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Full Length Article

Sucrose Synthase Genes Showed Genotype-Dependent Expression in Sugarcane Leaves in the Early Stage of Growth

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Abstract

Sucrose synthase (SuSy) is one of the key enzymes regulating sucrose metabolism in plants. There are at least five different SuSy genes exist in sugarcane, and their biological functions are not fully understood. In this paper, the phylogenetic analysis of all plant *SuSy* genes published to-date (109) has been grouped into three classes: SuSyI, SuSyII and SuSyIII. SuSyI was further divided into monocot and eudicot SuSyI, indicating their independent evolutionary trajectory paralleling monocot and dicot divergence. The leaf total sugar content of high-, medium- and low-sugar sugarcane genotypes varied at the early stage of plant growth. However, there was no strong correlation between early-stage leaf sucrose content and the final stem sugar content and sugar yield of mature crop. The proportion of leaf sucrose and fructose content in most sugarcane genotypes in the early stage of growth was about 30% each, and that of glucose was about 40%. The SuSy enzyme activity and gene expression of sugarcane sucrose synthase gene *ScSuSy1* and *ScSuSy4* in those genotypes also showed a remarkable variation during the same growth period. The expression profiles of these genes in high-sugar, medium- and low-sugar genotypes were complex with no clear association between their activity at the early stage of plant growth and final sugar yield of those genotypes. The biological role of *ScSuSy1* and *ScSuSy4* gene may change with sugarcane plant development, and may be involved in both the synthesis and decomposition of sucrose.

Key words: Sugarcane; Sucrose synthase; Sucrose metabolism; Gene expression; Phylogeny

Introduction

Sugarcane (*Saccharum* spp interspecific hybrids) is the most important sugar crop and the second largest energy crop in the world (FAO 2018; Available at: http://www.fao.org/faostat/en/#data/QC). Sucrose production from sugarcane accounts for about 80% of the total sugar produced globally. Sucrose synthase (SuSy) is one of the key enzymes regulating sucrose metabolism in plants (Qin *et al.* 2018). SuSy catalyzes the reversible conversion of sucrose and uridine diphosphate (UDP) to fructose and UDP-glucose (Koch 2004; Qin *et al.* 2018). Sucrose synthase thus plays dual function of sucrose synthesis and degradation, but it is generally considered as a sucrose degrading enzyme, providing precursors and substrates for many metabolic pathways, including the synthesis of various polysaccharides needed for cell wall and starch (Edurne *et al.* 2003, 2009; Takeda *et al.* 2017. There are several *SuSy* genes of different types that may exist in the same plant. For example, as many as 6, 7 and 15 *SuSy* genes were reported in *Arabidopsis* (Baud *et al.* 2004), rice (Hirose *et al.* 2008) and the tetraploid cotton (Zou *et al.* 2013), respectively. In sugarcane, at least five *SuSy* genes have been reported (Thirugnanasambandam *et al.* 2019).

By virtue of their involvement in carbohydrate metabolism, *SuSy* genes play a direct role in determining crop yield. For instance, wheat *SuSy* gene *TaSuSy2* influenced thousand kernel weight, and was positively correlated with yield (Jiang *et al.* 2011). Potato *SuSy* gene promoted transgenic cotton yield cotton through increaed fiber growth and seed production (Xu *et al.* 2012). Over-expression of *SuSy* gene significantly increased dry weight,

starch content and total yield of transgenic potato (Edurne *et al.* 2009). The potato *StSuSy4* gene increased starch content in corn seeds by 10–15% (Li *et al.* 2013). *SuSy* dynamically regulated cell division and starch accumulation, and significantly improved hull size and grain weight in transgenic rice (Fan *et al.* 2019). Further, *SuSy* genes are also related to plant quality. For instance, transgenic tomato with reduced *SuSy* gene expression grew slowly and became smaller in size (D'Aoust *et al.* 1999). The cotton sucrose synthase gene *SuSy3* affected cotton fibroblast differentiation and seed development (Ruan *et al.* 2003). Poplar (*Populus* L.) *SuSy* gene increased cellulose content in secondary cell wall and enhanced its wood density (Coleman *et al.* 2009). Poplar *SuSy* gene *PsnSuSy1* and *PsnSuSy2* increased tobacco secondary cell wall thickness, vegetative growth and mechanical strength of stem (Li *et al.* 2019). Furthermore, *SuSy* genes have been shown to be involved in plant abiotic stress response. For example, Cucumber *SuSy* gene *CsSuSy3* was implicated in hypoxia tolerance (Wang *et al.* 2014) and rubber tree *SuSy* gene *HbSuSy5* was associated with low temperature and drought response (Xiao *et al.* 2014). Tobacco sucrose synthase genes *Ntab0288750* and *Ntab0234340* regulated sucrose degradation under abiotic stress condition (Wang *et al.* 2015).

High sucrose content is the main target of sugarcane variety improvement. Elucidating the genes associated with sucrose accumulation can thus provide a molecular target for improving sugarcane varieties by molecular approaches. There are different *SuSy* genes in sugarcane, and their biological functions are not fully understood. Previous studies on the expression of sugarcane *SuSy* genes focused mainly on stem sugar accumulation during the middle and late stages of crop development. Little is known about physiological and molecular aspects of *SuSy* genes in the early stages (1–3 months) of sugarcane growth. In this study, we first conducted a phylogenetic analysis of all *SuSy* genes in plants published to-date to gain more insight on the relatedness of sugarcane *SuSy* genes. In our earlier study, the activity of sugarcane *SuSy* gene *ScSuSy4* (KM598653) in the leaves of low-sugar genotypes was found to be significantly higher than that of high-sugar clones during active stem elongation phase- i.e. at the middle stage of the crop life cycle (Chen *et al.* 2016). Because of this association we studied the activity of *ScSuSy4*, and another *SuSy* gene *ScSuSy1*, identified from our gene expression studies (unpublished data) to expand the knowledge of *SuSy* genes and their relationship with sugarcane growth and sugar accumulation. Understanding the relationship between *ScSuSy1* and *ScSuSy4* activity in leaf and crop sugar yield opens up the possibility of using them as a molecular surrogate for identifying high-sugar clones during selection. Thus, here we investigated the activity of *ScSuSy1, ScSuSy4* and sucrose synthase enzyme, along with the changes in soluble during the first 3 months of growth of sugarcane genotypes characterized by high-, medium- and low-sugar content. The results, presented below, are discussed from sugarcane growth and sugar accumulation perspective.

Materials and Methods

Sugarcane materials and experimental details

Four high-sugar commercial varieties, ROC22 (sucrose content 15%, ROC5×ROC69-463), GT42 (sucrose content 15%, ROC22×GT92-66), GT35 (sucrose content 17%, ROC23×CP84-1198) and GT28 (sucrose content 16%, CP80-1018×CP88-2032), one medium-sugar non-commercial genotype YT71-210 (sucrose content 12%, YT57-423×HN56-12) and one low-sugar non-commercial genotype GT86-877 (sucrose content 6.0%, GT82-10×GT73-11) were selected because of their similar growth habit and biomass production but with large differences in sugar content. They were planted in Dingdang Sugarcane Experiment Station (Longan County, Nanning, Guangxi), Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences (Nanning, Guangxi, China) on March 18, 2019. Each genotype was planted in rows in 5×7 meter (m) plots with 1.2 m inter-row spacing. Experimental set up followed Block Design with three replicates. Sugarcane was grown following local management practices for cultivation, pest and disease control, fertilizer application and irrigation. The youngest fully expanded leaves were collected from several plants from each replicate on May 9 (sugarcane seedling stage), May 22 (early tillering stage), June 4 (rapid tillering stage), and June 18 (early stalk emergence stage), respectively in 2019, and flash frozen in liquid nitrogen, then stored at -80°C for further use. These four stages will be collectively called as "the early stage of plant growth" hereafter. In March 2019, the maximum-minimum temperature in the Experimental Station was 23 and 16°C with 33–100% relative humidity, and those in June 2019 were 33 and 26°C with 43–100%, relative humidity.

Soluble sugar content analysis

Sucrose, glucose and fructose contents of the youngest leaves were quantified by High Performance Liquid Chromatography (HPLC) technique (Edurne *et al.* 2009). Frozen sugarcane leaf tissue was finely ground in liquid nitrogen and the powder (0.2 g) was thoroughly mixed with 5 mL of 80% ethanol in a 10 mL centrifuge tube and incubated in a water bath set at 80°C for 20 min with stirring every 5 min. The suspension was then centrifuged at 12000 rpm, 4°C for 10 min, and the clear supernatant was collected and filtered into a sampling bottle for analysis.

The HPLC used was equipped with a RID-10 differential detector and used HPLC grade water as the

mobile phase. The chromatography conditions were: 80℃ column temperature, 0.4 mL/min flow rate and 10 μL loading volume for 10 min. According to the peak area and concentration of the standard sample, the sugar content of the loading solution was calculated using the formula:

$$cU = \frac{rU}{rS} \times cS$$

Where cU sugar concentration of loading solution (μg/mL); rU is loading solution peak area; rS is standard sample peak area; cS is standard sample concentration (g/mL).

The sugar content of sugarcane samples was calculated based on the formula:

$$SC = \frac{cU \times V}{FW \times 1000}$$

Where SC is Sugar content (mg/g); cU is sugar concentration of loading solution (μ g/mL); V is volume of extracted solution (mL); FW is fresh weight of sample (g).

Assay of sucrose synthase activity

Frozen leaf tissue was shredded and ground to a fine powder in liquid nitrogen. Two grams of powdered tissue was mixed thoroughly with 10 mL extraction buffer (50 mmol/L $MgCl_2$, 2 mmol/L EDTA, 0.2 % (w/v) BSA, 2 % PVP) in a 10 mL centrifuge tube, incubated at 4°C for 30 min and centrifuged at 12000 rpm, 4°C for 15 min. The supernatant was transferred to a new centrifuge tube and precipitated with ammonium sulfate at 4°C overnight. It was then centrifuged at 15000 rpm, 4°C for 15 min and the precipitate was dissolved in 2 mL extraction buffer and transferred to a dialysis bag. After 24 h of dialysis at 4°C using a diluted (10% v/v) extraction buffer (dialysate was replaced several times during dialysis), the final protein sample was made up to 3 mL with extraction buffer. The enzyme reaction was started by adding 0.1 mL of reaction solution to 0.1 mL of dialyzed extract. The components of the reaction solution are 50 mmol/L Hepes, 15 mmol/L $MgCl_2$, 25 mmol/L fructose, 3 mmol/L uridine-5-diphosphate glucose (UDPG). There was no UDPG in the control reaction. The enzyme reaction mixture (enzyme extract plus reaction solution) was incubated at 37°C for 30 min and the reaction was stopped by boiling it for 10 min. The sucrose content in the reaction mixture was determined by measuring the absorption value at 480 nm by UV spectrophotometer (Thermo GENESYS 10S NUV-VIS) and the SuSy activity was calculated and expressed as μg sucrose produced/min/g leaf fw (fresh weight).

Expression analysis of *ScSuSy1* and *ScSuSy4* genes

The methods described by (Chen 2017, 2019) and Huang 2017 were used for *ScSuSy1* and *ScSuSy4* expression analysis. RNA for qPCR was extracted from sampled tissues using Spin Column Plant total RNA Purification Kit (Songon Biotech, Shanghai, China). DNA in RNA samples was treated by RQ1 RNase-Free DNase (Promega, USA). The first strand cDNA was synthesized using AMV Reverse Transcriptase First Strand cDNA Synthesis Kit (Life Science, Florida, USA). qRT-PCR was performed in the ABI StepOneTM Plus Real-Time PCR System with the SYBR Green PCR Master Mix (Takara), with three biological replicates for each gene and three technical repeats per experiment. Relative gene expression was normalized by comparison with the expression of sugarcane GAPDH (EF189713), and analyzed using the 2-ΔΔCT method. The primers used in this study are listed below:

ScSuSy1-qF :5'-ACAGCCAAACCAACACACACACT-3',
ScSuSy1-qR :5'-ACCCTGGTACGGTCAATGTGTG-3;
ScSuSy4-q-F :5'-GCAAGCAAGAACCAACCATAACTAACTAACTAACT-3,
ScSuSy4-q-R :5′-ACATACTTCCAGAAACTAGACC-3′;
GAPDH -F：5'-CTTGCCAAGGTCATCCATG -3',
GAPDH -R：5'-CAGTGATGGCATGAACAGTTG -3.

Sequences collection and phylogenetic analysis

The non-redundant amino acid database on NCBI website (http://www.ncbi.nlm.nih.gov) was searched with the term "sucrose synthase" and all the *SuSy* sequences from plants published to-date were obtained. The Vector NTI software was used to analyze all the sequences obtained. The repeated sequences and incomplete sequences were removed from the dataset to extract the full-length *SuSy* sequences. The phylogenetic tree was draw by the MEGA5 program with the maximum likelihood (ML) approach.

Statistical analysis

The data were processed using Microsoft Excel 2007 and, where relevant, were analysed for statistical significance by ANOVA using SPSS (22.0) statistical program.

Results

Phylogenetic analysis of *SuSy* genes in plants

Phylogenetic analysis showed that a total 109 *SuSy* genes reported in higher plants so far form three classes (types) namely, SuSyI, SuSyII and SuSyIII. SuSyI genes were further divided into monocotyledonous and dicotyledonous SuSyI, indicating their independent evolutionary trajectory following the divergence of monocotyledons and diccotyledons. There were both monocotyledonous and dicotyledonous SuSys in SuSyI and SuSyIII. Five full length *SuSy* genes, *ScSuSy1* (JX416283), *SoSuSy2* (AY118266), *SoSuSy3*, *ScSuSy4* (KM598653) and *SoSuSy5* had been identified in sugarcane (Shi *et al*. 2019). *ScSuSy1* (JX416283) and *SoSuSy2* (AY118266) were grouped in monocot SuSyI, while *ScSuSy4* (KM598653) was classified as a SuSyII memeber. *SoSuSy3* and *SoSuSy5* were grouped into SuSyIII cluster (Fig. 1).

Changes in leaf soluble sugars in different sugarcane genotypes during the early stage of plant growth

Changes in total soluble sugar content: The total soluble sugar content in the leaves of all sugarcane genotypes except GT28 and ROC22 gradually increased during the first 3 months of growth (Fig. 2). ROC22 showed a remarkable increase in total leaf sugar content at the last sampling compared to other clones. In contrast, GT28, which showed a significantly greater leaf sugar content than all other genotypes for the first 2.5 months, had the lowest leaf sugar level at the last sampling (June 18). In general there was no distinguishing pattern of leaf soluble sugar content in the studied genotypes in relation to their final sugar yield in the field, except a general upward trend for ROC22 and GT28, barring the last measurement for GT28 (Fig. 2).

Changes in different soluble sugar content: During the first three months of growth, the proportion of different soluble sugars changed considerably. The proportion of sucrose and fructose in most sugarcane genotypes was about 30% each, and that of glucose was about 40% at the first sampling when the plants were about 1.5 months old (Fig. 3). There was a general reduction in the percentage of leaf sucrose with a proportional increase in fructose for the first 2.5 months of growth in all genotypes studied except for ROC22, which showed no clear pattern of soluble sugars ratio throughout the experiment. The reduction in leaf sucrose to fructose ratio was more pronounced in the medium-sugar (YT71-210) and low-sugar (GT86-877) genotypes, but this trend was reversed at the last measurement. The sucrose content in the leaves of high-sugar genotype ROC22 increased significantly with tillering (Jun 4) and then declined (Fig. 4). However, in other genotypes studied, the sucrose content was decreased till tillering and showed a marked increase at the early stalk elongation period, except for GT35 (Jun 18). There was no significant relationship between the leaf sucrose content in the first three months of growth and the final sugar content of crop at maturity in tested genotypes (Fig. 4).

Sucrose synthase enzyme activity and gene expression in leaves of different sugarcane genotypes at early stage of plant growth

Sucrose synthase enzyme activity: The sucrose synthase enzyme activity in the leaves of GT86-877 (low-sugar), YT71-210 (medium-sugar) and ROC22 (high-sugar) genotypes was decreased significantly during the early stage of growth (Fig. 5). And the enzyme activity in leaves of low- and medium-sugar genotypes was higher than those of high-sugar genotypes when they were 2 months old but that trend turned around after a month (Fig. 5). No other distinct patterns were evident from the sucrose synthase enzyme activity data.

Expression of *ScSuSy1* and *ScSuSy4* genes: *SuSy* is an important regulatory gene in plant sucrose synthesis pathway. This study showed that both *ScSuSy1* and *ScSuSy4* genes were expressed in the leaves of all sugarcane genotypes analysed (Fig. 6; Fig. 7). *ScSuSy1* and *ScSuSy4* expression did not change much during the first 2.5 months of plant growth. However, the *ScSuSy1*enzyme activity picked up significantly in 2 of the high-sugar and the medium-sugar genotypes at the last sampling (Jun 16). The expression of *ScSuSy4* at the last sampling (Jun 16) was found declined in all genotypes except for GT42, which showed a sharp increase in activity (Fig. 6; Fig. 7).

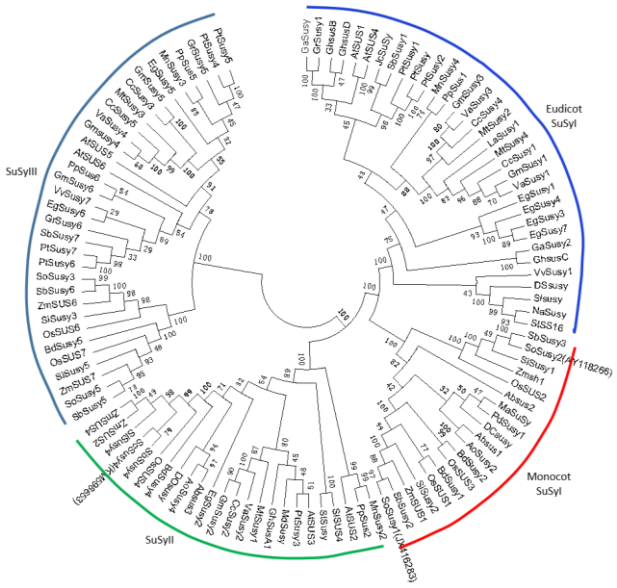

Fig. 1: Phylogenetic tree of *SuSy* genes in higher plants

Ab (*Albuca bracteata*); Ao (*Asparagus officinalis*); At (*Arabidopsis thaliana*); Bd (*Brachypodium distachyon*); Ga (*Gossypium arboretum*); Gh (*Gossypium hirsutum*); Gm (*Glycine max*); Gr (*Gossypium raimondii*); Hv (*Hordeum vulgare*); Na (*Nicotiana attenuate*); Os (*Oryza sativa*); Pd (*Phoenix dactylifera*); Pp (*Prunus persica*); Rc (*Ricinus communis*); Sb (*Sorghum bicolor*); Si (*Setaria italica*); So (*Saccharum officinarum*); St (*Solanum tuberosum*); Ta (*Triticum aestivum*); Vv (*Vitis vinifera*); Va (*Vigna angularis*); Zm (*Zea mays*)

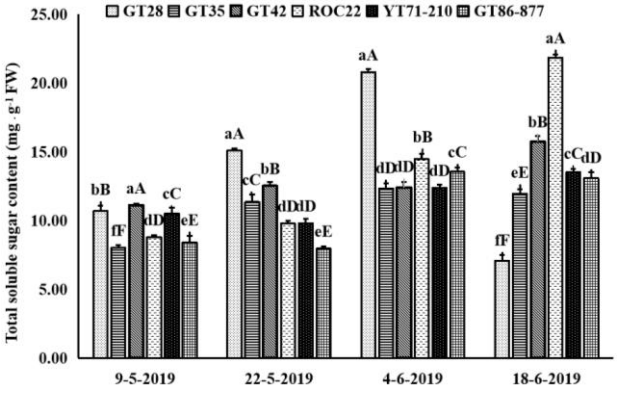

Fig. 2: Total soluble sugar content in the leaves of different sugarcane genotypes in the early stage of plant growth

Total soluble sugar = fructose + sucrose + glucose. Values presented are mean ±SD of 3 biological replicates. Different lowercase letters indicate significant difference at $P<0.05$, and different capital letters indicate highly significant difference at $P<0.01$

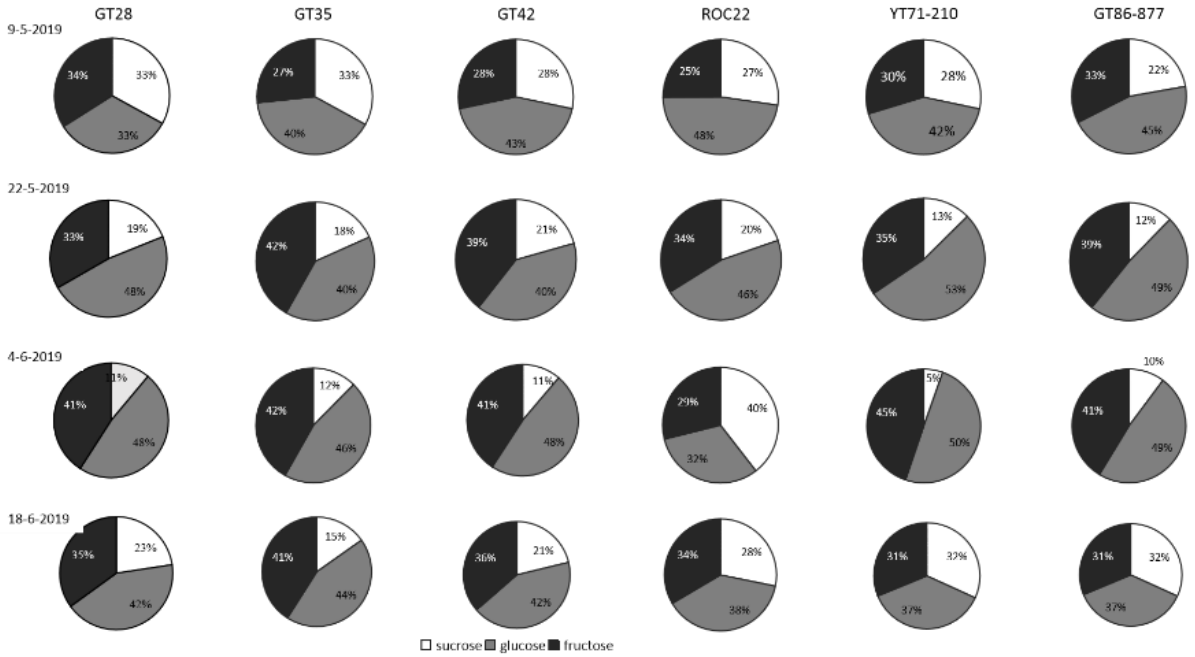

Fig. 3: The proportion of different soluble sugars in the leaves of different sugarcane genotypes in the early stage of plant growth

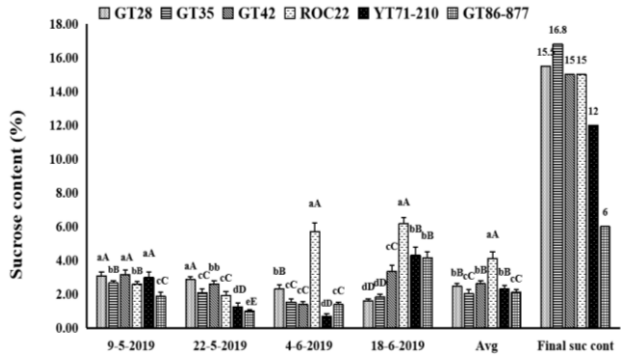

Fig. 4: Sucrose content in the leaves of different sugarcane genotypes in the early stage of plant growth

Values presented are mean ±SD of 3 biological replicates. Different lowercase letters indicate significant difference at P<0.05, and different capital letters indicate highly significant difference at P<0.01. Final suc cont: Final sucrose content of sugarcane at maturity

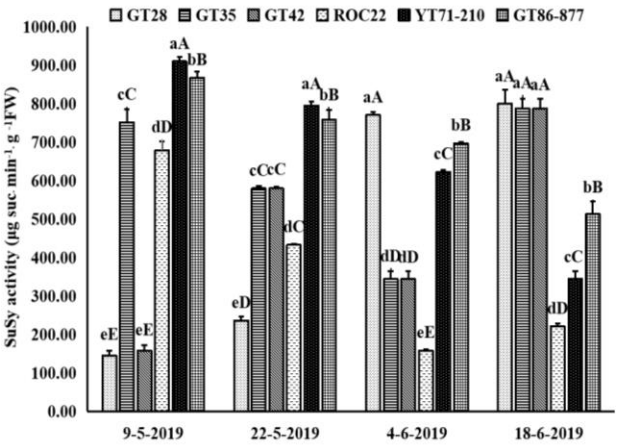

Fig. 5: Sucrose synthase (SuSy) activity in the leaves of different sugarcane genotypes in the early stage of plant growth

Values presented are mean ±SD of 3 biological replicates. Different lowercase letters indicate significant difference at P<0.05, different capital letters indicate highly significant difference at P<0.01

Correlation analysis between sucrose synthase and sugar content in leaves of different sugarcane genotypes

Data showed that in high-sugar genotypes sucrose content and SuSy enzyme activity showed both significant (P<0.01) positive and negative correlation, depending on the genotype (Table 1). There was no noticeable pattern for correlation between SuSy enzyme activity and the content of other soluble sugars or total sugars. A significant (P<0.01) positive correlation between the contents of glucose, fructose and total sugars and the expression of *ScSuSy1* in high-sugar clones GT42 and ROC22, and *ScSuSy4* in GT28 was observed. No such clear correlation was evident in medium- or low-sugar genotypes (Table 1). Significant (P<0.01) positive and negative correlation between SuSy enzyme activity and *ScSuSy1* and *ScSuSy4* expression was recorded for GT42 and GT28, respectively (Table 2). There were no correlations amongst other clones tested except for a significant (P<0.01) positive and negative correlations observed between SuSy enzyme activity and *ScSuSy4* expression in YT71-210 and GT35, respectively.

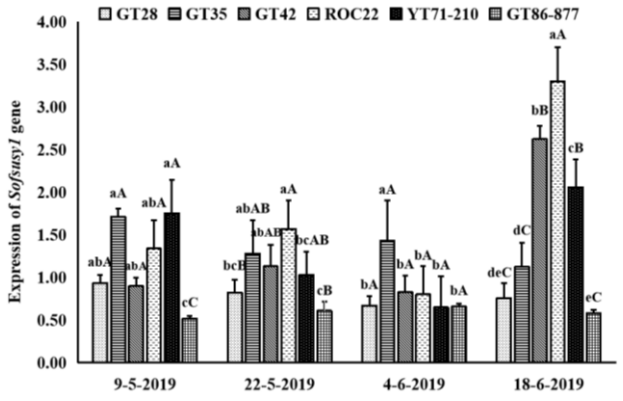

Fig. 6: Expression of *ScSuSy1* gene in the leaves of different sugarcane genotypes in the early stage of growth

Values presented are mean±SD of 3 biological replicates. Different lowercase letters indicate significant difference at P<0.05, different capital letters indicate highly significant difference at P<0.01

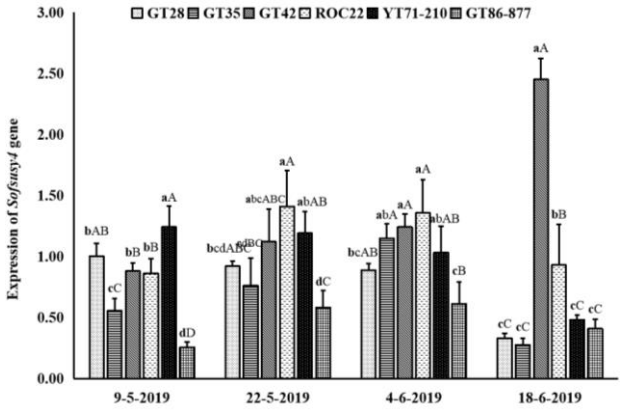

Fig. 7: Expression of *ScSuSy4* gene in the leaves of different sugarcane genotypes in the early stage of growth

Values presented are mean ±SD of 3 biological replicates. Different lowercase letters indicate significant difference at P<0.05, different capital letters indicate highly significant difference at P<0.01

Discussion

There are multiple *SuSy* genes in different plants species. Phylogenetic analysis showed that SuSy genes in plants formed three subfamilies: SuSyI, SuSyII and SuSyIII, and SuSyI was further divided into monocot SuSyI and dicot SuSyI (Komatsu *et al.* 2002; Hirose *et al.* 2008; (Chen *et al.* 2012; Zou *et al.* 2013). More recently each sub-family was again clustered into 3 groups: a monocot group, dicot group and basal angiosperm group, except for the SuSyI subfamily, in which the basal angiosperm group was missing, due to a second duplication event probably occurred in a common angiosperm ancestor (Stein and Granot 2019; Xu *et al.* 2019). In the current study, we analyzed the amino acid sequences of all the published full-length *SuSy* genes in plants. These results also showed that the *SuSy* genes in higher plants were divided into three subfamilies: SuSyI, SuSyII and SuSyIII (Fig. 1), and SuSyI could further be divided into monocot SuSyI and eudicot SuSyI. This finding from the broader analysis is consistent with the previous studies using smaller sample populations (Komatsu *et al.* 2002; Hirose *et al.* 2008; Chen *et al.* 2012. This means that the current classification of *SuSy* genes is accurate and reliable. Five full length *SuSy* genes had been identified in sugarcane. *ScSuSy1* (JX416283) and *SoSuSy2* (AY118266) were classed into monocot type SuSyI, indicating that the whole genome duplication (WGD) event happened in sugarcane after the monocot and eudicot divergence (Shi *et al.* 2019). *ScSuSy4* (KM598653) was grouped into type SuSyII, while *SoSuSy3* and *SoSuSy5* formed part of the SuSyIII cluster.

The correlation between final sucrose content of mature sugarcane and soluble sugar content in sugarcane leaf is rarely reported. In a previous study a positive correlation between the sucrose content in the leaves at seedling stage and cane yield and mature crop sugar content in 8 sugarcane genotypes was reported (Tan *et al.* 2003). This suggested that the sucrose content of seedling leaves could be used as an indirect predictor for the selection of high cane yield and high-sugar sugarcane clones in breeding. In the current study, although the total sugar content was higher in leaves of some high-sugar genotypes, the total sugar contents in the medium- and low-sugar genotypes were not always the lowest, and at times they were almost equal to those in high-sugar genotypes during the first three months of plant growth (Fig. 2). Our results indicate that the total sugar content in leaves can't be used to predict the final mature crop sugar content in sugarcane. Indeed, the sucrose content in leaves of different genotypes changed profoundly in different growth periods, and there was no significant correlation between the sucrose content in the early stage of plant growth and the sugar content of a mature crop (Fig. 4).

Although SuSy performs dual functions of sucrose synthesis and digestion, it is generally believed that its main function is decomposition of sucrose (Takeda *et al.* 2017; Qin *et al.* 2018). Therefore, the SuSy activity can be negatively correlated with sucrose content. For example, the activity of carrot SuSy enzyme DcSuSy showed strong negative correlation with sucrose concentration in carrot roots during five plant developmental stages (Liu *et al.* 2018). A similar phenomenon was found in sugarcane; however, SuSy activity was positively correlated with hexose sugars (Verma *et al.* 2010). In other examples, SuSy activity was positively correlated with sugar accumulation in some plants (Kalwade and Devarumath 2014). Also, in many studies no correlation between SuSy and sucrose accumulation was found in sugarcane (Lingle and Irvine 1994; Mirajkar *et al.* 2016). Our study showed that the SuSy activity in leaves of high, medium and low sugar genotypes changed greatly at the early growth stage and had no correlation with the final sucrose content of sugarcane (Fig. 5). During the early growing stage of sugarcane, sucrose appears to be mainly used for growth. Consequently, the tissue sugar composition also changed rapidly to meet the

demand generated by plant growth at the early stage of its life cycle. This may explain why there is no consensus on the role of SuSy in relation to sugar accumulation in sugarcane, and plants in general.

Various types of *SuSy* genes are found in the same plant species, and most of them have conserved expression pattern and function among different plant species. In rice, high expression of *SuSy1* was found in roots, young leaves and the elongation tissues of internodes, and the gene was thought to be involved in cellulose synthesis (Hirose *et al.* 2008). *SuSy2* is expressed widely in various tissues, suggesting that it may be a house-keeping gene in rice (Hirose *et al.* 2008). *SuSy3* and *SuSy4* are expressed highly in caryopsis in rice, showing that they are potentially involved in the distribution of carbon during the grain filling stage (Hirose *et al.* 2008). In our study, the expression analysis of two genes, *ScSuSy1* and *ScSuSy4* showed that their expression in the leaves were genotype-dependent, and they might be involved in sucrose degradation more so than sucrose accumulation (Fig. 5, 6, 7; Table 1, 2).

It is reported that at least five *SuSy* genes were found in sugarcane (Thirugnanasambandam *et al.* 2019). There are about 10 alleles of each type of sugarcane *SuSy* gene due to the complex polyploid (about 10×) background, making the functional characterization and mechanistic studies of sugarcane *SuSy* genes challenging. Various alleles of *SuSy* genes showed SuSy activity (Shi *et al.* 2019); (Thirugnanasambandam *et al.* 2019). Thus, the specific function of these genes during plant growth very likely varies temporarily as well as spatially. It is expected that the growing genomic and phenomic data and the detailed characterization of spatial and temporal expression pattern of sucrose synthase enzyme activity should unravel *SuSy* gene functions in sugarcane.

Conclusion

The SuSy enzyme activity and gene expression of *ScSuSy1* and *ScSuSy4* showed remarkable variation during the early growth period and did not show any correlation with the final sugar yield potential of sugarcane. The biological role of *ScSuSy1* and *ScSuSy4* gene may change with the time of sugarcane plant development but more research is needed to validate this finding in different genetic backgrounds grown in diverse agro-climatic conditions.

Acknowledgements

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Author Contributions

ZLC and YYG performed the field experiment, analyzed the enzyme activity and gene expression. MW and AML supported the collection of samples and analyzed the several sugars content. DLH and CXQ designed the experiment and wrote the manuscript. PL revised the manuscript. All authors reviewed the manuscript.

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