Global Transcriptome Changes of Elongating Internode of Sugarcane in Response to Mepiquat Chloride

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Abstract

**Background:** Mepiquat chloride (DPC) is an extensively used chemical to control internode growth and compact canopies in cultured plants. Previous studies suggested that DPC could inhibit gibberellin biosynthesis in sugarcane. Unfortunately, the molecular mechanism underlying the suppressive effects of DPC on plant growth is still largely unknown.

**Results:** In the present study, we first obtained the high-quality long transcripts from internode of sugarcane by PacBio Sequel System. A total of 72,671 isoforms with N50 as 3,073 were generated. These long isoforms were used for the following RNA-seq as reference. Subsequently, short reads generated from Illumina Hiseq 4000 platform were used for comparing the differentially expressed genes in DPC and control groups. The transcriptome profiling showed the 6 days post DPC treatment had the most significant changed genes. These genes were related to plant hormone signal transduction and biosynthesis of several metabolites, indicating DPC affected multiple pathways beside depressed gibberellin biosynthesis. The network of DPC on the key stage was illustrated by weighted gene co-expression network analysis (WGCNA). Among the constructed 36 modules, the top positive correlated module with stage of 6 day post spraying DPC was sienna3. Stf0 sulphotransferase, cyclin-like F-box and HOX12 were the hub genes in sienna3 that had high correlation with other genes in this module. The qPCR demonstrated the high accuracy of RNA-seq result.

**Conclusion:** Taken together, we demonstrated the key role of these genes in DPC-induced growth inhibition in sugarcane.

1. Introduction

Hormone regulation in plant culturing has been widely used to control quality agricultural and horticultural products [1]. Several hormones have effect on regulation and co-ordination of plant growth [2]. Up to date, Auxin [3], gibberellin (GA) [4], Cytokinins (CTK) [5], abscisic acid (ABA) [6], ethyne (ETH) [7] and brassinosteroid (BR) [8] are the most popular hormones to stimulate organism growth in crops. However, growth appearance is not the only performance that matches the increasing demand made by farmers. For example, with excessive vegetative growth, crops such as cotton and sugarcane could hardly be controlled and lead to irregular height in farmland which result in low productivities [9, 10]. Thus, other regulated chemicals have been introduced to inhibit the hormonal pathways.

Mepiquat chloride (DPC) is one famous chemical that control organism growth via suppressing the GA pathways [11, 12]. As an exogenous plant growth regulator, DPC is a water-soluble material which facilities the spraying in farmland [13]. With low dose DPC, the internode elongation and plant height were reduced [13, 14]. Recent studies showed DPC could also regulated the synthesis of endogenous hormones, carbohydrates, enzymes and other organic molecules [15, 16]. The DPC treatment increased concentrations of chlorophyll, free proline and soluble proteins but depressed malondialdehyde levels contributing to improve resistance to stress [17–19]. In addition, DPC promoted the calcium and
phosphorus levels in leaves to strengthen ability to anti-disease [20, 21]. By regulating CTKs and GAs synthesis as well as controlling the ratios of CTKs:GA, DPC mediated rhizogenesis [22]. Therefore, the function and its regulatory role of DPC is far away from systematically understand.

Sugarcane is a major agricultural crop for sugar production over the world [23–25]. Globally, about 80% sugar is isolated from this crop and sugarcane thus, becomes the critical bioenergy crop [26]. Sucrose is primarily generated in sugarcane stem and higher shoot [27, 28]. The internode elongation of stem is associated with the deposition of sucrose [29]. In this situation, GA is employed to stimulate internode elongation [30]. However, the rapid growth of stem may lead to lower sucrose accumulation [31, 32]. The balance of stem growth is the key question for sugar production. DPC is introduced to control the negative effects of GA treatment [33]. Although, DPC is a widely recognized as regulator for GA and promotes the ability of resistance to stress [34, 35], the underlying molecular mechanism is still unknown. The widely scanning of systematic regulation of DPC on plant is needed.

Previous study showed that during internode elongation, the regulation by microRNA-mRNA network in zeatin biosynthesis, nitrogen metabolism and plant hormone signal transduction pathways participated in stem growth in sugarcane [36, 37]. These effects may be mediated by GA20-oxidase (GA20-OX1) and gibberellin receptor (GiD1). The DPC has shown the inhibited effects on GA generation by suppressing the activities of copalyl diphosphate synthase and ent-kaurene synthase [13]. These results revealed the molecular mechanism in controlling growth performance by DPC. Still, large information about DPC roles in growth and e resistance to stress regulation is unknown. Herein, we used Weighted Gene Co-expression Network Analysis (WGCNA), a mathematical method to identify key gene network and hub genes [38–40]. The present study was conducted which focused on the transcriptome changes by DPC treatment using Illumina Hiseq 4000 platform. These evidences presented here provide new insights on DPC function in controlling stem growth as well as regulating resistance to stress which are the two most economically important traits, in sugarcane.

2. Materials And Methods

2.1 Sugarcane preparation

All the tested sugarcanes were bred in the Sugarcane Research Institute (SRI), Guangxi Academy of Agricultural Sciences, Nanning, China. The sugarcane variety was GT42 sourced from SRI Experimental Farm at Nanning, China. The 10 months mature cane stalks were selected to obtain buds in the middle internodes and the buds were cut into setts from single-bud. The setts were incubated in 52 °C for 30 min to eliminate pathogenic bacteria. The setts were planted into a moist sandbox and maintained artificial climate box (Essenscien, USA). The culturing conditions were temperature 28.0 ± 0.1 °C, humidity: 75 ± 1.5% RH, photoperiod: 12 h light and 12 h dark with 100% full light (light intensity 25000 LX). The 2-leaf stage seedlings were transferred to plastic pots (35 cm width × 35 cm length × 50 cm height). In each pot, two seedlings were planted. After 5 days, the seedlings were divided into two replicates randomly. The seedlings were cultivated to the to pre-elongation stage which contained 9–10 leaves defined as the early
elongation stage. In this stage, the DPC group was sprayed with 200 mg/L DPC (Solarbio life science, Beijing, China) until the solution drops down from leaves. Water was sprayed for the control group in similar pattern. All the sugarcane pots were settled on a greenhouse in 18 rows with 1.2 m width. The first three columns belonged to control and the last three columns belonged to DPC group. At 0, 3, and 6 days post spraying, the third internodes were obtained for further assays. The control samples from 0, 3, and 6 days post spraying were named C1, C2 and C3, respectively. The samples of DPC group from 0, 3, and 6 days post spraying were named D1, D2 and D3, respectively. All the samples were store at − 80 °C until RNA isolation. For each group at different time point, 3 biological replicates were collected for analyses.

2.2 Determination of growth performance

The sugarcane growth performance was measured from control and DPC groups. At the 0, 3 and 6 days post spraying, the stalk height from the soil surface to the dewlap of youngest fully expanded leaf and the length of the internodes. For each group, five plants were randomly chosen for measuring. The whole height and the first seven internode length (from shoot top of 10 matured plants) were measured as well.

2.4 PacBio Iso-Seq

For obtaining the accurate reference for genes in sugarcane, the full-length transcriptome sequencing was performed in the present study. The RNA libraries of internode from one mature sugarcane at ten months old were prepared. The mRNAs were first enriched by Oligo (dT) magnetic beads and the full-length cDNAs were synthesized using Clontech SMARTer PCR cDNA Synthesis Kit (Pacific Biosciences, USA). Three libraries with different length (1–2 kb, 2–3 kb and 3–6 kb) were constructed. The sequencing was performed on PacBio Sequel System (Pacific Biosciences, USA). The raw sequences were analyzed using SMRT Link v5.0.1 software. Based on the primer at 5’ and 3’ as well as ploy-A, the full-length, non-full-length, chimeric and non-chimeric categories were identified. The non-full-length sequences were polished by Quiver algorithm. The following Illumina RNA-seq data were used to correct the low-quality sequences. The sequences were annotated by Nr, SwissProt, COG/KOG, GO and KEGG pathway. The un-annotated sequences were further performed CDS prediction.

2.5 Preparation of RNA-seq libraries

Total RNAs from 3 plants in each group were isolated using RNA Trizol (Invitrogen, Carlsbad, CA, USA) following the manufacturer’s instructions. A total of 6 RNA-seq libraries (3 from control group and 3 from DPC group) were prepared for the next-generation sequencing. The quantity and integrity of the total RNAs were assayed using an Agilent 2100 bioanalyzer (Agilent, Santa Clara, CA, USA). The mRNAs were enriched by oligo (dT) magnetic beads and fragmented by fragmentation buffer. The first-strand cDNA was synthesized using 6-base random primers. The second-strand cDNA was then synthesized by DNA polymerase I. The cDNAs were then isolated using a Qiaquick PCR extraction kit (Qiagen, Hilden, Germany) and ligated with Illumina sequencing adapters. The cDNAs were finally purified using agarose gel electrophoresis and amplified by PCR to generate the RNA-seq libraries. The RNA-seq were performed on Illumina HiSeq™ 4000 by Gene Denovo Biotechnology Co. (Guangzhou, China).
2.6 Transcriptome mapping and differentially expressed genes (DEGs) identification

The sequencing adaptor were trimmed first. Then, the low-quality reads with unknown nucleotides (N) ratio > 10% or Q-value ≤ 20 were removed. The retained reads were high-quality clean read which were used for the following analyses. The clean reads were mapped to reference transcriptome sequence using the full-length transcriptome by TOPHAT (version 2.0.9) [41]. The relative gene expression was calculated and normalized by Fragments Per Kilobase of transcript per Million mapped reads (FPKM). The principal component analysis (PCA) was performed with R package (http://www.r-project.org/) to evaluate the reproducibility of biological replicates. When the genes with a false discovery rate (FDR) < 0.05 and log2(fold change) > 1 or <-1 compared between the control group and DPC group, the genes were determined as DEGs.

2.7 Functional annotation of DEGs

To define the function of DEGs, enrichment analysis of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways was performed. The DAVID online tools (http://david.ncifcrf.gov/) were employed to perform the enrichment analysis. The GO items with adjust p ≤ 0.001 and KEGG pathways with p ≤ 0.001 were considered as significantly enriched.

2.8 WGCNA

The WGCNA was performed to identify key gene groups and hub genes based on the FPKM using the R package [42]. The data were first filtered according to the 25% variation based on variance (Standard Deviation/Mean) across samples. The FPKM matrix of the retained genes was using for creating weighted adjacency matrix. The soft threshold power (β) set at 10 was selected to perform scale-free topology. The parameters for construction of gene module were power = 8, min ModuleSize = 30, branch merge cut height = 0.25. The correlations between gene modules and treatment groups were evaluated using correlation coefficients. The D2 group was the most concerned treatment group due to the large numbers of DEGs. We choose the top correlated module (Sienna3) of D2 for further analysis. First, GO and KEGG pathway enrichment analyses of the module were performed using KOBAS v3.0 (http://kobas.cbi.pku.edu.cn/). The top three hub genes were identified using Cytohubba (http://apps.cytoscape.org/apps/cytohubba) and the network was plotted by Cytoscape v3.7.1.

2.9 Real-time Quantitative PCR Analysis of Genes (qPCR)

Total RNAs from internode tissues in control group and DPC group were isolated and tested as shown in 2.1. The first-strain cDNAs were synthesized using PrimeScript RT Reagent Kit with gDNA Eraser (Takara, Japan). Primers used for qPCR were designed by Primer premier 5.0 (Applied Biosystems, Waltham, MA, USA) according to the gene sequences from the PacBio Iso-Seq (Additional file 1). The qPCR was performed on an Analytikjena-qtower2.2 fluorescence quantitative PCR instrument (Germany). The PCR program was as following: 95 °C for 5 min for initial denaturation, followed by 45 amplification cycles at 95 °C for 10 s and 60 °C for 20 s. Five biological replicates was conducted for the amplification of each
sample. EF1A was used as control gene. To confirm the specificity of the PCR reaction, melt curve
analysis was performed. The relative expression of gene was calculated by the $2^{-\Delta\Delta CT}$ method.

### 2.10 Data Statistical Analysis

The growth rate, sugarcane height, internode length and relative expression were presented as means ±
standard deviations (SD). The significant differences among the groups were determined using one-way
analysis of variance (ANOVA) and post hoc Tukey’s test on SPSS statistical software package (V19.0,
SPSS, Chicago, IL, USA). P < 0.05 was confirmed as significant difference.

### 3. Results

#### 3.1 Growth performance

At the beginning of the experiment (0 days), no significant difference could be found compared the
control and DPC group (p > 0.05). However, the sugarcane heights on day 3, 6, and 12 as well as in mature
sugarcane were significantly higher in control compared to DPC groups (p < 0.05) (Fig. 1A). Similar to the
sugarcane height, the growth rates of DPC groups were significantly lower on day 3, 6, and 12 compared
to the control (p < 0.05) (Fig. 1B). All of the internodes were significantly longer in control (Figure. 1C).

#### 3.2 Full-length transcriptome of sugarcane

To generate a high accuracy reference for reads mapping data, full-length mRNA sequence was
performed using PacBio Sequel platform using internodes from mature sugarcane. A total of 17 billion
raw reads were obtained. The average length was 2,718 bp and N50 was 3,011 bp, respectively. After
circular-consensus sequence (CCS) extraction, 428,444 reads were identified. Among these reads,
348,840 accounting for 81.42% reads were full-length reads contained 5′ adaptor, poly(A) tail signals, and
3′ adaptor. Meanwhile, 999 million full-length non-chimeric (FLNC) reads with average length of 2,906 bp
were identified. These FLNC reads of cDNA library contain repetitive isoforms which provided data for
analysis of isoforms by alignment and assigning to different cluster. The present full-length
transcriptome generated 72,671 isoforms. The average length was 2888.94 bp and the N50 was 3,073 of
these isoforms (Additional file 2).

The isoforms were annotated by aligning the protein and nucleotide databases. In total, 69,803, 56,843,
47,438 and 30,240 isoforms were annotated from Nr, Swissport, KOG and KEGG, respectively. Combining
these results, a total of 69,867 isoforms were annotated (Additional file 3). The isoforms were aligned to
the different species. The five species with the most hit sequences were *Zea mays*, *Setaria italica*, *Oryza
sativa Japonica* Group, *Dichanthelium oligosanthes* and *Sorghum bicolor*. These isoforms were also
annotated by GO terms assigned to three categories—biological process (50,805 isoforms), cellular
component (32,922 isoforms) and molecular function (26,696 isoforms). In the biological process
categories, metabolic process (13,462 isoforms) and cellular process (12,836 isoforms) were the two
most functional terms. Cell (7,598 isoforms) and cell part (7,597 isoforms) were the two most functional
terms in the cellular component category. In the molecular function category, catalytic activity (13,086 isoforms) and binding (11,642 isoforms) were the two most functional terms (Fig. 2C).

3.3 DEGs by DPC treatment

The 150 pair-end reads were obtained for the DEGs analysis. In total, 1,404,530,300 raw reads were generated from 18 cDNA libraries using Illumina Hiseq 4000 platform. After trimming the adopter and removing the low-quality reads, 1,380,323,402 (98.28%) reads were retained as high-quality clean reads. These clean reads were mapped to the reference as full-length transcriptome. The mapping ratios for the 18 cDNA libraries covered from 73.97–83.78%. Using these data, the normalized expression data were calculated and the normalized gene expression were analyzed by PCA (Fig. 3A). The result showed that two clusters were clearly defined by PCA which contained DPC group and control for each cluster. The first principal component PC1 summarized 30.7% of the whole variability and discriminated samples according to the treatment. The second principal component PC2 and the third principal component PC2 summarized 25.1% and 17.4% of the whole variability and discriminated samples, respectively. The DEGs analysis showed that the comparison between C2 and D2 groups had the most DEGs (A total of 6,012 genes, contained 3,227 up-regulated genes and 2,785 down-regulated genes). The D1 showed more up-regulated genes compared to D2 and D3 groups while less down-regulated genes were found in D1 when compared to D2 and D3 groups. Besides the most DEGs in C2-vs-D2, C1-vs-C2 (2,895 DEGs) and D1-vs-D2 (3,157 DEGs) also showed large amount of differentially expressed genes.

3.4 Functional analyses of DEGs between C2 and D2 groups

For illustrating the functions of the DEGs after DPC treatment, GO enrichment and KEGG enrichment analyses of the comparison of C2 and D2 with the most DEGs were performed. The up-regulated genes and down-regulated genes were annotated in 29 and 37 in GO terms. The GO enriched terms with four most up-regulated genes were DNA metabolic process, negative regulation of biological process, regulation of translation, and regulation of cellular amide metabolic process were the most while the GO enriched terms with two most down-regulated genes were single-organism transport and single-organism localization (Additional file 4). KEGG enrichment analysis showed that 17 and 30 pathways were enriched in the up-regulated and down-regulated genes, respectively. Either for the up-regulated genes and down-regulated genes, metabolic pathways and biosynthesis of secondary metabolites were the top two enrichment KEGG pathways with the most genes. In the up-regulated genes, 55 genes were found increased in plant hormone signal transduction pathway. Phenylpropanoid biosynthesis, flavonoid biosynthesis, favone and flavonol biosynthesis, and glucosinolate biosynthesis were enriched in the down-regulated genes (Additional file 5). These KEGG pathways were associated with internodes growth and development.

3.5 WGCNA and hub genes

The WGCNA divided the genes into 36 modules. Based on the DEGs identification, we focus on the D2 group which contained significant gene expression changes which is the crucial stage for internode
elongation. We found that sienna3 was the top module that significantly correlated with the D2 stage (p = 1e-4) (Additional file 6). The sienna3 module contained 33 genes. The top three hub genes including Stf0 sulphotransferase, cyclin-like F-box and HOX12 were identified in this module. These three hub genes were correlated with 30 genes (Additional file 7).

### 3.6 Validation of RNA-seq result

The qPCR was used to validate the results of RNA-seq. Randomly, 9 genes were selected for the analysis. Except GID2 and PBS1, the other 6 tested genes including GA2OX1, GID1, MPK4, CML49, PRPF8 and ACO2 showed similar qPCR results with RNA-seq. Namely, expression trend of 6 in 8 genes from qPCR and RNA-seq had high consistence indicating the majority of genes had same tendency. These results showed the high reliability of the RNA-Seq data.

### 4. Discussion

Sugarcane is the main source for sugar industry accounting for 79% of sugar production over the world. Developing techniques for controlling growth of sugarcane accelerate the yields and culture biotechnology for sugarcane. GA and DPC are two pairs of chemicals that regulate plant growth in sugar farming with different effects. GA stimulates sugarcane internode elongation by regulating genes associated to zeatin biosynthesis, nitrogen metabolism and plant hormone signal transduction pathway \[43\] while the DPC depressed sugarcane growth. However, compared to the clear mechanism of GA stimulated growth, the molecular mechanisms of DPC is blur. Thus, in the present study, we focus on the transcriptomic regulation by DPC on sugarcane and discuss the key genes mediated the growth depressed effect.

First, to obtain high quality reference for gene annotation, we generate full-length transcriptome from sugarcane. The full-length transcriptome from sugarcane was sequenced by PacBio Sequel platform generated 72,671 isoforms. Compared to illumine platforms, PacBio Sequel platform could gain longer transcripts which is facility to construction of high-quality reference for short sequence analysis. The present study generated the reads with N50 as 3,011 bp. These long reads guarantee longer contigs and isoforms for the following transcriptome analysis \[44\]. It turns out that the N50 was 3,073 for the isoforms in the present study. Sugarcane is a widely cropped plant. Up to date, large amount of different varieties has been developed. The Guitang varieties developed from Guangxi which have become a series of varieties planted in southern China \[45\]. GT42 belonging to the Guitang varieties is a new breeding line with higher productivities for sugar \[45\]. Although the genome of sugarcane is reported until 2018, the genome data may differ from varieties \[46\]. Our present first reported the full-length transcriptome GT42. These data would accelerate the studies on the new high yield crop and provide high-quality reference when analyzed the illumine short reads. Meanwhile, these global transcriptome data gave a chance to illustrate the function of internodes in GT42. The most abundant biological process GO term of GT42 isoforms contained metabolic process and cellular process. Thus, this functional isoform showed similar function assignment with previous results from sugarcane \[46\–48\]. Based on these data, the GT42 had similar functional constitution of genes with other sugarcane varieties. The present full-length
transcriptome first generated the general information of GT42 and provided high-quality reference transcriptome for further investigation on this variety.

DPC is one of the most successful and widely used chemicals to regulated plant growth. Internode length and leaf size could be depressed by DPC treatment in cotton and sugarcane [12]. The present study also suggested that DPC inhibited internode length in GT42 which was similar to previous results. After understanding the effects of DPC on the internode growth, the next question is to find out the molecular mechanism of the function of DPC in sugarcane. We used RNA-seq to show the whole profile of regulation on gene expressions in the present study. Using hi-seq technique, we obtained millions of short reads to reveal the expression in different stages by DPC treatment. Thanks to the high-quality full-length transcriptome data, the mapping ratios for these libraries covered 73.97–83.78%. The comparison between C2 and D2 had the most DEGs which was 6,012 genes. This numbers of DEGs was much higher than that in C1-vs-D1 and C3-vs-D3 suggesting the gene expression changes between control and DPC treatment were mainly in the second stages, namely, on 6 days post spraying. In a study in cotton spraying by DPC, the 96 h post spraying significantly had the most DEGs compared to the 48 and 72 h stages. It seems that the DPC resulted changes gene expression could be in a long-term until 4 to 6 days. The gene expression regulation by DPC is not an acute effect. Meanwhile, after 10 days, the effects of DPC on gene expression were diminished. We supposed the best effect period of DPC regulated gene expression is 6 days.

The KEGG enrichment analysis showed that 55 genes in plant hormone signal transduction pathway were increased by DPC treatment. Internodes growth is controlled by several hormones such as G biosynthesis genes, auxin related genes, and ethylene genes. It had been reported that GA treatment significantly up-regulated these genes. Meanwhile, DPC may suppressed hormone expression. In Agapanthus praecox, auxin-related genes could be inhibited by DPC treatment [49]. Surprisingly, the present study also indicated that DPC increased several hormonal genes. This different may due to the different species. The sugarcane may have different response to DPC in molecular levels. We also found that several key pathways could be down-regulated by DPC, such as phenylpropanoid biosynthesis, flavonoid biosynthesis, favone and flavonol biosynthesis, and glucosinolate biosynthesis were enriched. The phenylpropanoid pathway provides metabolites for plant growth which contributes to the requirement of lignin biosynthesis [50]. Favone, flavonol and glucosinolate are key metabolites for internode growth [51, 52]. Flavonol biosynthesis could be affected by light intensity and led to different growth appearances in Ginkgo (Ginkgo biloba) [53]. The glucosinolate concentration influenced by sulfur and nitrogen supplement was associated with growth of broccoli [54]. The down-regulated of genes in these pathways may lead to the shorten effects of sugarcane internodes.

To find out the key gene modules and hub genes by DPC treatment, WGCNA was performed. The most correlated gene module with D2 group was sienna3 containing only 33 genes. Therefore, the most critical genes play key role in the module. Hub genes are the gene that correlate with other genes in expression levels which could be identified by mathematical method. The top three identified in this study were Stf0 sulphotransferase, cyclin-like F-box and HOX12. Stf0 belongs to sulphotransferase family which affects
root development processes, elongation growth, and gravitropism [55]. In several plants, including *Medicago truncatula*, *Lotus japonicus*, and *Arabidopsis thaliana*, cyclin-like F-box genes were expressed in all the tissues contained high actively dividing cells. Meanwhile, knockdown of this gene resulted in accumulation of CYCB1:1 suggesting that cyclin-like F-box gene could regulate cell cycle in the dividing cells [56]. It had been reported that HOX12 regulated panicle exsertion via modulating EUI1 gene expression [57]. These three hub genes were correlated with the other genes in the sienna3 modules. Based on this information, it could be concluded that Stf0 sulphotransferase, cyclin-like F-box and HOX12 mediated a gene group and constituted as a gene network which contributed to the DPC effects on sugarcane growth.

In conclusion, the full-length transcriptome of GT42 was first reported providing an informative resource for sugarcane breeding and transcriptome analysis. The RNA-seq suggested that the main effects of DPC on sugarcane gene expression was 6 days post spraying. The significant enriched gene function categories contained several pathways related to internode growth including multiple pathways that participated in production of Metabolic products. The gene modules included 33 genes were high correlated with the stage of 6 days post spraying in DPC group showing a potential role in response to DPC. Among these genes, Stf0 sulphotransferase, cyclin-like F-box and HOX12 were hub genes that may regulate all the other genes in this module. Further studies should focus on determination the function in detail of these key genes, especially in controlling internode growth affected by DPC.

**Abbreviations**

DPC
Mepiquat chloride; WGCNA:weighted gene co-expression network analysis; GA:gibberellin; CTK:Cytokinins; ABA:abscisic acid; ETH:ethyne; BR:brassinosteroid; GA20-OX1:GA20-oxidase; GID1:gibberellin receptor; SRI:Sugarcane Research Institute; GO:Gene Ontology; KEGG:Kyoto Encyclopedia of Genes and Genomes; FPKM:Fragments Per Kilobase of transcript per Million mapped reads; CCS:circular-consensus sequence; FLNC:full-length non-chimeric; PCA:principal component analysis; SD:standard deviations; ANOVA:one-way analysis of variance; DEGs:differentially expressed genes.

**Declarations**

**Acknowledgments**

Not applicable.

**Authors’ contributions**

RC, LQ and JW conceived and designed the experiments. YF, HZ and SM collected the samples and cultured the sugarcane. ZZ, HY and TL analyzed the data. XH, MW, PL and YL performed the experiments. RC, YF, LQ and JW wrote the manuscript. All authors reviewed and approved the final manuscript.
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**Availability of data and materials**

Data for the sugarcane used in the RNA-Seq analysis are accessible at NCBI under BioProject accession number PRJNA633918.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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Figures

Figure 1

Effects of DPC on sugarcane growth performance on different days after treatment. (A) The height of sugarcane on different days after DPC treatment (n = 4). (B) The growth rate of sugarcane on different days after DPC treatment (n = 4; mature period, n = 10). (C) The internode length of sugarcane in mature sugarcane after DPC treatment. * indicated p < 0.05.
Figure 2

Full-length transcriptome of internode of sugarcane. (A) Length distribution of reads generated from PacBio Sequel System sequencing. (B) Length distribution of isoforms generated from PacBio Sequel System sequencing. (C) Distribution of annotated genes from NR database in different species. (D) GO annotation of the isoforms.
Figure 3

Expression profile analysis based on RNA-seq result. (A) Principle component analyses of the eighteen transcriptomes from the internodes of sugarcane on different days in the control and DPC treatment groups based on the FPKM. (B) Number of up-regulated and down-regulated genes of pairwise comparisons.
**Figure 4**

GO enrichment analysis result of up-regulated genes (A) and down-regulated genes (B) from C2-vs-D2 comparison.

**Figure 5**

KEGG enrichment analysis result of up-regulated genes (A) and down-regulated genes (B) from C2-vs-D2 comparison.
Figure 6

WGCNA analysis of internode transcriptomes. (A) The influence of soft-thresholding power on scale-free fit index. (B) The influence of soft-thresholding power on the mean connectivity. (C) Cluster dendrogram of the clustering of dissimilarity using a consensus topological overlap. Modules were assigned to different colors.
Figure 7

The heatmap of module-trait relationship between different groups and gene modules. The sienna3 is the top positive module that correlated with D2 group. Values in each box represented the correlation coefficient between modules and traits. Values in brackets from each box represented the P-values for the correlation test.
Figure 8

Identification of hub gene in sienna3 by Cytoscape. Blue cycles represented the hub genes while green cycles showed other genes. Grey lines showed the correlations between the genes.
Figure 9

Validation of RNA-seq results by qPCR. The line charts showed the log₂(FPKM) values of the genes and bar charts showed the relative expression from qPCR results.

Supplementary Files
This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile7sienna3threehubgenes.csv
- Additionalfile6enrichmentmoduleTraitPvalue.csv
- Additionalfile5DEGsgoenrichment.xlsx
- Additionalfile4DEGsgoenrichment.xlsx
- Additionalfile3ISOannotation.xlsx
- Additionalfile2Summary.xlsx
- Additionalfile1Theprimersusedinthisstudy.xlsx