Transcriptomic exploration of a high sucrose mutant in comparison with the low sucrose mother genotype in sugarcane during sugar accumulating stage

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Abstract
Sugarcane (Saccharum spp. hybrid) is a noteworthy crop in the world for sugar and bioenergy production. In the present study, transcriptomic analysis was steered for a high sucrose mutant Guixuan B9 (GXB9) and the low sucrose mother genotype B9 of sugarcane during sugar accumulating stage. A total of 112,170 unigenes were obtained, and 106,026 (94.52%) were annotated by using the available public databases nonredundant, Gene ontology, COG, Kyoto Encyclopedia of Genes and Genomes, Swiss-Prot, and Pfam. Functional assignment analysis of unigenes exhibited that they were active in a diversity of metabolic pathways. The vital unigenes for sucrose metabolism and accumulation were relatively characterized in the immature internodes (int. 5, 6) and maturing internodes (int. 13, 14) of GXB9 in comparison with B9. The differentially expressed unigenes (DEGs) encoding sucrose phosphate synthase (SPS, EC2.4.1.14), sucrose synthase (SuSy, EC2.4.1.13), and invertase (EC3.2.1.26), which are the vital enzymes functioning in the biosynthesis and splitting of sucrose, in the internodal tissues were revealed by using Illumina Hiseq 2000 platform, and verified by quantitative reverse transcription-polymerase chain reaction analysis. The DEGs encoding SPS proteins SPS1, SPS2, SPS4, and SPS5 were upregulated in the maturing internodes of GXB9 compared with B9. The DEGs encoding SuSy proteins were majorly upregulated in the immature internodes of GXB9, and those encoding the enzymes involved in trehalose synthesis were downregulated in GXB9 compared with B9. The results in this study have revealed the opportunities for future sugarcane improvement by concentrating on the DEGs linked to sucrose metabolic pathways by applying omics technologies.

Keywords
cell wall invertase, cell wall synthase, sucrose content, sucrose phosphate synthase, sucrose synthase, sugarcane, trehalose

[Correction added on 12 July 2021, after first online publication: Yang-Rui Li, Yong-Xiu Xing and Xiu-Peng Song have been added as the corresponding authors. ORCID has been added to Yang-Ru Li. Qaisar Khan has been removed from the corresponding authorship.]
1 | INTRODUCTION

Sugarcane (Saccharum spp. hybrid) is a C₄ plant belonging to family Gramineae, and is well cultivated commercially at least in 106 countries of tropical and subtropical areas (Barnes, 1974). Sugarcane is the most important sugar and bioenergy crop in the world (Bonnett & Henry, 2011; Cheavegatti-Gianotto et al., 2011). About 80% of sugar production is from sugarcane which has significant capability for sucrose accumulation in the stalks, chiefly in ripen internodes, and its quantity drives aloft to 0.7 M (Moore, 1995). Besides, many useful by-products such as falernum, bagasse, cachaça, molasses, rum spentwash, ethanol, pressmud, trash, yeast, and subsequent derivatives are produced from sugar factories (Cesnik & Mioque, 2004; Kumar et al., 2018; Toppa et al., 2010). Sucrose is produced by the carbohydrate from photosynthesis in green leaves of sugarcane plants, and then transferred to sink organs, and consuming and storage sinks. In consuming sinks, mainly growing roots, stems, and flowers, sucrose is hydrolyzed to glucose and fructose for growth need, while in accumulation sink, that is mature stalks, it is stored stably (Bihmidine et al., 2013; Braun & Slewinski, 2009; Braun et al., 2014; Yadav et al., 2015). Sucrose accretion in the parenchyma cells of sugarcane stalk is achieved via incessant cleavage and resynthesis (McCormick et al., 2009), whereas its metabolism is catalyzed by several crucial enzymes, sucrose phosphate synthase (SPS), neutral invertase (NI), sucrose synthase (SuSy), soluble acid invertase, and cell wall invertase (CWIN; Sturm, 1999).

Parent nature of genotypes, environmental circumstances, and ripening time of sugarcane varieties have an imperative part in the level of sucrose storage in internodes (Jackson, 2005; Moore, 1995). At sugar accumulating stage of sugarcane, the bottom internodes convert to maturity earlier while the top internodes of the stalk continue growth. As the time passes, the sugarcane crop continues ripening, and more and more internodes receive higher sucrose amount. Based on the time of sucrose accumulation in cane, sugarcane varieties are divided into early, mid-late, or late maturing types. Sucrose content in cane is a significant trait of sugarcane varieties and a central focus of sugarcane scientists (de Morais et al., 2015).

Sucrose content improvement has remained an eventual purpose in sugarcane (Cheavegatti-Gianotto et al., 2011), and sugarcane breeders are employing modern genomic strategies to insert the multiplicities of alleles into the breeding program through gene extracting from wild relatives (Abberton et al., 2016). Innovation in high-throughput DNA sequencing technologies has appreciably magnified the volume of data easily available for phylogenetic implications (McCormack et al., 2013; Steele et al., 2012; Straub et al., 2012). It is importantly useful for complex groups resulting from fast evolutionary radiation, like the grass tribe Andropogoneae (Arthan et al., 2017; Estep et al., 2014; Welker et al., 2015).

Recently, omics technologies have played significant roles in comprehensive understanding of sugarcane genome, and raising of commercial varieties with essential qualities. These omics advances have a widespread range of applications in genomics, transcriptomics, metabolomics, and proteomics. The functional interpretations are built on logical approaches, bioinformatics, computational scrutiny, and numerous successive interdisciplinary biological acuities (Casu et al., 2010; Mustafa et al., 2018). Transgenic technology has been applied to produce crops with extra nutritional prominence in short time and with durable resistance to biotic and abiotic challenges like insects, drought, fungal pathogens, herbicides, salinity, and cold stress (Van Emon, 2016).

Transcriptomic study of sugarcane has arisen as a powerful approach for functional assignment of unknown genes. It reduces the complication of information statistics, and purely functional genes in the target tissue or organ are measured at the time and location of specimen. Transcriptomic technique can absolutely compare the identical tissues at varied developing stages in different sugarcane varieties which are flourishing in diverse conditions (Casu et al., 2010). Transcriptome analyses have been used to discover the molecular mechanism of regulation of sucrose content in sugarcane (Thirugnanasambandam et al., 2017), and have also been predominantly applied in various crops to explain numerous discrete traits (Chen et al., 2020; Habib et al., 2018; Shu et al., 2019). Unigenes with differential expression in sugarcane leaf abscission were investigated via RNA-seq transcriptomic analysis, and RNA-seq technology was also used to sequence the transcriptome of six sugarcane genotypes, a large quantity of transcripts, simple-sequence repeats (SSRs), single nucleotide polymorphisms (SNPs) were obtained and annotated (Cardoso-Silva et al., 2014; Li et al., 2016).

The present study was designed to explore the transcriptomic difference between a high sucrose content sugarcane mutant Guixuan B9 (GXB9) and its low sucrose content mother variety B9 at sugar accumulating stage. Sugarcane variety B9 was introduced from Brazil to China in October 1999, which showed good ratooning, wide adaptability, high tillering rate, many millable stalks, medium to medium large stalks, good defoliation, and high yield but relatively low sugar content (Wang, Yang, et al., 2016; Wang, Duan, et al., 2016). On October 15, 2013, a high sugar mutant clone was observed in the B9 population, and named as GXB9. Since then, several years of field experiments have been conducted to compare the performances of GXB9 and its original mother variety B9, the obtained results proved that the two clones have similar morphological characteristics but significant difference in sucrose content (Figure 1; File S1). We
further conducted SSR marker-based test which showed variations between them, which confirmed the mutation.

On the basis of phenotypic observation, sugar content, and SSR analysis, the present study was conducted to detect the transcriptomic differences in the immature internodes (int.5, 6) and maturing internodes (int.13, 14) of GXB9 and B9. This is the first transcriptomic analysis of the genes involved in sucrose synthesis and accumulation in internodal tissues of the mutant GXB9 in comparison with its mother variety B9.

2 | MATERIALS AND METHODS

2.1 | Plant materials and sample collection

The experiment materials of this research were a high sucrose content sugarcane mutant GXB9 and its low sucrose content mother variety B9 selected by Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences. The seedcanes were planted in early March 2019 in the experiment field of College of Agriculture, Guangxi University, Nanning, China, and the crop was managed with conventional practices. Compound fertilizer (N–P₂O₅–K₂O: 22–8–12), carbendazim fungicide, and metsulfuron as herbicide were applied as common practice. Compound fertilizer (N–P₂O₅–K₂O: 22–8–12), carbendazim fungicide, and metsulfuron as herbicide were applied as common practice. For transcriptomic analysis, the samples of immature internodes (int. 5, 6) and maturing internodes (int. 13, 14) of stalks were taken in the middle of October, November, and December, 2019, respectively, instantly frozen into liquid nitrogen, and stored at −80°C for upcoming use.

2.2 | RNA extraction

Total RNA was extracted from the sampled sugarcane internode tissues using TRIZol® Reagent (Plant RNA Purification Reagent for plant tissue) according to the manufacturer's instructions (Invitrogen) and genomic DNA was removed using DNase I (TaKaRa). Then, the integrity and purity of the total RNA quality were determined by 2100 Bioanalyzer (Agilent Technologies, Inc.) and quantified using ND-2000 (NanoDrop Thermo Scientific). Only high-quality RNA sample (OD260/280 = 1.8–2.2, OD260/230 ≥ 2.0, RIN ≥ 8.0, 28S: 18S ≥ 1.0, >2 μg) was used to construct the sequencing library.

2.3 | Preparation of RNA-Seq library and sequencing

RNA purification, reverse transcription, library construction, and sequencing were performed at Shanghai Majorbio Biopharm Biotechnology Co., Ltd. according to the manufacturer's instructions (Illumina). The RNA-seq transcriptome libraries were prepared using Illumina TruSeq™ RNA sample preparation kit. Poly (A) mRNA was purified from total RNA using oligo-dT-attached magnetic beads, and then fragmented by fragmentation buffer. Using these short fragments as templates, double-stranded cDNA was synthesized with SuperScript double-stranded cDNA synthesis kit (Invitrogen) and random hexamer primers (Illumina). Then, the synthesized cDNA was subjected to end-repair, phosphorylation, and “A” base addition according to Illumina's library construction protocol. Libraries were size selected for cDNA target fragments of 200–300 bp on 2% low range ultra-agarose followed by PCR amplification using Phusion DNA polymerase (New England Biolabs) for 15 PCR cycles. After quantified by TBS380, two RNAseq libraries were sequenced in a single lane on an Illumina Hiseq xten/NovaSeq 6000 sequencer (Illumina) for 2 × 150 bp paired-end reads.

2.4 | De novo assembly and annotation

The raw paired end (PE) reads were trimmed and the quality was controlled by SeqPrep (https://github.com/jstjohn/SeqPrep) and Sickle (https://github.com/najoshi/sickle) with
default parameters. The clean data from the sample were used to do de novo assembly with Trinity (http://trinityrnaseq.sourceforge.net/; Grabherr et al., 2011). All the assembled transcripts were searched through the databases NCBI protein nonredundant (NR), COG, Kyoto Encyclopedia of Genes and Genomes (KEGG), Gene ontology (GO), Swissprot, and Pfam using BLASTX to identify the genes that had the highest sequence similarity with the given transcripts to retrieve their function annotations, and a typical cut-off E-value less than 1.0 × 10^{-5} was set. BLAST2GO (http://www.blast2go.com/b2ghome; Conesa et al., 2005) was used to get GO annotations of unique assembled transcripts for describing biological processes, molecular functions, and cellular components. Metabolic pathway analysis was performed using the KEGG (http://www.genome.jp/kegg/; Ogata et al., 1999).

2.5 | Differential expression analysis and functional enrichment

To identify the differentially expressed unigenes (DEGs) between the two different genotypes, the expression level of each transcript was calculated according to the transcripts per million reads method. RSEM (http://deweylab.biost at.wisc.edu/rsem/) (Li & Dewey, 2011) was used to quantify the gene abundances. Essentially, differential expression analysis was performed using the DESeq2 (Love et al., 2014) with \( p \leq 0.05 \), and the transcripts with \( \log_{2} FC > 1 \) were considered to be differentially expressed genes. In addition, functional enrichment analyses including GO and KEGG were performed to identify the DEGs significantly enriched in GO terms and metabolic pathways at Bonferroni-corrected \( p \leq 0.05 \) compared with the whole transcriptome background. GO functional enrichment and KEGG pathway analysis were carried out by Goatools (https://github.com/tanghaibao/Goatools) and KOBAS (http://kobas.cbi.pku.edu.cn/home.do; Xie et al., 2011).

2.6 | Quantitative reverse transcription-polymerase chain reaction authentication

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was carried out to validate the reliability of differentially expressed genes by using TSINGKE Biological Technology (www.tsingke.net) with PCR primers in Light Cycler®480 II (Roche). 1 μg RNA of separate sample was converted into cDNA using first-strand cDNA synthesis kit (Vazyme Biotech Co., Ltd.) according to the directions of manufacturer. The qRT-PCR reaction was performed in a volume of 20 μl comprising of 2 μl of cDNA (template), 10 μl of 2× ChamQ Universal SYBR qPCR Master Mix (Vazyme Biotech Co., Ltd.), 7.2 μl of sterile water, 0.4 μl of primer mix (10 μm each of forward and reverse primers). The amplification procedure was as follows: one cycle of 30 s at 95°C, followed by 40 cycles of 5 s at 95°C and 15 s at 60°C, one cycle of 15 s at 95°C, 1 min at 60°C, 15 s at 95°C. The sugarcane housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase, served as the internal reference gene to normalize the gene expression level. Three biological and three technical replications were set for each sample. The data were analyzed using LightCycler® 480 software version 1.5.1 provided by Roche. The comparative fold change of the designated genes was calculated using \( 2^{-\Delta\Delta Ct} \) algorithm.

3 | RESULTS

3.1 | Sequencing and de novo assembly of transcriptome

Two cDNA libraries were created using equivalent volumes of RNA extracted from the immature and maturing internodes of both sugarcane genotypes. To illustrate the selected sugarcane transcriptome, the cDNA libraries were exposed to PE read sequencing using the Illumina HiSeq2000 platform. After removing the primers, adapter sequences, and poor quality reads, we gained an overall of 119,756,557 clean reads, with mean guanine-cytosine content of 47.56%. The mean mapped percentage, trans rate evaluation score, and benchmarking universal single-copy orthologs evolution score were 71.831%, 0.15109%, and 64.6%, respectively (Table 1).

Table 1 Summary of assembled transcripts and unigenes in stalk internodes of sugarcane

| Term          | Unigene | Transcript |
|---------------|---------|------------|
| Total base    | 119,756,557 | 268,440,272 |
| Total number  | 112,170 | 224,576 |
| Largest length (bp) | 18,353 | 18,353 |
| Smallest length (bp) | 201 | 201 |
| Average length (bp) | 1067.63 | 1195.32 |
| N50 length (bp) | 1960 | 2007 |
| E90N50 length (bp) | 3245 | 2516 |
| Mean mapped percent (%) | 71.831 | 82.699 |
| GC percent (%) | 47.56 | 48.07 |
| Trans rate score | 0.15109 | 0.23576 |
| BUSCO score   | 64.6% (1.7%) | 64.6% (1.7%) |

Abbreviations: BUSCO, benchmarking universal single-copy orthologs; GC, guanine-cytosine.
The unigenes were divided into different groups based on sequence length, for example, those with 200–500 bp were 49,573 (44.19%), and those with 501–1000 bp were 28,507 (25.41%). 10,675 (9.51%) unigenes fell in a range from 1001 to 1500 bp in length, and 6478 (5.77%) unigenes were 1501–2000 bp in length. The unigenes ranging 2001–2500, 2501–3000, 3001–3500, 3501–4000, and 4001–4500 bp were 4611 (4.11%), 3429 (3.05%), 2572 (2.29%), 1967 (1.75%), and 1273 (1.13%), respectively, and those with a length above 4500 bp were 3085 (2.75%; Table 2).

3.2 | Functional annotation of unigenes

The absence of a reference genome is a serious balk for gene function prediction and utilization of the transcriptome data in sugarcane. An overall of 58,995 (52.59%) unigenes were annotated with functions through available public databases (Table 3). A total of 57,708 unigenes (51.45%) showed similarity to the NR database, 50,673 (45.18%) unigenes had resemblance with COG, 46,293 unigenes (41.27%) harmonized with GO, 31,347 unigenes (27.95%) had homology with Pfam, 30,832 unigenes (27.49%) had similarity with Swiss-Prot, 18,762 unigenes (16.73%) had affinity with KEGG, while 53,175 (47.41%) unigenes were not functionally annotated in any selected database. These non-matched unigenes could be novel; however, some of them may be characterized as non-coding RNA molecules.

3.3 | Species homology distribution

All the assembled unigenes were hit up on NR public database for species homology search by using the BlastX with a cut-off E-value of $10^{-5}$. A total of 57,708 unigenes were annotated in NR database. Among them, 28,009 (48.54%) were homologous with *Sorghum bicolor*, 11,283 (19.55%) were equated with *Zea mays*, 2965 (5.14%) had similarity with *Panicum miliaceum*, 2601 (4.50%) matched to *Oryza sativa*, 1798 (3.12%) were alike to *Panicum hallii*, 1555 (2.69%) matched with *Saccharum* hybrid cultivar, 1182 (2.04%) had homology with *Dichanthelium oligosanthes*, 959 (1.66%) were the same as *Setaria viridis*, 540 (0.93%) had congruity with *Vitis vinifera*, 362 (0.62%) showed compatibility with *Aegilops tauschii*, 332 (0.57%) had conformity to *Brachypodium distachyon*, 256 (0.44%) were the same to *Oryza brachyantha*, 251 (0.43%) matched to *Triticum urartu*, while 3640 (6.30%) showed similarity to other species, respectively (Figure 2). The unigenes showed 48.53% homology with *S. bicolor*, but only 2.69% with *Saccharum* hybrid cultivar. This minor homology of the unigenes with those of other *Saccharum* hybrid (R570) may be due to the big genetic difference in different sugarcane varieties, or the nonavailability of reference genome sequences, and insufficient public data related to sugarcane.

### Table 2

Unigenes and transcripts length distribution in the stalk internodes of sugarcane

| Unigene length | Number | Percentage | Transcript length | Number | Percentage |
|----------------|--------|------------|-------------------|--------|------------|
| 200–500        | 49,573 | 44.19      | 200–500           | 81,226 | 36.17      |
| 501–1000       | 28,507 | 25.41      | 501–1000          | 56,455 | 25.14      |
| 1001–1500      | 10,673 | 9.51       | 1001–1500         | 27,510 | 12.25      |
| 1501–2000      | 6478   | 5.77       | 1501–2000         | 18,532 | 8.25       |
| 2001–2500      | 4611   | 4.11       | 2001–2500         | 12,861 | 5.73       |
| 2501–3000      | 3429   | 3.05       | 2501–3000         | 8833   | 3.93       |
| 3001–3500      | 2572   | 2.29       | 3001–3500         | 6266   | 2.79       |
| 3501–4000      | 1967   | 1.75       | 3501–4000         | 4242   | 1.89       |
| 4001–4500      | 1273   | 1.13       | 4001–4500         | 2796   | 1.25       |
| >4500          | 3085   | 2.75       | >4500             | 5855   | 2.60       |
| Total          | 112,170| 100        | Total             | 224,576| 100        |
transport, and metabolism (1045, 1.88%); lipid transport and metabolism (766, 1.39%); energy production and conversion (724, 1.32%); inorganic ion transport and metabolism (644, 1.17%); secondary metabolites biosynthesis, transport, and catabolism (551, 1%); cell wall/membrane/envelope biogenesis (502, 0.91%); cytoskeleton (385, 0.70%); coenzyme transport and metabolism (367, 0.67%); nucleotide transport and metabolism (345, 0.63%); cell cycle control, cell division, chromosome partitioning (326, 0.59%); RNA processing and modification (247, 0.45%); defense mechanisms (245, 0.44%); chromatin structure and dynamics (228, 0.41%); cell motility (6, 0.01%); and nuclear structure (2, 0.003%), respectively.

3.5 | Gene ontology

Gene ontology database was used to predict the functions of all the assembled unigenes. In GO, the functions of the unigenes were divided into three types: molecular function, cellular component, and biological process. A total of 46,293 (41.27%) unigenes were annotated and classified into 52 subfunctional classes. Collectively, 191,591 GO terms were gained from all the three categories including molecular function (terms: 58,201, 30.38%), cellular component (terms: 72,623, 37.91%), and biological process (terms: 60,767, 31.72%; Figure 4; File S2). Molecular function category included 17 discrete subfunctional types, cellular component function category included 13 different subfunctional groups, and biological process category included 22 individual subfunctional groups. In molecular function category, the unigenes related to binding (28,023, 48.14%) and catalytic activity (23,150, 39.78%) were obviously dominant. In cellular component category, the unigenes related to cell part (22,457, 30.92%), membrane part (16,208, 22.318%), and organelle (1922, 16.42%) terms had significant expression intensity, correspondingly. In biological process category, the unigenes related to cellular process (20,554, 33.82%), metabolic process (17,930, 29.51%), biological regulation (6214, 10.23%), and response to stimulus (4470, 7.36%) were highly expressed. The unigenes allocated to subfunctional sets such as molecular carrier activity, signal transducer, carbon utilization, membrane part, cellular component organization or biogenesis, binding, and developmental process could be strictly connected to growth, disease, and sucrose content, which may be helpful to provide significant data for upcoming studies.

3.6 | Kyoto Encyclopedia of Genes and Genomes

To investigate the complicated biological and metabolic pathways in the stalk internodes of sugarcane, the unigenes

TABLE 3  Functional annotation of unigenes through public databases

| Database  | Unigenes | Percentage |
|-----------|----------|------------|
| NR        | 57,708   | 51.45      |
| COG       | 50,673   | 45.18      |
| GO        | 46,293   | 41.27      |
| Pfam      | 31,347   | 27.95      |
| Swiss-Prot| 30,832   | 27.49      |
| KEGG      | 18,762   | 16.73      |
| Total annotation | 58,995 | 52.59 |
| Total number | 112,170 | –          |

Abbreviations: GO, Gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; NR, nonredundant.

FIGURE 2  Homology of unigenes in stalk internodes of sugarcane with those in different species
were elucidated by applying the KEGG database. As a result, 17,317 (17.27%) unigenes were grouped into 148 pathways (File S3). This classification ranged from the lowest enriched pathway, mannose type O-glycan biosynthesis (1, 0.006%), to the highest enriched pathway, ribosome (779, 4.50%). The considerably enriched pathways were ribosome (779, 4.50%), protein processing in endoplasmic reticulum (538, 3.10%), plant–pathogen interaction (512, 2.95%), spliceosome (451, 2.60%), RNA transport (441, 2.54%), endocytosis (403, 2.32%), purine metabolism (389, 2.24%), plant hormone signal transduction (388, 2.24%), thermogenesis (375, 2.16%), MAPK signaling pathway plant (319, 1.84%), pyrimidine metabolism (315, 1.81%), phenylpropanoid biosynthesis (312, 1.80%), glycolysis/gluconeogenesis (309, 1.78%), ubiquitin-mediated proteolysis (294, 1.69%), oxidative phosphorylation (284, 1.64%), amino sugar and nucleotide sugar metabolism (259, 1.49%), phagosome (253, 1.46%), starch and sucrose metabolism (244, 1.40%), and carbon fixation in photosynthetic organisms (233, 1.34%), respectively. The pathways involved in starch and sucrose metabolism (244, 1.40%), glycolysis/gluconeogenesis (309, 1.78%), and amino sugar and nucleotide sugar metabolism (259, 1.49%) could
be manipulating sucrose synthesis/storage, and might be the key emphasis of the upcoming researches to improve sugar productivity in sugarcane.

3.7 | Putative SSR marker discovery

Microsatellites or SSR markers are stretches of DNA in which the same short nucleotide sequence is repeated over and over. SSR markers are considered highly polymorphic because the number of repeats can vary greatly in plants. SSRs are widely and profusely spread in most nuclear eukaryotic genomes, and have been extensively used as molecular markers to elucidate genetic multiplicity, create genetic maps, and execute comparative genomics in recent years. In this study, advantageous SSR markers were attained from the investigation of unigenes by applying microsatellite identification tool (MISA software). All the assembled unigenes were subjected to MISA for SSR analysis, and a total of 23,592 SSRs were mined. Among them, 3060 sequences had more than one SSR. The frequently existing repeat motifs were mononucleotide (11,278, 47.80%) and trinucleotide (7395, 31.35%), followed by dinucleotide (4190, 17.60%), tetranucleotide (403, 1.71%), pentanucleotide (187, 0.79%), and hexanucleotide (139, 0.59%), respectively (Figure 5A; File S4). Together, 173 types of nucleotide motif repeats were found among the SSR loci. The most repeat type was A/T (10744, 45.54%), followed by CCG/CGG (2911, 12.34%), AG/CT (2081, 8.82%), AC/GT (1059, 4.49%), AGG/CCT (902, 3.82%), AAC/GTT (881, 3.73%), AT/AT (801, 3.39%), C/G (534, 2.26%), ACG/CGT (481, 2.03%), and ACC/GGT (405, 1.72%), respectively. Still, there were 1869 (7.92%) other repeat motifs (Figure 5B). Based on these results, the compulsory primer pairs were designed using Primer3.0.

3.8 | SNPs

A total of 1,048,575 putative SNP sites were documented from 1,12,170 diverse unigenes in this study (File S5). Through GO explanation of particular unigenes which owned unique SNPs in individual groups, several vital types were found to be related to the biological functions of sugarcane. For example, 31 unigenes with SNPs were involved in phosphatase activity category, 107 unigenes having SNPs were linked to response to abiotic stimulus category, 10 unigenes containing SNPs involved in amino sugar catabolic process category, 32 unigenes with SNP were found in photosynthesis category (File S5). The SNPs inferred from transcriptome sequences could be affiliated to the differentially expressed genes, which could be beneficial to amplify meaningful markers connected to important agronomic traits of different sugarcane genotypes.

3.9 | DEGs evaluation

Differential unigene expression was assessed between the high sucrose sugarcane mutant GXB9 and its low sucrose content mother variety B9 at sugar accumulating stage. A total of 106,026 DEGs were gained from the immature internodes and maturing internodes of GXB9 compared with B9. Among them, 48,169 (45.43%) were upregulated, and 57,857 (54.57%) were downregulated (Figure 6; Files S6A, and 6B).

3.10 | Sucrose metabolism

Comparison of the two sugarcane genotypes produced 14 DEGs encoding SuSy in the immature internodes: Three (one for SuSy2; two for SuSy) were upregulated, and 11 (six for SuSy, two for SuSy2, three for SuSy4) were downregulated in GXB9 compared with B9. Sixteen SuSy transcripts were differentially expressed in the maturing internodes: Nine (five for SuSy, three for SuSy2, one for SuSy4) were upregulated, and seven (two for SuSy2, five for SuSy) were downregulated in GXB9 compared with B9. It was found that there were 17 DEGs encoding CWIV in the immature internodes: Six (one for INVA, two for CINV2, two for NIN1, one for NIN3) were upregulated and 11 (three for NIN3, two

FIGURE 5  The SSR number (a) and repeat types (b) in the unigenes in stalk internodes of sugarcane
for NIN1, two for INVC, two for INVD, two for CINV2) were downregulated in GXB9 compared with B9. In the maturing internodes, there were 16 DEGs encoding CWIV: Eight (two for CWIN, one for INVA, one for CINV2, four for NIN1) were upregulated and eight were (one for INVD, one for INVC, one for CINV2, three for NIN3, one for NIN1, one for INV4) downregulated in GXB9 compared with B9. A total of nine DEGs encoding SPS were observed in the immature internodes: Four (three for SPS1, one for SPS4) were upregulated, five (three for SPS5, one for SPS4, one for SPS2) were downregulated in GXB9 compared with B9. There were 12 DEGs encoding SPS in the maturing internodes: Five (three for SPS1, two for SPS2) were upregulated and seven (five for SPS5, two for SPS4) were downregulated in GXB9 compared with B9.

### 3.11 Cell wall

It was noticed that a total of 80 DEGs involved in cellulose synthase (EC2.4.1.12) in the immature internodes of GXB9 compared with B9: 42 were upregulated and 38 were downregulated. The DEGs included those encoding CesA 1, CesA 2, CesA 3, CesA 4, CesA 5, CesA 6, CesA 7, CesA 9, CesA 10, cellulose synthase-like protein CSLH 1, 2, 3, CSLD 1, 2, and CSLE 1, 2. The DEGs encoding CesA 2, 6 were upregulated, and those encoding CSLG2, CSI3 were downregulated. In the maturing internodes, 71 DEGs connected to cellulose synthase were detected: 31 were upregulated and 40 were downregulated in GXB9 compared with B9. The DEGs encoding CSLG2 was upregulated, and those encoding CSLH1, CSLH3, and CSI3 were downregulated.

### 3.12 Sugar transporter

Sugar transporters are significant proteins in plants for carrying sucrose in various portions, mainly in source, and consuming and storage sinks. A total of 243 DEGs were detected in the immature internodes of GXB9 compared with B9: 109 were upregulated and 134 downregulated. These DEGs included those involved in sugar transporter families like nucleotide/sugar transporter family protein, sugar transporter ERD6-like 4, 5, 6, 16, bidirectional sugar transporter SWEET11, 14, 15, 6b, sugar transport protein MST1, MFS1, 2, sugar and sugar efflux transporter for intercellular exchange. Those involved in sugar transporter MST2, 6, SWEET 13, 1a were upregulated, whereas those laboring in sugar transporter SWEET10, 1b, and MFS5, YbfB were downregulated. In the maturing internodes of GXB9 compared with B9, 262 DEGs were found: 111 were upregulated and 151 were downregulated. The DEGs included those synthesizing SWEET 11, 13, 14, 15, sugar transporter ERD6-like 6, 4, sugar transport protein MST1, 6, and MFS1, 2, 5. The DEGs encoding sugar transporter like SWEET10, sugar transporter family protein, sugar carrier protein C, MST2, and other sugar transporters, nucleotide-diphospho-sugar transferase, sugar efflux transporter for intercellular exchange, sugar transport protein 7 were upregulated, while those encoding ERD6-like 16, 5, bidirectional sugar transporter SWEET 1b, 1a, NSUT2, STP14, 8, and POT were downregulated.

### 3.13 Sucrose signaling

A total of 44 DEGs were discovered to be involved in trehalose-phosphate phosphatase/synthase: 17 were downregulated and 27 were upregulated in the immature internodes of GXB9 compared with B9. The DEGs included those active in TPP4, 9, TPS7. Those involved in TPP1, 3, 6, 7, D, TPS5, 11 were upregulated, and those encoding TPP, TPP2, TPS6 were downregulated. The same 44 DEGs were observed in the maturing internodes of GXB9 compared with B9: 25 were upregulated and 19 were downregulated. The DEGs included those encoding TPS6, 9, TPP6, 7. Those working in TPS7, 11, TPP1, 3 were upregulated, and those functioning in TPS5, TPP2, 4, 9, D were downregulated.

**FIGURE 6** Venn diagram showing DEGs in high sucrose sugarcane mutant Guixuan B9 (GXB9) versus low sucrose variety B9.
3.14 | Transcription factor

Transcription factors (TF) play a vital role in plant growth, and respond to various biotic and abiotic stresses and signal transduction. Collectively, 3653 DEGs involved in TF were obtained from the immature and maturing internodes of GXB9 competed with B9: 1824 were upregulated and 1829 were downregulated. These DEGs showed associations with 36 diverse TF families particularly WRKY, bHLH, basic leucine zipper (bZIP), B3-superfamily, NAC, zf-B-box (C2C2), HSF, C3H, GRAS, AP2/ERF, FAR1, NF-Y, BBR-BPC, MYB_superfamily, LBD (AS2/LOB), GRF, LOB, C2H2, BES1, GbEP, TCP, CAMTA, EIL, Zf-Dof (C2C2), MADS, SBP, SRS, ZF-HD, GATA-zf (C2C2), RWP-RK (Nin-like), E2F/DP, TCR (CPP), Zf-NF-X1 (NF-X1), Whirly, Zf-LSD1 (C2C2), and S1Fa-like.

3.15 | Protein kinase

Protein kinases (PTKs) are the enzymes that order the biological activity of proteins by phosphorylation of specific amino acids with ATP as the source of phosphate, thus resulting in a conformational alteration from an inactive to an active form of the protein in plants. Sugarcane PTK (EC2.7.11.1) displayed varied expressions of these proteins. In this study, a sum of 3954 DEGs were found contributing to the synthesis of disparate PTK types: 1874 were upregulated and 2080 were downregulated in the immature and maturing internodes of GXB9 compared with B9. These DEGs encode a wide range of PTKs especially the families and groups PRKAA, AMPK, MAPKKK 17, 18, PK, pyk, SYMPK, MKP1,2,4, MAPK7, SYMPK, RABEPK, CMPK2, 1, CPK, glpK, GK, MPK8, UMPK, hisIE (PK), pyk, ITPK1, PDPK1, THI80COPB2, SEC27, IPPK, SRPK3, STK23, IPMK, TP53RK, PRPK, BUD32, MPK3, GPKOW, and TAZ(thiN).

3.16 | Hormone signaling

The immature and maturing internodes showed a large amount of DEGs associated with different plant hormones between GXB9 and B9 genotypes. It was observed 404 DEGs connected to auxin: 189 were upregulated and 215 downregulated in GXB9 compared with B9. They mainly included those encoding IAA14, IAA17, IAA20, IAA26, IAA30, auxin transporter-like protein 2, ARFI, ARFB, ARFD, AIR12, SAU76, ARFW, ARFS, auxin efflux carrier component 1a, ARF15, 12, auxin-repressed 12.5 kDa protein, auxin-responsive protein SAUR40, 32, auxin response factor 4, 8, 24, 25, 7, auxin response factor, DNA-binding domain, and AUX/IAA family.

It was noticed that there were 63 gibberellin (GA)-related DEGs in the immature and maturing internodes of GXB9 compared with B9: 41 were upregulated and 22 were downregulated. These DEGs were found mainly active in GA-GID1L2, GID1, GA2-beta-dioxygenase1, 3, 8, 6, GA-regulated protein (GASA) 4, CIGR2, GA20 oxidase 3, GASA, and 6, 14 signaling molecules.

In the immature and maturing internodes, 384 DEGs were found linked to abscisic signaling hormone-like abscisic acid 8 hydroxylase 1, abscisic acid receptor PYL5, PYL4, PYL9, glycine-rich RNA-binding, abscisic acid inducible protein, abscisic acid insensitive 5 like protein 3, ABA receptor SnRK2.6, and abscisic stress-ripening protein-like 1, 2; among them, 183 were upregulated and 201 downregulated in GXB9 compared with B9.

There were 248 ethylene-related DEGs detected in the immature and maturing internodes: 149 were upregulated and 99 were downregulated in GXB9 compared with B9. These DEGs encode the proteins related to ethylene signaling, mainly including ethylene responsive TF ERF024, ERF026, ERF13, ERF014, ERF2, ERF3, ERF5, RAP2-11,13, BBM1, ABR1, PLT1, AP2-AIL1, ERF-TINY, ERF-RAP2-4, ERF-ERN1, ERF-RAP2-6, ERF060, ERF-AIL5, ERF071, ethylene insensitive 2, 3, reversion to ethylene sensitivity 1.

3.17 | Relative expression of selected genes

The genes SPS (SPS5), SuSy, CWIN, and CeS were selected for relative expression detection with qRT-PCR. The results showed that these genes expressed in the internodal tissues of both sugarcane clones, and all these genes were upregulated in GXB9 whereas downregulated in B9 (data not shown). The upregulated genes showed variation in relative expression between immature and maturing internodes of GXB9 (Figure 7). The results exhibited that the expression level of SPS was significantly higher in the maturing internodes than the immature internodes, while those of SuSy, CWIN, and
SuSy cleaves sucrose and provides the glucose moiety to form uridine 5′-diphosphate glucose (UDPG) or adenosine diphosphate glucose whereas the fructose portion remains behind (Wu et al., 2017). SuSy is energetically functional in immature parts of sugarcane stalks (Goldner et al., 1991), and is negatively correlated with sucrose and positively linked with hexose levels (Verma et al., 2011). Sucrose broken down by SuSy yields subsequent metabolites which are used in numerous metabolic pathways like creation of energy, and plants with improved SuSy expression showed healthier growth, better xylem parts and cell wall girth (Stein & Granot, 2019).

Overexpression of cotton SuSy gene enhanced C-partition in direction of cellulose biosynthesis and altered the cell wall crystallinity in poplar (Coleman et al., 2009). The flower and fruit growth was negatively affected by less cellulose, starch, and hexose contents due to the downregulation of SuSy gene in cucumber (Fan et al., 2019). In this study, upregulation of SuSy genes was detected in the immature internodes of GXB9 compared with B9, which perhaps contributed to the growth and development of the growing tissue, and that is why immature stalks have no noteworthy sucrose storage. The qRT-PCR analysis of SuSy verified the same pattern. In the maturing internodes, SuSy genes were downregulated, and this was negatively correlated with the higher sucrose content in cane in GXB9.

Cell wall invertase (CWIN1: EC3.2.1.26) is the key enzyme in sucrose metabolism which catalyzes the irreversible hydrolysis of sucrose into hexoses (glucose, fructose) and consecutive ingress of hexoses with assistance of sugar transporters into cells of growing tissues. This seems to be critical for proper metabolism, development, and differentiation of plant cells. Furthermore, CWIN also has numerous pleiotropic functions like response to biotic and abiotic stresses, sugar signaling, development of flower, fruit, seed, meristematic tissue, and environmental indications (Li et al., 2012; Proels & Hückelhoven, 2014; Ru et al., 2017; Su et al., 2018). The alkaline/NI expression has been described in immature and maturing tissues of sugarcane where it is positively correlated with hexoses concentration and negatively correlated with sucrose accumulation (Rossouw et al., 2007). In this study, the DEGs encoding CWIN1 and A/NINV were upregulated in the immature internodes while downregulated in the maturing internodes of GXB9 compared with B9. Analysis of CWIN through qRT-PCR also showed higher expression in immature internodes, which supported the transcriptomic results. Obviously, the downregulation in the maturing internodes is beneficial to the sucrose synthesis and accumulation in the stalks of sugarcane.

SPS (EC2.4.1.14) is a significant enzyme that has energetic contribution to sucrose synthesis from UDPG and fructose-6 phosphate in different species of plants (Ma et al., 2020; Wind et al., 2010). Moreover, SPS is also connected to numerous valuable agronomic characters including increased plant height and yield (Ishimaru et al., 2004; Sarquís et al., 1998). Numerous SPS studies in sugarcane unveiled that the transcriptomic expression of SPS genes was higher in mature internodes than immature internodes (Verma et al., 2011). Grof et al. (2006) described that A, B, C, and D families of SPS are present in sugarcane, which have varied expression in stalks of sugarcane throughout sugar accumulation. In this study, the DEGs encoding SPS1, SPS2, SPS4, and SPS5 were upregulated in the immature and maturing internodes of the high sucrose sugarcane mutant GXB9 compared with B9 at sugar accumulating stage. It has been reported that overexpression of SPS1 gene along with sucrose enhancement has also contributed to other agronomical traits in sugarcane (Anur et al., 2020). Expression of SPS4 gene in stem of sugarcane was found positively affecting the growth and development of cane stalk as well as sucrose accumulation (Park et al., 2008). The activities of SPS2 and SPS5 were recorded in the internodes of high sweet sorghum than grain sorghum (Ghate & Bhargava, 2017). In this study, SPS genes were upregulated in the internodes of GXB9 and downregulated in B9. The relative expression of SPS in qRT-PCR analysis was also higher and upregulated in the internodes of GXB9 than in B9, and maturing internodes had significantly higher SPS expression level as compared with immature internodes. The result of upregulation of SPS gene had positive correlation with the higher sucrose accumulation in the internodes, which was consistent with previous reports (Grof et al., 2006).

Cellulose synthase catalyzes the biosynthesis of cellulose which is a chief constituent of plant cell wall. This enzyme functions mainly in algae and plants. Cellulose is composed of glucose linear polymer attained from an activated sugar
donor UDPG which is available as a result of sucrose breakdown by SuSy (Coleman et al., 2006, 2009, 2010; Geisler-Lee et al., 2006; Nishiyama et al., 2003). In an alternative opinion, glucose precursor could not be gained entirely from the sucrose in stalk, and possibly will thus be supplied by the depolymerization of cell wall, enhanced photosynthesis production, or other metabolic pathways, so sucrose content is not affected particularly in mature internodes (Kawaoka et al., 2006; Liu et al., 2015; McCarthy et al., 2009). In this study, the upregulation of CesA complex in the immature internodes reflected a sign of plentiful prerequisite of cellulose in actively growing tissues, and some DEGs encoding CesA were slightly upregulated in the maturing internodes of GXB9 compared with B9, signifying that cellulose synthesis was still going on to make the tissue fully matured.

Pectinesterases catalyze the demethylation of pectin and influence the biological structure of plant cell wall (Moustacas et al., 1991). They also order various growth processes, like fiber and pollen formation, fruit ripening, plant pathogen interactions, and vegetative reproduction (Pelloux et al., 2007). In this study, abundant DEGs associated with pectinesterase encoding were found in the mature and immature internodes of GXB9 compared with B9, and they were predominantly upregulated in the immature internodes and downregulated in the maturing internodes. It might suggest that they have possible connection to the internode development and fiber formation during internode elongation in sugarcane (Oikawa et al., 2010).

Lignin is important in the formation of cell walls, which fills the spaces in the cell wall between cellulose, hemicellulose, and pectin components. In immature internodes of sugarcane, lignification starts first and lignin incorporation in other tissues occurs later (Casu et al., 2007). In this study, the immature internodes showed a greater number of DEGs encoding the proteins for lignin biosynthesis compared to the maturing internodes and the numbers of upregulated DEGs were more in immature internodes than maturing internodes.

Sugarcane has elevated sucrose accumulation in stalks. Sucrose is mainly formed in leaves by photosynthesis and donated to consuming and storing sinks, but the mechanism of sucrose translocation and accumulation between sources and sinks is yet not very clear. This transportation is aided by sucrose transporters and SWEET proteins. Overexpression of unigenes linked to transporters and SWEETs is positively interrelated with unloading of sucrose and sink strength (Cheng et al., 2018; Julius et al., 2017; Leggewie et al., 2003; Li et al., 2017; Rosche et al., 2002). It was reported that SWEETs are adhered in the sucrose movement across cell membrane in Lotus japonicas (Sugiyama et al., 2017), Sorghum (Mizuno et al., 2016), and Arabidopsis (Chen et al., 2012). SWEETs enable pathogen nutrition via sugar efflux, and nectar secretion is also reliant on alliance between SWEET and sucrose synthesis catalyzed by SPS (Chen et al., 2010; Lin et al., 2014). It has recently affirmed that N-terminal truncated SPS expresses noteworthy activity disregarding regulation through allosteric effectors (Sawitri et al., 2016). So, it is presumed that by using management approaches to regulate the expressions SWEETs and SUT genes, and boosted SPS activity, sucrose content in cane could be further improved in sugarcane. Furthermore, N-terminal removed SPS would be the goal of research and breeding in future to develop sugarcane varieties with higher sucrose content (Anur et al., 2020). The differential expression of SUTs infers that various SUTs are vigorous in sucrose accumulation in sugarcane.

Trehalose is a disaccharide made of two glucose molecules, and trehalose-6-phosphate (T6P) is its metabolic precursor (Paul et al., 2018). The T6P is obtained from a reaction between UDPG and G6P in the presence of T6P synthase (TPS), which is lastly transformed into trehalose catalyzed by a T6P phosphatase (TPP; Cabib & Leloir, 1958). Eventually trehalose is sliced into two glucose molecules by trehalase (Elbein et al., 2003). Corresponding to the specific needs and circumstances of discrete tissues, T6P functions as signal transduction and negative feedback regulator of sucrose in source leaves. It affects sucrose concentration in plants mostly through manipulating sucrose biosynthesis. In sink tissues, it is active in sucrose consumption for growth and development including embryo development and leaf senescence (Figueroa & Lunn, 2016). Both TPS and TPP genes are existing in species of all plant taxa (Avonce et al., 2006, 2010; Lunn, 2007; Lunn et al., 2014; Oszvald et al., 2018). In this study, downregulation of the DEGs encoding the proteins involved in trehalose synthesis in the immature and maturing internodes of GXB9 compared with B9 suggests that the expression of trehalase gene was negatively correlated to the sucrose accumulation in sugarcane.

The growth and development of plants frequently face biotic and abiotic stresses such as severe cold, pathogen, drought, and salinity. Numerous intricate metabolic regulatory mechanisms support the plants to resist in the adverse environments through physiological fluctuations driven at molecular level. It was stated that WRKY TFs have extensive responses to different stresses, including drought (Laxa et al., 2019), water logging (Hsu et al., 2013), and cellulose and lignin biosynthesis (Wang et al., 2010; Wang, Yang, et al., 2016; Wang, Duan, et al., 2016; Zhao et al., 2010). All these conditions affect crop growth, development, sugar signaling, sucrose metabolism, and product quality (Chen et al., 2017; Zandalinas et al., 2018). NAC TF family has also various roles, including protection from pathogens via hypersensitive responses (Yuan et al., 2019). MYB is a diversely operational TF group contributing to stress response, cell morphogenesis, protein organization, DNA binding, protein–protein interaction, and sucrose storage (Ambawat et al., 2013; Roy, 2016). AP2/ERF TFs have also been described to be involved in plant response to abiotic stresses such as temperature,
drought, and salt stresses (Niu et al., 2020; Sakuma et al., 2002). The bZIP TFs are sensitive to shifts in nutrients, abiotic stresses, and sucrose signaling (Kang et al., 2010). MADS-box gene seems to be related to plant development processes and responses to oxidative stress and environmental stresses such as salt and drought (Castelán-Muñoz et al., 2019). The transcripts encoding C_{2}H_{2} zinc finger TFs are also participating in secondary cell wall synthesis (Hu et al., 2017). In the present study, a large number of DEGs involved in TF synthesis were found in GXB9 compared with B9, reflecting TFs might play important roles in the growth and sucrose accumulation in sugarcane.

Plant PTKs work in amalgamation with growth controllers, nutrient signaling pathways and influence cell cycling and proliferation. CDPK, MAPK, CIPKs, and CBLs are usually linked to many stresses and sugar signaling in plants (Hu et al., 2017). SWR1 and SWI2/SNF2 are involved in plant development and response to environmental variations including biotic and biotic stresses (Crozet et al., 2014). AMPK involved in glucose balance (Dolphin, 2012). The alliance between T6P and SnRK1 (SNF1/AMPK) meaningfully affect the control of carbon division and consumption in plants (Paul et al., 2018). SnRK1 plays also a significant role in the acclimation of plants to an extensive range of circumstances (Hao et al., 2003; Lee et al., 2009; Solomon et al., 2010). In the current study, MAPK kinase member SNF1 (SnRK1_1,2) was downregulated in the maturing internodes of GXB9 compared with B9, which suggested a negative regulation on sucrose accumulation in different sugarcane genotypes. Previous studies have proven the negative expression of CDPK with high sucrose content in plants (Fedosejevs et al., 2016).

Plant growth hormones have been proven to be closely connected to sugar signaling in mutants of Arabidopsis (Ljung et al., 2015). In the present study, the DEGs encoding auxin-related enzymes were much more dynamic in the immature internodes compared with maturing internodes. It is stated that auxin signaling is linked with plant cell expansion, which proposes to enable higher sucrose accumulation. Members of SAURs group are effective in growing young tissues serving growth and development through auxin-induced acid (Stortenbeker & Bemer, 2019). Aldehyde dehydrogenases are involved in detoxification, abiotic stresses, and a diversity of functions (Kotchoni et al., 2006). GA_{2}-oxidase has a function in regulation of plant growth (Sakamoto et al., 2001). ABA has constructive effects on plant growth and rises sugar level in sugarcane (Hayamichi, 1997). In this study, the immature internodes showed more DEGs encoding the key enzymes for auxin, ABA, GA, and ethylene synthesizes than the maturing internodes in the high sucrose sugarcane mutant GXB9. No straight sign was found that ABA, GA, and ethylene signaling act in response to significant sucrose accumulation in sugarcane stalks. Plant height was improved by application of GA on sugarcane leaves at elongation time (Qiu et al., 2019; Rai et al., 2017; Verma et al., 2017; Wu et al., 2010). The application of ethephon on leaves also improved cane yield and sucrose content in sugarcane (Abberton et al., 2016; Li et al., 2007). These hormones along with growth and development contribute in sucrose signaling, too, and their roles in sucrose accumulation would be the emphasis of forthcoming research.

### 5 | CONCLUSION

The current research explored two sugarcane genotypes with similar genetic background, a high sucrose mutant GXB9 and its low sucrose mother genotype B9, to identify the DEGs linked to sucrose metabolism and accumulation in the immature and maturing internodes. It was found that the DEGs encoding SPS proteins SPS1, SPS2, SPS4, SPS5 were upregulated in GXB9 compared with B9, signifying that the SPS gene might have an important role to increase the sucrose accumulation capability in sugarcane. Comparative downregulation of the genes SuSy, CWIN and A/NINV, little expression of cellulose synthase gene, and mostly downregulation of trehalose pathway genes in the internodes of GXB9 also correlated with the higher sucrose accumulation in the stalk. The variation of SNP observed in this study also proposed that there were numerous low effect regulatory single nucleotides in sugarcane. Based on the results of this study, it is suggested to adapt a collective variation-based approach for genetic enhancement evaluation in sugarcane.

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### AUTHOR CONTRIBUTION

Qaisar Khan, Jiao-Yun Chen, Xu-Peng Zeng, Ying Qin, Amir Mahmood, and Qiang Liang performed the experiment and did bioinformatics analysis. Li-Tao Yang, Xiu-Peng Song, Yong-Xiu Xing, Dao-Jun Guo, and Yang-Rui Li supervised the project. Qaisar Khan, Li-Tao Yang, Xiu-Peng Song, Yong-Xiu Xing, Dao-Jun Guo, and Yang-Rui Li designed the study. Qaisar Khan, Xiu-Peng Song, Yong-Xiu Xing, and Yang-Rui Li participated in writing the manuscript. All authors have read and agreed to the published version of the manuscript.
DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available in the Supporting Information of this article.

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SUPPORTING INFORMATION

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