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Comparative transcriptome analysis provides insight into differentially expressed genes related to bud dormancy in grapevine (*Vitis vinifera*)

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ABSTRACT

Bud dormancy is an important adaptive strategy of perennial woody plants that benefits survival of a species under unfavorable conditions. Grapevine is one of the most important fruit species with wide distribution all over the world. There are two kind of buds, latent buds and prompt buds, along shoots of grapevine, which show different phenotypes in terms of dormancy. To better understand the molecular mechanisms behind this, the transcriptomes of grapevine (*Vitis vinifera*) latent buds and prompt buds were analyzed using RNA-sequencing (RNA-seq) technology and compared. Of all the genes detected, 4864 were identified as differentially expressed genes (DEGs), with 2613 being upregulated and 2251 being downregulated in latent buds than in prompt buds. Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis revealed that these DEGs were mostly associated with biological process, single-organism transport, lipid metabolic process and transporter activity, and also involved in biosynthesis of secondary metabolites, metabolism of phytohormone, flavonoids, phenylpropanoid, sugar and fatty acid. Lots of transcription factors (TFs), especially some members of MYB, TCP, and DAM, were identified to be candidates regulating grapevine bud dormancy. Results of transcriptome profiling were validated using real-time PCR in 27 selected genes. The results revealed that interactions of phytohormone, sugars and flavonoids and their regulation to the cell-cycle genes via the mediation of TFs may contribute greatly to grapevine bud dormancy. Consequently, this study provides abundant genetic resources and lays a foundation for further research on the molecular mechanisms underlying bud dormancy in grapevine.

1. Introduction

Bud dormancy is an important adaptive strategy of perennial woody plants that benefits survival of a species under unfavorable conditions. It refers to the latent state of meristem during late-autumn or winter when the temperature becomes low and the photoperiod becomes short, within buds, which makes it possible to resist to adverse conditions (Rios et al., 2014). It is of great importance because the timing of bud dormancy can affect the phenological period and thus may have a fatal effect on the yield of fruit tree.

Grapevine is one of the most important fruit species with wide distribution all over the world. Unlike other perennial woody plants, there are two kind of buds, latent buds and prompt buds, along shoots of grapevine (Morrison, 1991), which are located adjacently between the leaf axils, but show different phenotypes in terms of dormancy: prompt buds can sprout and then develop into shoots in the same year; while latent buds remain dormant to safely overwinter, until the following year, when the environmental condition is suitable for bud sprout (Morrison, 1991). It was found in the process of viticulture that the sublateral shoots derived from prompt buds would always need to be removed, which not only wasted lots of plant nutrients, but also greatly increased labor costs in grape production process; In addition, the ripen period of grape was dependent on the timing of bud break. In some places of China, it was frequently happened that grapes ripen period coincided with the rainy season, which can affect the production and quality of grape seriously and bring huge economic losses to farmers. Therefore, it is necessary to have a better understanding of grapevine bud dormancy, especially the key factors and genes regulating it. This will not only be used as basis for genetic engineering to reduce sublateral shoots thereby reducing labor costs, but also help to modulate the timing of bud dormancy through molecular biology thus reducing economic losses in grapevine cultivation.

Abbreviations: LB, latent bud; PB, prompt bud; DEGs, differentially expressed genes; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; RNA-Seq, RNA sequencing; ABA, abscisic acid; SA, salicylic acid; JA, jasmonic acid; CTK, cytokinins; GA, gibberellin; BR, brassinosteroid; TF, Transcription factor; FDR, false discovery rate

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The mechanism of grapevine bud dormancy induction and release, and photoperiod and chilling perception, has been explored mainly on the aspects of physiological, biochemical and genetic responses (Halaly et al., 2008; Halaly et al., 2011; Or et al., 2000; Pang et al., 2007). It was suggested that grapevine bud dormancy is implicated with the external factors such as temperature, photoperiod and water status (Schrader et al., 2004) and the resulting internal factors such as hormone (Zheng et al., 2015), sugars (Anderson et al., 2005; Horvath et al., 2008), Ca^{2+} (Pang et al., 2007) and enzymes (Or et al., 2002). Several lines of evidence suggested corrections between absisic acid (ABA) (Zheng et al., 2015), auxin (IAA) (Prusinkiewicz et al., 2009), jasmonic acid (JA) (Christov et al., 1992) and bud dormancy. Strigolactone, a novel caroteroid-derived hormone (Gomez-Roldan et al., 2008; Umehara et al., 2008), was also reported to play a role in repressing bud outgrowth. Ca^{2+} (Pang et al., 2007) and oxidative stress (Ophir et al., 2009; Or et al., 2000; Pérez et al., 2008) were also suggested to be part of the grapevine bud dormancy release machanism. Many genes induced by these factors were identified and several flowering related genes were proved to have a dual effect on bud dormancy (Sreekantan et al., 2010). Though researchers have paid much effort to elucidate the genetic network and to explain the mechanism at molecular level, bud dormancy is such a complex trait that thousands of genes are involved in, the mechanism of grapevine bud dormancy still remains unclear. Transcriptomic studies have arisen during the past few years with the rapid development of the high-throughput sequencing technology. RNA sequencing (RNA-seq), based on determination of overall mRNA abundance at different status, has become a more routine experimental method in transcriptome analyses. To address the global changes in gene expression triggered by bud dormancy or dormancy release events, RNA-seq was performed among many perennial species, including Japanese apricot (Zhuang et al., 2013), Chinese white pear 'Suli' (Liu et al., 2012a), Japanese pear (Bai et al., 2013), Chinese cherry (Zhu et al., 2015) and grapevine (Fennell et al., 2015; Sreekantan et al., 2010), detecting differentially expressed genes associated with photoperiod (Olsen, 2010), circadian clocks (Bohlenius et al., 2006) and different hormones (Dogramaci et al., 2013; Mornya and Cheng, 2011; Olsen, 2010). Though transcriptome analysis on grapevine bud dormancy and dormancy release was ever performed as mentioned above (Fennell et al., 2015; Sreekantan et al., 2010), study from the perspective of different dormant characteristics of grapevine latent buds and prompt buds via RNA-seq technology was seldom documented.

In this study, RNA-seq technology was used to acquire a comprehensive view of comparison of transcriptome profiling between grapevine latent buds and prompt buds, in the purpose of identifying the key genes and pathways related to grapevine bud dormancy, thus elucidating the molecular mechanism of grapevine bud dormancy and release and laying foundation for the modulation of sublateral shoots and the timing of bud break via genetic manipulation.

2. Materials and methods

2.1. Sample collection

All the samples were collected from the grapevine experimental station of Northwest Agriculture & Forestry University (Yangling, China). Thirteen-year-old trees of wine grape cultivar 'Cabernet Sauvignon' (*Vitis Vinifera*.L) were used as material. During the sampling period, plants were not pruned or chemically treated. Prompt buds were collected on at anthesis stage May 25 in 2015, when some prompt buds starts to develop into sublateral shoots. Latent buds were collected on November 25 in 2015, when the buds were defined to be endodormant based on our previous work (Chunying et al., 2009; Zhang et al., 2012). Buds from one cane of five vines were incised carefully and pooled as one unit of biological replicate, and named LB (latent bud) and PB (prompt bud), respectively. This was repeated on separate vines for replicates 2 and 3. The buds were frozen in liquid nitrogen immediately after collection and stored at −80 °C for subsequent experiments.

2.2. RNA extraction, quantification and qualification

Total RNA was extracted from 3 biological replicates of buds using Plant RNAout (TIANDZ, Beijing, China) according to the manufacturer's instruction. Genomic DNA contamination was removed from total RNA using RNase-free DNase I (Thermo Scientific, USA). RNA degradation and contamination was monitored on 1% agarose gels. RNA purity was checked using the NanoPhotometer® spectrophotometer (IMPLEN, CA, USA). RNA concentration was measured using Qubit® RNA Assay Kit in Qubit® 2.0 Flurometer (Life Technologies, CA, USA). RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA).

2.3. Library preparation for transcriptome sequencing

3 μg total RNA per sample was used for RNA preparations. These high quality RNA samples were sent to Beijing Novogene Bioinformatics Technology Co., Ltd. (Beijing, China). NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) was used to construct sequencing libraries according to the manufacturer's recommendations and index codes were added to sequences to distinguish one sample from another. Quality of these libraries were assessed using the Agilent Bioanalyzer 2100 system. The index-coded samples were clustered on a cBot Cluster Generation System using TruSeq PE Cluster Kit v3-cBot-HS (Illumia) following the manufacturer's instructions. Then the library were sequenced using Illumina Hiseq platform (Hiseq 4000, 150PE).

2.4. Quality control and reads mapping

All the clean reads have been deposited in the National Center for Biotechnology Information (NCBI) Short Read Archive (SRA) Sequence Database under accession number SRP092034. Raw data (raw reads) of fastq format were filtered by removing reads containing adapter, reads containing ploy-N and low quality reads, thus clean reads were obtained. At the same time, Q20 (The percentage of bases with a Phred value > 20), Q30 (The percentage of bases with a Phred value > 20) and GC (base G and C) content of the clean data were calculated. All the subsequent analyses were based on the clean data with high quality. Genome of the sequenced grapevine 'Pinot noir' (12Xv1) and gene model annotation files were downloaded from genome website (ftp://ftp.ensemblgenomes.org/pub/release-23/plants/gtf/vitis_vinifera/). Bowtie v2.2.3 was used to build the index of the reference genome and paired-end clean reads were aligned to the reference genome using TopHat v2.0.12. We selected TopHat as the mapping tool for that TopHat can generate a database of splice junctions based on the gene model annotation file and thus a better mapping result than other non-splice mapping tools.

2.5. Quantification of gene expression level and differential expression analysis

HTSeq v0.6.1 was used to count the reads numbers mapped to each gene. And then FPKM of each gene was calculated based on the length of the gene and reads count mapped to this gene. FPKM, expected number of fragments per kilobase of transcript sequence per millions base pairs sequenced, considers the effect of sequencing depth and gene length for the reads count at the same time, and is currently the most commonly used method for estimating gene expression levels (Cole et al., 2012).

DESeq R package (1.18.0) was used to analyze the different expression of latent buds and prompt buds. DESeq provide statistical routines for determining differential expression in digital gene