RNA sequencing analysis of gene expression regulated by the transcription factor SlZFP2 during early fruit development

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Abstract

The transcription factor SlZFP2 (Solanum lycopersicum Zinc Finger Protein 2) regulates ABA biosynthesis during fruit development. To reveal the regulatory network of this transcription factor, we conducted a high-throughput RNA-seq to identify differentially expressed genes in 2 dpa (days post anthesis) fruits from a representative RNAi line in Solanum pimpinellifolium LA1589 background and the wild type. The transcriptome analysis revealed that expression of 2722 genes was regulated by SlZFP2 during early fruit development and further helped to narrow down its direct targets to 193 genes. Here, we provide a detailed description of the experimental procedure associated with our transcriptome sequencing data deposited in the National Center for Biotechnology Information (accession no. GSE63838).

Keywords:
RNA-seq
Transcription factor
ABA
Fruit development
Tomato

1. Direct link to deposited data

The raw reads and gene expression data have been deposited in the National Center for Biotechnology Information (accession no. GSE63838) [http://www.ncbi.nlm.nih.gov/bioproject/?term=GSE63838].

2. Experimental design, materials and methods

2.1. Experimental design

Transcription factors regulate transcription of a number of genes either directly or indirectly. The transcription factor SlZFP2 negatively regulates ABA synthesis during tomato fruit development and ripening, and further protein-DNA binding assays identified the (A/T)(G/C)TT motif as its core DNA binding sequences. In the wild type, ABA production is drastically reduced after fruit set, usually completed around 2 dpa, in tomato. When suppressing SlZFP2 expression in tomato by RNA interference (RNAi), the young developing fruits accumulated significantly higher ABA content [1]. To better understand the impact on global gene expression by down-regulation of SlZFP2 expression and also to identify its direct targets during early fruit development, we conducted a high-throughput RNA-seq analysis on 2 dpa fruits from the SlZFP2 RNAi line and its nontransgenic sibling (as wild type control). The whole fruits collected from hand-pollinated inflorescences of 3–5 plants were used for RNA-seq analysis and the experiment was conducted in three biological replicates.

2.2. Plant materials

The representative RNAi line (207) showed significant reduction of SlZFP2 expression in the fruits as analyzed by quantitative RT-PCR [1]. The RNAi line 207 and its nontransgenic sibling (207N) were grown in the phytotrons at 20 °C to 25 °C under a humidity of 70% to 80%, with daily illumination for 16 h by 150 mE m–2 s–1 light from metal halide and high-pressure sodium lamps. The whole fruits from hand-pollinated inflorescences were collected and immediately frozen in liquid nitrogen and stored at −80 °C.

2.3. Total RNA extraction and quality control

Total RNA was extracted whole fruits with Trizol reagent (Invitrogen) based on the method previously described [2].
integrity of isolated total RNA was checked by gel electrophoresis in RNA MOPS (3-N-morpholino-propanesulfonic acid) buffer, then quantified using NanoDrop 2000c (Thermo Fisher Scientific Inc., USA) and Qubit®2.0 (Thermo Fisher Scientific Inc., USA).

2.4. Transcriptome sequencing and annotation

Barcoded paired-end sequencing libraries were constructed for the six samples using the TrueSeq stranded mRNA kit (RS-122-2101, Illumina) based on the manufacturer’s instructions. We sequenced the six libraries in one run on an Illumina’s Miseq system using the 500-cycles Miseq reagent kit (MS-102-2003). After in-house quality control and removal of the index sequences, the 250-bp paired-end reads were mapped to the tomato reference genome (ITAG2.5) using the Tophat program version 2.0.12[3]. The mapping was guided by current genome annotation and following parameters were used: –read-mismatches 5 –read-gap-length 3 –read-edit-dist 5 –library-type=fr-firststrand –splice-mismatches 0 and default settings for other parameters. In total, the numbers of read pairs mapped for each replicate were as follows: 1,497,635 (207N, replicate 1; 80.7% mapped), 1,191,334 (207N, replicate 2; 80.6%), 1,279,658 (207N, replicate 3; 83.2%), 573,982 (207, replicate 1; 80.2%), 749,210 (207, replicate 2; 80.2%), and 1,158,542 (207, replicate 3; 80.5%).

Uniquely mapped reads were then assembled by Cufflinks program 2.2.1 [4] using following parameters: –GTF-guide –frag-bias-correct –min-frags-per-transfrag 10. Then, differentially expressed genes (adjusted p value of 0.05 or less) were selected by comparison between the RNAi line and its nontransgenic sibling (wild type) using Cuffdiff command in the Cufflinks program with following parameters used: –frag-bias-correct –multi-read-correct –min-alignment-count 10 –FDR 0.05. The analysis identified a total of 2722 differentially expressed genes in the 2 dpa fruits between the SIZFP2 RNAi line 207 and the wild type. After further comparison with the gene list of putative SIZFP2 targets based on bacterial one hybrid screening, 193 genes were identified as direct targets of SIZFP2 in early fruit development.

Acknowledgments

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