, and the remaining trees were used to calculate Bayesian posterior probabilities (BPP) in a 50% majority-rule consensus tree (Li, Ren, et al. 2019). According to the phylogenetic tree (Figure 1), the *Hordeum vulgare* L. var. *trifurcatum* exhibited a close relationship with *Hordeum vulgare* subsp. *spontaneum* (Bdolach et al. 2019) and *Hordeum vulgare* subsp. *vulgare* (Zeng et al. 2017).

Disclosure statement

The authors have declared that no competing interests exist.

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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. MW017635. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA717119, SRR14082750, and SAMN18478699, respectively.

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