Comparative transcriptome analysis of two sugarcane varieties in response to diazotrophic plant growth promoting endophyte Enterobacter roggenkampii ED5

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1. Introduction

Sugarcane (Saccharum officinarum L.) is an important sugar and energy crop, accounting for more than 70% of the global sugar production (Brumbley et al. 2009; Li and Yang 2015). Sugarcane production requires a large amount of fertilizer, especially nitrogen (N) (Bokhtiar et al. 2005). Whereas the continuous application of chemical fertilizers and pesticides leads to contaminated agricultural land and environmental pollution (Srivastav 2020; Raheem et al. 2020; Sharma and Singhvi 2017; Wang et al. 2018). Plant growth-promoting bacteria (PGPB) can improve the soil micro-ecological environment and have various plant growth promoting functions such as ammonia production, nitrogen fixation, biocontrol, resistance to abiotic stress, secretion of plant hormones, and siderophore (Jha and Saraf 2015; Kuan et al. 2016; Mehmood et al. 2018; Arora and Verma 2017; Mhatre et al. 2019; Kumar et al. 2018). The application of PGPB in agricultural production can significantly reduce the application of chemical fertilizers and pesticides. Previously, some PGPB were isolated from sugarcane rhizosphere soil and plant tissues, including Bacillus xiamensis, Klebsiella pneumoniae, Burkholderia anthina, Kosakonia radicicantans, Stenotrophomonas maltophilia, Pseudomonas, Enterobacter cloacae, Streptomyces chartreusis, and Klebsiella variicola (Xia et al. 2020; Bhardwaj et al. 2017; Malviya et al. 2020; Singh et al. 2020a; Singh et al. 2020b; Li et al. 2017; Safirzadeh et al. 2019; Wang et al. 2019; Lin et al. 2015).

Biological nitrogen fixation (BNF) is a vital function of some PGPB to enhance plant growth, which reduces dinitrogen of the atmospheric air to ammonia and provides nitrogen to the plants for proper growth (De Bruijn 2015). Using the nitrogen-fixing potential of PGPBs is an effective way to reduce the application rate of chemical nitrogen fertilizer and improve the nitrogen utilization efficiency and yield of sugarcane. In this study, we investigate the molecular mechanism of the interaction between diazotrophic plant growth-promoting endophytic bacterium (DPGPEB) E. roggenkampii ED5 and sugarcane. This reported strain was previously isolated from Saccharum sinense and showed high nitrogenase activity along with various plant growth-promoting (PGP) and biocontrol properties (Guo et al. 2020). Additionally, the nitrogen fixing and PGP genes were predicted by whole gene sequencing (WGS) and proved E. roggenkampii ED5 was able to enhance the plant growth, and improve the agronomic traits and photosynthetic leaf gas exchange capacity in sugarcane under greenhouse experiment (Guo et al. 2020). Hence, E. roggenkampii ED5 has...
significant application potential in sugarcane production. However, the molecular mechanism of the interaction between *E. roggenkampii* ED5 and sugarcane is unclear.

RNA-seq technology is effective for studying gene functions and expressions at the transcriptional level, and revealing the molecular mechanism of specific biological processes (Kukurba and Montgomery 2015; Stark et al. 2019; Simoneau et al. 2021). Earlier, transcriptomics technology was mainly used to explore the interaction mechanisms between microorganisms and plants in response to water-deficit, root exudates, and biotic and abiotic stresses (Zhao et al. 2020; Zafar-ul-Hye et al. 2019; Fan et al. 2012; Gao et al. 2020; Khan et al. 2020; Moradi et al. 2021). Transcriptome microarray analysis found that some differentially expressed genes (DEGs) were enriched in auxin regulatory metabolism and plant defense response in *Arabidopsis thaliana* inoculated with *Bacillus subtilis* FB17, to enhance the plant stress resistance (Lakshmanan et al. 2013). Inoculation with *Bacillus subtilis* RR4 inhibited the transcription of the genes encoding defense response enzymes, cell wall modifying enzymes, transport, and secretion of phytochemicals, indicating that PGPB could regulate plant gene expression to promote its colonization and plant growth in rice (Rekha et al. 2018). However, limited information has been found for exploring the molecular interaction mechanism between PGPB and sugarcane using RNA-seq technology.

In the present study, two sugarcane varieties with different nitrogen-fixing abilities were grown and the seedlings were inoculated with *E. roggenkampii* ED5 at greenhouse conditions. Based on the Illumina Novaseq 6000 sequencing platform, the Illumina PE library was constructed for 2×150 bp sequencing, and all the mRNAs from sugarcane leaves were sequenced after inoculation. The transcriptomic data were analyzed to explore the DEGs and their functions using bioinformatics approaches, to reveal the molecular basis of *E. roggenkampii* ED5 interaction with sugarcane. To our best knowledge, this is the first study of the molecular mechanism of interaction between *E. roggenkampii* ED5 and sugarcane-based on transcriptomic analysis, which would provide a reference for the commercial application of *Enterobacter roggenkampii* ED5 in the near future.

### 2. Materials and methods

#### 2.1. Plant materials and bacterial inoculation

Two sugarcane varieties (GT11, which requires a high concentration of nitrogen for growth; and B8, which requires a low concentration of nitrogen for growth) were used in this study. The seedcane was cut into segments and grown in trays with sand as described by (Li et al. 2017a). The bacterial strain *E. roggenkampii* ED5, isolated from *Saccharum sinense* roots and exhibited in *vitro* higher nitrogenase activity (Guo et al. 2020), was used for inoculation. The experiment was conducted in the greenhouse of Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences (GXAS), Nanning, Guangxi, China. The soil was taken from the field and autoclaved for 30 min under 121 °C. The 18 cm high pots with 28 and 25 cm in upper and lower diameter were used for plant culture. The strain *E. roggenkampii* ED5 was incubated in Luria–birtani (LB) broth for 48 h and centrifuged, and diluted into 1×10⁶ CFU mL⁻¹ with sterilized water. The sugarcane seedlings were soaked in the bacterial solution for 1 h and then transplanted in the pots. The plants soaked in sterile water were used as the control.

#### 2.2. Colonization of *E. roggenkampii* ED5 in sugarcane variety GT11

Green Fluorescent Protein (GFP) was used to detect the colonization of *Enterobacter roggenkampii* ED5 in sugarcane plant tissues, *i.e.* roots, stems, and leaves. The pPROBE-Ptet-7T plasmid having GFP gene and the plantlets of sugarcane variety GT11 were provided by Sugarcane Research Institute, GXAS. The strain ED5 and plasmid vector were incubated at 32 °C (48 h) in an orbital shaker at 160 rpm, then mixed (1:2 ratio) and continued to culture for 48 h under the same conditions. The cells were centrifuged at 6,000×g for 5 min and the supernatant was discarded, and then added with sterile water. The sugarcane plantlets were put into a flask with the bacterial suspension and kept under light at 30 °C for 72 h. The plant tissues were cut into small pieces by blade and observed under a confocal laser scanning microscope (CLSM). The ED5 colonization in sugarcane tissues was assessed according to Singh et al. (2020).

#### 2.3. Extraction of RNA

Total RNA was isolated from the plant leaves at 30 days after inoculation with strain ED5 using TRIzol® Reagent (plant RNA purification reagent for plant tissue) as per the manufacturer’s guidelines (Invitrogen, Carlsbard, CA, USA), and genomic DNA was removed with TaKara DNase I (Biotechnology, Dalian, China). The integrity and purity of the total RNA quality were detected by 2100 Bioanalyser (Agilent Technologies, Inc., Santa Clara, CA, USA) and measured using the ND-2000 (NanoDrop Thermo Scientific, Wilmington, DE, USA). 15 μg of high-quality RNA samples (RIN value ≥ 8.0, OD260/280 ≥ 1.8, OD260/230 ≥ 1.0, 28S:18S ≥ 1.0) were used to construct the sequencing cDNA library.

#### 2.4. Library preparation, and Illumina NovaSeq 6000 sequencing

The RNA library was established using the TruSeq TM RNA sample preparation kit (Illumina, San Diego, CA, USA). Initially, magnetic beads with Oligo(dT) were used to enrich the mRNA with poly-A tail from 5 μg of total RNA. A six-base random primer (Invitrogen, CA, USA) and the template mRNA were used to reversely synthesize the one-strand cDNA, and the two-strand cDNA synthesis was performed by using SuperScript double-stranded DNA synthesis kit (Invitrogen, CA, USA) to form a stable double-strand structure. The double-stranded cDNA structure had a sticky end. End Repair Mix was added to make a blunt end, and then a base was added to the 3’ end to connect to the Y-shaped linker. After the cDNA was purified by PCR, a 200–300 bp band was retrieved by electrophoresis using 2% agarose gel. After being quantified by TBS380 (Picogreen, CA, USA), the library was used for Illumina NovaSeq 6000 sequencing platform for high-throughput sequencing. The sequencing read length is PE 150.
2.5. De novo assembly and annotation

The raw paired-end readings were trimmed and the quality was regulated with default parameters using SeqPrep (https://github.com/jstjohn/SeqPrep) and Sickle (https://github.com/najoshi/sickle). Trinity (Version v2.8.5, http://trinityrnaseq.sourceforge.net/) was used to perform de novo assembly of the clean data of the samples (Grabherr et al. 2011). All the transcripts obtained by transcriptomic sequencing were compared with six major databases, Swiss-Prot, Pfam, Non-Redundant Protein Sequence (NR), Cluster of Orthologous Groups of Proteins (COG), Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). The annotation information in each database was obtained, and statistical analysis on the annotation status was performed.

2.6. Differentially expressed genes and functional enrichment analysis

Differentially expressed genes (DEGs) between treatment and control group were identified with DESeq2 (Version v2.8.5, http://bioconductor.org/packages/stats/bioc/) software after obtaining the read counts of genes. The transcripts per million reads (TPM) method was employed to calculate the expression level of each transcript. The DESeq2 with Q value ≤ 0.05, DEGs with |log2FC|>1 were considered to be significant for differentially expressed genes. In addition, GO and KEGG analyses were performed to analyze the functional enrichment. A Bonferroni-corrected P-value of 0.05 was used to determine which DEGs were significantly enriched in GO terms and the metabolic pathways as compared to the whole-transcriptome background. KEGG pathway and GO KOBAS were carried out for functional enrichment analysis (http://kobas.cbi.pku.edu.cn/home.do) and Goatools (https://github.com/tanghaibao/Goatools) (Chen et al. 2011).

2.7. Quantitative real-time-PCR

Quantitative real-time polymerase chain reaction (qRT-PCR) was performed to confirm the transcripts obtained from high-throughput sequencing. The total RNA was isolated by TRizol1 (Cinow Biosciences, Beijing, China) and NanoDrop 2000 was used to assess the RNA quality. The TAKARA PrimeScriptTM RT reagent kit (Biotechnology, Dalian, China) was used for cDNA synthesis. SYBR Premix Ex TapTM II was used for qRT-PCR with LightCycler1480 II (Roche Applied Science, Germany) following the PCR conditions described by Zhu et al. (2021). The 2^(-ΔΔCt) procedure was performed to analyze the relative expression levels of genes (Livak and Schmittgen 2002). The primers for the internal reference gene (Glyceraldehyde-3-phosphate dehydrogenase, GAPDH) and candidate genes were designed and produced by Tsingke Biotechnology (Nanning, China) using Primer 5.0 software (Premier, Canada), they were presented in Table S1.

3. Results

3.1. Colonization of E. roggenkampii ED5 in sugarcane

Fluorescent confocal microscopy images showed that strain E. roggenkampii ED5 labeled with GFP had been successfully colonized in sugarcane plant tissues such as roots, stems, and leaves (Figure 1).

3.2. De novo assembly and transcriptome sequencing

Illumina NovaSeq 6000 platform with pair-end sequencing techniques were used to examine the response of two sugarcane varieties GT11 and B8 to E. roggenkampii ED5 inoculation at the transcriptome level. We performed RNA-Seq for the RNA isolated from leaves of both inoculated and non-inoculated sugarcane plants. In this study, a total of 113125765 bp of unigenes were obtained from de novo assembly of clean reads, including 129781 unigenes. The unigenes ranged in length from 201 to 17710 bp with an average of 871.67 bp. The N50 and E90N50 lengths of the unigenes were 1574 and 2798 bp, respectively. The fragments mapped and GC percent of unigenes was 64.68 and 48.01%, respectively (Table S2).

3.3. Functional annotation of unigenes

Sugarcane cultivars lack a reference genome. Unigene annotation in GO, KEGG, COG, NR, Swiss-prot, and Pfam public databases indicated that 47991 unigenes (36.98%) were annotated in GO, 19820 (15.27%) in KEGG, 53781 (41.44%) in COG, 32742 (25.23%) in SwissProt, 34311 (26.44%) in pfam, and 59916 (46.17%) in NR (Figure S2).

3.4. Species homology distribution

BlastX search was performed to analyze the species homology for all the assembled unigenes in the NR public database. The results showed that 29788 (49.29%), 10579 (17.50%), 3011 (4.98%), 2761 (4.57%), 2189 (3.62%), 1982 (3.28%), 1642 (2.72%), 1569 (2.60%), 1310 (2.17%), 1107 (1.83%), 423 (0.70%), 331 (0.55%), 297 (0.49%), 251 (0.42%), and 3198 (5.29%) unigenes matched to Sorghum bicolor, Zea mays, Panicum miliaceum, Oryza sativa, Setaria italica, Panicum hallii, Saccharum hybrid cultivar, Quercus super, Dichanthelium oligosanthes, Setaria viridis, Aegilops tauschii, Brachypodium distachyon, Hordeum vulgare, Oryza brachyantha, and other species, respectively (Figure S3). Sorghum bicolor and Zea mays had closer homology with sugarcane, which further attested to the reliability of the assembly.

3.5. GO, COG, and KEGG annotation

The functions of all the assembled unigenes were predicted by the GO database, and the results showed that the unigenes functions were categorized into three parts, that is, biological process (BP), molecular function (MF), and cellular
component (CC). In this study, a total of 198,593 GO terms with 52 subfunctional groups were annotated in the three functional categories, including BP (terms-61846, 31.14%), CC (terms-74608, 37.57%), and MF (terms-62139, 31.29%) (Figure S4).

The entire assembled unigenes from sugarcane leaves were re-edited and labeled with COG database. The results showed that all the unigenes were grouped into 23 subfunctional categories in COG. Most of the unigenes were found to have unknown functions (32565 unigenes, 55.36%) whereas the least annotation was in nuclear structure (1 unigene) (Figure S5).

To investigate the metabolic pathways, the unigenes were predicted by using the KEGG database. The top five pathways were found to be metabolism (with 11-second category), genetic information processing (with 4-second category), environmental information processing (with 2-second category), cellular processes (with 2-second category), and organismal systems (with 2-second category). Among them, the carbohydrate metabolism pathway included the most unigenes (1619) (Figure S6).

3.6. DEGs analysis

The DEGs in sugarcane leaves in response to strain ED5 inoculation were identified with DESeq2 using a gathering cut-off value of fold change. The DEGs between the treatment inoculated with strain ED5 and the non-inoculated control in the two sugarcane varieties GT11 (GE, GC) and B18 (BE, BC) were compared. The volcano plot showed that a total of 1905 DEGs (1093 up-regulated and 812 down-regulated) were identified in GT11, and 6214 (4627 up-regulated and 1587 down-regulated) were found in B8 (Figure 2).

3.7. GO enrichment of DEGs

After E. roggenkampii ED5 inoculation, the DEGs (FPKM>10) were enriched in GO terms and KEGG pathways for functional analysis. Among the significant GO terms in GT11, the DEGs mainly involved in plant resistance to biotic or abiotic stress, for example, 20 were found in ‘hydrogen peroxide metabolic process,’ 22 in ‘peroxidase activity (GO:0004601),’ 20 in ‘hydrogen peroxide catalytic process (GO:0042744),’ and 22 in ‘oxidoreductase activity, acting on peroxide as acceptor (GO:0016684),’ respectively. Some DEGs were enriched in plant growth-related metabolism, for example, 10 in ‘plant-type secondary cell wall biogenesis (GO:0009834),’ 17 in ‘plant-type cell wall biogenesis (GO:0009832),’ 22 in ‘cell wall biogenesis (GO:0042546),’ and 31 in ‘cell wall organization or biogenesis (GO:0071554),’ respectively (Figure 3(a)). For the DEGs in B8, some DEGs were found to be related to the plant disease resistance metabolic pathway, for example, 19 to ‘response to jasmonic acid (GO:0009753)’ and 17 to ‘regulation of jasmonic acid-mediated signaling pathway (GO:2000022).’ For Plant drought resistance-related terms, ‘response to water (GO:0009415)’ had 37 DEGs, and ‘response to water scarcity (GO:0009414)’ had 37 DEGs in GO enrichment. Additionally, some plant growth related DEGs were also found, for example, 16 DEGs were associated with ‘cytokinin metabolic process (GO:0009690),’ 12 with ‘photosynthesis, light
harvesting in photosystem I (PS-I) (GO:0009768). For other important GO terms, ‘secondary metabolic process (GO:0019748)’ had 35 DEGs, ‘cellular hormone metabolic process (GO:0034754)’ had 16 DEGs, and ‘secondary metabolite biosynthetic mechanisms (GO:0044550)’ had 22 DEGs (Figure 3(b)).

3.8. KEGG enrichment of DEGs

Bubble plots were used to show the significance of the DEGs enriched KEGG pathways in two sugarcane varieties (Figure 4). It showed that the 30 DEGs were involved in ‘phenylpropanoid biosynthesis (map00940),’ 21 in ‘MAPK signaling pathway-plant (map04016),’ 14 in ‘glutathione metabolism (map00480),’ and 17 in ‘Plant hormone signal transduction (map04075),’ respectively (Figure 4(a)). Similarly, some key pathways for DEGs enrichment were found in B8, for example, 15 DEGs were involved in ‘nitrogen metabolism (map00910),’ 35 in ‘starch and sucrose metabolism (map00500),’ 79 in ‘plant hormone signal transduction (map04075),’ 65 in ‘phenylpropanoid biosynthesis (map00940),’ and 60 in ‘MAPK signaling pathway-plant (map04016),’ respectively. In addition, ‘photosynthesis-antenna proteins (map01850)’ had 13 DEGs, ‘amino sugar and nucleotide sugar metabolism (map00520)’ had 34 DEGs, ‘phenylalanine metabolism (map00360)’ had 18 DEGs, and ‘carotenoid biosynthesis (map00906)’ had 14 DEGs, respectively (Figure 4(b)).

3.9. Nitrogen metabolism-related DEGs

Nitrogen utilization is essential for plant growth and development in sugarcane. In this study, it was found that, after *E. roggenkampii* ED5 inoculation, 15 DEGs (seven up-regulated, eight down-regulated) related to nitrogen metabolism were expressed in B8 while only one DEG was upregulated in GT11. Further analysis showed that, out of these nitrogen metabolism-related DEGs, five were involved in ‘glutamine synthetase,’ five in ‘nitrate reductase,’ five in ‘alpha carbonic anhydrase,’ and another gene was related to ‘hypothetical protein’ and ‘uncharacterized protein’ (Table S3.)

3.10. DEGs related to metabolism of starch and sucrose

Metabolism of starch and sucrose is the fundamental way to accumulate sugar in sugarcane. KEGG enrichment analysis found that there were 11 and 35 DEGs associated with the ‘starch and sucrose metabolism’ pathway (map00500) in GT11 and B8, respectively, after ED5 inoculation. The functions mainly included alpha-amylase isozyme, probable trehalose-phosphate phosphatase, probable trehalose-phosphate phosphatase, soluble starch synthase, glucose-1-phosphate adenyltransferase small subunit, granule-bound starch synthase, beta-glucosidase, starch branching enzyme, phosphate phosphatase, glucan endo-1,3 beta-glucosidase, and beta-amylase (Table S4).

3.11. Validation by qRT-PCR

qRT-PCR was employed to validate the accuracy of the RNA-Seq results. A total of 15 sugarcane growth-related genes were randomly selected for the analysis, including seven up-regulated, three down-regulated, and two non-changed genes. The results showed that 13 of the selected genes had similar expression patterns as identified by RNA-Seq, 2 genes (TRINITY_DN17037_c0_g1, TRINITY_DN2872_c1_g2) did not (Figure 5), reflecting the results of RNA-seq are generally reliable.

4. Discussion

In the present study, we used the RNA-seq method to analyze the molecular basis of interaction between two sugarcane varieties in response to N-fixing endophytic strain ED5 inoculation at the transcriptome level. A total of 1905 DEGs were found between treatment and control group in sugarcane variety GT11, and 6214 DEGs were identified in B8. Both sugarcane varieties showed a significant difference
in DEGs expression profiles after *E. roggenkampii* ED5 inoculation, which may be due to genetic differences between them (Yang et al. 2019). There were 15 DEGs linked to the nitrogen metabolism processes in B8, however, only one was in GT11 in this study. The nitrogen-fixing effect of PGPB on sugarcane has been confirmed by some researchers (Dong et al. 1994; Lin et al. 2009; Li et al. 2017). Additionally, it has been reported that the PGPB have different responses to different sugarcane varieties (Hari and Srinivasan 2005). In previous study, 15N dilution method was used to detect the nitrogen utilization rate of both sugarcane varieties and found that variety B8 had a higher nitrogen utilization rate and showed better nitrogen fixation capacity than variety GT11 (Ting et al. 2010), which supported the finding in this study. We found three NR-related DEGs (TRINITY_DN1933_c0_g1, TRINITY_DN2372_c0_g1, TRINITY_DN35828_c0_g1) were up-regulated after *E. roggenkampii* ED5 inoculation in variety B8. Nitrate is the main nitrogen source in plants, and nitrate assimilation is a highly regulated process (Ferrario-Méry et al. 1998). The regulation of NR activity in plants plays a vital role in regulating primary nitrogen assimilation and significantly affects the growth and development of plants (Reyes et al. 2018; Yu et al. 2017). The reduction of NR activity in plant roots will change the distribution of degraded nitrogen compounds and carbohydrates (Hänisch et al. 2001), and over-expression of the NR gene can increase the mRNA level (Vincentz and Caboche 1991). Similarly, over-expression of the NR genes can increase the mRNA level and enhance nitrogen absorption in the plant system (Rosales et al. 2012). After inoculation of *E. roggenkampii* ED5, NR-related genes were up-regulated in variety B8, indicating the strain ED5 might stimulate the activity of sugarcane nitrate reductase, which indirectly affects the sugarcane nitrogen metabolism pathway. Additionally, the present research showed that there were five DEGs related to glutamine synthetase (GS) in both sugarcane varieties GT11 and B8. Among them, two genes (TRINITY_DN118680_c0_g1, TRINITY_DN91009_c0_g1) were up-regulated and three genes (TRINITY_DN101512_c1_g1, TRINITY_DN 9631_c0_g1, TRINITY_DN2872_c1_g2) were down-regulated. NO3− and NH4+ in the soil are the main sources of plant nitrogen, and among them, NO3− must be converted into NH4+ before being assimilated. In biological nitrogen fixation, nitrogenase in nitrogen-fixing microorganisms reduces N2 to form NH3, which is further assimilated into glutamine (Gln) (Hirel et al. 2001).
Previous studies showed that the over-expression of glutamine synthetase-related genes could promote plant nitrogen utilization and growth (Oliveira et al. 2002; Fuentes 1987). The results in this study confirmed that the strain ED5 has the potential for biological nitrogen fixation in sugarcane.

Additionally, this study showed that the some DEGs in two sugarcane varieties were enriched in starch and sucrose metabolism in the KEGG database. Starch mainly functions as the unit of energy storage and the products of photosynthetic carbon assimilation in most crops; however, sucrose is the unit of energy storage (Ma et al. 2019). Eleven DEGs related to starch and sucrose metabolism pathway were involved in GT11 (four up-regulated and seven down-regulated) in our study. At the same time, 35 DEGs were enriched in B8 (25 up-regulated and 10 down-regulated). Soluble starch synthase (SSS) is a starch synthase family and plays a key role in starch biosynthesis. The non-reducing ends of glucose chains in starch synthesis are elongated at starch synthases actions (Zeeman et al. 2010; Leterrier et al. 2008). This study observed that all the four soluble starch synthase-related DEGs were up-regulated in sugarcane B8, however, only one down-regulated DEG was detected in GT11 after inoculated strain ED5, suggesting that strain ED5 has different effects on the soluble starch synthase pathway in different sugarcane varieties. For β-amylase related DEGs, we found another type of starch and sucrose metabolism genes in sugarcane variety B8 but none in variety GT11. Previous studies reported that β-amylase is related to sugar production of sugarcane and abiotic stresses (Ukoskit et al. 2019; Nawae et al. 2020). The active center of β-amylase contains at least three special gene groups X, A, and B. These gene groups participate in the combination of enzyme and substrate and involve in the reaction process of enzyme–substrate complex conversion into a product (Dicko et al. 1999; Kaplan et al. 2006). The results in this study showed that the strain ED5 had an impact on sugarcane starch and sucrose metabolism.

Additionally, the DEGs related to trehalose-phosphate phosphatase, granule-bound starch synthase, β-glucosidase, and glucan endo-1,3 β-glucosidase were also found between the two sugarcane varieties. These enzymes are vital in sugarcane starch and sucrose metabolism, and they have an indirect impact on sugarcane growth (Du et al. 2000). Using PGPB to promote plant resistance to biotic or abiotic stress is an effective way to develop ecological agriculture. The present study showed that the E. roggenkampii ED5 could induce sugarcane to produce these hydrolytic enzymes which are helpful to resist various abiotic stresses in vitro conditions. Whole-genome analysis of this strain confirmed the presence of different coding genes (CDS) related to biotic or abiotic tolerance (Guo et al. 2020).

Some DEGs between the two sugarcane varieties were observed to be linked to the MAPK signaling pathway-plant, phytohormone signaling, biosynthesis of phenylpropanoid, and photosynthesis. Some reports showed that these metabolic pathways would be changed in plants under biotic or abiotic stresses (Danquah et al. 2014; Vogt 2010; Zhu et al. 2021). However, E. roggenkampii ED5 was isolated from healthy sugarcane plants, and DEGs were enriched in these pathways, indicating strain ED5 enhanced the sugarcane immune response.

In this study, phenylpropanoid biosynthesis (KEGG, map00940) related DEGs were enriched in both sugarcane varieties. The biosynthesis of the phenylpropanoid pathway will be changed under biotic or abiotic stresses (Geng et al. 2020). The phenylpropanoid biosynthesis pathway plays an important role in plant development and in response to adverse conditions such as improving plant disease resistance, avoiding plant damage from stresses, and serving as a signal transduction molecule (Xu et al. 2014; Colquhoun et al. 2011). For different varieties, however, 30 DEGs of the phenylpropanoid biosynthesis pathway were enriched in GT11 and 65 in B8, which reflected the genetic variance in different sugarcane varieties.

Mitogen-activated protein kinase (MAPK) signaling pathway-plant (map04016) was found to be enriched in both sugarcane varieties in this study. MAPK signaling pathway is an important pathway for plants to respond to stresses (Xu and Zhang 2015). The MAPK cascade pathway transmits signals through MAPKKK→MAPKK→MAPK phosphorylation step by step. Then the downstream products (cytoskeleton proteins, protein kinases, apoptosis factors, nuclear receptors, phospholipases, and transcription factors) are phosphorylated by MAPK. They regulate the expression of corresponding genes and prompt plants to respond to

![Figure 5. Validation of 15 selected differentially expressed genes by qRT-PCR. Error bar represents the SD.](image-url)
adversity (Zhang et al. 2018). In addition, MAPK has also transmitted signals through interaction with ethylene, auxin, jasmonic acid, abscisic acid, and phospholipid signaling pathways (Zhang et al. 2018). In this study, 21 DEGs related to the MAPK signaling pathway in sugarcane variety GT11 and 60 in B8 were detected after ED5 inoculation, which suggested that ED5 would open the immune response system of sugarcane against stresses.

In our previous study, whole-genome analysis of *E. rogenkampii* ED5 showed the presence of some key plant growth-promoting genes such as nitrogen metabolism, siderophore, plant hormones, synthesis of resistance inducers, root colonization, biofilm formation, oxidoreductase, cold-shock protein, heat shock proteins, heavy metal resistance, and drought resistance in its genome. In addition, it was found that all growth parameters in GT11 were significantly enhanced after ED5 inoculation (Guo et al. 2020). We speculated that strain ED5 promotes sugarcane growth in multiple ways. Previous studies also showed that PGPB promotes crop growth in various ways (Goswami et al. 2016; Basu et al. 2021). Our findings also confirmed that strain ED5 has multiple pathways in interaction with sugarcane at the transcriptome level. The transcriptomic DEGs enrichment pathways in sugarcane variety B8 were significantly more than GT11 after *E. rogenkampii* ED5 inoculation might be due to the genetic differences between the two sugarcane varieties.

5. Conclusions
This study used RNA-seq method to compare the key gene expressions in two sugarcane varieties GT11 and B8 at transcriptome level after DPGPEB *E. rogenkampii* ED5 inoculation. Some DEGs were found in the ED5 inoculation treatment as compared to control. These DEGs were enriched in starch, sucrose, and nitrogen metabolism, phenylpropanoid biosynthesis, phytohormone signaling transduction, MAPK signaling pathway-plant, secondary metabolic process, cell wall biogenesis, and photosynthesis in GO and KEGG databases. These findings provided a reference for further research in the interactions between *E. rogenkampii* ED5 and sugarcane.

Disclosure statement
No potential conflict of interest was reported by the author(s).

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Author contributions
DJG, RS, PS, and YRL designed the experiments. DJG, DPL, RS, PS accomplished the experiments. KKV, YQ, AS, QK, XPS, and MM analyzed the data. DJG, DPL, RS and PS drafted the manuscript. YRL and YXX critically revised the article. All authors reviewed the article and approved it for publication.

Availability of data and material
All the data supporting the findings of this study are available within the article and its supplementary materials.

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