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Research article

Culm transcriptome sequencing of Badila (*Saccharum officinarum* L.) and analysis of major genes involved in sucrose accumulation

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ABSTRACT

Sugarcane is an important sugar and energy crop worldwide. It utilises highly efficient C4 photosynthesis and accumulates sucrose in its culms. The sucrose content in sugarcane culms is a quantitative trait controlled by multiple genes. The regulatory mechanism underlying the maximum sucrose level in sugarcane culms remains unclear. We used transcriptome sequences to identify the potential regulatory genes involved in sucrose accumulation in *Saccarum officinarum* L. cv. Badila. The sucrose accumulating internodes at the elongation and mature growth stage and the immature internodes with low sucrose content at the mature stage were used for RNA sequencing. The obtained differentially expressed genes (DEGs) related to sucrose accumulation were analysed. Results showed that the transcripts encoding invertase (beta-fructofuranosidase, EC: 3.2.1.26) which catalyses sucrose hydrolysis and 6-phosphofructokinase (PFK, EC: 2.7.1.11), a key glycolysis regulatory enzyme, were downregulated in the high sucrose accumulation internodes. The transcripts encoding key enzymes for ABA, gibberellin and ethylene synthesis were also downregulated during sucrose accumulation. Furthermore, regulated protein kinase, transcription factor and sugar transporter genes were also obtained. This research can clarify the molecular regulation network of sucrose accumulation in sugarcane.

1. Introduction

Sugarcane (*Saccharum* sp.) is a tropical and subtropical economic crop that produces more than 70% of the world's sugar supply and is a perennial C4 grass plant that belongs to Saccharinae, Andropogoneae of Poaceae (Moore and Botha, 2014). In sugarcane stalks, sucrose can remarkably accumulate in the storage parenchyma cells at a high concentration of up to 12%–16% of culm fresh weight (FW) (Bull and Glasziou, 1963; Moore, 1995), thus establishing the economic value of sugarcane. Theoretical sucrose content can reach 30% of FW in sugarcane stalk (Bull and Glasziou, 1963). However, the sucrose content in sugarcane cultivar culms is less than 20% of the FW (Moore and Botha, 2014). The genetic mechanism underlying the maximum sucrose level in sugarcane stalks remains unclear.

Sucrose is the main product of photosynthesis and used for translocation from source to sink tissues in sugarcane (Hartt et al., 1963). Sucrose is synthesised in sugarcane source mesophyll cells and transported through the phloem to sink organs to support growth and development (Hartt et al., 1963). Sucrose phosphate synthase (SPS, EC: 2.4.1.14) is the key enzyme which catalyses sucrose synthesis. Invertase (INV, EC: 3.2.1.26) breaks sucrose into glucose and fructose (Ruan, 2014). Sucrose synthase (Susy, EC: 2.4.1.13) reversibly catalyses sucrose synthesis and hydrolysis from or into uridinediphosphoglucose and fructose (Ruan, 2014). The enzyme activities for sucrose synthesis and hydrolysis control the cellular sucrose level. In sugarcane sucrose storage internodes, the activities of SPS, Susy and INVs are relatively low (Zhu et al., 1997). Additionally, the transcriptional regulation mechanisms on sucrose accumulation have been studied in sugarcane culms. Genes relative to sucrose accumulation have been analysed in the immature and mature internodes of hybrid sugarcane genotypes

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Abbreviations

ABF	ABA responsive element binding factor
ACO	Aminocyclopropanecarboxylate oxidase
ACS	1-aminocyclopropane-1-carboxylate synthase
ARF	Auxin response factor
AUXIAA	Auxin/indole-3-acetic acid
BAK	BRI-association receptor kinase
BR	Brassinosteroid
CDS	Coding regions
CesA	Cellulose synthase complex
CIN	Cytoplasmic neutral invertase
CIPK	CBL-interacting protein kinase
COG	Clusters of orthologous groups
CPK	Calcium dependent protein kinase
CTR	Constitutive triple response
CWIN	Cell wall invertase
DEG	Differently expressed genes
EBF	EIN3-binding F-Box
ERF	Ethylene responsive factor
ETR	Ethylene receptor
FW	Fresh weight
G6PD	Glucose-6-phosphate 1-dehydrogenase
GA	Gibberellin
GID	GA-insensitive dwarf
GO	Gene ontology
HEX	Hexose transporter
HXK	Hexokinase
INV	Invertase
KEGG	Kyoto Encyclopedia of genes and genomes
MPK	Mitogen activated protein kinase
NCED	9-cis-epoxycarotenoid dioxygenases
NI	Neutral invertase
NGS	Next generation sequencing
NR	Non redundant protein database
PFK	6-phosphofructokinase
PFP	Pyrophosphate-fructose-6-phosphate phosphotransferases
PYR/PYL	Pyrabactin resistance/pyrabactin resistance like
PP2C	Protein phosphatases type 2C
RLK	Receptor like kinase
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
RLK	Receptor like kinase
SAI	Soluble acid invertase
SAUR	Small auxin-up RNA
SnRK	Sucrose non-fermenting 1-related protein kinase
SPS	Sucrose phosphate synthase
STK	Serine/threonine protein kinase
STP	Sugar transporter protein
Susy	Sucrose synthase
WAK	Wall associated kinase

with high and low sucrose contents, respectively (Carson and Botha, 2002; Casu et al., 2003, 2004; Papini-Terzi et al., 2009; Thirugnanasambandam et al., 2017). The specially regulated genes in storage parenchyma, vascular bundles and rind of mature internodes from Q117 hybrids have already been analysed (Casu et al., 2015). Multiple genes were found to be associated with sucrose content, such as in sugar metabolism and transport, signal transduction, fibre synthesis and abiotic stress tolerance. However, the key points and master regulators controlling sucrose content in sugarcane must be investigated.

Sugarcane is a highly polyploid plant species with complex genomic composition (Piperidis et al., 2010). The taxonomy of sugarcane is divided into six species: two wild types of *S. spontaneum* and *S. robustum* and four domesticated types of *S. officinarum*, *S. barberi*, *S. sinense* and *S. edule* (Moore and Botha, 2014). *S. officinarum* is used for sugar production before nobilisation breeding, the interspecific hybridisation between *S. officinarum* and *S. spontaneum* and back crossing to noble canes (*S. officinarum*) to produce an adaptable and stress-resistant nobilised cultivar (Bremer, 1963). Nobilised cultivars are crossed to produce additional hybrid cultivars. The breeding program reassembles the germplasms to broaden the genetic base via constant hybridisation. During breeding, the genetic bases of sugarcane hybrids are changed to be as complicated as interspecific aneuploids. Sugarcane commercial cultivars form complex genomic compositions (Piperidis et al., 2010). Studies indicate that 70%–80% of chromosomes in commercial cultivars are inherited from *S. officinarum*; among these chromosomes, 10%–20% are from *S. spontaneum* and 10% are recombinant (Cuadrado et al., 2004; D'Hont et al., 1996; Piperidis et al., 2010). The genetic parts from *S. officinarum* contribute to maintain the high sucrose content (Cuadrado et al., 2004; D'Hont et al., 1996; Piperidis et al., 2010).

Rapid progress in transcriptome sequencing and next-generation sequencing (NGS) accelerates the identification of genes and gene networks involved in plant metabolism and regulation (Varshney et al., 2009). These methods can systematically analyse relative multigenes in a metabolic pathway. Alternatively, large-scale transcriptome sequencing can provide high-throughput information on the genes expressed in a special organism and identify the effective genes or gene networks associated with certain plant traits (Varshney et al., 2009). In this study, the culm transcriptomes were analysed using RNA sequencing (RNA-seq) in *S. officinarum* species Badila, which has been used as parent for breeding a series of cultivars in China, to clarify the gene networks associated with sucrose content in sugarcane. De novo transcriptome sequencing of mature internodes from Badila at elongation stage and immature and mature internodes at mature stage was performed using the Illumina RNA-Seq platform. Many transcripts were obtained, and the genes involved in sucrose accumulation and regulation were identified. The validities of sequences and expression patterns of candidate genes were confirmed using RT-PCR. The culm transcriptome analysis from *S. officinarum* will aid in discovering gene-regulated networks that control sucrose accumulation.

2. Materials and methods

2.1. Plant material

Badila plants were grown in natural conditions in the experimental field (19°59′6″N, 110°19′21″E) of the Chinese Academy of Tropical Agricultural Sciences, Haikou, Hainan province, China. The high- and low-sucrose content internodes from Badila were selected base on the previous sucrose content testing data (Unpublished data). The mature internodes 10 (The internodes attaching the first fully expanded leaf is numbered as No.1) from Badila plants at 150 and 300 days were sampled as ShMN1 and ShMN2, respectively. The immature internode 5 from Badila plants at 300 days was sampled as ShYN1. Each samples were treated with three replicates. These samples were quickly frozen in liquid nitrogen and stored at −80 °C until use.

2.2. Soluble sugar assay

Approximately 1 g of leaf and culm tissues was ground into powder in liquid nitrogen, and soluble sugars were extracted with ethanol (Hartt et al., 1963). Sucrose content were analysed using a high-performance liquid chromatography system (Waters e2695, USA). The NH2 column (3.5 μm, 4.6 mm × 250 mm; Waters, USA) was used to separate sugars in extraction samples. The samples (100 μL) were mixed with acetonitrile (1:1, V/V), and the mixture solution was filtered through a 0.45 μm syringe filter. Ten microliters of mixture solution were injected with a mobile phase in an 80% acetonitrile solution at a speed of 1 mL/min^{-1}, and each sample was experimented in triplicate.

2.3. RNA extraction and sequencing

Total RNA of the samples was isolated using E.Z.N.A.™ Plant Total RNA Kit (Omega, USA). RNase-free DNase I (Takara, Japan) was used to remove genomic DNA contamination. The quality and quantity of the extracted RNA were confirmed using a spectrophotometer (Eppendorf, Germany) and agarose gel electrophoresis, respectively. For RT-PCR, the first strand cDNA was synthesised from 10 ng of total RNA with the First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA). For transcriptome sequencing, mRNA was purified with Sera-Mag Magnetic Oligo (dT) Beads (Thermo Fisher Scientific) and then broken into short fragments by using a fragmentation solution (Ambion, USA). These short fragments were applied for double-stranded cDNA preparation using the SuperScript Double-Stranded cDNA Synthesis Kit (Invitrogen, USA). Adaptors were connected with these short cDNA fragments. Fragments with lengths of 200–700bp were selected for PCR amplification and further sequencing. Finally, the constructed libraries were sequenced using Illumina HiSeq™ 2000 in the Beijing Genomics Institute (BGI, Shenzhen, China). The paired-end reads length was 150bp.

2.4. Sequence data processing and assembly

Raw read data were firstly generated using Illumina HiSeq™ 2000. Low-quality reads were removed, and clean reads were obtained. The clean reads were assembled using Trinity (http://trinityrnaseq.sourceforge.net/) (Grabherr et al., 2011), and those with over lapping sequences were integrated to form contiguous sequences (contigs). Based on the paired-end reads, the contigs from the same transcript and the distance amongst them were detected. Finally, the contigs were assembled to generate unigenes. The reads data has been submitted in the National Centre for Biotechnology Information (NCBI) database and the accession number is SRA9669713–SRA9669715.

2.5. Sequence functional annotation

The function and classification of the assembled sequences were predicted by comparing them against the NCBI non redundant protein database (NR), Swiss-Prot protein, Clusters of Orthologous Groups (COGs) and Kyoto Encyclopedia of Genes and Genomes (KEGG) using BLASTX with a cut-off E-value of 10^{-5}. If the unigenes are not annotated in all the protein databases, then their orientations and coding regions are confirmed using ESTScan (Iseli et al., 1999).The NR blast results were analysed using Blast2GO to determine the gene functions based on the Gene Ontology (GO) terms (Conesa et al., 2005). The GO data were then processed using the WEGO software to determine the gene distribution in each category (Ye et al., 2006).

2.6. Gene expression analysis

Different expression genes (DEGs) were analysed between the mature internodes ShMN1 from plants at elongation stage and the ShMN2 from plants at mature stage to clarify the gene regulatory network during sucrose accumulation. DEGs were also analysed between the mature internodes ShMN2 and the immature internodes ShYN1 at mature stage. The expression levels of all unigenes were calculated using the reads per kb per million reads (RPKM) method (Mortazavi et al., 2008). The ratios of $\mathrm{RPKM_{ShMN2}/RPKM_{ShMN1}}$ and $\mathrm{RPKM_{ShMN2}/RPKM_{ShYN1}}$ were calculated. The threshold of FDR (false discovery rate) < 0.001 and $|\log_2 \mathrm{Ratio}| \geq 1$ were used to determine the significance of DEGs (Benjamini et al., 2001). The upregulated and downregulated genes were clarified with GO terms, and their enriched pathways were analysed using the KEGG database.

2.7. qRT-PCR

First strand cDNA synthesised from total RNA was diluted 50 times before being used as the amplification template. The gene-specific primers used for RT-PCR were designed with Primer3 software (Untergasser et al., 2012) and are listed in Supplemental Table 1. The qRT-PCR mixture (10 μL) contained 5 μL of SYBR®Premix Ex Taq (Takara), 0.2 μL of forward primer (10 μM), 0.2 μL of reverse primer (10 μM), 0.2 μL of ROX Reference Dye, 3 μL of cDNA and 1.4 μL of sterile distilled water. The reactions were performed on the Mx3000P (Stratagene) with three technical and three biological replicates. The amplification program was as follows: initial denaturation at 95 °C for 30s, followed by 40 cycles of 95 °C for 15s and 60 °C for 60s. GADPH was used as an internal control.

3. Results

3.1. Sucrose content in Badila internodes

The results showed that the sucrose content in mature internodes ShMN1 of sugarcane plants at the elongation stage was 86 mg/g FW. In young internodes ShYN1 and mature internodes ShMN2 of mature sugarcane plants, sucrose content were 41 mg/g FW and 151 mg/g FW, respectively. To clarify the gene regulatory network during sucrose accumulation in sugarcane culms, these internodes with different sucrose content were used for RNA-seq. DEGs were analysed between low- and high-sucrose-content internodes including ShYN1 versus ShMN2 and ShMN1 versus ShMN2 to discover the potential key genes regulating sucrose accumulation in sugarcane culms.

3.2. De novo assembly and sequence quality

Three culm cDNA libraries ShMN1, ShMN2 and ShYN1 were sequenced using the Illumina Hiseq2000 system. The obtained sequences were assembled into unigenes separately for each sample. It generated more than 60 million raw paired-end reads for each sample. After low-quality reads were removed, more than 53 million clean reads (equivalent to 8 Gb of data) were obtained with Q20 of > 97% for each sample. Transcriptome sequences of each sample were assembled from approximately 8.0 GB of data. More than 88,000 unigenes for each sample were assembled with an N50 of 1250–1,476bp and mean length of 668–796bp. Finally, total 112,337 all-unigenes were generated from combined unigenes of three samples. A total of 48,909 unigenes were longer than 1 kb, and 19,271 unigenes were longer than 2 kb. The length distribution of unigenes, which mainly ranges from 200 bp to 1,800bp, from each library is shown in Supplemental Fig. 1. The CDS of all-unigenes was analysed using ESTScan. A total of 73,636 unigenes presented CDS.

3.3. Function annotation of unigenes

A total of 112,337 all-unigenes were compared against NR, Swiss-Prot, KEGG, COG and GO protein databases with a cut-off E-value of 10^{-5} to identify the putative functions of the assembled unigenes. The results showed that 85,983 unigenes were annotated in at least one of these databases. The orientations and CDS of 70,241 unigenes were confirmed using BLASTX, and the CDS of 3395 unigenes was further confirmed by ESTScan. The predicted minimum CDS length of unigenes was 200bp and the CDS length distribution is listed in Supplemental Fig. 1.

A total of 72,564 transcripts of 112,337 all-unigenes corresponded with known proteins in the NR database. Statistical data indicated that 58.7% of 72,564 transcripts showed high homology with E-value < $1.0\mathrm{e}^{-45}$, and the rest showed homology with E-value < $1.0\mathrm{e}^{-5}$. Based on the similarity statistical results, 64.2% of the transcripts had high similarity (> 80%), and 17.2% of which showed 60%–80% similarity with the available sequences. When the sequence homology of plant species was classified, the majority of the transcripts corresponded with *Sorghum bicolor* and *Zea mays* at 58.9% and 24.6%, respectively. Additional sequences were matched other plants such as Poaceae, Japanese rice (6.1%), *Brachypodium distachyon* (1.7%) and Indian rice (1.6%).

GO classifications of the unigenes were analysed using the Blast2GO software. A total of 51,646 unigenes (46%) were assigned to 56 categories in three parts: biological process, cellular component and molecular function. For the biological process, most transcripts were enriched in the cellular (55.3%), metabolic (55.5%) and single-organism (31.4%) processes. In the cellular component, most transcripts enriched in the top three groups were cell (71.4%), cell part (71.4%) and organelle part (61.7%). With respect to molecular function, most unigenes were enriched in binding (54%), catalytic (48.1%) and transporter (6%) activities.

According to the COG function annotation of unigenes, 29,009 unigenes were matched into 25 clusters. Many sequences were classified in the 'General function prediction only' (37.9%), 'Translation, ribosomal structure and biogenesis' (27.3%), 'Transcription' (26.5%) and 'Replication, recombination and repair' (25.2%) clusters.

The metabolic pathways of these assembled transcripts were searched in the KEGG database. A total of 45,163 unigenes (40.2%) were annotated in 128 KEGG pathways. The top five largest pathways were

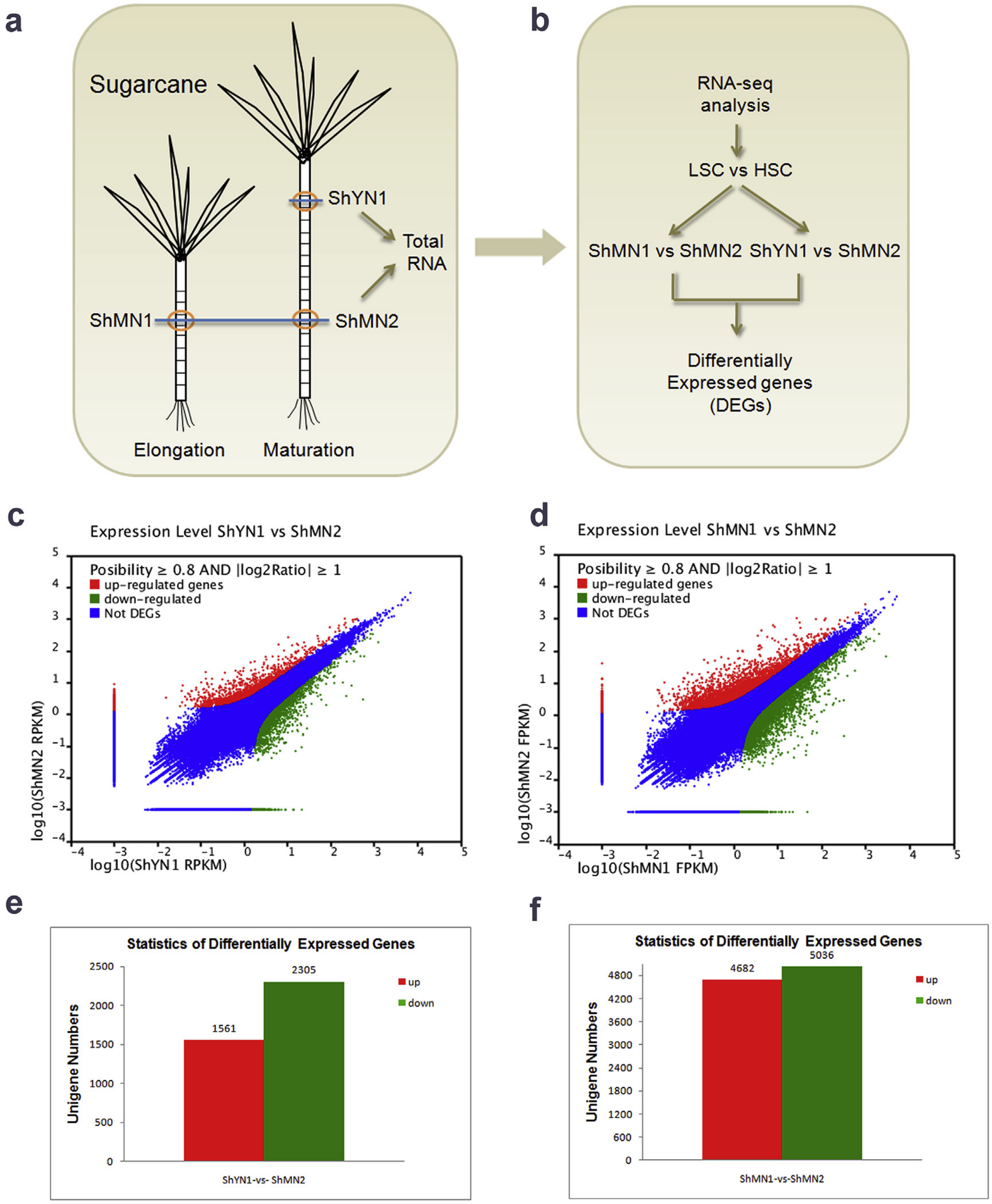

Fig. 1. Overview of transcriptiom analysis from sugarcane internodes. (a) Internodes samples harvesting from sugarcane plants at elongation and mature growth stage. (b) RNA-seq analysis. HSC, high sugar content internode; LSC, low sugar content internode. (c) (d) Expression level distribution of genes for ShYN1-vs-ShMN2 and ShMN1-vs-ShMN2, respectively. (e) (f) The numbers of different expression genes for ShYN1-vs-ShMN2 and ShMN1-vs-ShMN2, respectively.

'metabolic pathways' (10.6%), 'RNA transport' (6.6%), 'mRNA surveillance pathway' (5.7%), 'biosynthesis of secondary metabolites' (3.9%) and 'glycerophospholipid metabolism' (3.3%).

3.4. Analysis and annotation of DEGs

The DEGs of ShMN1 versus ShMN2 and ShYN1 versus ShMN2 were analysed, annotated and selected based on two criteria: FDR < 0.001 and $|\log_2\text{Ratio}| \geq 1$. A total of 9718 DEGs were identified in ShMN1 versus ShMN2. The expression levels of 4682 DEGs were upregulated, whereas those of 5036 DEGs were downregulated in ShMN2 (Fig. 1). A total of 3866 DEGs were identified in ShYN1 versus ShMN2. The expression levels of 1561 DEGs were upregulated, whereas those of 2305 DEGs were downregulated in ShMN2 (Fig. 1). There were 1499 DEGs regulated in the two comparative groups.

Based on the GO assignments of DEGs, the top five biological process terms are metabolic, cellular and single-organism processes, response to stimulus and biological regulation in both comparative groups. The top five cellular component terms are cell, cell part, organelle, membrane and membrane part in both comparative groups. The top five molecular function terms are binding, catalytic, transporter, electron carrier and nucleic acid binding transcription factor (TF) activities in both comparative groups.

DEGs were compared in the KEGG database to identify their specific pathways. A total of 3963 DEGs in ShMN1 versus ShMN2 were assigned to 122 KEGG pathways, which are dominantly enriched in included mRNA surveillance pathway (983, ko03015), RNA transport (1059, ko03013), biosynthesis of secondary metabolites (487, ko01110), metabolic pathways (1092, ko01100) and plant–pathogen interaction (308, ko04626). A total of 1663 DEGs in ShYN1 versus ShMN2 were assigned to 108 significantly enriched KEGG pathways, including the mRNA surveillance pathway (378, ko03015), RNA transport (396, ko03013), biosynthesis of secondary metabolites (258, ko01110), metabolism pathway (508, ko01100) and plant–pathogen interaction (186, ko04626).

3.4.1. DEGs involved in sugar metabolism

Sucrose accumulates in the culms of mature sugarcane plants and is the main product of photosynthesis in sugarcane source leaves (Moore, 1995). Synthesised sucrose is transported to sink tissues for plant growth and development and mainly supports sugarcane plant growth at the early stages (Moore, 1995). Additional sucrose accumulates in the culm parenchyma cells at later stages (Moore, 1995). In sugarcane culms, the sucrose content dynamically changes, partitioning between sucrose and hexose pool (Whittaker and Botha, 1997). Sucrose can be converted into other metabolites through the hexose pathway (Glasziou and Gayler, 1972; Whittaker and Botha, 1997). Many enzymes catalyse these multistep metabolism processes (Moore, 1995). The numbers of DEGs involved in carbohydrate metabolism were analysed (Fig. 2). The DEGs involved in sucrose metabolism were involved in starch and sucrose metabolism pathways (ko00500). We found 157 and 51 DEGs in the starch and sucrose metabolism pathways in ShMN1 versus ShMN2 and ShYN1 versus ShMN2, respectively (Fig. 2). Thirty-one DEGs in the starch and sucrose metabolism pathways were regulated in both comparative groups including enzymes involving in sucrose, trehalose, cellulose and pectin metabolisms. The regulated transcripts encoding key enzymes involved in sucrose and hexose metabolism were showed in Fig. 3.

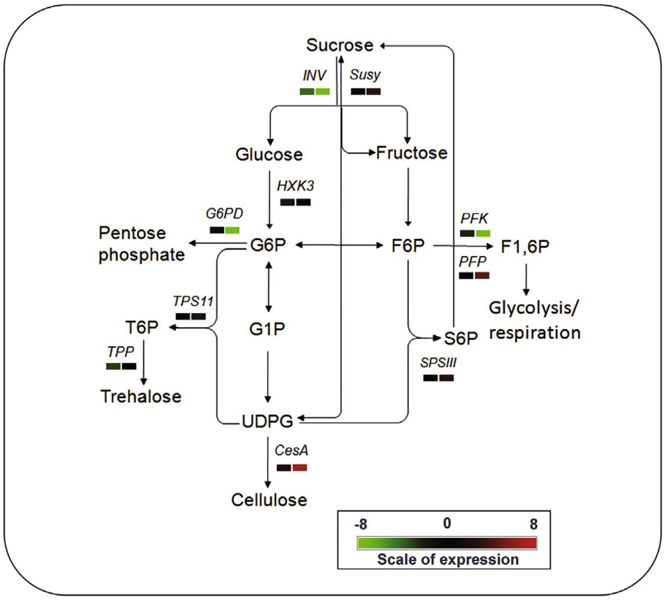

Fig. 3. The expression levels of regulatory genes involved in sucrose and hexose metabolism. The regulatory levels were showed in colour shades and indicated by $\log_2$ fold change. Red colour shades represent upregulation and green colour shades represent downregulation. The two boxes beneath represent the regulatory levels for ShYN1-vs-ShMN2 and ShMN1-vs-ShMN2, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.4.1.1. Sucrose metabolism.

The DEGs which encode sucrose metabolism enzymes were analysed between the sucrose-

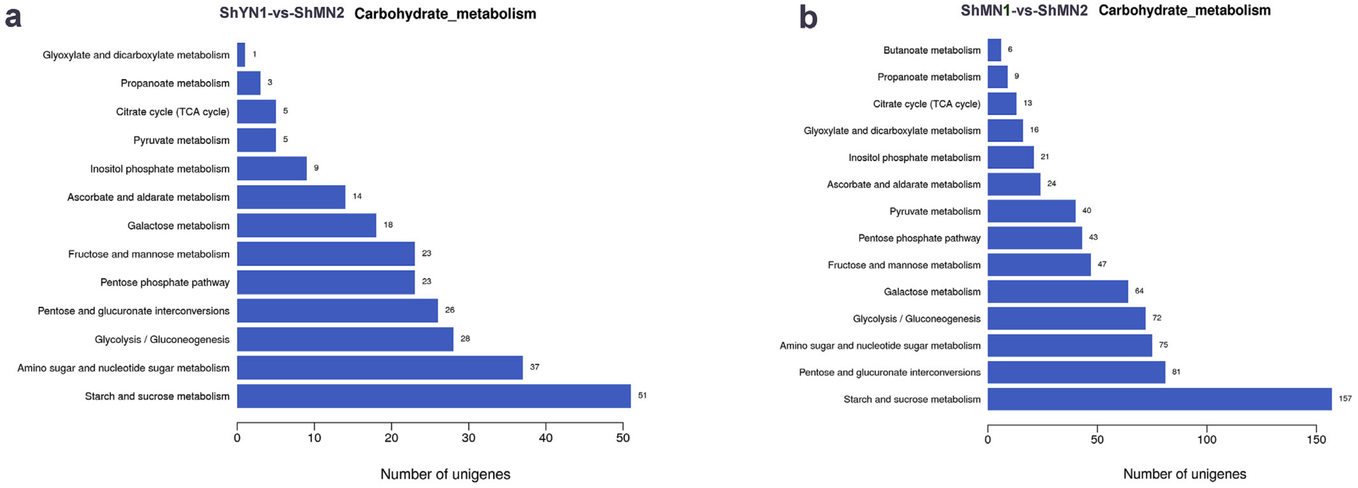

Fig. 2. Different expression genes statistics involved in carbohydrate metabolism pathways for ShYN1-vs-ShMN2 and ShMN1-vs-ShMN2, respectively.

accumulating internodes at elongation and mature stages and between the immature and sucrose-accumulating internodes at mature stages. The transcript levels of SPS III, Susy 1 and Susy 2 were upregulated, whereas those of Susy 6, three cell wall INVs (CWIN), two vacuole soluble acid INVs (VIN) and two neutral/alkaline INVs (CIN) were downregulated in the sucrose-accumulating internodes at the mature stage compared with those at the elongation stage (Table 1, Fig. 3). The transcript levels of one CWIN and two CINs were downregulated compared with those of the immature internodes with low sucrose content (Table 1).

3.4.1.2. Sucrose conversion. Sucrose is the main photosynthesis product in sugarcane and is unloaded from the phloem in sink tissues to provide carbon and energy resource (Moore, 1995). This compound is converted into other carbohydrates or precursors to synthesise many metabolites through glycolysis and pentose phosphate pathways (Whittaker and Botha, 1997). Thus, glycolysis and pentose phosphate affect sucrose accumulation in sugarcane culms. According to the analysis of DEGs encoding the enzymes involved in glycolysis, the transcripts encoding the key glycolysis regulatory enzymes of four 6-phosphofructokinases (PFK, EC: 2.7.1.11) and hexokinase 3 (HXK 3, EC: 2.7.1.1) were downregulated in ShMN1 versus ShMN2, and one of the four PFKs was also downregulated in ShYN1 versus ShMN2 (Table 1, Fig. 3). Four and one pyrophosphate-dependent pyrophosphate-fructose-6-phosphate-phosphotransferases (PFP, EC: 2.7.1.90) were upregulated in ShMN1 versus ShMN2 and ShYN1 versus ShMN2, respectively (Table 1, Fig. 3). Three transcripts encoding PFPα were upregulated in ShMN1 versus ShMN2. One transcript encoding PFPβ was slightly upregulated in the two comparative groups. Moreover, the DEGs encoding glucose-6-phosphate 1-dehydrogenase (G6PD, EC: 1.1.1.49), the key regulatory enzyme of the pentose phosphate pathway, were also downregulated in the two comparative groups (Table 1, Fig. 3).

3.4.1.3. Cell wall metabolism. The cell wall is composed of cellulose, hemicelluloses and pectin which are major carbon sinks in addition to sucrose (Whittaker and Botha, 1997). Cellulose is synthesised by the cellulose synthase complex (CesA, EC: 2.4.1.12) (Li et al., 2016). CesA4, CesA7, CesA9, CesA10, CesA11 and CesA12 were upregulated in ShMN1 versus ShMN2 (Table 2, Fig. 3). Only CesA10 was upregulated in ShYN1 versus ShMN2 (Table 2, Fig. 3). Four 1,4-β-D-xylan synthases (EC: 2.4.2.24) were upregulated in the two comparative groups (Table 2). Many pectinesterases (EC: 3.1.1.11) were also differently regulated in ShMN1 versus ShMN2 (13-up, 42-down) and ShYN1 versus ShMN2 (3-up, 17-down) (Table 2).

3.4.2. DEGs involved in signalling transduction

3.4.2.1. Sucrose signalling. Trehalose 6-phosphate (Tre6P) senses sucrose signals, and the feedback regulates sucrose levels in plants (Figueroa and Lunn, 2016). Tre6P is synthesised from UDP-glucose and glucose 6-phosphate by Tre6P synthase (TPS, EC: 2.4.1.15) and is dephosphorylated by trehalose-phosphate phosphatase (TPP, EC: 2.4.1.216) to produce trehalose (Figueroa and Lunn, 2016). TPS11 was downregulated in ShMN1 versus ShMN2, whereas TPP1 and TPP4 were downregulated in ShYN1 versus ShMN2 (Table 2, Fig. 3).

3.4.2.2. Hormone signalling. Hormone signalling is linked with sugar signalling (Ljung et al., 2015). Through KEGG pathway analysis, the DEGs involved in plant hormone synthesis and signal transduction were obtained, and those involved in auxin, gibberellin (GA), abscisic acid (ABA), ethylene and brassinosteroid (BR) synthesis and signalling pathways were analysed (Table 3). Four and two transcripts encoding aldehyde dehydrogenase (EC:1.2.1.3) involved in auxin synthesis were upregulated in ShMN1 versus ShMN2 and ShYN1 versus ShMN2, respectively (Table 3). The auxin response genes, including *Auxin/indole-3-acetic acid (AUXIAA)*, *auxin response factor (ARF)*, *GH3* and small auxin-up RNA *(SAUR)*, were differently regulated in the two comparative groups (Table 3). Three GA 20-oxidases (EC: 1.14.11.12), a key GA synthesis enzyme, were downregulated in ShMN1 versus ShMN2 (Table 3). Moreover, three and two GA 2-oxidases (EC: 1.14.11.13), another key GA synthesis enzyme, were significantly downregulated in ShMN1 versus ShMN2 and ShYN1 versus ShMN2, respectively (Table 3). For GA signal transduction, the receptor GA-insensitive dwarf 1 (GID1) and DELLA proteins were also significantly downregulated in high-sucrose-content internode ShMN2 (Table 3). The key ABA synthesis enzymes 9-cis-epoxycarotenoid dioxygenases (NCED, EC: 1.13.11.51) were also significantly downregulated in ShMN2 (Table 3). For ABA signal transduction, the transcripts encoding pyrabactin resistance/pyrabactin resistance like (PYR/PYL, 1-up), protein phosphatases type 2C (PP2C, 3-up, 19-down), sucrose non-fermenting 1-related protein kinase 2 (SnRK2, 1-up, 4-down) and ABA responsive element binding factor (ABF, 5-down) were differently regulated in ShMN1 versus ShMN2 (Table 3). Two PP2Cs and three SnRK2s were downregulated in ShYN1 versus ShMN2. The 1-aminocyclopropane-1-carboxylate synthase (ACS, EC: 4.4.1.14) and aminocyclopropanecarboxylate oxidase (ACO, EC:1.14.17.4) are the key enzymes for ethylene synthesis. Three transcripts encoding ACS and seven transcripts encoding ACO were significantly downregulated in ShMN1 versus ShMN2 (Table 3). Five transcripts encoding ACO were significantly downregulated in ShYN1 versus ShMN2 (Table 3). The different transcriptional expression of ACS was not found in ShYN1 versus ShMN2. The transcript of ACO (Unigene27747_All) was not expressed in the high-sucrose-content internode ShMN2. Ethylene transduction pathway analysis showed that the genes involved in ethylene signalling, including *ethylene receptor* (*ETR*), *constitutive triple response 1 (CTR1)*, *mitogen-activated protein kinase 6 (MPK6)*, *EIN3-binding F-Box 1/2 (EBF1/2)* and *ethylene responsive factor 1/2 (ERF1/2)*, were differently regulated in ShMN1 versus ShMN2, and a few were regulated in ShYN1 versus ShMN2 (Table 3). Additionally, the multiple transcripts of BR insensitive BRI1 and BRI-association receptor kinase BAK1 were differently expressed in the two comparative groups (Table 3).

3.4.2.3. Protein kinases. Protein kinases are important signalling molecules in plants. DEG analysis showed that 346 and 196 protein kinases were differently expressed in ShMN1 versus ShMN2 and ShYN1 versus ShMN2, respectively. Seventy-four protein kinases were regulated in the two comparative groups. These protein kinases consisted of wall-associated kinases (WAKs), receptor-like kinases (RLKs), calcium-dependent protein kinase (CPK), CBL-interacting protein kinase (CIPK), mitogen-activated protein kinase (MPK) and serine/threonine-protein kinase (STK) (Table 4). Remarkably, the transcripts of a cysteine-rich, receptor-like protein kinase (Unigene58520_All), three WAKs (CL12584.Contig2_All, Unigene58507_All and CL8561.Contig1_All) and two L-type lectin-domain containing receptor kinase (LecRLKs; Unigene17952_All and

Table 1
Unigene Statistics from transcriptoms of Badila internodes ShYN1, ShMN1 and ShMN2.

Number of Unigene	112,337
Unigene length > 1 kb	48,909
Unigene length > 2 kb	19,271
Mean length (nt)	1084
Unigene with CDS	73,636
N50 value (nt)	1749
NR annotations	72,564
NT annotations	80,359
Swiss-Prot annotations	46,141
KEGG annotations	45,163
COG annotations	29,009
GO annotations	51,646

Table 2
Different expression genes associated with sucrose, hexose, trehalose and cell wall carbohydrates metabolisms in high-sucrose-content internode ShMN2 compared with low-sucrose-content ShYN1 and ShMN1, respectively. The fold change is $\log_2$ fold change ≤ -1.00 or ≥ 1.00. –, no fold change.

Gene name	Unigene numbers	Regulation	Fold change	
			ShYN1 vs. ShMN2	ShMN1 vs. ShMN2
SPSIII	1	up	–	2.9
Susy1	1	up	1.3	3.1
Susy2	1	up	–	2.6
Susy6	1	down	–	2.0
CWIN	4	down	2.3 (1)	7.5 (3)
CWIN	1	up	2.6	–
VIN	2	down	–	3.5
CIN	2	down	4.5	3.5
PFK	4	down	7.6 (4)	2.8 (1)
PFPa	3	up	4.3	1.7
PFPb	1	up	1.2	1.5
HXK3	1	down	2.2	–
G6PD	2	down	7.4 (1)	1.3 (1)
CesA	6	up	2.0 (1)	6.5 (6)
Pectinesterase	15	up	3.2 (3)	4.7 (13)
	50	down	4.4 (18)	7.7 (41)
Xylan synthase	4	up	5.7	5.9
TPS11	1	down	–	1.5
TPP	3	down	3.0	–

Table 3
Different expression genes associated with hormone synthesis and signalling transduction in high-sucrose-content internode ShMN2 compared with low-sucrose-content ShYN1 and ShMN1, respectively. The fold change is $\log_2$ fold change ≤ -1.00 or ≥ 1.00. –, no fold change.

Pathway	Gene name	Unigene numbers	Regulation	Fold change	
				ShYN1 vs. ShMN2	ShMN1 vs. ShMN2
Auxin synthesis	Aldehyde dehydrogenase	3	up	2.9	5.0
Auxin signalling	AUXIAA	4	up	–	3.2
		2	down	1.6 (1)	1.2 (1)
	ARF	4	up	1.6 (1)	2.0 (3)
		2	down	2.1	1.9
	GH3	1	up	2.0	3.6
		2	down	2.3	2.6
	SAUR	3	up	3.3 (1)	2.0 (2)
		5	down	2.7	4.2
GAs synthesis	gibberellin 20-oxidase	2	down	–	7.6
	gibberellin 2-oxidase	3	down	7.2 (2)	8.6 (3)
GAs signalling	GID1	15	down	3.5 (4)	7.6 (15)
	DELLA	4	down	2.8 (1)	3.1 (3)
ABA synthesis	NCED	11	down	6.4 (10)	9.4 (7)
ABA signalling	PYR/PYL	1	up	–	2.4
	PP2C	3	up	–	3.4
		24	down	2.7 (2)	8.6 (22)
	SnRK2	3	down	2.0 (2)	3.2 (2)
	ABF	5	down	–	4.3
Ethylene synthesis	ACS	3	down	–	7.7
	ACC	6	down	10.4 (6)	7.1 (4)
Ethylene signalling	ETR	2	up	–	2.3
	CTR1	2	up	–	1.9
		5	down	–	2.8
	MPK6	1	up	–	1.6
	EBF1/2	2	up	–	2.7
	ERF1/2	2	up	2.5 (1)	8.9 (2)
		1	down	1.5	2.5

Table 4
Different expression genes of protein kinases, transcription factors and sugar transporters in high-sucrose-content internode ShMN2 compared with low-sucrose-content ShYN1 and ShMN1, respectively. The fold change is $\log_2$ fold change ≤ -1.00 or ≥ 1.00. –, no fold change. MPK, Mitogen-activated protein kinase; MPKKK, Mitogen-activated protein kinase kinase kinase; RLSTK, Receptor-like serine/threonine-protein kinase; RLP, Receptor-like protein kinase; CIPK, CBL-interacting protein kinase; LECRK, L-type lectin-domain containing receptor kinase; STK, Serine/threonine-protein kinase; WAK, Wall-associated receptor kinase; LRR-RLSTK, LRR receptor-like serine/threonine-protein kinase; G-type lectin S-receptor-like STK, G-type lectin S-receptor-like serine/threonine-protein kinase; CRK, Cysteine-rich receptor-like protein kinase; CPK, Calcium-dependent protein kinase.

Gene name	Unigene numbers	Regulation	Fold change	
			ShYN1 vs. ShMN2	ShMN1 vs. ShMN2
Protein kinases				
MPK	3	down	2.4	3.1
MPKKK	2	down	5.1	2.6
RLSTK	1	down	3.5	2.9
RLP	3	up	5.1	2.2
	5	down	3.7	3.0
CIPK	4	down	3.7 (4)	2.0 (2)
	3	up	3.3 (1)	7.4 (3)
LECRK	4	up	8.2	8.2
STK	2	up	3.8	1.5
	4	down	3.7	3.1
WAK	1	down	2.0	2.2
	5	up	7.7	7.7
LRR-RLSTK	7	down	3.8	3.8
	6	up	8.8	3.6
G-type lectin S-receptor-like STK	1	up	1.2	1.5
	1	down	3.4	3.0
CRK	1	up	7.6	7.6
CPK	2	down	2.3	2.5
Transcription factor				
DREB	6	down	10.8	7.7
ERF	1	up	8.9	2.5
	13	down	8.6	9.0
WRKY	6	down	2.8	4.4
HSP	3	down	4.1	2.1
NAC	2	down	6.9	2.0
GATA	1	up	1.7	1.9
MADS	1	up	3.4	5.8
HOX	1	down	5.0	1.5
Sugar transporters				
Sugar carrier	3	up	4.6 (3)	2.5 (2)
	2	down	2.9 (1)	4.6 (2)
ERD6-like Sugar transporter	1	up	–	2.2
STP	6	up	2.3 (1)	3.7 (5)
	4	down	2.1 (2)	2.5 (3)
HEX	2	down	–	7.6
SWEET	9	down	7.4 (8)	3.0 (3)

Unigene12543_All) were specifically expressed in high-sucrose-accumulating internode ShMN2.

3.4.2.4. Transcription factors (TFs). TFs respond to development and stress signals to control downstream gene transcription. The DEGs encoding for TFs during sucrose accumulation were analysed. A total of 216 and 80 DEGs of TFs were annotated in ShMN1 versus ShMN2 and ShYN1 versus ShMN2, respectively. Forty-six TFs, including *dehydration responsive element binding proteins* (DREB), ERFs, WRKYs, NAC8, MADS13 and HOX24, were regulated in the two compared groups (Table 4). All DREBs were down regulated in the high-sucrose-accumulating internode ShMN2. The ERFs were almost all downregulated in the mature internode ShMN2, except for ERF1b

(Unigene12663_All), which was upregulated in ShMN2. Additionally, the transcripts of NAC8 and MADS13 were upregulated.

3.4.3. DEGs of sugar transporters

Sugar transporters mediate sugar transport and distribution. Table 4 shows that 18 and 15 sugar transporter transcripts were differently expressed in ShMN1 versus ShMN2 and ShYN1 versus ShMN2, respectively. Seven sugar transporters were regulated in the two compared groups. These sugar transporters include sugar transporter proteins (STPs), hexose transporter (HEX), sugar carrier protein (Sc) and SWEETs. The sugar transporter SWEETs were downregulated in high-sucrose-content internode ShMN2 (Table 4). Eight transcripts encoding *SWEETs* were downregulated in ShYN1 versus ShMN2 (Table 4). The DEG transcripts encoding *STPs* and *Scs* were differently regulated in the two comparative groups (Table 4). Two transcripts of hexose transporter *HEX6* were also downregulated in ShMN1 versus ShMN2.

3.5. Validation of expression patterns of genes involved in sucrose accumulation

The expression profiles of 15 genes, which were regulated in two comparative groups, were analysed using RT-PCR to confirm the accuracy of the gene expression patterns by RNA-seq. These genes were listed in Supplemental Table 1. The results showed that the expression patterns of the selected genes were consistent between qRT-PCR and RNA-seq (Supplemental Fig. 2).

4. Discussion

Further understanding the molecular mechanism underlying the high sucrose accumulation in sugarcane culms can facilitate efficient sugar production to meet the growing demand for human consumption. In sugarcane, sucrose, which is synthesised in source leaves and transported into sink organs, provides energy for plant growth and development and is stored in culm parenchyma cells (Glasziou and Gayler, 1972; Moore, 1995). Sucrose accumulates in sugarcane culms from low level in mature internodes of plants at elongation stage to high level in mature internodes of plants at mature stage (Glasziou and Gayler, 1972; Moore, 1995). Moreover, sucrose accumulates from low level in immature internodes to high level in mature internodes during sugarcane culms growth and development (Whittaker and Botha, 1997). The molecular mechanism on maintaining high sucrose level in sugarcane culms remains unknown. Multiple genes are predicted to contribute to maintain cellular sucrose and osmotic homeostasis in plants (Ruan, 2014; Tognetti et al., 2013). In this study, we utilised RNA-seq to discover transcripts in sugarcane culm tissues from *S. officinarum* variety, Badila. We compared the gene expression differences between mature internodes of sugarcane plants at elongation stage and mature internodes of sugarcane plants at mature stage and the gene expression differences between immature internodes and mature internodes of sugarcane plants at mature stage. It aimed to clarify the gene regulatory network underlying high sucrose accumulation in sugarcane culms. The regulated genes involved in sucrose metabolism, carbon partitioning, sugar and hormone signalling and sugar transportation during sucrose accumulation were analysed.

Sucrose storage in sugarcane culm is regulated by sucrose synthesis and hydrolysis (Whittaker and Botha, 1997). DEG analyses showed that transcripts encoding SPSIII were upregulated in high-sucrose-content internodes from mature plants compared with low-sucrose-content internodes from plants at elongation stage. The transcripts encoding INVs were downregulated in the high-sucrose-content internodes. However, the transcripts encoding INVs were differently regulated in the two comparative groups. CWINs, VINs and CINs were downregulated in high-sucrose-content internodes from mature plants compared with low-sucrose-content internodes from plants at elongation stage, whereas CWINs and CINs were regulated in high-sucrose-content mature internodes compared with low-sucrose-content immature internodes from mature plants. In the two comparative groups, the transcripts encoding two CINs were both downregulated. The transcripts encoding two VINs were downregulated in high-sucrose-content internodes from mature plants compared with low-sucrose-content internodes from plants at elongation stage. SPS activity was high in mature internodes in some sugarcane cultivars (Zhu et al., 1997; Verma et al., 2011) The upregulated *SPSIII* in high-sucrose-content internodes from mature plants compared with low-sucrose-content internodes from plants at elongation stage might be beneficial for sucrose accumulation in sugarcane mature internodes. Sucrose hydrolysis is catalysed by INVs and Susy (Ruan, 2014). CWIN catalyses apoplasmic sucrose hydrolysis, which involves sucrose apoplasmic unloading and hexose supply for development; VIN plays important roles in hexose accumulation, cell expansion and abiotic stress response; CIN maintains cytoplasmic sugar homeostasis, regulating root and reproductive development (Ruan, 2014). Fourteen INV genes have been cloned from sugarcane, including six CINs, seven CWINs and one VIN (Wang et al., 2017). CWINs and CINs in sugarcane were differently regulated under draught, cold stress and sugar treatment, whereas VIN was induced by fructose treatment (Wang et al., 2017). It indicates that different INVs play versatile roles in sugar metabolism and signalling in sugarcane. The high expression levels of VINs in mature internodes at the elongation stage might support internodes elongation and rapid growth, whereas the downregulation of CWINs and CINs in high-sucrose-content internodes at the mature stage are likely to reduce sucrose hydrolysis and facilitate sucrose storage. The downregulation of CIN activity in sugarcane leads to decreased respiration and increased sucrose to hexose ratio (Rossouw et al., 2010), demonstrating that CIN is involved in sucrose partition and storage in sugarcane. Susy has been proven to be associated with sink strength and is involved in cellulose, starch, protein and lipid syntheses (Ruan, 2014). The upregulation of Susy in poplar increased cellulose content, according to Coleman et al. (2009) (Coleman et al., 2009). Susy also provides UDP-glucose for cellulose synthesis (Amor et al., 1995). The upregulated Susy1 and Susy 2 expression in high-sucrose-content internodes from mature plants compared with low-sucrose-content internodes from plants at elongation stage might function in carbon partition into cellulose synthesis. The activity of Susy is similar in mature sugarcane at different internodes (Zhu et al., 1997). Only the transcript-encoding Susy 2 slightly increases in high-sucrose-content mature internodes compared with low-sucrose-content immature internodes from mature plants, showing that Susy might not directly control high sucrose storage in mature internodes. We therefore propose that INVs are the key regulatory genes for sucrose accumulation in sugarcane culms.

In addition to sucrose metabolism, sucrose storage in sugarcane culms is affected by carbon partitioning (Whittaker and Botha, 1997). The DEGs analysis showed that four transcripts encoding PFK were downregulated, and four transcripts encoding three PFPα and one PFPβ were upregulated in high-sucrose-content internodes from mature sugarcane plants. The respiration and synthesis of amino acids, organic acids and water-insoluble matter were decreased in high-sucrose-content mature internodes (Whittaker and Botha, 1997). Glycolysis is the control point of carbon flux to provide the precursor for energy metabolism and other carbohydrate synthesis. In the glycolysis pathway, three limited steps are catalysed by hexokinase, phosphofructokinase and pyruvate kinase (Fernie et al., 2004). In plants, two kinds of phosphofructokinase are present: ATP-dependent phosphofructokinase (PFK; EC 2.7.1.11) and diphosphate-dependent phosphofructokinase (PFP; EC 2.7.1.90) (Fernie et al., 2004). The downregulation of PFK genes showed their potential role in reducing carbon partitioning from sucrose to facilitate sucrose storage. The PFP activity was negatively correlated to sucrose content in sugarcane (Whittaker and Botha, 2010). The down-regulation of PFP activity in sugarcane culms increased the sucrose content in immature internodes, whereas the sucrose content in mature internodes was not increased (Enomoto et al., 1991), suggesting that PFP might function in gluconeogenic direction flux in mature internodes. The upregulation of PFP genes in mature internodes might be associated with sucrose storage from gluconeogenesis. Cell wall polysaccharides are the competitive carbon pool for sucrose storage in sugarcane. The significant upregulation of multi-CesA genes, including CesA4, 7, 9, 10, 11 and 12 in high-sucrose-content internodes from mature plants compared with low-sucrose-content internodes from plants at elongation stage, indicated that cellulose synthesis is active in mature sugarcane culms. However, CesA10 was upregulated by two fold in high-sucrose-content mature internodes compared with low-sucrose-content immature internodes from mature plants. Pectinesterases belong to large multigene families in plants that catalyse the demethoxylation of pectin and affect the biological status of plant cell walls (Moustacas et al., 1991). These enzymes are also involved in the regulation of many development processes, including fibre and pollen formation, vegetative reproduction, fruit ripening and plant pathogen interactions (Pelloux et al., 2007). Numerous pectinesterase-encoding genes were differently expressed, showing their possible involvement in sugarcane culm growth and fibre formation during maturation. The upregulated genes encoding xylan synthase was consistent with the higher xylan content in the mature internodes (Oikawa et al., 2010).

Sugars are not only taken as metabolites that provide carbon and energy resource but also function as signal molecules that regulate plant growth and development (Rolland et al., 2002). A homology transcript of *Arabidopsis* TPS11 was slightly decreased in high-sucrose-content internodes from mature plants compared with low-sucrose-content internodes from plants at elongation stage. The transcripts encoding the homologies of rice TPP1 and TPP4 were downregulated in high-sucrose-content mature internodes compared with low-sucrose-content immature internodes from mature plants. Tre6P is a signal molecule, whose feedback regulates sucrose levels to maintain an optimal concentration in plants (Figueroa and Lunn, 2016). Tre6P regulates sucrose synthesis in source leaves and sucrose hydrolysis in sink organs to maintain an optimal sucrose level (Figueroa and Lunn, 2016). TPS catalyses Tre6P synthesis from UDP-glucose and glucose 6-phosphate, whereas TPP dephosphorylates Tre6P to produce trehalose (Cabib and Leloir, 1958). The regulated TPS and TPP genes showed their potential role on Tre6P signalling during sucrose accumulation in sugarcane culms.

Genetic analysis in *Arabidopsis* mutants showed that plant hormone signals extensively interact with sugar signalling (Ljung et al., 2015). In the mature, high-sucrose-accumulating internodes of sugarcanes, the transcripts that encode key enzymes for auxin synthesis were upregulated, and the transcripts encoding key enzymes for ABA, GA and ethylene synthesis were downregulated. No evidence shows how ABA, GA and ethylene signalling responds to high sucrose accumulation in sugarcane culms. High sugar can enhance ABA synthesis and signalling in plants (Arenas-Huertero et al., 2000; Rook et al., 2001). Auxin biosynthesis is also regulated by soluble carbohydrates (Sairanen et al., 2012). GA, sprayed on leaves of sugarcane plants at elongation stage, increased the plant's height (Wu et al., 2009). Ethephon, sprayed on sugarcane leaves, also increased cane yield and sucrose content (Li et al., 2007). Auxin and GA_3 increased the stability of INV mRNA, and ABA increased the synthesis rate of INV in immature sugarcane internodes (Gayler and Glasziou, 1968), thus indicating that hormones are involved in sucrose metabolism in sugarcane. The downregulated genes encoding key synthetase of GA, ABA and ethylene in high-sucrose-accumulating internodes may adjust to sucrose concentration. DEGs involved in ABA, GA and BR signalling pathways indicated that these hormones may respond to changes in the sucrose level. In sugarcane, it has been proved that a DELLA protein ScGAI was a key negative regulator for culm growth and sugar accumulation (Garcia Tavares et al., 2018). Over expression of *ScGAI* in sugarcane reduced the sucrose content in mature internodes (Garcia Tavares et al., 2018). DELLAs are GA-signalling repressors that regulate plant development and stress responses (Sun, 2010). DELLA protein functions in sucrose-GA interaction and also connect sucrose to BR signalling via interaction with BRASSINAZOLE RESISTANT 1 (Loreti et al., 2008; Li et al., 2012). The downregulation of DELLA genes in high-sucrose-content internodes may facilitate sucrose accumulation.

The family of protein kinases in sugarcane is divided into five groups, whereas the RLKs are clustered into four subgroups, including RLCKs, L-lectin, WAK and BRI1 (Rocha et al., 2007). The tissue-specific, upregulated L-type LecRLKs and WAKs in high-sucrose-content internodes of sugarcane might be involved in culms development and potential sugar signal transduction. It has been proved that sucrose affects gene expression by regulating TFs, bZIP11 (Rahmani et al., 2009), MYB75/PAP1 (Teng et al., 2005) and WRKY (Ishiguro and Nakamura, 1994; Nagata et al., 2015; Sun et al., 2003). The expression levels of SUSIBA2 and AtWRKY20, which belong to the WRKY family, are induced by sucrose (Nagata et al., 2015; Sun et al., 2003). WRKYs that are homologous to WRKY18, WRKY40, WRKY41 and WRKY50 in *A. thaliana* are downregulated in high-sucrose-content internodes, showing their potential role in culm development and sucrose accumulation. ERFs and DREBs which belongs to APETALA2/ethylene response factor (AP2/ERF) superfamily influence development processes and stress response (Lata and Prasad, 2011; Srivastava and Kumar, 2018). An ERF TF, the homology of Arabidopsis *ERF1B*, which is highly induced by salinity and drought stress, was upregulated in high-sucrose-content internodes (Cheng et al., 2013). Two homologies of *Oryza sativa DREB1E* and *DREB1G* (CL8024.Contig1_All and CL15326.Contig1_All) were downregulated. The over expression of OsDREB1G improved the tolerance of water deficit stress in rice, whereas the over expression of OsDREB1E did not (Chen et al., 2008). In sugarcane, the over expression of AtDREB2A CA improved drought tolerance and increased the sucrose level of transgenic plants (Reis et al., 2014). It suggested that AP2/ERF TFs were the key potential regulators involved in sugarcane culm sucrose accumulation.

Several sugar transporter genes encoding STPs and SWEETs were regulated in high-sucrose-content internodes. Sugar transporters play pivotal roles in carbon partitioning, regulate plant growth and development and affect crop yield (Doidy et al., 2012). The expression levels of STPs were regulated by development stages and environment factors (Hofmann et al., 2009; Norholm et al., 2006; Truernit et al., 1996). In sink tissues, STPs are responsible for the uptake of glucose and fructose from sucrose hydrolysis by INV (Doidy et al., 2012). SWEETs are a recently identified family of STPs and function as sugar facilitators (Chen et al., 2010, 2012). SWEETs are involved in phloem loading (Chen et al., 2012), plant–pathogen interactions (Liu et al., 2011; Yuan et al., 2009), pollen growth (Engel et al., 2005; Guan et al., 2008) and vacuole fructose transport (Chandra et al., 2015). SWEETs are suggested to play crucial roles in carbohydrate partition and pathogenesis. These regulated STP and SWEET genes showed their potential role in sucrose accumulation in sugarcane internodes.

Finally, the key candidate genes involved in regulating sucrose accumulation in sugarcane culm were summarized in Fig. 4. It provides the potential targets for genetic manipulation to improve sugarcane sugar yield by conventional and molecular techniques.

Contributions

Shu-Zhen Zhang, Wen-quan Wang and Zu-xing Zhang conceived and designed the experiments. Jun-gang Wang and Ting-ting Zhao performed the experiments, result analysis, and manuscript drafting. Wen-zhi Wang, Cui-lian Feng, Xiao-yan Feng, Guo-Ru Xiong and Lin-bo Shen revised the manuscript. All authors have read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial

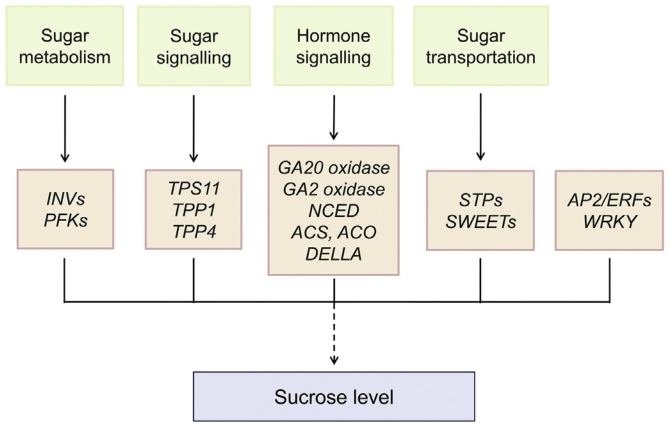

Fig. 4. The key candidate regulator genes during sucrose accumulation in Badila internodes. INV, invertase; PFK, 6-phosphofructokinase; TPS11, trehalose-phosphate synthase 11; TPP1,4, trehalose-phosphate phosphatase 1,4; GA 20 oxidase, gibberellins 20 oxidase; GA 2 oxidase, gibberellins 2 oxidase; NCED, 9-cis-epoxycarotenoid dioxygenases; ACS, 1-aminocyclopropane-1-carboxylate synthase; ACO, aminocyclopropanecarboxylate oxidase; STP, sugar transporter protein; AP2/ERF, APETALA2/ethylene response factor.

interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.10.016.

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