Identification and Characterization of the Tomato UGT Gene Family and Effects of GAME 17 Overexpression on Plants and Growth and Development under High-CO$_2$ Conditions

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Abstract: Steroidal glycoalkaloids (SGAs), the nitrogen-containing compounds produced primarily by Liliaceae and Solanaceae species, are toxic to animals and humans and have putative roles in defense against pests. UDP-glycosyltransferases (UGTs) catalyze the final glycosylation steps of SGA biosynthesis. Although previously published studies focused on the effect of UGT proteins on SGA biosynthesis, research to understand the effects of constitutive overexpression of UGTs on plant phenotype and fruit development is limited. The constitutive overexpression of a UGT encoding gene, GAME 17, may provide an alternative method to study the role of UGTs on the fruit development. In this study, we have identified 162 SlUGT proteins in tomato that are classified into t 23 groups. Gene structure and motif analyses have demonstrated that all SlUGTs have similar intron/exon distribution and motif compositions. RNA-seq data analysis has shown that SlUGTs exhibit differential expression patterns in different organs or different stages of fruit development. When the constitutive promoter 35S is used to control the expression of GAME 17, we have observed significant differences in growth parameters (i.e., plant height, leaf length, leaf width, internode length, and stem diameter) between WT and transgenic plants under high-CO$_2$ conditions, and slight differences in growth parameters between WT and transgenic plants have been detected. In addition, the contents of glucose, fructose, and soluble sugar of transgenic plants are significantly higher than those of WT plants. The increases in glucose, fructose, and soluble sugar in transgenic tomato fruits at three developmental stages under high-CO$_2$ conditions are significantly higher than under natural conditions. This study provides additional evidence that the GAME 17 gene plays an important role in controlling plant phenotype and sugar homeostasis, especially in environments with high concentration of CO$_2$.

Keywords: tomato; UGT gene family; GAME 17; high-CO$_2$ conditions; expression analysis

1. Introduction

Tomato (Solanum lycopersicum), as the second-largest vegetable crop in the world, originates from Peru, Ecuador, and Bolivia based on numerous wild and cultivated varieties [1,2], which contain bioactive key components, including phenolic compounds (phenolic acids and flavonoids), carotenoids (lycopene, α and β carotene), vitamins (ascorbic acid and vitamin A), and steroidal glycoalkaloids (SGAs) [3,4]. SGAs are nitrogen-containing toxic compounds occurring primarily in Liliaceae (Veratrump californicum) and Solanaceae species such as potato (S. tuberosum), tomato (S. lycopersicum), and eggplant (S. melongena) [5,6]. SGAs are widely distributed and expressed in plant organs, including roots, leaves, flowers, and fruits [7–10]. To date, about 100 SGAs have been detected in various organs and developmental stages of tomato fruits [11–13]. The α-tomatine and dehydrotomatine are the major SGAs in green tissues, whereas esculeosides are predominant in red ripe fruits [14–16].
Previous studies have focused on discovering the structure and composition of SGAs in different plants and systematically revealing the corresponding biosynthetic pathway [13,17]. Cholesterol is a precursor for SGAs biosynthesis, and undergoes several hydroxylation, oxidation, transamination, and glycosylation steps during the SGAs biosynthesis [18]. Numerous studies have demonstrated that UGTs can catalyze final glycosylation steps of SGAs biosynthesis [18–20]. In potato, three UGTs genes (SGT1, SGT2, and SGT3) are involved in glycosylating SGA [21,22]. The first UGT gene involved in the SGA biosynthesis in tomato is the potato SGT1 homolog, GLYCOALKOID METABOLISM 1 (GAME1), which adds a galactose to the aglycone tomatidine [17]. Thus, four glycosyl transfer seen coding genes (GAME1, GAME2, GAME18 and GAME 17) are involved in catalyzing the final glycosylation steps of SGA biosynthesis in tomato [17]. In addition, Itkin et al. (2013) identified a set of GAME genes involved in the core pathway producing SGAs in potato and tomato, and found that GAME genes are located physically close to each other on the same chromosome to form a gene cluster [17]. In tomato, genes encoding cytochrome P450s (GAME6, GAME7, GAME8a, and GAME8b located on Chr. 7 and GAME4 located on Chr. 12) and a dioxygenase (GAME11 located on Chr. 7) are involved in the hydroxylation and oxidation of the cholesterol skeleton, and a gene encoding transaminase (GAME12 located on Chr.12) incorporates the nitrogen atom into the SA aglycone. Finally, four glycosyltransferases encoding genes (GAME1, GAME2, GAME 17, and GAME18 located on Chr.7) can add sugar moieties to tomatidine to form tomatine [17]. Thus, UGTs play a key role in SGAs biosynthesis and participate in the regulation of sugar homeostasis.

Carbon dioxide (CO$_2$) is known to play important roles in plant growth and carbon assimilation [23,24]. Carbon assimilated via the Calvin cycle is partitioned, with a fraction exported to the cytosol for sucrose biosynthesis and a fraction retained in the chloroplast for starch biosynthesis [25]. Endogenous soluble sugars and starch are the main carbohydrates accumulating in tomato fruits [26]. However, the effect of UGTs on carbon assimilation and plant development remains unclear. In this study, we have first identified the SIUGT family genes in tomato, constructed a phylogenetic tree, and performed protein motif organization and gene structure analysis. Moreover, we have investigated the expression pattern of SIUGT family genes in different tissues or different developmental stages of tomato fruits by using previous RNA-seq data. Finally, we have generated transgenic tomato plants overexpressing the GAME 17 gene to explore the growth parameters and sugar content in wild type(WT) and transgenic plants under natural and high-CO$_2$ conditions. The results provide valuable information on the roles of the GAME 17 gene in the growth and development of tomato under high-CO$_2$ conditions.

2. Materials and Methods

2.1. Identification of SIUGT Proteins in S. lycopersicum

The UGT protein sequences of Arabidopsis and rice that were functionally characterized in previous studies were obtained from the NCBI (http://www.ncbi.nlm.nih.gov (accessed on 6 April 2022)) [27,28]. The UDPGT (PF00201) domain HMM (Hidden Markov Model) profile, which was obtained from the Pfam database, was used to identify SIUGT family genes from the S. lycopersicum genome by using the HMMER3 software package [29]. Moreover, redundant sequences in the same chromosome location and short proteins (length < 100 aa) were removed on the basis of physical localizations of all candidate genes. In addition, Pfam and SMART databases were used to verify the presence of the UDPGT domain for all candidates [29]. Finally, the identified candidate protein sequences containing the UDPGT domain were considered as SIUGT genes used in this study.

2.2. Phylogenetic Analysis

The UGT protein sequences, including SIUGTs, OsUGTs, and AtUGTs from tomato, rice, and Arabidopsis, were aligned using the MUSCLE algorithm with default parameters in MEGA X software [30]. The Model Generator program was used to select the best fitting model for UGT family protein evolution [31]. An unrooted neighbor-joining phylogenetic
tree was constructed through multiple sequence alignments of these UGTs by using the MEGAX software [30]. UGT family members were further grouped into different classes based on the topology of the phylogenetic tree. Parameters were as follows: pairwise deletion, Poisson model, and 1000 bootstrap replications.

2.3. Chromosomal Localization, and Motif and Gene Structure Analyses

The physical location and gene structure of SlUGT genes were acquired in the genome annotation database of S. lycopersicum, and the isodose distribution of SlUGT genes was plotted by using the MapInspect software (http://mapinspect.software.informer.com/, Version number: MapInspect 1.0, Developer: MapInspect, New York, USA (accessed on 6 April 2022)). The conserved motifs in SlUGT protein sequences were identified with the online program MEME5.0.1 (https://meme.nbcr.net/meme/intro.html, Version number: MEME5.0.1, Developer: Timothy L. Bailey, Brisbane, Australia (accessed on 6 April 2022)). The optimized parameters were as follows: maximum motif width, 50 bp; minimum motif width, 6bp; and maximum numbers of different motifs, 15 bp [32].

2.4. Expression Analysis

The expression patterns of SlUGTs in different tissues or different developmental fruit stages of S. lycopersicum were investigated using the published RNA-seq data from TFGD database (http://ted.bti.cornell.edu/cgi-bin/TFGD/digital/home.cgi, accessed on 7 April 2022)). The transcriptional abundance of SlUGTs was calculated as fragments per kilobase of exon per million reads (FPKM). The deduced FPKM value of these genes acquired from published data were normalized with Log2 for further visualization in Pretty Heatmap.

2.5. Construction of Plasmids and Plant Transformation

The full-length coding sequence of the GAME 17 gene (Solyc07g043480) was amplified from the cDNA of tomato leaves by using primers (forward primer: 5′-CCCGGGATGGATAACAAAGATGATGTAGC-3′ and reverse primer: 5′-GACTAGTCTATCTTGTGATATGC
GGAT-3′) and inserted into the SMA1 and SPE1 sites of binary vector pCAMBIA1300. Transgenic ‘Micro-Tom’ tomato plants were generated by Agrobacterium tumefaciens-mediated transformation (strain EHA105) by using previously described methods [33].

2.6. Detection of Transgenic Tomato Plants

The genomic DNA (gDNA) was extracted from the leaves of T0 transgenic tomato plants by using the DNA secure plant kit (Tiangen Biotech, Beijing, China). PCR (polymerase chain reaction) was used to detect transgenes in genetically modified (GM) tomato by using specific primers (HygB-F: 5′-TAGCGAGAGCCTGACCTATT-3′, HygB-R: 5′-GATGTTGCGACCTCGTATT-3′).

2.7. RNA Isolation and Real-Time Quantitative PCR Assays

Total RNA was extracted using the RNAasy kit (Qiagen, Inc., Hilden, Germany) and treated with RNase-free DNase accordance with the manufacturer’s instructions. The Primer script RT Reagent Kit was used to synthesize cDNA by using total DNA-free RNA as template. For real-time quantitative PCR (RT-PCR), the SYBR Premix ExTag was used on the Bio-Rad CFX96 (Bio-Rad, Shanghai, China) instrument. PCR reactions was performed in a 96-well iCycler. The melting temperature of products was determined to verify the specificity of the amplified fragments. The sequences of primers used for real-time quantitative PCR are listed in Supplementary Table S1. Results were analyzed by the ΔΔCT method by using SlActin as the control locus [34]. All real-time quantitative PCR data points were the average of 3 biological and 3 technical replicates.

2.8. Determination of Plant Morphological Indices

Transgenic tomato and WT plants were grown in a growth chamber on a 16 h light (22 °C)/8 h dark (20 °C) cycle with 65% relative humidity. Light (~160 µmol·m⁻²·s⁻¹) was
supplied by sodium lamps. 30 d (day) seedlings were used in the following research. Plant morphological indices, including plant height, leaf length, leaf width, and stem diameter of transgenic tomato plants overexpressing GAME 17 and WT plants were measured at 1, 5, 10, 15, 20, 25, and 30 d under the different concentrations of CO$_2$ (natural condition: 400µmol m$^{-2}$ s$^{-1}$ and high-CO$_2$ condition: 800µmol·mol$^{-1}$).

2.9. Determination of Glucose, Fructose, and Soluble Sugar Contents in Fruits

The soluble sugar (fructose and glucose) content in fruits of transgenic tomato and WT plants was determined using HPLC. Fruit samples (50 g) were mixed and homogenized with 200 mL ddH$_2$O and then heated in a shaking water bath maintained at 50 °C for 30 min. The tube was cooled to 25 °C in an iced water bath and then centrifuged at 8000× g and for 15 °C 25 min. The supernatant was collected and used to determine the contents of sucrose, fructose, and glucose in samples by HPLC. The mobile phase was ddH$_2$O and the flow rate was 0.4 mL-min$^{-1}$ for 35 min. A sample loop (20 mL) was used for injection. Quantification was carried out using external standards (i.e., HPLC-grade sucrose, glucose, and fructose (Sigma-Aldrich Co., LLC., Taufkirchen, Germany)). Results were expressed as grams per 100 g of sample.

2.10. Statistical Analysis

The data are presented as the means ± SD of three replicates. The statistical analyses, such as Student’s t-test and analysis of variance, were performed in R.

3. Results

3.1. Genome-Wide Identification and Analysis of SlUGT Family Genes in S. lycopersicum

Several UGT genes of Arabidopsis and rice were identified and functionally analyzed [27,28]. The Pfam database was used to detect the conserved domain [UDPGT (PF00201)] in all identified UGTs proteins. The full-length of the UDPGT domain obtained from the Pfam database was used as query to search the genome-wide protein sequence data of S. lycopersicum [35]. A total of 162 putative candidate proteins were identified after removing redundant proteins. SIUGT proteins ranged in length from 61 aa (Solyc07g043440) to 952 aa (Solyc04g079030), with an average length of approximately 438 aa. The molecular weight (MW) of the identified SIUGT proteins ranged from 6.94 kDa (Solyc07g043440) to 109.59 kDa (Solyc04g079030), and the calculated isoelectric points (PI) of these proteins ranged from 4.68 (Solyc11g010813) to 9.56 (Solyc04g026160). In addition, the subcellular localization of the identified SIUGT proteins was predicted. Of the 162 SIUGTs, 58, 4, and 41 were localized in cell membrane, chloroplast, and cell membrane/chloroplast, respectively (Table S1).

In accordance with the information of gene annotation, the predicted 162 SIUGT genes were localized on chromosome 12 in S. lycopersicum (Figure 1). As a result, the congrate region and the number of SIUGTs were unevenly distributed on 12 chromosomes (Figure 1). Chromosome 4 contained the largest number of SIUGT genes (total: 22), and only eight SIUGT genes were present on chromosomes 8 and 9. Moreover, four identified SIUGT genes (GAME1, GAME2, GAME 17, and GAME18) were located in a cluster on chromosome 7. In addition, 67 SIUGT genes were derived from the tandem duplication events (Figure 1). Chromosome 4 and 8 contained eight and two tandem duplicated genes, respectively, suggesting that the tandem duplication event slightly contributed to the expansion of SIUGT family genes in S. lycopersicum.
respectively, suggesting that the tandem duplication event slightly contributed to the expansion of SlUGT family genes in *S. lycopersicum*.

Figure 1. The distribution of SlUGT genes on the chromosomes in *S. lycopersicum*. The two chromosome sizes are indicated by their relative length, and the genes marked by the curve in the figures are tandem-duplicated genes, the GAME 17 gene (Solyc07g043480) was marked by prink color.

3.2. Phylogenetic Analysis of the UGT Gene Family among Arabidopsis, Rice, and *S. lycopersicum*

An unrooted phylogenetic tree of UGT family genes from *S. lycopersicum*, rice, and *Arabidopsis* was constructed using the maximum likelihood method (ML) implemented in the PhyML program (v.3.0) to further explore the evolutionary relationship among SlUGT genes in *S. lycopersicum* (Figure 2 and Table S2). SlUGT family genes in *S. lycopersicum* were clustered into 23 groups on the basis of the phylogenetic tree topology and functional proteins were identified in *Arabidopsis* and rice (Figure 2). The distribution of SlUGT genes in different group varied. Group XXIII, the largest group among the 23 groups, contained 17 SlUGT genes, followed by groups VI and X. Group V, the smallest group among 23 groups, contained only one SlUGT gene. Moreover, groups VI and XXIII contained more UGT genes in *S. lycopersicum* than those in *Arabidopsis* and rice, indicating that the species-specific expansion of the SlUGT gene family occurred in *S. lycopersicum* after the divergence of core eudicots. In addition, group V contained many more OsUGT genes in rice than in *Arabidopsis* and *S. lycopersicum*, suggesting that the species-specific expansion of this gene family occurred in rice after the differentiation of monocots and dicots.
Figure 2. Maximum-likelihood (ML) phylogenetic tree of SlUGT family proteins among *S. lycopersicum*, rice, and *Arabidopsis*. An unrooted phylogenetic tree was built with the full-length sequences of UGT proteins with 100 bootstrapping replicates. The bootstrap values are shown on the nodes. Different color arcs indicate different groups of UGT proteins.

3.3. Gene Structure and Motif Analyses of the SlUGT Gene Family in *S. lycopersicum*

The gene-structural diversity and the protein motif composition of SlUGT family members were also analyzed in this study. GSDS software (v. 2.0) was used to investigate and visualize the intron/exon structure of SlUGTs, and results found that the SlUGT family-genes possessed exons varying from 1 to 15 (Figure 3a). Of the 162 SlUGT family members, 88 lacked introns and had only one exon, 70 possessed 1 to 5 introns, and 4 contained more than 10 introns. One SlUGT gene (Solyc04g071540) contained the largest number of exons (15 exons, Figure 3a). In addition, the conserved motifs in SlUGTs were identified in the present study, and 15 conserved motifs, namely, motifs 1 to 15, were identified through the MEME analysis (Figure 3b and Figure S1). Ten motifs, including motifs 1, 2, 3, 5, 6, 7, 8, 9, 13, and 14, corresponding to the conserved UDPGT domain, were widely distributed in all members of the SlUGT family (Figure 3b).
1, 2, 3, 5, 6, 7, 8, 9, 13, and 14, corresponding to the conserved UDPGT domain, were widely distributed in all members of the SlUGT family (Figure 3b).

Figure 3. Schematic diagram of conserved motifs and gene structures of SlUGT family members in *S. lycopersicum*. (a) The intron and exon structures of the SIUGT genes were obtained by Perl scripts and visualized by GSDS software. (b) Motif analysis was conducted using MEME online program.
3.4. Expression Pattern of SlUGT Family Genes in Different Organs of S. lycopersicum

The expression levels of SlUGT genes in different tissues of *S. lycopersicum* were evaluated through the published RNA-seq data in the TFGD database to reveal the potential functions of SlUGTs in plant growth and development (Figure 4). All SlUGT genes had RPKM values larger than 1 in at least one organ (Figure 4). Thus, we considered all SlUGTs to be expressed during tomato growth and development (see Materials and Methods). By contrast, RPKM values for 25 (15.4%) SlUGTs were lower than 1 in different organs or different developmental stages of tomato. A total of 28 genes showed high expression levels among all the examined tissues. Moreover, the expression levels of 11 SlUGTs (Solyc10g086240, Solyc12g057080, Solyc04g016210, Solyc12g057070, Solyc02g091370, Solyc03g078490, Solyc04g080010, Solyc02g091350, Solyc04g016230, Solyc11g010740, and Solyc10g079320) in vegetative organs (leaf and root) were significantly higher than those in reproductive organs (flowers and fruits), where the expression levels of three SlUGTs (Solyc10g085230, Solyc10g085240, and Solyc10g085280) were significantly higher in reproductive organs than in vegetative organs. Then, the expression levels of 20 SlUGT genes in the early-stage fruit were significantly higher than those in the late-stage fruit. In addition, several SlUGT genes displayed tissue-specific expression patterns. For example, 20 (Solyc02g066960, Solyc06g062330, Solyc04g074330, Solyc07g008230, Solyc07g043150, Solyc07g043170, Solyc05g052870, Solyc03g071850, Solyc04g074380, Solyc03g078770, Solyc08g014050, Solyc03g078500, Solyc06g072880, Solyc01g105350, Solyc04g074390, Solyc07g006800, Solyc11g007370, Solyc04g082860, and Solyc12g006430), 3 (Solyc12g098590, Solyc06g082300, and Solyc05g010710), and 4 (Solyc04g074340, Solyc09g092480, Solyc02g085660, and Solyc10g079350) SlUGT genes were specifically expressed in roots, leaves, and flowers, respectively, suggesting that these genes play important roles in regulating organ growth and development.

Figure 4. Expression pattern of SIUGT genes in different organs of *S. lycopersicum* based on the published RNA-seq data, GAME 17 was marked by red.
3.5. Generation and Identification of Transgenic Tomato Plants

On the basis of the previous study and the expression pattern of GAME 17 in vegetative organs and green fruits, we further investigated the effect of increasing GAME 17 expression levels on tomato growth and development. The full-length CDS of GAME 17 was inserted into the pCAMBIA1305.1 vector, which contained the cauliflower mosaic virus (CaMV) 35S promoter to generate the 35S::GAME 17 over-expression vectors (Figure 5a). Transgenic tomato plants overexpressing the GAME 17 gene were generated using the Agrobacterium-mediated transformation method (Figure 5b). A pair of primers for hygromycin resistance gene (HYG; see Materials and Methods) was used to verify the presence of the target transgene in T0 generation of transgenic plants by using PCR (Figure 5c). The seeds, collected from three transgenic tomato lines and WT plants, were germinated in a culture dish at 28 °C in the dark overnight. Then, seedlings were grown in a growth chamber with 16h days (25 °C) and 8h nights (18 °C). Upon the two true-leaf stage, T1 generation transgenic tomato seedlings were transferred into a standard glass greenhouse at the experimental field of Shanxi Agricultural University. Compared with WT plants, three transgenic tomato lines with higher GAME 17 gene expression were used in the following research (Figure 5d).

![Figure 5](image)

Figure 5. Generation and identification of transgenic tomato plants. (a) Schematic diagram of vectors for tomato transformation. (b) The construction of tomato transformation. (c) Characterization of transgenic tomato lines. Maker: DL2000 DNA marker. WT: the wild-type tomato leaves. Line 1, 3 and 6 represent three representative transgenic lines. (d) The phenotypic differences between WT and transgenic tomato plants.

3.6. Effects of the Constitutive Overexpression of GAME 17 on Plant Growth and Development

GAME 17 is responsible for adding sugar moieties to tomatidine to form tomatine. The constitutive promoter 35S-GAME 17 cDNA fusion construct was transformed into the tomato cultivar ‘Micro-Tom’ to further determine whether the overexpression of GAME 17 gene in transgenic tomato plants caused plant phenotypic changes. Thirty-day-old seedlings, including three transgenic tomato lines and WT, were used to detect the expression level of the GAME 17 gene. Compared with that in WT plants, the expression level of the GAME 17 gene in transgenic tomato plants was higher by 4.37~4.73 times under natural conditions, and 2.25~2.43 times under high-CO2 conditions (Figure 6), suggesting that a high concentration of CO2 could significantly inhibit the expression level of the GAME 17 gene. Moreover, the plant morphological indices, including plant height, internode length, leaf length, leaf width, and stem diameter, of transgenic tomato overexpressing GAME 17 and WT plants were measured under natural and high-CO2 conditions, respectively (Figure 7). The plant height, internode length, leaf length, and leaf width of transgenic
tomato plants were higher than those of WT plants under natural and high-CO$_2$ conditions (Figure 7, Table S3). Compared with WT plants, the average plant height in transgenic tomato plants was slightly increased by 4.9%, 5.3%, 2.8%, 0%, 1.4%, and 2.1% under natural conditions on days 5, 10, 15, 20, 25, and 30, respectively, and these values were further increased by 10.8%, 10.9%, 9.3%, 10.9%, 10.3%, and 13.3%, respectively, in the high-CO$_2$ environment (Figure 7a). The average internode length in transgenic tomato plants was increased by 3.6%, 2.9%, 0, 2.2%, 3.9%, and 3.9% under natural conditions on days 5, 10, 15, 20, 25, and 30, respectively, and these values further increased by 11.1%, 9.1%, 7.7%, 12.2%, 10.9%, and 10.2%, respectively, under high-CO$_2$ conditions (Figure 7b). Moreover, the average leaf length in transgenic tomato plants displayed a dynamic changing pattern at different time points under natural conditions and in the high-CO$_2$ environment (Figure 7c). The average leaf width in transgenic tomato plants under natural conditions was slightly increased by 1.0%, 0.4%, 0.4%, 1.5%, 1.4%, and 1.8% on days 5, 10, 15, 20, 25, and 30, respectively, compared with that in WT plants, and these values were further increased by 14.0%, 16.7%, 13.4%, 12.8%, 12.1%, and 11.6%, respectively, under high-CO$_2$ conditions (Figure 7d). In addition, the average stem diameter of transgenic tomato plants was increased by 4.2%, 4.2%, 4.1%, 3.3%, 3.2%, and 3.1% on days 5, 10, 15, 20, 25, and 30, respectively, under natural conditions in comparison with that of WT plants, and these values were further increased by 7.3%, 9.1%, 9.0%, 8.6%, 12.1%, and 11.6%, respectively, under high-CO$_2$ conditions (Figure 7e). These results suggest that the GAME 17 gene could increase the plant height, internode length, leaf width, and stem diameter under natural and high-CO$_2$ conditions, and the increases in plant growth parameters of transgenic tomato plants under high-CO$_2$ conditions were significantly higher than those under natural conditions.

**Figure 6.** The expression level of GAME 17 in transgenic tomato and WT plants. (a) The expression level of GAME 17 in transgenic tomato and WT plants under natural conditions. (b) The expression level of GAME 17 in transgenic tomato and WT plants under high-CO$_2$ conditions. Data are means ±SD, n = 3.
Figure 7. Comparison of the growth parameters between transgenic tomato plants. (a) The average plant height in transgenic tomato plants; (b) the average internode length in transgenic tomato plants; (c) the average leaf length in transgenic tomato plants; (d) the average leaf width in transgenic tomato plants; (e) the average stem diameter in transgenic tomato plants.

3.7. Effects of the Constitutive Overexpression of SigAME 17 Gene on the Content of Glucose, Fructose, and Soluble Sugar

Three developmental stages of tomato fruits (i.e., expansion stage, break stage, and ripening stages) were used to detect the contents of glucose, fructose, and soluble sugar.
and further investigate the effect of SlGAME 17 overexpression in transgenic tomato plants on the content of glucose, fructose, and soluble sugar in tomato fruits (Figure 8a–c). At the expansion stage, the contents of glucose, fructose, and soluble sugar followed the order: transgenic tomato plants in high-CO₂ conditions > WT plants in high-CO₂ conditions > transgenic tomato plants in natural conditions > WT plants in natural conditions (Figure 8a–c). At the break stage, the contents of glucose, fructose, and soluble sugar followed the order: transgenic tomato plants in high-CO₂ conditions > transgenic tomato plants in natural conditions > WT plants in high-CO₂ conditions > WT plants in natural conditions (Figure 8a–c). In addition, the contents of glucose, fructose, and soluble sugar were highest in transgenic tomato plants in high-CO₂ conditions at the ripening stage, followed by WT plants in high-CO₂ conditions and transgenic tomato plants in natural conditions, and lowest in WT plants in natural conditions (Figure 8a–c). These results indicated that the overexpression of the SlGAME 17 gene in transgenic tomato plants could promote the accumulation of glucose, fructose, and soluble sugar in tomato fruit under natural and high-CO₂ conditions, and that the increases in glucose, fructose, and soluble sugar contents in transgenic tomato plants under high-CO₂ conditions were significantly higher than under natural conditions.

Figure 8. Comparison of the content of glucose, fructose, and soluble sugar in WT and transgenic tomato fruits. The fructose content in tomato fruits at three developmental stages of transgenic tomato and WT plants (a); the glucose content in tomato fruits at three developmental stages of transgenic tomato and WT plants (b); and the soluble sugar content in tomato fruits at three developmental stages of transgenic tomato and WT plants (c). Values represent the means ± SD (n = 3) of three plants per line.

4. Discussion

SGAs, a class of nitrogen-containing steroidal glycosides, are commonly found in Solanum plants and are important components of plant resistance against pests and pathogens, but can be toxic to humans at high levels [6,36,37]. SGA biosynthesis requires UGT genes encoding uridine 5′-diphosphate (UDP)-glycosyltransferases that decorate the steroidal alkaloid skeleton with various sugar moieties [18,38]. Although UGT family genes have been identified in many plants, the identification and molecular characterization of the UGT gene family in S. lycopersicum have not been reported. In this study, 162 SlUGT genes were identified, characterized, and divided into 23 groups on the basis of the topology of the phylogenetic tree and the functions of AtUGT genes. The number of UGT family genes in S. lycopersicum (162 UGT family members) was higher than in Citrus grandis (145 UGT genes) and Lotus japonicus (94 UGT genes), but lower than in G. max (212UGT genes) and M. truncatula (243 UGT genes). This result suggests that the UGT family did not exhibit significant expansion in S. lycopersicum [39–41]. Moreover, gene structure and motif analyses further revealed that SlUGT family genes were highly conserved during evolution (Figure 3).

UGT genes have important roles in plant growth and development and interactions with the environment; play an essential role in regulating glucose metabolism and homeostasis; and are involved in the biosynthesis, storage, and transport of secondary metabo-
lites [42–45]. Previous studies revealed that a UDP-glucosyltransferase gene (SaGT4A) from *S. aculeatissimum* glucosylates sapogenins, solasodine, and tomatidine [46,47]. The potato enzyme StGT1 can utilize either UDP-Gal or UDP-Glu to catalyze galactosylation or glucosylation, respectively [48]. Moreover, some *Solanum* UGT genes (SGT2, SGT3, GAME1, GAME2, GAME 17, and GAME18) are involved in constructing the 3β-OH oligosaccharide portion of SGAs, which glycosylate nonalkaloidal sapogenins [17]. Although have studies reported that tomato GAME 17 is involved in the glycosylation of SGAs [49], studies that reported the effect of tomato GAME 17 on plant growth and development and soluble sugar content in tomato fruits are few. In the present study, we generated transgenic tomato plants overexpressing SIGAME 17 derived by 35S promoter to investigate plant phenotypic changes under natural and high-CO\textsubscript{2} conditions. The results showed that the plant morphological indices (i.e., plant height, internode length, leaf width, and stem diameter) in transgenic tomato plants were slightly higher than in WT plants under natural conditions and were significantly higher than in WT plants under high-CO\textsubscript{2} conditions. These results suggested that the SIGAME 17 gene played important roles in promoting the plant growth and development under high-CO\textsubscript{2} conditions. This finding was consistent with that of a previous study [50]. Soluble sugars in tomato fruits, including sucrose, fructose, and glucose, affected the quality of fruits [51,52]. Previous studies have revealed that the contents of fructose, glucose, and soluble sugars in plants under high-CO\textsubscript{2} conditions increase [53,54].

In the present study, the contents of fructose, glucose, and soluble sugars in tomato fruits at three different developmental stages under high-CO\textsubscript{2} conditions were higher than under natural conditions, which is consistent with results observed in previous studies [55,56]. For example, CO\textsubscript{2} can maintain the quality of fresh-cut pears by regulating the conversion of various sugar components to enhance the soluble sugars content [55,56]. Moreover, the contents of fructose, glucose, and soluble sugars in fruits at three developmental stages of transgenic plants were higher than in fruits of WT plants. The magnitude of increase in transgenic tomato plants under high-CO\textsubscript{2} conditions was significantly higher than that under natural conditions, suggesting that high-CO\textsubscript{2} could increase the activity of UDP-glucosyltransferase to promote the production of soluble sugars by feedback regulation. Previous studies revealed that the content of soluble sugar in transgenic rice plants overexpressing UGT3 gene and transgenic *Arabidopsis* overexpressing WssgtL3.1 is higher than that in WT plants [57].

In the present study, 162 SIUGTs were identified in tomato. Motif and gene structure analyses suggest that SIUGT family genes are highly conserved during evolution. The expression pattern of SIUGT genes suggests that SIGAME 17 is predominantly expressed in the vegetative organs and green fruits of tomato plants. Moreover, UGTs likely play an important role in tomato growth and development and soluble sugar metabolism.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12091998/s1, Figure S1. Sequence logos of SIUGT proteins. Table S1. Detailed information of SIUGT genes in tomato. Table S2. The distribution of UGT family genes in *Arabidopsis*, rice, and tomato. Table S3. The magnitude of the increase in growth parameters of transgenic tomato and WT plants under natural and high-CO\textsubscript{2} conditions.

**Author Contributions:** G.-M.X. designed the experiment. S.-W.Z. and Z.-F.C. conducted the experiment. Z.-Y.Z. and T.-M.L. analyzed the data. S.-W.Z. wrote the manuscript. G.-M.X. and T.-T.L. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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