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Research paper

Transcriptome analysis of the Chinese bread wheat cultivar Yunong 201 and its ethyl methanesulfonate mutant line

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ABSTRACT

Roche 454 next-generation sequencing was applied to obtain extensive information about the transcriptomes of the bread wheat cultivar Yunong 201 and its EMS mutant line Yunong 3114. Totals of 1.43 million and 1.44 million raw reads were generated, 14,432, 17,845 and 27,867 isotigs were constructed using the reads in Yunong 201, Yunong 3114 and their combination, respectively. Moreover, 29,042, 34,722, and 48,486 unigenes were generated in Yunong 201, Yunong 3114, and combined cultivars, respectively. A total of 50,382 and 59,891 unigenes from the Yunong 201 and Yunong 3114 were mapped on different chromosomes. Of all unigenes, 1363 DEGs were identified in Yunong 201 and Yunong 3114. qRT-PCR analysis confirmed the expression profiles of 40 candidate unigenes possibly related to abiotic stresses. The expression patterns of four annotated DEGs were also verified in the two wheat cultivars under abiotic stresses. This study provided useful information for further analysis of wheat functional genomics.

1. Introduction

Wheat (*Triticum aestivum* L.) is an important cereal crop alongside maize and rice; more than 600 million tons of wheat are harvested annually (Shewry, 2009). A total of 704, 723, and 883 million tons of wheat, rice, and maize were produced in 2011, respectively (http://faostat.fao.org/). These data show that wheat yield increases more slowly than other crop yields. The genome sizes of the three cereal crops are also remarkable; for instance, the genome of rice is approximately 400 Mbp (Goff et al., 2002; Yu et al., 2002), and that of the ancient allotetraploid maize is approximately 2.3 Gbp (Schnable et al., 2009). By contrast, the genome size of wheat is 16 Gbp. Such a large and complex genome complicates research on wheat genome; the depth and breadth of such a research are inferior to those of rice, maize, barley, and other crops. Modern biotechnologies have been applied to improve wheat yield, nutritional content, and salinity, drought, and biotic tolerance (Tester and Langridge, 2010). However, information on the genome sequences and transcriptome of wheat remain insufficient.

The next-generation sequencing (NGS) has been successfully applied in wheat and its closely related species for several years; other extensive applications and studies were conducted in the past 2 years. For instance, the first homolog-specific sequence assembly of wheat transcriptome provides a reference transcriptome for future wheat studies based on Roche 454 and Illumina GAIIx (Schreiber et al., 2012). A high-throughput RNA sequencing has been performed using Illumina NGS to characterize the transcriptome of Wangshuibai during *Fg* infection (Xiao et al., 2013). RNA sequencing (RNA-seq) methods have also been applied to generate the transcriptome profiles of the wheat cultivar Chinese Spring in response to 10 d of phosphate (P_i) starvation and to elucidate the molecular mechanisms associated with such conditions (Oono et al., 2013). RNA-seq can accurately measure the transcript levels of *Pina*, *Pinb*, and each of the four *Pinb-2* variants in developing wheat seeds, whereas Northern blots cannot accurately quantify *Pinb-2* transcript levels because of cross-hybridization (Giroux et al., 2013). Interactions between stem rust and wheat were studied using NGS of rust genomes and transcriptomes of infected wheat tissues; RNA-seq expression profiling demonstrated race- and host-specific responses in different combinations of stem rust and wheat genotypes (Akhunov, 2013).Nowadays, ethyl methanesulfonate(EMS) mutation has also reached a mature stage, in which damages in plants are reduced and abundant plant mutations are generated by controlling the usage of EMS. EMS mutants have been employed as basic materials in extensive studies.

Until now, RNA-seq and EMS mutants were rarely combined in wheat transcriptome studies. The Chinese winter wheat cultivar Yunong 201 we used in this study, developed by Agronomy College of Henan Agricultural University, was released as a high-quality noodle

Abbreviations: EMS, ethyl methanesulfonate; NGS, next-generation sequencing; qRT-PCR, quantitative real-time PCR; EST, expressed sequence tag; DEG, differentially expressed gene; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; FDR, false discovery rate; ddH_2O, double distilled H_2O; RLK, receptor-like protein kinase; RLK, receptor-like protein kinase; TLP, thaumatin-like protein; TF, transcription factor; Hsp, heat-shock protein; RWC, relative water content.

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wheat cultivar by Henan province in 2006. An elite M_2 line was screened from the EMS mutagenized population encompassing 2000 lines because of its different plant architecture, large kernel size, and high grain weight. This line was self-crossed thrice into the M_5 line Yunong 3114.Compared with Yunong 201, Yunong 3114 showed relatively larger kernel size, higher thousand grain weight and higher yield per plot. Yunong 201 and Yunong 3114 also showed obvious difference on tolerance to abiotic stresses (e.g. drought, coldness, dry-hot wind). Therefore, comparison of transcriptomes of Yunong 201 and Yunong 3114 could provide valuable information for further dissection of molecular and genetics basis of the phenotypes related to abiotic stresses as well as yield and quality in bread wheat. In the study, Roche 454 sequencing was applied in bread wheat Yunong 201 and Yunong 3114 to generate their transcriptomes. The gene profiles of Yunong 201 and Yunong 3114 were obtained by *de novo* sequencing. Differentially expressed genes (DEGs) related to the growth, stimuli, and photosynthesis of Yunong 201 and Yunong 3114 were comprehensively analyzed. Subsequently, the expression patterns of four annotated DEGs were verified in the two varieties under abiotic stresses. This study provided important information for future understanding the transcriptome of hexaploid wheat.

2. Materials and methods

2.1. Plant materials

The Chinese winter wheat cultivar Yunong 201 (released No. Yushenmai 2006006) and its EMS-derivative Yunong 3114 as mentioned above were planted and grown at the Zhengzhou Scientific Research and Education Center of Henan Agricultural University (N34.9°, E113.6°) during 2011 to 2012 cropping seasons at non-stressed conditions. The leaves and stems at the three-leaf stages were collected to generate the cDNA libraries of Yunong 201 and Yunong 3114.

2.2. RNA extraction, library construction, and Roche 454 sequencing

Full-length cDNA libraries were sequenced using a GS FLX sequencer (Roche) in accordance with the standard single read shotgun 454 sequencing protocol with titanium chemistry (Roche). The total RNAs of Yunong 201 and Yunong 3114 were extracted using Invitrogen Trizol Reagent. The two 454 libraries of Yunong 201 and Yunong 3114 were constructed using a GS FLX titanium general library preparation kit. The quantity and quality of the total RNA were analyzed via spectrophotometry (Ultrospec™ 2100 pro UV/Visible spectrophotometer) and gel electrophoresis. mRNA was then isolated from the total RNA by using oligo (dT)-attached magnetic beads. cDNA was synthesized using a random Roche primer and via PCR amplification. The quality of the double-stranded cDNA was checked by running on a 2% 1× TAE agarose gel with 0.1 mg/mL ethidium bromide (carcinogen) for approximately 30 min. cDNA concentration was determined using a Bioanalyzer 7500 kit. cDNA samples sheared by ultrasonication should be >80 ng/μL (total > 1 μg) and should range from 100 bp to 800 bp, which is the appropriate fragment size range for 454 sequencing.

RL adaptors or RL MID adaptors were ligated to the fragmented cDNA, and the small fragments were removed. A TBS 380 fluorometer (Turner Biosystems) was used to quantify the cDNA library. The quality of the cDNA library was assessed, and the average fragment length ranged between 600 and 1200 bp with a low size cut-off <10% of 500 bp.

2.3. Pretreatment of data

A total of 5 Gb raw data were generated from each cultivar. Raw reads were trimmed using Newbler 2.5 (Margulies et al., 2005). Low-quality bases and vector sequences were filtered; reads less than 50 bp were also removed. The trimmed reads were then subjected to polygenetic analysis with CD-HIT version 4.0 (Huang et al., 2010) at a sequence identity threshold of 99%. The trimmed reads without redundancy were analyzed by comparing with the NT library. Assembly and quantification were conducted by 454 software Newbler and RPKM algorithm. Trimmed reads assembled using Newbler 2.5 were called contigs; isotigs were formed by the assembly of one or more contigs. Three groups comprising Yunong 201, Yunong 3114, and their combination were finally generated.

2.4. Sequence annotation

Isotigs + singlets sequences were subjected to polygenetic analysis by using CD-HIT version 4.0 (Huang et al., 2010) with an identity of 95%. Chromosome mapping using ssahaSNP software and comparing with known genome segments were also performed. The BLAST program (Camacho et al., 2009) (a searched threshold of 1e–10) was used to search for unigenes in EST libraries of 10 species related to NCBI (http://www.ncbi.nlm.nih.gov/) and DFCI (ftp://occams.dfci.harvard.edu/pub/bio/tgi/data). Nucleic acid and protein were separately annotated by comparing the unigenes with those in the NT library (E-value <1e–5) and the NR library (BLASTX; E-value <1e–5; similarity of protein >30%).

2.5. GO and KEGG orthology

The gene ontology (GO) project is a major bioinformatics initiative that aims to standardize the representation of gene and gene product attributes across species and databases (http://www.geneontology.org/). We identified the annotated unigene sequences for the possible functions by using this method. We mapped sequences to the reference authoritative pathways in Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.genome.jp/kegg/) to determine the active biological pathways in annotated unigene sequences. KEGG is a database used to understand the high-level functions and utilities of a biological system.

2.6. Analysis of gene expression

The total unigenes of the combined Yunong 201 and Yunong 3114 were divided into three sections to analyze the expression difference in unigenes: unique unigenes in Yunong 201 and Yunong 3114, and common unigenes in the first two groups. False discovery rate (FDR) statistical test was conducted in accordance with the RPKM value of the common unigenes in Yunong 201 and Yunong 3114. At FDR < 0.05, DEGs were present; at FDR > 0.05, no differences were present between the expression levels of common unigenes.

2.7. Validation of DEGs by quantitative real-time PCR (qRT-PCR)

Based on the functional annotation of unigenes, the 44 DEG we selected are possibly related to stresses, and corresponding specific primers were designed by software Primer 3.0. We verified the expression profiles of 40 wheat candidate unigenes that were chosen from the 1363 DEGs.

2.8. Stress treatments and validation of DEGs by qRT-PCR

Seeds of Yunong 201 and Yunong 3114 were immersed sterilized with 0.01% (*w*/*v*) H_2O_2 for 0.5 h and then thoroughly washed with distilled water. Sterilized seeds were grown in glass dishes (9 cm diameter) with double distilled H_2O (ddH_2O). Seedlings were maintained in illuminated incubator at 25/15 °C day/night temperatures under 16/8 h light/dark photoperiod and 250 μmol m^{-2} s^{-1} light intensity. Two-week old seedlings with similar heights were used to analyze the effects of different abiotic stresses. In order to investigate abiotic stress of the Yunong 201 and Yunong 3114 plants, ten seedlings for each cultivar