Plant Physiology and Biochemistry 132 (2018) 95–103

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Full-length RNA sequencing reveals unique transcriptome composition in bermudagrass

Bing Zhang[a,*], Jianxiu Liu[b], Xiaoshan Wang[a], Zhenwu Wei[a]

[a] College of Animal Science and Technology, Yangzhou University, Yangzhou 225009, China
[b] Institute of Botany, Jiangsu Province and Chinese Academy of Sciences, Nanjing 210014, China

ARTICLE INFO

Keywords:
Bermudagrass
Transcriptome
PacBio sequencing
C_4 photosynthesis
Alternative isoform

ABSTRACT

Bermudagrass [*Cynodon dactylon* (L.) Pers.] is an important perennial warm-season turfgrass species with great economic value. However, the reference genome and transcriptome information are still deficient in bermudagrass, which severely impedes functional and molecular breeding studies. In this study, through analyzing a mixture sample of leaves, stolons, shoots, roots and flowers with single-molecule long-read sequencing technology from Pacific Biosciences (PacBio), we reported the first full-length transcriptome dataset of bermudagrass (*C. dactylon* cultivar Yangjiang) comprising 78,192 unigenes. Among the unigenes, 66,409 were functionally annotated, whereas 27,946 were found to have two or more isoforms. The annotated full-length unigenes provided many new insights into gene sequence characteristics and systematic phylogeny of bermudagrass. By comparison with transcriptome dataset in nine grass species, KEGG pathway analyses further revealed that C_4 photosynthesis-related genes, notably the phosphoenolpyruvate carboxylase and pyruvate, phosphate dikinase genes, are specifically enriched in bermudagrass. These results not only explained the possible reason why bermudagrass flourishes in warm areas but also provided a solid basis for future studies in this important turfgrass species.

1. Introduction

Bermudagrass [*Cynodon dactylon* (L.) Pers., 2n = 4x = 36] is an important perennial warm-season turfgrass species and is widely used to produce uniform and superior turf for home lawns, public parks, sport fields and golf courses in tropical and subtropical regions (Zheng et al., 2017). In addition to its great economic value, as a worldwidely distributed grass species, bermudagrass is a good material to study the adaptions of plants to diverse environments (Shi et al., 2014b). Furthermore, the coexistence of erect stems (shoots) and prostrate stems (stolons) makes bermudagrass a good material to study plant architecture formation and regulation (Zhang et al., 2017).

In the past several years, high-throughput transcriptome analyses have successfully provided many new insights into the growth, development and stress responses of bermudagrass. For example, genes related to leaf and root growth in bermudagrass were analyzed using cDNA microarray and Illumina sequencing, respectively (Kim et al., 2008; Hu et al., 2015). Comparative transcriptome analysis revealed the gene expression difference between two bermudagrass wild accessions with different stem growth directions (Zhang et al., 2017). Furthermore, transcriptome analyses also identified key genes and metabolic pathways that are important to cold and salt tolerance of specific wild bermudagrass accessions (Zhu et al., 2015; Chen et al., 2015; Hu et al., 2018). Many transcriptome analyses were also performed to explore the mechanisms how bermudagrass adapt to other stress conditions (Kim et al., 2009; Shi et al., 2014a; Melmaiee et al., 2015). However, the deficiency of reference genome sequence makes the assembly and annotation of the bermudagrass transcriptome incomplete and error-prone, which severely impedes in-depth molecular breeding and gene functional studies of this important turfgrass species.

Recently, single-molecule long-read sequencing technology from Pacific Biosciences (PacBio) has provided an efficient approach to sequence full-length cDNA molecules, which has been successively used for whole-transcriptome profiling in many animal and plant species (Sharon et al., 2013; Dong et al., 2015; Abdel-Ghany et al., 2016; Wang et al., 2016a; Kuo et al., 2017; Chen et al., 2017; Xu et al., 2017; Cheng et al., 2017). In comparison with Illumina and other second-generation sequencing (SGS) techniques, the methodological advantages of PacBio transcriptome sequencing mainly include better sequence completeness to both the 5′ and 3’ ends of cDNA molecules and higher accuracy to identify alternative isoforms (Abdel-Ghany et al., 2016; Wang et al., 2016a).

* Corresponding author.
E-mail address: bingzhang@yzu.edu.cn (B. Zhang).

https://doi.org/10.1016/j.plaphy.2018.08.039
Received 25 June 2018; Accepted 29 August 2018
Available online 30 August 2018

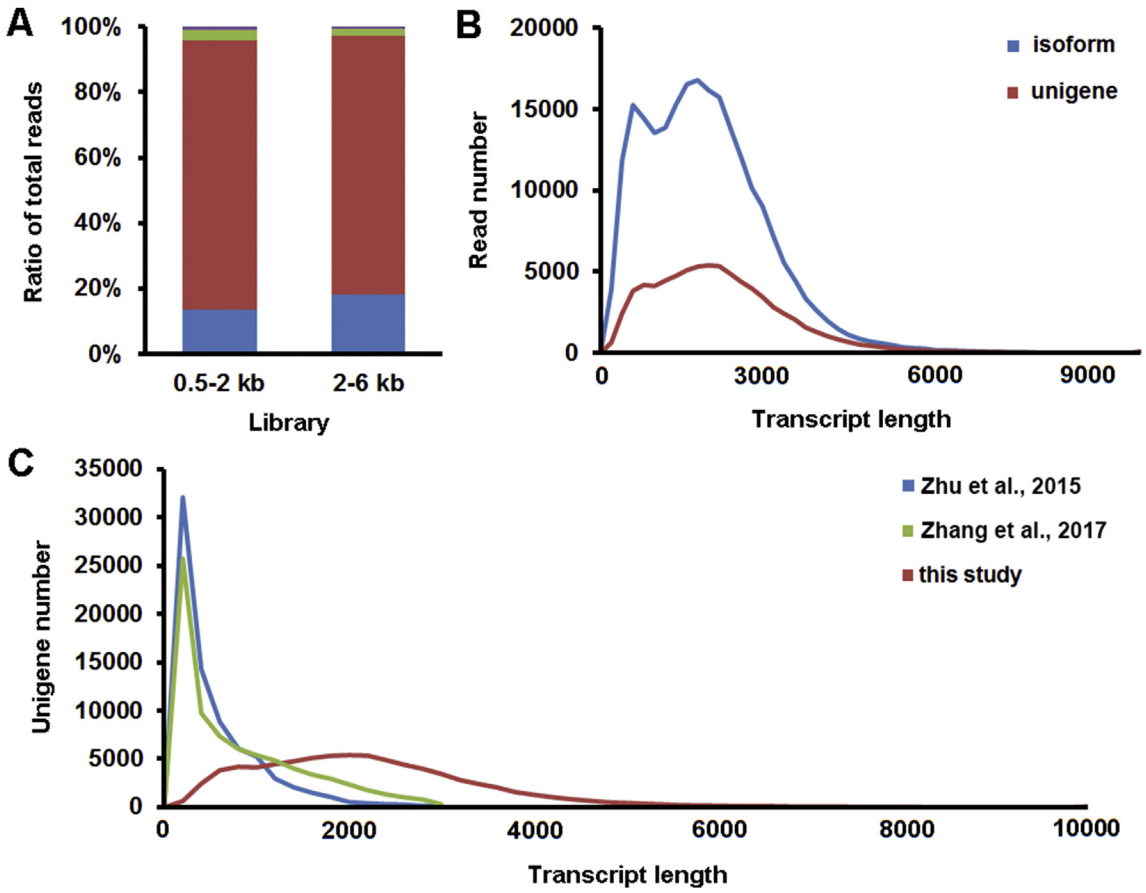

Fig. 1. Overview of the *C. dactylon* full-length transcriptome dataset.
(A) Distribution of different types of ROIs. Blue, nFL reads; red, FL non-chimera reads; green, FL chimera reads; purple, filtered short reads. (B) Length distribution of isoforms and unigenes. (C) Comparison of unigene length in this study and two other studies reporting the assembly of *C. dactylon* unigenes from Illumina sequencing. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

In this study, we reported the first PacBio transcriptome sequencing of bermudagrass. A total of 78,192 unigenes with an average length of 2317 bp were successfully identified from the 173,369 mRNA sequences, whereas 27,946 unigenes were further analyzed to have at least two isoforms. The results of the study not only provided many new insights into gene sequence characteristics and systematic phylogeny of bermudagrass but also pointed out the possible reason why bermudagrass has a strong photosynthetic capacity.

2. Materials and methods

2.1. Plant material

C. dactylon cultivar Yangjiang was used in this study. As a cultivated wild germplasm selected from thousands of Chinese bermudagrass wild germplasm, cultivar Yangjiang has typical characteristics of bermudagrass (asexual reproduction through stolons, tolerance to heat and drought but sensitive to cold stress, etc.) and is widely used in turf establishment in China, making it a good material for different studies of bermudagrass (Zhang et al., 2016, 2018). The cultivar was grown at the turfgrass plots of the Nanjing Botanical Garden (latitude and longitude: 32°02′ N, 118°28′ E; altitude: 30 m a.s.l.; average maximum/minimum temperatures: 38.1 °C/-5.7 °C; average annual precipitation: 1106 mm; soil type: 80% river sand mixed with 20% peat soil) under normal management conditions (irrigation: as required to keep the soil moist; fertilization: four times/year with compound fertilizer (N: P: K = 15: 15: 15; Lvkang Fertilizer Corporation, Nanjing, China) in a concentration of 5 g/m^2; mowing: two times/month) for more than ten years.

2.2. RNA extraction

Leaves, stolons, shoots, roots and flowers of the *C. dactylon* cultivar Yangjiang were collected randomly from different plants. Specifically, the four vegetative tissues were collected from plants at tillering stages (about 30 days after regenerating from stolon nodes), whereas flowers were collected from mature plants at flowering stages. All of the collected tissues were frozen in liquid nitrogen and then stored at −80 °C until use. Total RNA was extracted from the five tissues using the RNeasy Micro Kit (Qiagen, Hilden, Germany). RNA quality and quantity were determined by gel electrophoresis and an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA), respectively.

2.3. Library construction and PacBio sequencing

The library construction and PacBio sequencing were performed according to the official protocol as described by Pacific Biosciences (Pacific Biosciences, Menlo Park, CA, USA). Briefly, 1 μg of total RNA extracted from each of the five tissues were equally pooled together and used as input for cDNA synthesis using SMARTer PCR cDNA Synthesis kit. A total of 23 PCR cycles of amplification were performed using KAPA HiFi PCR Kits. Amplification was followed by size selection using the BluePippin Size Selection System with two bins: 0.5–2 and 2–6 kb. After size selection, another amplification was performed using 12 PCR cycles. The cDNA products were then subjected to the construction of SMRTbell template libraries using the SMRTbell Template Prep Kit.