Environmental and Experimental Botany 155 (2018) 619–627

Contents lists available at ScienceDirect

Environmental and Experimental Botany

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

Comparative *de novo* transcriptomic profiling of the salinity stress responsiveness in contrasting pearl millet lines

Harshraj Shinde[a], Keisuke Tanaka[b], Ambika Dudhate[a], Daisuke Tsugama[c], Yoko Mine[d], Takehiro Kamiya[e], Shashi K. Gupta[f], Shenkui Liu[g], Tetsuo Takano[a,*]

[a] *Asian Natural Environmental Science Center (ANESC), The University of Tokyo, Nishitokyo-shi, Tokyo, 188-0002, Japan*
[b] *NODAI Genome research center, Tokyo University of Agriculture, Setagaya-ku, 156-8502, Tokyo, Japan*
[c] *Laboratory of Crop Physiology, Research Faculty of Agriculture, Hokkaido University, Sapporo-shi, Hokkaido, Japan*
[d] *Tokyo University of Agriculture, Funako, Atsugi, 243-0034, Kanagawa, Japan*
[e] *Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Japan*
[f] *International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, 502 324, India*
[g] *State Key Laboratory of Subtropical Silviculture, Zhejiang A and F University, Hangzhou, 311300, China*

A R T I C L E I N F O

Keywords:
Pearl millet
Salinity
Differentially expressed genes
Metabolic pathways
Transcription factors

A B S T R A C T

Pearl millet (*Pennisetum glaucum* (L) R. Br.) is a staple crop for more than 90 million poor farmers. It is known for its tolerance against drought, salinity, and high temperature. To understand the molecular mechanisms underlying its salinity tolerance, physiological analyses and a comparative transcriptome analysis between salinity tolerant (ICMB 01222) and salinity susceptible (ICMB 081) lines were conducted under control and salinity conditions. The physiological studies revealed that the tolerant line ICMB 01222 had a higher growth rate and accumulated higher amount of sugar in leaves under salinity stress. Sequencing using the Illumina HiSeq 2500 system generated a total of 977 million reads, and these reads were assembled *de novo* into contigs corresponding to gene products. A total of 11,627 differentially expressed genes (DEGs) were identified in both lines. These DEGs are involved in various metabolic pathways such as plant hormone signal transduction, mitogen-activated protein kinase signaling pathways, and so on. Genes involved in ubiquitin-mediated proteolysis and phenylpropanoid biosynthesis pathways were upregulated in the tolerant line. In contrast, unigenes involved in glycolysis/gluconeogenesis and genes for ribosomes were downregulated in the susceptible line. Genes encoding SBPs (SQUAMOSA promoter binding proteins), which are plant-specific transcription factors, were differentially expressed only in the tolerant line. Functional unigenes and pathways that are identified can provide useful clues for improving salinity stress tolerance in pearl millet.

1. Introduction

Salinity stress severely limits crop production. Low precipitation, irrigation with saline water, a rising water table, and poor irrigation practices generally cause salinity stress. More than 6% of the world's total land area is affected by soil salinity (Munns and Tester, 2008). The adverse effects of salinity on plants includes ion toxicity, nutrient constraints, oxidative stress, and osmotic stress (Shrivastava and Kumar, 2015).

Salinity tolerance involves complex responses at the molecular, cellular, metabolic, and physiological levels. At the molecular level, genes encoding ion transporters, transcription factors, protein kinases, and osmolytes are able to confer salinity tolerance (Tuteja, 2007; Kasuga et al., 1999). Pathways such as plant hormone signaling pathway, SOS (salt overly sensitive) pathway, calcium-signaling pathway, MAPK (mitogen-activated protein kinase) signaling pathway, and proline metabolism also have key roles in the salinity stress tolerance (Zhu, 2002; Danquah et al., 2014; Ji et al., 2013; Knight, 1999; Kavi Kishor et al., 2005).

Pearl millet is an important grain crop grown in adverse agro-climatic conditions where other crops fail to produce sufficient yields. It is grown mostly in arid and semi-arid tropical regions of Asia and Africa (Vadez et al., 2012). It is a glycophyte but has the inbuilt capacity to withstand soil salinity. The pearl millet variety named "HASHAKI I" has

* Corresponding author.
E-mail addresses: hsshinde@anesc.u-tokyo.ac.jp (H. Shinde), kt205453@nodai.ac.jp (K. Tanaka), ambika_dudhate@anesc.u-tokyo.ac.jp (A. Dudhate), tsugama@res.agr.hokudai.ac.jp (D. Tsugama), y3mine@nodai.ac.jp (Y. Mine), akamiyat@mail.ecc.u-tokyo.ac.jp (T. Kamiya), S.Gupta@cgiar.org (S. K. Gupta), shenkuiliu@nefu.edu.cn (S. Liu), takano@anesc.u-tokyo.ac.jp (T. Takano).

https://doi.org/10.1016/j.envexpbot.2018.07.008
Received 1 February 2018; Received in revised form 11 July 2018; Accepted 11 July 2018
Available online 15 August 2018
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been released to be grown in salinity affected areas of Uzbekistan (Shivhare and Lata, 2017). Limited information is available on the responses of pearl millet to salinity stress. According to previous studies, the reduced shoot nitrogen content and increased K+ and Na+ contents are associated with the salinity tolerance in pearl millet (Krishnamurthy et al., 2007; Dwivedi et al., 2011). A transcriptome study of pearl millet using the suppression subtractive hybridization approach discovered salinity stress-related genes (Mishra et al., 2007). Functions of only a small number of salinity stress-responsive genes such as *PgDREB2A* (dehydration responsive element binding), *PgNHX1* (Na+/H+ antiporter), *PgDHN* (dehydrin), *PgVDAC* (voltage-dependent anion channel), and *PgLEA* (late embryogenesis abundant) have been studied (Agarwal et al., 2010; Verma et al., 2007; Reddy et al., 2012; Desai et al., 2006; Singh et al., 2015). Recently, comprehensive transcriptome analysis for drought stress response has been performed in pearl millet (Dudhate et al., 2018; Jaiswal et al., 2018). However, a comprehensive understanding of salinity stress tolerance in pearl millet still remains to be obtained.

Among the different transcriptome analysis methods, RNA sequencing (RNA-Seq) has become a widely used method to study gene expression and identify novel genes and pathways. RNA-Seq can efficiently detect unknown genes and novel transcripts (Hrdlickova et al., 2017).

In this study, we conducted a comparative transcriptome analysis of the pearl millet salinity tolerant line and the salinity susceptible line using the high-throughput Illumina HiSeq platform. Genome sequences of pearl millet have been published (Varshney et al., 2017) but the genome has only been partially annotated. Thus, we performed *de novo* assembly of our transcriptome data. We identified many genes and metabolic pathways involved in the salinity stress tolerance of pearl millet. Comparative physiological studies of the two lines were also conducted. To our knowledge, this is the first study conducted to understand the molecular basis of salinity tolerance of pearl millet using the RNA-Seq approach.

2. Materials and methods

2.1. Plant material and stress treatment

Seeds of two pearl millet lines, ICMB 01222 and ICMB 081 were provided by the International Crop Research Institute of Semi-Arid Tropics (ICRISAT), India. ICMB 01222 had been evaluated as a salinity-tolerant line in ICRISAT and hardly withered under a salinity stressed condition in our study, whereas ICMB 081 has been evaluated as a salinity-susceptible line and did wither under the stressed condition (see Fig. 1). Seeds were sown in composite soil in a greenhouse at 28 °C during the day and at 25 °C during the night with a relative humidity between 55%–75%. After 18 days, 250 mM salinity (NaCl) stress was imposed for 6 days.

2.2. Physiological responses of contrasting pearl millet lines against salinity stress

Chlorophyll content were measured using SPAD 502 plus chlorophyll meter, relative water content (RWC) was calculated as previously described (Smart and Bingham, 1974). Total soluble sugar was determined using the anthrone reagent method using the glucose as the standard (Yemm and Willis, 1954), Na+ contents in leaves were determined using inductively coupled plasma-mass spectrometry [ICP-MS (Agilent 7800, Agilent Technologies, U.S.)].

2.3. RNA isolation, library construction, and sequencing

The total RNA was isolated from leaves of ICMB 01222 and ICMB 081 under control and salinity stress condition (250 mM NaCl for 18 h) with three biological replications. The RNA was extracted with a Trizol reagent (Invitrogen). RNase free DNase (Qiagen, Germany) was used to eliminate genomic DNA contamination. To check the purity of the RNA, gel electrophoresis, nanodrop, and the Agilent 2100 bioanalyzer were used. Highly pure Messenger RNA (mRNA) was isolated from the total RNA using oligo (dT) beads. The Illumina TruSeq RNA Library Prep Kit v2 was used to synthesize the second strand cDNAs library. The Illumina HiSeq 2500 platform was used to sequence the constructed cDNA libraries. Sequencing results were obtained as paired-end reads (2 × 100 bp each) in the FASTQ format.

2.4. De novo assembly, ORF detection, and clustering

Raw reads were subjected to quality control by fastaQC (an online tool). Any poor-quality reads and adaptor sequences were filtered by the Trimmomatic and the FASTX-toolkit (Bolger et al., 2014; Gordon et al., 2014). The clean reads were deposited in the sequence read archive (SRA) of the National Center for Biotechnology Information (NCBI) under the accession number SRP128956. The obtained clean reads were assembled into transcriptome, *de novo*, by Bridger (Chang et al., 2015). After the transcriptome was assembled, a TransDecoder was used for the identification of long open reading frames (ORFs) within the transcripts and to score them according to their sequence similarity (Haas et al., 2013). In order to filter redundancies and to reduce noise in the generated contigs, clustering was performed by the CD-HIT program (Li and Godzik, 2006).

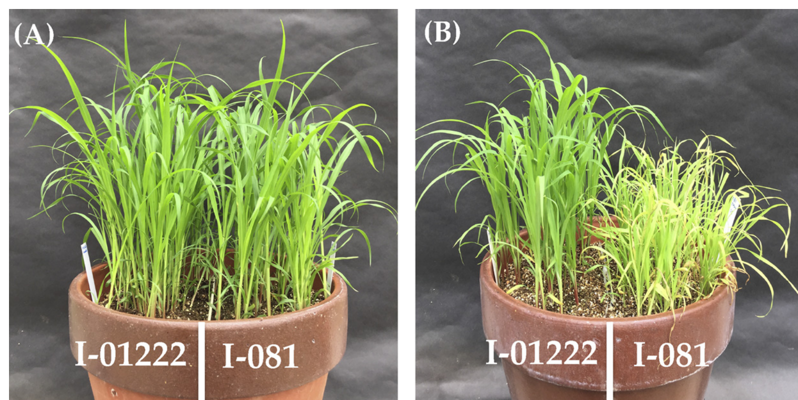

Fig. 1. Differential responses of two pearl millet lines to salinity stress. The left and right sides of the pots contained the ICMB 0122 (tolerant) and ICMB 081 (susceptible) lines, respectively. (A) Control condition; (B) Salinity stressed condition (250 mM NaCl for 6 days).

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