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PLASTOME ANNOUNCEMENT

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The complete chloroplast genome sequence of a warm season ornamental grass, *Cenchrus alopecuroides* Thunb. 1794 (Poaceae)

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ABSTRACT

Cenchrus alopecuroides Thunb. 1794 is a warm-season ornamental grass with glossy foliage and showy inflorescence, which is widely distributed from eastern Asia to Australia. Although this species is of great economic importance, little genomic sequence data are available. Here, we report the complete chloroplast genome sequence of *C. alopecuroides*, which provides valuable plastid genomic resources for further studies on this ornamental grass. The chloroplast genome of *C. alopecuroides* was 138,053 bp in length and exhibited a typical quadripartite structure, which comprised a pair of inverted repeat regions (22,331 bp) separated by a large (81,177 bp) and a small single copy (12,214 bp) region. In total, 110 unique genes were annotated in the chloroplast genome, containing 76 protein-coding genes, 30 transfer RNAs and four ribosomal RNAs. The overall GC content of the chloroplast genome was 38.6%. Phylogenetic analysis based on 11 whole-chloroplast genome sequences of *Cenchrus* species suggested that *C. alopecuroides* and *C. compressus* were sisters to each other and joint sisters to *C. clandestinus*.

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KEYWORDS
Cenchrus alopecuroides; chloroplast genome; phylogenetic analysis

1. Introduction

Cenchrus alopecuroides Thunb. *1794, formerly* known as *Pennisetum alopecuroides*, belongs to the Poaceae family and is widely distributed from eastern Asia to Australia (Thomas and Taylor 2021). This species is an attractive and widely popular, moderately sized ornamental grass (Figure 1) that forms dense clusters of narrow leaves and tall, upright, foxtail-shaped inflorescences (Thomas and Taylor 2021). Since it produces long flower spikes that erupt and curve out from the plant like a fountain of water, *C. alopecuroides* is also called swamp foxtail grass and/or foxtail fountain grass (Toon et al. 2018). Currently, many cultivars of this species are commercially available for horticulture practices, with a wide range of plant height (0.30–1.52 m), various flower colors (e.g. purple, pink and white), and autumn foliage (http://www.missouribotanicalgarden.org). Despite its high economic importance, there is little genomic sequence data available for *C. alopecuroides*. Here, we report the chloroplast genome sequence of *C. alopecuroides* and reconstruct its phylogenetic relationship with other *Cenchrus* species. The main objective of this study was to provide a basis for further research and utilization of this economically important species.

2. Materials and methods

2.1. Plant material, DNA extraction, and genome sequencing

Fresh leaves of *C. alopecuroides* (Figure 1, taken by the author Zhiyong Wang) were sampled from the Xinyang Agricultural and Forestry University (E114°12′, N32°16′). A voucher specimen was deposited at the College of Horticulture, Xinyang Agricultural and Forestry University (https://www.xyafu.edu.cn/en/, contact Peiling Li, lxmlpl@163.com) under voucher number Cal20211202. DNA was extracted using DNA Plantzol Reagent (Invitrogen Trading Co., Ltd, Shanghai, China). DNA library construction and 150 bp paired-end sequencing were performed on the Illumina HiSeq^{4000} platform, and approximately 6 GB of raw data was obtained. Library construction and sequencing were conducted by Novogene Bioinformatics Technology Co., Ltd. (Beijing, China).

2.2. Chloroplast genome assembly and annotation

The chloroplast genome was assembled using the GetOrganelle pipeline (Jin et al. 2020), with the suggested default parameters. To evaluate the quality and completeness of the genome assembly, the genome coverage was

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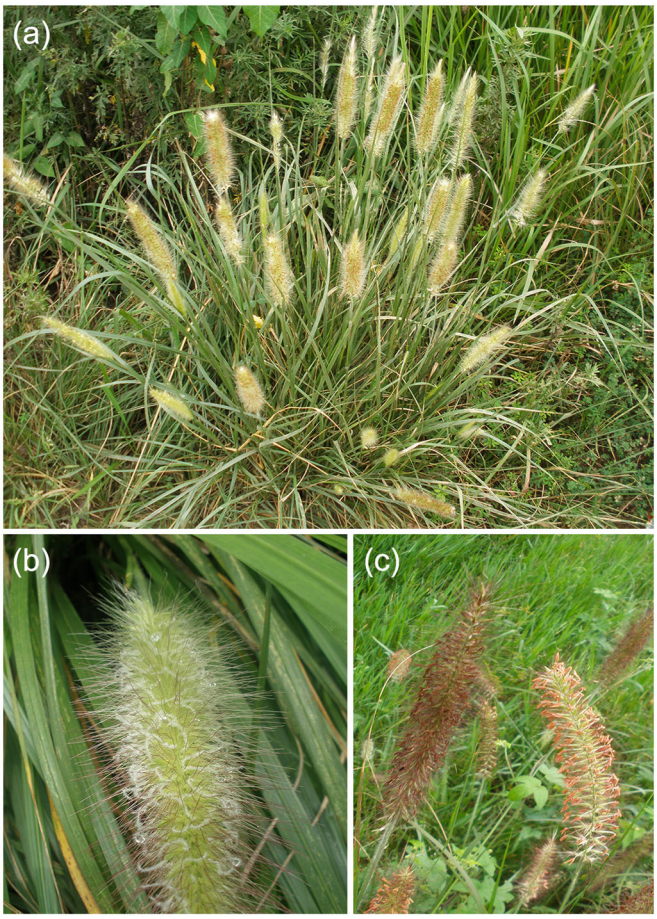

Figure 1. The morphological characteristics of the *Cenchrus alopecuroides*. (a) the characteristics of whole plant during flowering; (b) the flowering period of *C. alopecuroides*; (c) the fruiting period of *C. alopecuroides*.

estimated by mapping all the clean reads back to the chloroplast genome sequence, using CLC Genomics Workbench 23.0.1 (https://digitalinsights.qiagen.com). The assembled plastome sequence was annotated using the PGA (Plastid Genome Annotator) program (Qu et al. 2019), with the annotated chloroplast genome sequence of *C. americanus* (MN180104) as a reference. The exon/intron boundaries and the start and stop codons were further verified by comparison with the reference genome using Geneious Prime® 2022.0.1. software (http://www.geneious.com). The annotated chloroplast genome sequence of *C. alopecuroides* has been deposited in GenBank (accession number: ON206984). A chloroplast genome map was drawn using CPGview (http://www.1kmpg.cn/cpgview) (Liu et al. 2023).

2.3. Phylogenetic analyses

Phylogenetic analyses were conducted on *C. alopecuroides* and 10 *Cenchrus* species, with *Stenotaphrum secundatum* (KY432796) and *Setaria italica* (MK348605) as outgroups. The chloroplast genomes of these 13 species were aligned in Geneious Prime® 2022.0.1 using the MAFFT v.7 plugin (Katoh and Standley 2013). The best nucleotide substitution model was determined using jModelTest v2.1.4 (Posada 2008) under the Akaike Information Criterion (AIC) criterion, and the GTR + G + I substitution model was selected. Maximum likelihood (ML) analysis was carried out using RAxML-HPC v.8.2.8 (Stamatakis 2014) on the CIPRES Science Gateway (http://www.phylo.org/portal2/). Clade support values were derived from 1,000 bootstrap replicates.

3. Results and discussion

3.1. Chloroplast genome features

A total of 1,111,437 clean reads representing 2.5% of the total whole genome sequencing data were mapped to the chloroplast genome sequence of *C. alopecuroides*, with an average coverage depth of 1207.6× (Figure 2(a)). The chloroplast genome sequence of *C. alopecuroides* was 138,053 bp in length, and exhibited a typical quadripartite structure consisting of a pair of inverted repeat regions (IRs) of 22,331 bp separated by a large single-copy (LSC) region of 81,177 bp and a small single copy (SSC) region of 12,214 bp (Figure 2(b)). The chloroplast genome encoded 130 genes, of which 110 (76 protein-coding genes, 30 tRNA genes, and 4 rRNA genes) were unique and 20 (8 protein-coding genes, 8 tRNA genes, and 4 rRNA genes) were duplicated in the IRs (Figure 2(b)). Eight protein-coding genes (*rps*16, *atp*F, *pet*B, *pet*D, *rpl*16, *rpl*2, *ndh*B, and *ndh*A) and six tRNA genes (*trn*K-UUU, *trn*G-UCC, *trn*L-UAA, *trn*V-UAC, *trn*I-GAU, and *trn*A-UGC) contained a single intron, whereas three genes (*ycf*3, *rps*12, and *clp*P) contained two introns. The structure of cis-/trans-splice genes were shown in Figure S1. The overall GC content was 38.6%, whereas the CC content in the IR, LSC, and SSC were 44.1, 36.5, and 33.2%, respectively.

3.2. Phylogenetic analyses

Comparative chloroplast genome analyses of *C. alopecuroides* and 10 previously reported chloroplast genomes of *Cenchrus* showed that the chloroplast genomes within this genus were conserved in terms of genome size, genome structure, and gene content (e.g. Bhatt and Thaker 2018; Wu and Zhou 2019; Zhang et al. 2021). The phylogenetic results using 100% bootstrap values for almost all nodes, strongly supported the monophyly of *Cenchrus*, and also indicated that *C. alopecuroides* and *C. compressus* formed a sister group, which was further sister to *C. clandestinus* (Figure 3).

4. Conclusion

In this study, the chloroplast genome of *C. alopecuroides* was reported for the first time, and its organization was described. The complete chloroplast genome was 138,053 bp in length and encoded a total of 130 genes. Comparative chloroplast genome analyses revealed that the chloroplast genomes within this genus were conserved in terms of genome size and structure, and gene content. Phylogenetic analysis of the *Cenchrus* species suggested that *C. alopecuroides* and *C. compressus* are sisters to each other and joint sisters

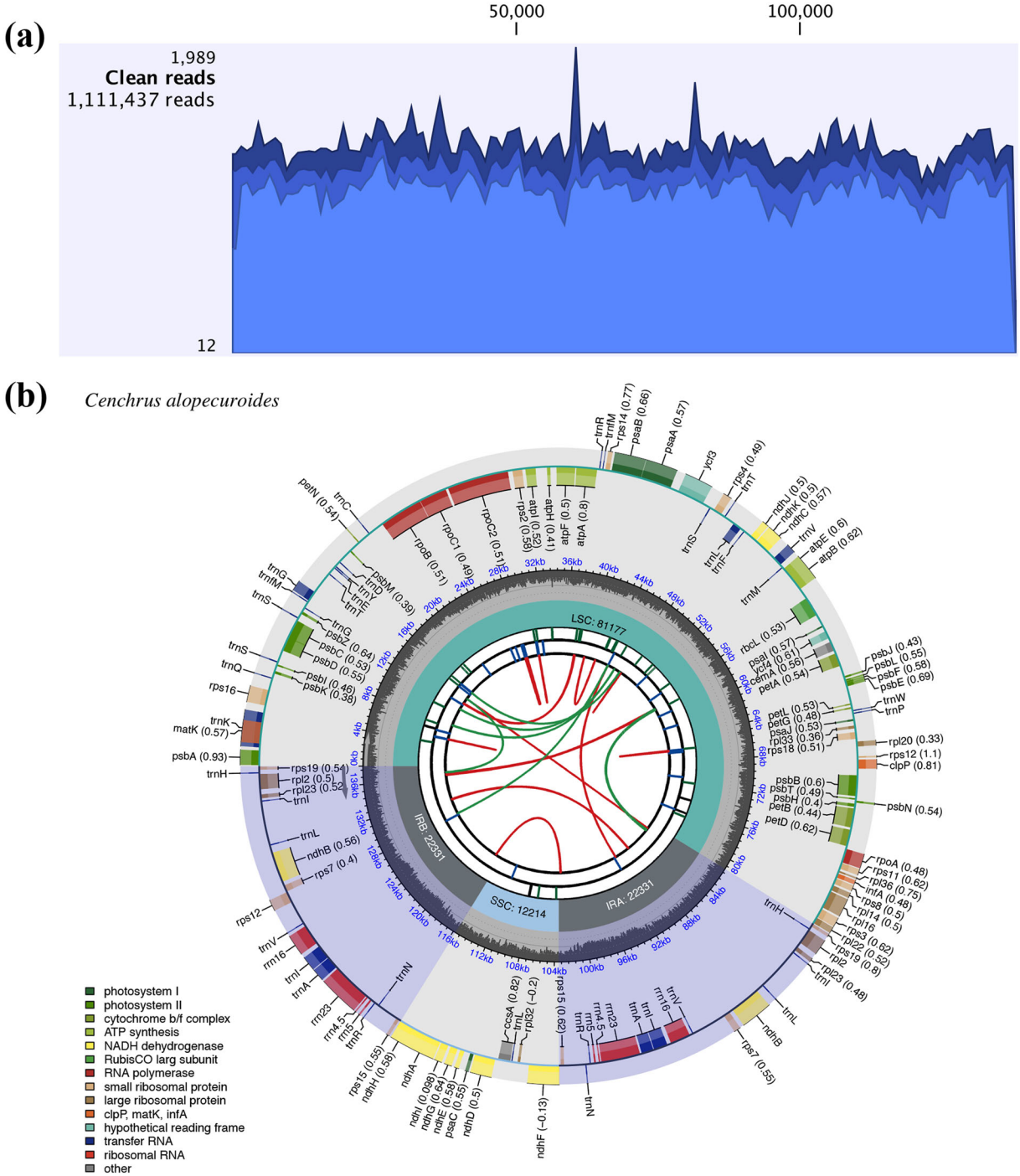

Figure 2. The chloroplast genome coverage depth and map of *Cenchrus alopecuroides*. (a) The mapped read depth of *Cenchrus alopecuroides* chloroplast genome. (b) The chloroplast genome map of *Cenchrus alopecuroides*. The map contains six circles. From the center to outward, the first circle shows the distributed repeats connected with red (forward) and green (reverse) arcs. The second circle shows the tandem repeats as short bars. The third circle shows the microsatellite sequences marked with short bars. The fourth circle shows the size of the LSC, SSC, and IRs. The fifth circle shows the GC contents along the plastome. The sixth circle shows the genes having different colors based on their functional groups. Genes shown outside the circle are transcribed clockwise, and those inside counter-clockwise.

to *C. clandestinus*. The genomic resources presented here will not only offer valuable information for further genetic and evolutionary studies on *C. clandestinus* but will also be useful for breeding, cultivation and utilization of this economically important species.

Ethical approval

We confirm that all the research meets ethical guidelines and adheres to the legal requirements of the Xinyang Agricultural and Forestry University (China).

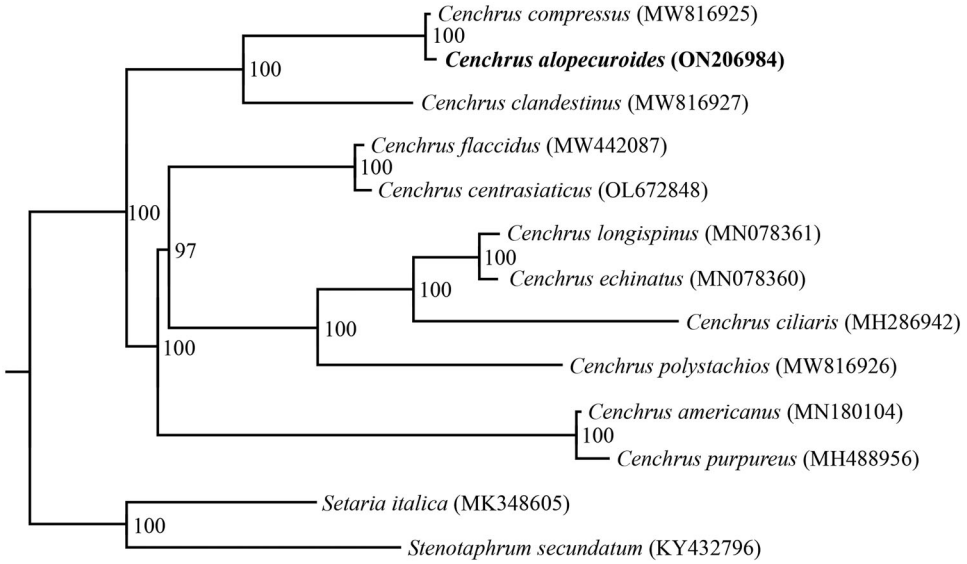

Figure 3. Phylogenetic tree inferred by the maximum likelihood (ML) method based on complete chloroplast genomes of 11 *Cenchrus* species, with *Stenotaphrum secundatum* (KY432796) and *Setaria italica* (MK348605) as outgroups. Numbers near the nodes represent ML bootstrap values. The following sequences were used: MW816925–MW816927 (Xu et al. 2021), MW442087 (Liu, W., Direct submission), OL672848 (Zhang, L., Direct submission), MN078360–MN078361 (Hyun et al. 2019), MH286942 (Bhatt and Thaker 2018), MN18014 (Xu et al. 2019), MN488956 (Bhatt and Thaker 2018), MK348605 (Liu et al. 2019), KY432796 (Gallaher et al. Unpublished).

Author contributions

ZW and PL designed the project. LY, JY and YT collected samples and analyzed data. ZW and PL wrote the manuscript. JY and PL revised the manuscript. All authors read and approved the manuscript. All the authors meet the criteria for authorship as per the ICMJE criteria.

Disclosure statement

No potential conflict of interest was reported by the author(s). The study was permitted under permission from Xinyang Agricultural and Forestry University.

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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. ON206984. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA871033, SRR21166642, and SAMN30399920, respectively.

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