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COMPLETE CHLOROPLAST GENOME SEQUENCE OF A VEGETATIVE BERMUDAGRASS CULTIVAR 'TIFEAGLE' (*CYNODON DACTYLON* × *CYNODON TRANSVAALENSIS*)

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Abstract

Hybrid (*Cynodonn dactylon* × *C. transvaalensis*) is a widely distributed turfgrass and shows a great value of environment, horticulture and economic. Though, the chloroplast genome of *C. dactylon* has been reported, it might be helpful finding reasons that triploid bermudagrass shows a better drought and trampling tolerance than common bermudagrass through comparing chloroplast genome analysis. The present results showed the complete chloroplast genome of the *C. dactylon* × *C. transvaalensis* is 134655 bp in length. The tetramerous genome contained a large single copy (LSC) region (79,998 bp), a small single copy (SSC) region (12,517 bp), and a pair of inverted repeat (IR) regions (42,140 bp). In the chloroplast genome, 116 genes were predicted, including 83 protein-coding, 29 tRNA and 4 rRNA genes. Furthermore, a total of 80 repeat sequences were identified. Only 0.23% intergenicnon-collinear sequences were found between the chloroplast genome of *Cynodon dactylon* × *C. transvaalensis* and *Cynodon dactylon*.

Introduction

Cynodon dactylon × *Cynodon transvaalensis* is widely used in sports fields, lawns, parks, golf courses (Harlan *et al*. 1970). The triploid hybrid bermudagrass showed higher turf quality with limited irrigation than common bermudagrass (Hanna 1998). Hu *et al*. (2009, 2010) reported that the better ability of drought tolerance due to the hybrid bermudagrass had more active ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) enzyme, RuBisCO activase and more stable proteins for carbon assimilation which made a greater capability of photosynthesis. By comparative proteomic analyzing the difference between triploid and common bermudagrass under water-deficit stress, Zhao *et al*. (2011) found a inhibition of expression of Chl a-b binding proteins, oxygen-evolving enhancer protein, ATP synthase and RuBisCO large subunit in common bermudagrass under drought stress. These results demonstrate there exist a differential gene expression and enzymatic activity in chloroplast under drought stress between triploid and common bermudagrass. It is speculated that chloroplast sequence variation happened in the process of triploid bermudagrass formation. If so, the genomic resources of the sequence of *C. dactylon* (Genbank accession number KY024482.1) (Huang *et al*. 2017) and hybrid (*C. dactylon* × *C. transvaalensis*) will be of great value for new bermudagrass germplasm generation and genetic conservation.

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Materials and Methods

Fresh plant was collected from Laboratory for Turfgrass Science, College of Life Science, South China Agricultural University. The genomic DNA was extracted according to the method of CTAB (Porebski *et al*. 1997). The quality and concentration of the DNA products were assessed using agarose gel electrophoresis and a NANODROP 1000 spectrophotometer (Thermo Scientific).

Fifteen pair of primers (Table 1) was designed based on *Cynodon dactylon*, according to the method mentioned by Zhang *et al*. (2016). Amplification of target gene regions was carried out using the PCR in Eppendorf Mastercycler nexus (Hamburg, Germany).

Table 1. Sequences of 15 pairs of primers used for *Cynodon dactylon* × *C. transvaalensis* chloroplast genome amplification.

Primer name	Primer sequence
CPUP_1F	GCACTTAAAAGCCGAGTACTCTACCA
CPUP_1R	CAAAGGTTTAGAAGACCTCTGTCCTATCCA
CPUP_2F	CCATTGTCTAATGGATAGGACAGAGGTC
CPUP_2R	AGGACAAATGATTGATTTACCTATTCAAAG
CPUP_3F	TGCTTTGAATAGGTAAATCAATCATTTGT
CPUP_3R	CTGTCAAGGCGGAAGCTGCGGG
CPUP_4F	GAACCCGCAGCTTCCGCCTTGAC
CPUP_4R	TTAAAAGTTGCTCCTGCTACTCAGCC
CPUP_5F	CAAGGCTCTAGGCTGAGTAGCAGGAG
CPUP_5R	ACCAGATTTGAACTGGTGACACGAGGA
CPUP_6F	ACTGAAAATCCTCGTGTCACCAGTTCA
CPUP_6R	ATGCATACCATGATTTTTCTGTCTATCA
CPUP_7F	GAGCAATGCATGCAGTTATTGATAGA
CPUP_7R	AGGTTCAAATCCTACAGAGCGTGAT
CPUP_8F	ATCACGCTCTGTAGGATTTGAACC
CPUP_8R	TTAATAATTCAAGTCACACACTCCCA
CPUP_9F	TGGGAGTGTGTGACTTGAATTATTAATT
CPUP_9R	ATCCATGGCTGAATGGTTAAAGCGCC
CPUP_10F	TTTACCAATTATGAGTTGGGCGCTTT
CPUP_10R	AGGAAGAGCACTTGCCATTCGTTGGT
CPUP_11F	CCATATTTCGACCCGGACGCTTTTGG
CPUP_11R	GATGCCTCAGCTGCATACATCACTGC
CPUP_12F	ATGCAGCTGAGGCATCCTAACGAACG
CPUP_12R	TGCTTCCTAAGAGCAGCGTGTCTACC
CPUP_13F	GGTAGACACGCTGCTCTTAGGAAG
CPUP_13R	CACCAATAAGATACGGAGACTTGCTTCACA
CPUP_14F	TGTGTAATTCCAAATGTGAAGCAAGTCT
CPUP_14R	GGTTCGAATCCCTCCTCGCCCA
CPUP_15F	ATCCATGGCTGAATGGTTAAAGCGCC
CPUP_15R	CTCAATGGTAGAGTACTCGGCTT

PCR product was extracted with the SDS method. The harvested DNA was detected by the agarose gel electrophoresis and quantified by Qubit. Whole-genome sequencing was performed on the IlluminaHiSeq 2500-PE125 platform with MPS (massively parallel sequencing) Illumina technology. A-tailed, ligated to paired-end adaptors and PCR amplified with a 500 bp insert and a mate-pair library with an insert size of 5 kb were used for the library construction at the Beijing Novogene Bioinformatics Technology Co., Ltd.

Illumina PCR adapter reads and low-quality reads from the paired-end and matepair library were filtered by the step of quality control using our own compling pipeline. All good quality paired reads were assembled using the SOAPdenovo (Li *et al*. 2008, 2010) (http://soap.genomics.org.cn/soapdenovo.html) into several scaffolds. Then the filter reads were handled by the next step of the gap-closing.

Preliminary gene prediction was performed with the online program DOGMA (Wyman *et al*. 2004). All tRNA genes were predicted by tRNA scanSE search server (Schattner *et al*. 2005). Repeats were detected by Tandem Repeats Finder (Department of Biomathematical Sciences, New York, NY, USA) (https://tandem.bu.edu/trf/trf.html) and REPuter (Benson 1999 and Kurtz *et al*. 2001). Annotated genome was submitted to online server Organellar GenomeDRAW for visualization (Lohse *et al*. 2013).

The blast results between triploid and common bermudagrass chloroplast genome were inputted into MCscan56 with default parameters to compute multiple synteny. The final gene collinearity results were generated from the MCscan output file by swapping the gene order number of each gene with their names using a Perl script.

Results and Discussion

The complete chloroplast genome of the *Cynodon dactylon* × *C. transvaalensis* is 134655 bp in length. The whole genome sequence data reported in this paper have been deposited in the Genome Warehouse in BIG data center (2017), Beijing Institute of Genomics (BIG), Chinese Academy of Sciences, under accession number GWHAAAN00000000 that is publicly accessible at http://bigd.big.ac.cn/gwh. The genome contains a pair of inverted repeat (IR) regions (42, 140 bp), which separated by two single copy regions (LSC 79, 998 bp and SSC 12, 517 bp). The GC content of the sequence is 38.38%, with the corresponding values of 36.3, 32.58, 44.03% for the LSC, SSC and IR regions, respectively. In the chloroplast genome, there are 116 genes predicted, including 83 protein-coding, 29 tRNA and 4 rRNA genes (Table 2). A total of 80 repeats were identified in the chloroplast genome of *Cynodon dactylon* × *C. transvaalensis*, containing 21 forward repeats, 26 palindromic repeats, 3 reverse repeats and 30 tandem repeats. The length of repeats mainly lies between 21 and 30 bp (53.75%). The tandem repeats have a wide length range and the length of forward and palindromic repeats mostly lies between 21 and 50 bp.

From the results of the collinear analysis, the two genomes are almost identical and only 0.23% of them are non-collinear (Fig. 2), which are intergenic. Also, sequences of genes associated with photosynthesis were compared and only three different bases were found in *ndh*A (TCC/TCT), *psa*A (GTT/GTA) and *psa*J (CCC/CCG). However, they encode the same amino acids, TCC and TCT encode serine, GTT and GTA encode valine, CCC and CCG encode proline.

It is well known that chloroplast gene is matrilineal inheritance. Theoretically, *Cynodon dactylon* × *C. transvaalensis* and *Cynodon dactylon* have the same chloroplast genome. The present hypothesis is based on the chloroplast genome variation in the process of triploid bermudagrass formation. Comparative analysis result demonstrated that there was almost no difference between the chloroplast genome between *Cynodon dactylon* × *C. transvaalensis* and *Cynodon dactylon*. The higher expression of photosynthesis-related genes, more active RuBisCO

enzyme, and stable proteins may occur mainly due to the different nuclear genome.

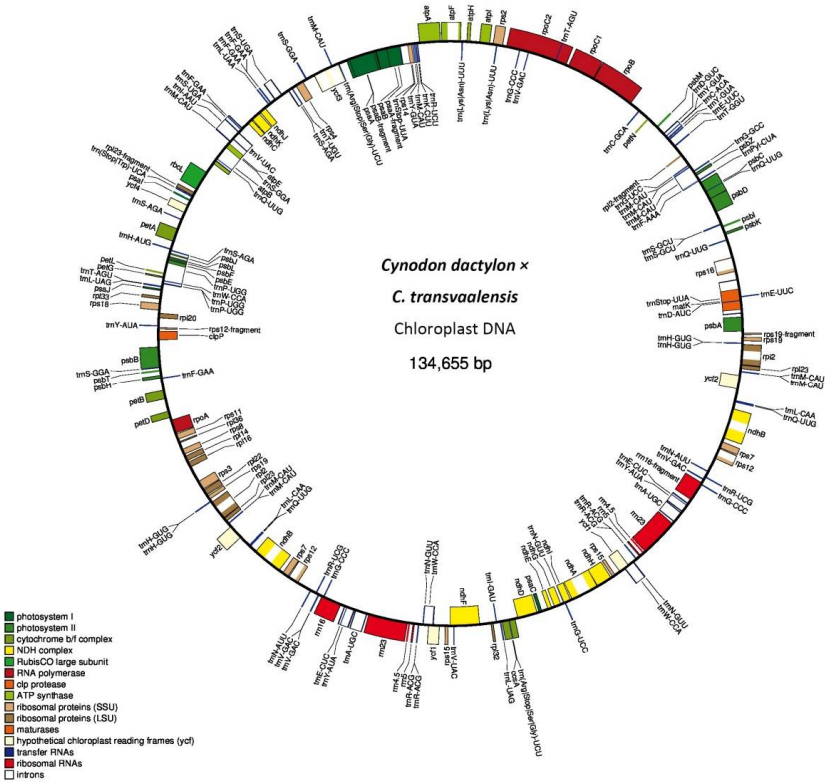

Fig. 1. Gene map of the *Cynodon dactylon* × *C. transvaalensis* chloroplast genome. Genes shown on the inside of the circle are transcribed clockwise, and genes outside are transcribed counter-clockwise. Genes belonging to different functional groups are color-coded.

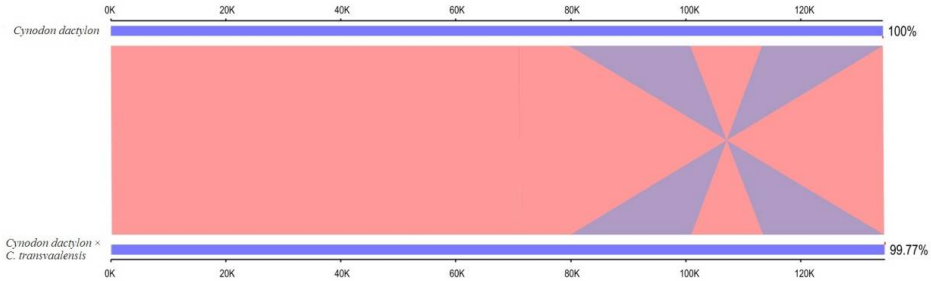

Fig. 2. Comparative analysis between *Cynodon dactylon* and *Cynodon dactylon* × *C. transvaalensis*. Dark blue region represents collinear sequences, red region indicates positive collinearity, light blue indicates negative collinearity.

Nuclear genes are involved in the regulation of chloroplast gene expression. NEP polymerase, which is encoded by nuclear gene is responsible for the expression of rRNAs, tRNAs, clpP and accD and so on (Krause *et al*. 2000). Specific nuclear products can affect the processing and stability of specific mRNA transcripts. The mutation of single nuclear gene in *Chlamydomonas*

also affects the stability of specific chloroplast mRNA (Gruisserm and Zwrawski 1985). Barkan (1989) thought nuclear encoded proteins may be related to the editing activity of plastid transcripts.

Table 2. List of genes in the *Cynodon dactylon* × *C. transvaalensis* chloroplast genome.

Gene category	Groups of genes	Name of genes
Self-replication	DNA-dependent RNA polymerase	rpoA, rpoB, rpoC1, rpoC2
	Large subunit of ribosomal proteins	rpl2[a,b], rpl14, rpl16[a], rpl20, rpl22, rpl23[b], rpl32, rpl33, rpl36
	rRNA genes	rrn16[b], rrn23[b], rrn4.5[b], rrn5[b]
	Small subunit of ribosomal proteins	rps2, rps3, rps4, rps7[b], rps8, rps11, rps12[b], rps14, rps15[b], rps16[a], rps18, rps19[b]
	tRNA genes	trnC-GCA,trnD-GUC,trnE-UUC,trnF-GAA,trnM-CAU[b], trnG-GCC,trnH-GUG[b], trnI-CAU,trnL-CAA[b], trnL-UAG, trnM-CAU,trnN-GUU[b], trnP-UGG, trnQ-UUG, trnR-ACG,trnR-UCU, trnS-GCU, trnS-GGA, trnT-GGU, trnT-UGU, trnV-GAC[b], trnW-CCA, trnY-GUA
Photosynthesis	ATP synthase	atpA, atpB, atpE, atpF[a], atpH, atpI
	Cytochrome b6/f complex	petA, petB[a], petD[a], petG, petL, petN
	NADH oxidoreductase	ndhA[a], ndhB[a,b], ndhC, ndhD, ndhE, ndhF, ndhG, ndhH, ndhI, ndhJ, ndhK
	Photosystem I	psaA, psaB, psaC, psaI, psaJ
	Photosystem II	psbA, psbB, psbC, psbD, psbE, psbF, psbH, psbI, psbJ, psbK, psbL, psbM, psbN, psbT, psbZ
	Rubisco	rbcL
Other genes	Conserved open reading frames	ycf4
	c-type cytochrome synthesis gene	ccsA
	Envelope membrane protein	cemA
	Maturase	matK
	Protease	clpP
	Translation initiation factor	infA

[a]Genes containing introns; [b]Duplicated gene (Genes present in the IR regions)

Some proteins encoded by the nuclear gene can interact with the specific stem-and-loop structure of the 5 'end of the mRNA. The composite can change the binding capacity of mRNA and ribosome, which activates or depresses translation (Gillham *et al*. 1994, Hirose and Sugiura 1996 and Rochaix 1996). Although plastid DNA can encode the protein needed, most of its protein is encoded by nuclear genes. For example, chlorophyll a-b binding protein (cab) and small subunit of RuBisCO are encoded by nuclear gene (Schreier *et al*. 1985). Besides, changes of DNA structure (Mullet 1988) and selective DNA methylation (Ngernprasirtsir *et al*. 1989) may also be the mechanisms of chloroplast gene transcription regulation.

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