

SUCROSE BIOSYNTHESIS-RELATED TO GENE EXPRESSION IN *Beta vulgaris* L. ACROSS DIFFERENT GROWTH PERIOD

Woon Ji Kim¹, Baul Yang², Seung Hyeon Lee¹, Jae Hoon Kim¹,
Sang Hoon Kim¹ and Jaihyunk Ryu¹

ABSTRACT

Understanding the molecular mechanisms of sugar accumulation in the taproot of sugar beet (*Beta vulgaris* L.) is essential for enhancing sugar production. In this study, we analysed growth parameters and gene expression profiles of taproots collected at 50 (S1), 90 (S2), 160 (S3), and 330 (S4) days after sowing (DAS). Growth analysis revealed that the length, width, and weight of the taproots increased over time, with a particularly rapid weight gain observed between 50 and 160 DAS. Sugar content increased sharply from 50 to 90 DAS, gradually rose until 160 DAS, and then tended to decrease up to 330 DAS. Differential expression analysis identified 4,560, 4,764, and 4,781 differentially expressed genes (DEGs) in comparisons between S1:S2, S1:S3 and S1:S4, respectively. Among these, 3,255 DEGs were common across all comparisons, with 1,345 genes upregulated and 1,909 genes downregulated. Focusing on sugar metabolism, we identified 48 DEGs related to sucrose metabolism enzymes and sugar transport proteins. Cluster analysis divided these DEGs into two groups based on their expression patterns: Class I genes including those encoding sucrose synthase, sucrose transporter proteins, fructokinase, and hexokinase were downregulated compared to S1, while Class II genes including sucrose transporter proteins, sucrose-phosphate synthase, fructokinase, and hexokinase were upregulated. These findings contribute to understanding of the gene expression associated with sugar accumulation during sugar beet taproot development and provide valuable insights for future genetic improvement aimed at increasing sugar content in sugar beet crops.

Additional Keywords: Differentially expressed genes, gene ontology, taproot

RESUMEN

Expresión genética relacionada con la biosíntesis de sacarosa en *Beta vulgaris* L en diferentes periodos de crecimiento

Comprender los mecanismos moleculares de la acumulación de azúcar en la raíz pivotante de la remolacha azucarera (*Beta vulgaris* L.) es esencial para mejorar la producción de azúcar. En este estudio se analizaron los parámetros de crecimiento y perfiles de expresión génica de las raíces recolectadas a los 50 (S1), 90 (S2), 160 (S3) y 330 (S4) días después de la siembra (DDS). La longitud, ancho y peso de las raíces pivotantes aumentaron con el tiempo, con una ganancia de peso rápida entre los 50 y 160 DDS. El contenido de azúcar aumentó notoriamente desde los 50 a 90 DDS, y gradualmente hasta 160 DDS, para luego tender a disminuir hasta 330 DDS. El análisis de expresión diferencial identificó 4560, 4764 y 4781 genes expresados diferencialmente (GED) en comparaciones entre S1:S2, S1:S3 y S1:S4, respectivamente. Entre estos, 3255 GED fueron comunes en todas las comparaciones, con 1345 genes sobreexpresados y 1909 subexpresados. Referente al metabolismo del azúcar, se identificaron 48 GED relacionados con las enzimas del metabolismo de la sacarosa y las proteínas transportadoras de azúcar. El análisis de conglomerados dividió estos GED en dos grupos según sus patrones de expresión: los genes de clase I, que incluyen los que codifican la sacarosa sintasa, las proteínas transportadoras de sacarosa, la fructoquinasa y la hexoquinasa, mostraron una subexpresión en comparación con S1; mientras que los genes de clase II, que incluyen las proteínas transportadoras de sacarosa, la sacarosa-fosfato sintasa, la fructoquinasa y la hexoquinasa, mostraron una sobreexpresión. Estos hallazgos contribuyen a la comprensión de la expresión génica asociada con la acumulación de azúcar durante el desarrollo de la raíz pivotante de la remolacha azucarera y proporcionan información valiosa para futuras mejoras genéticas destinadas a aumentar el contenido de azúcar en los cultivos de remolacha azucarera.

Palabras clave adicionales: Genes de expresión diferencial, ontología génica, raíz pivotante

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INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is one of the most important industrial crops, significantly

contributing to the global sugar supply by accounting for approximately 30 % of the world's sugar production (Mutasa *et al.*, 2012). This vital

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¹Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongseup 56212, Republic of Korea. e-mail: wjkim0101@kaeri.re.kr; lshyeon@kaeri.re.kr; jaehun@kaeri.re.kr; shkim80@kaeri.re.kr; jhryu@kaeri.re.kr (corresponding author)

²Imsil Cheese & Food Research Institute, Imsil-gun, Jeonbuk State 55918, Republic of Korea. e-mail: byang@icf.re.kr

commercial crop is not only used in the food industry but also plays a crucial role in producing bioethanol, a renewable energy source (Magaña *et al.*, 2011). In recent years, with global warming and environmental concerns, the production of ecofriendly biodegradable plastics has gained attention, and sugar beet can be utilized as a valuable resource (Dohm *et al.*, 2014; Turesson *et al.*, 2014). Belonging to the genus *Beta*, sugar beet is a biennial crop that develops a storage root accumulating sugars in the first year and produces seeds in the second year (Hoffmann *et al.*, 2021). Approximately 70 % of the dry weight of sugar beet taproots is composed of sugar, and the weight and sucrose content of the taproots are important factors in determining yield and quality (Atiweh *et al.*, 2021).

Eco-friendly biodegradable plastics are made by processing polylactic acid (PLA). The most important ingredient in PLA production is sugar, as lactic acid serving as a precursor to synthesize PLA is produced during the fermentation of sugar (Singhvi and Gokhale, 2013; Lambrechts, 2020). The process of fermenting sugars to produce lactic acid, which is then polymerized to make PLA, is currently the most economical and efficient way to produce PLA on a large scale (Inkinen *et al.*, 2011). Therefore, understanding the sugar biosynthesis pathway and identifying the genes involved is of great industrial importance, as well as for sugar beet breeding.

The most important component of sugar beets is sucrose, which is determined by sucrose synthesis, sucrose transport, and sink strength (Ahmad *et al.*, 2020). The synthesis of sucrose begins with photosynthesis, and the initial product is mainly triose phosphates, which are subsequently converted to monosaccharides and used as precursors to sucrose (Getz, 2000). Monosaccharides are ultimately synthesized into sucrose by sucrose-phosphate synthase (SPS) and sucrose-phosphate phosphatase (SPP) (Elliott and Weston, 1993). The sucrose produced by photosynthesis is transported into sink tissues via sucrose transport proteins and stored or transported to growing tissues for growth and development. Some of the key genes involved in sucrose synthesis and storage in the above process are SPS, sucrose synthase (SuSy), invertase (INV), sugar will eventually be exported transporters (SWEET), sucrose transporters

(SUT/SUC), fructokinase (FRK), and glucose-6-phosphate/phosphate translocator (GPT). Understanding and regulating their functions may lead to more efficient production and improved yield in sugar beet (Verma *et al.*, 2019; Pavlinova *et al.*, 2002).

Recent advancements in next-generation sequencing technologies have facilitated the establishment of efficient, affordable, and dependable methods for generating extensive expression datasets, which are crucial for functional genomic analysis. Consequently, RNA sequencing (RNA-Seq) has become a proven tool for detecting gene expression, discovering novel transcripts, identifying differentially expressed genes (DEGs), and expanding its application beyond a limited range of model organisms to a much broader array. The sugar beet genome has been reported to be sequenced at approximately 731 Mb, and over 27,000 protein-coding genes have been identified, including genes involved in carbohydrate metabolism, photosynthesis, and stress response (Lyu *et al.*, 2020). A reference transcriptome assembly has been presented for the sucrose accumulation pathway, gibberellin treatment, and vernalization in sugar beet (Mutasa *et al.*, 2012). The availability of sugar beet genome information has provided an efficient method with higher spatial resolution for studying the molecular mechanisms underlying taproot growth and sucrose accumulation. In this study, we compared the differential expression of genes involved in the sucrose synthesis metabolic pathway at different growth stages of sugar beet.

MATERIALS AND METHODS

Plant materials and RNA extraction. For the experiment and analysis, seeds (ASIA SEED KORA) of a Turkish sugar beet variety (*Beta vulgaris* var. *saccharifera* Alef) with the best yield in the Korean climate were sown. After sowing in early February, samples were collected 50 days (spring), 90 days (spring), 160 days (summer), and 330 days (late fall-early winter). Three replicate taproot samples were collected at 50, 90, 160, and 330 days after seedling (DAS) for phenotyping and RNA-seq analysis. The weight (kg.plant⁻¹), length (cm), width (cm) of the taproot at each growth period were investigated. The taproot sugar content was measured using a handheld

digital refractometer (PAL-1; ATAGO, Tokyo, Japan). Taproots were sliced lengthwise, and juice was obtained by lightly pressing the tissue. A small volume of the juice was applied to the prism surface of the refractometer, and sugar content was expressed as the Brix percentage (%). Total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany).

Sequence pre-processing and read mapping.

Short reads were preprocessed using Trimmomatic (v0.39) (Bolger *et al.*, 2014) to remove adapter sequences and improve data quality. The following options were applied for trimming and quality control: (1) SLIDINGWINDOW with a window size of 4 and a mean quality of at least 15; (2) LEADING and TRAILING options set to a minimum quality score of 3; and (3) a minimum read length set at 36 bp. The cleaned reads were then mapped to the reference genome using HISAT2 software (Kim *et al.*, 2015) to determine gene expression levels as read counts. Gene expression quantification was conducted using HTSeq (v0.11.0) (Anders *et al.*, 2015) by counting reads mapped to each gene.

Identification of differentially expressed genes (DEGs) and annotation. A brief overview of the general pipeline for differential gene expression (DEG) analysis is provided. DEG analysis typically begins with preprocessing of raw RNA-seq data to remove low-quality reads and sequencing adapters, followed by alignment of the cleaned reads to a reference genome to determine the origin of each transcript. Quantification of gene expression was then performed by counting the number of reads that align to each gene. These raw counts served as input for statistical models that identify genes showing significant differences in expression between samples or conditions. Statistical significance was assessed using fold-change thresholds and corrections for multiple testing, such as the false discovery rate (FDR). The identified DEGs were further explored through clustering and functional enrichment analysis to understand their biological relevance. In this study, DEGs between samples were identified using a twofold change threshold and an adjusted p-value (false discovery rate, FDR) less than 0.01 through a binomial test. Genes were classified as upregulated if the log₂ (fold change) was greater than 1 and downregulated if less than -1.

Normalization of gene expression levels to account for data variability across samples was performed using the DESeq (Anders and Huber, 2010) library in R. For gene annotation, sequences were compared against the Viridiplantae database from NCBI NR using BLASTP, with a significance threshold set at an e-value of $\leq 1e-10$.

Clustering analysis of selected DEGs. Gene expression patterns of the significantly expressed genes were analyzed using hierarchical clustering. This analysis was carried out using the *amap* (Lucas, 2014) and *gplots* (Warnes *et al.*, 2015) libraries in R, employing Pearson's correlation for measuring similarity and the complete linkage method for gene grouping.

Functional analysis of selected DEGs. For the functional analysis of DEGs, Gene Ontology (Ashburner *et al.*, 2000) analysis was conducted. Candidate genes were aligned against sequences from the GO database, and functional categories were assigned into biological process (BP), cellular component (CC), and molecular function (MF) using in-house scripts. The significance level was set at 0.05 for determining enriched GO terms among the DEGs.

RESULTS

Agronomic characteristics. Taproot weight, length, width, and sugar content were determined in sugar beet taproots collected at 50, 90, 160, and 330 DAS. As shown in Figures 1 and 2, the length, width, and weight of the sugar beet taproot increased over time, with weight in particular showing a rapid increase from 50 to 160 DAS. In addition, the sugar content increased rapidly from 50 to 90 DAS, then slowly increased from 90 to 160 DAS, and then tended to decrease from 160 to 330 DAS.

Sequence pre-processing, annotation, and read mapping. To understand the molecular mechanisms of genes related to sugar at each growth stage, we analyzed genome-wide changes in gene expression. Total RNAs from taproots at four stages were used to construct 12 cDNA libraries that were sequenced using the Illumina HiSeq X Ten platform. The number of trimmed reads ranged from 13,451,854 to 21,213,806, and the total length was 1,950,261,392 bp to 3,089,720,072 bp, with an average read length of 142.00 bp to 145.66 bp. After trimming, the

percentage of remaining data from the total raw data ranged from 88.74 % to 94.67 %. Reads less than 25 bp in length and those with a base quality score of less than 20 were excluded from the data. Of the 29,088 genes used in the analysis, 21,451 genes were expressed, of which 21,402 (99.77 %)

genes had functional descriptions, resulting in a high percentage of annotated genes (Table 1). Trimmed reads were mapped to the transcript reference genome to obtain gene expression values. The average mapping rate was 93.48 % (Table 2) based on HISAT2 software.

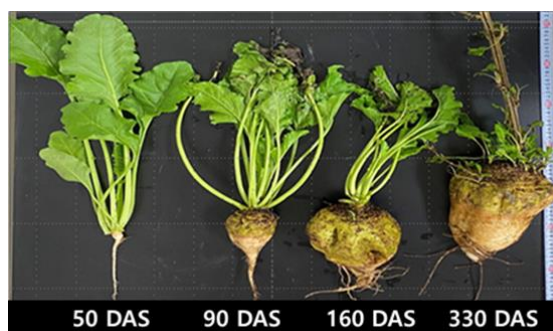


Figure 1. Sugar beet taproot morphology in different growth period. DAS: days after seedling.

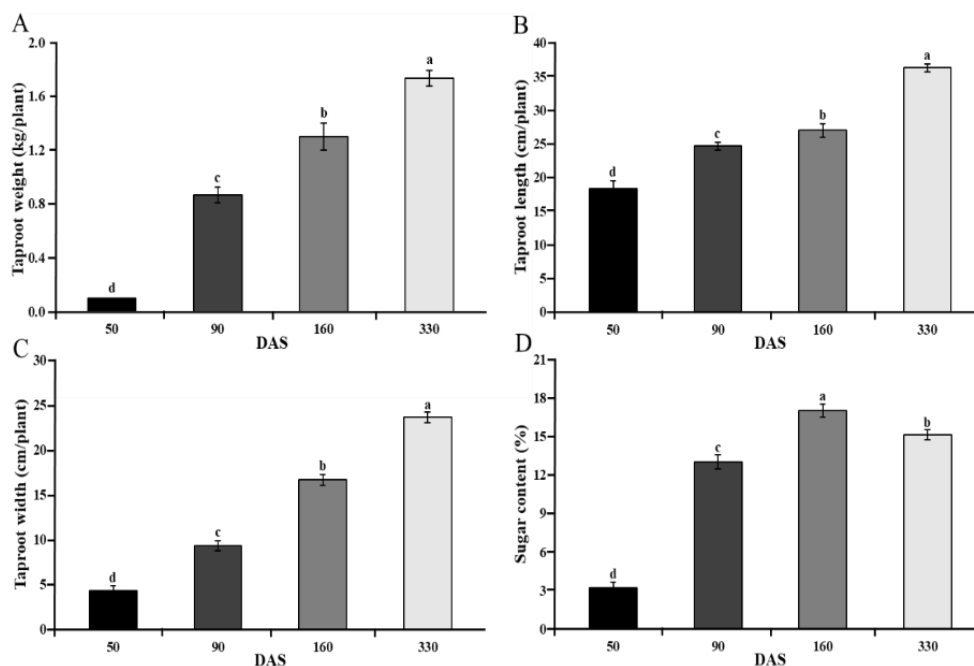


Figure 2. Taproot weight, taproot length, taproot width, and sugar content in root sample at 50, 90, 160, and 330 DAS. a: taproot weight (kg/plant), b: taproot length (cm/plant), c: taproot width (cm/plant), d: sugar content (%). Vertical bars are means \pm SDs ($n=3$). Bars labelled with lower-case letters are significantly different by Duncan's test at the $p \leq 0.05$ level.

Identification of DEGs in sugar beet with different growth periods. Differentially expressed genes among the four stages S1 (50 DAS), S2 (90 DAS), S3 (160 DAS), and S4 (330 DAS) were identified ($p \leq 0.05$ and $|\log_2$ fold

change > 1.5). A total of 4,991 differentially expressed transcripts were uncovered: 4,560, 4,764, and 4,781 DEGs in S1 vs. S2, S1 vs. S3, and S1 vs. S4, respectively (Figure 3). Among these DEGs, 2,048 genes were upregulated and

2,512 genes were downregulated in S1 compared with S2; 2,213 genes were upregulated and 2,551 genes were downregulated in S1 compared with S3; 2,123 genes were upregulated and 2,658 genes were downregulated in S1 compared with S4.

There were 3,255 genes that were commonly upregulated or downregulated in each comparison (Figure 4a). Of these, 1,345 genes were commonly upregulated and 1,909 genes were commonly downregulated (Figure 4b).

Table 1. Annotation statistics of 4 samples of sugar beet for growth period.

No. of genes used in the analysis	No. of genes with expression value	No. of genes with functional annotation information
29,088	21,451	21,402 (99.77 %)

Table 2. Statistics of reads mapping to reference genome.

Sample ID	Total reads	Aligned 0 times		Aligned exactly 1 time		Aligned > 1 times		Aligned (discordantly or single)		Mapping rate	
		Reads	Percent (%)	Reads	Percent (%)	Reads	Percent (%)	Reads	Percent (%)	Reads (ea)	Percent (%)
S1_1	15,910,948	1,046,091	6.57	13,962,191	87.75	319,751	2.01	582,914	3.66	14,864,857	93.43
S1_2	14,093,111	981,536	6.96	12,326,876	87.47	286,580	2.03	498,118	3.53	13,111,575	93.04
S1_3	21,213,806	1,520,505	7.17	18,505,592	87.23	474,059	2.23	713,649	3.36	19,693,301	92.83
S2_1	14,356,469	914,280	6.37	12,717,348	88.58	320,209	2.23	404,631	2.82	13,442,189	93.63
S2_2	13,838,828	920,899	6.65	12,244,692	88.48	262,650	1.90	410,587	2.97	12,917,929	93.35
S2_3	14,161,136	1,039,117	7.34	12,266,466	86.62	323,768	2.29	531,785	3.76	13,122,019	92.66
S3_1	13,733,971	958,481	6.98	11,939,342	86.93	368,831	2.69	467,316	3.40	12,775,490	93.02
S3_2	14,039,989	850,166	6.06	12,442,135	88.62	318,400	2.27	429,288	3.06	13,189,823	93.94
S3_3	19,781,039	1,190,499	6.02	17,530,335	88.62	424,590	2.15	635,614	3.21	18,590,540	93.98
S4_1	13,486,854	883,893	6.55	11,848,662	87.85	305,429	2.26	448,870	3.33	12,602,961	93.45
S4_2	15,066,970	861,731	5.72	13,360,143	88.67	319,359	2.12	525,736	3.49	14,205,239	94.28
S4_3	13,451,854	786,193	5.84	11,909,751	88.54	286,207	2.13	469,702	3.49	12,665,661	94.16
Total	183,134,975	11,953,391	6.52	161,053,533	87.95	4,009,833	2.19	6,118,210	3.34	171,181,584	93.48

Aligned 0 times: Number of reads that were not mapped to the reference gene sequence after pre-processing. Aligned exactly 1 times: Number of reads in which the number 1 mapping operates in the reference gene sequence after pre-processing. Aligned > 1 times: Number of reads in which mapping operates in the reference gene sequence after pre-processing over 1 time. Aligned (discordantly or single): Number of reads mapped to different chromosome between pairs in the reference gene sequence after pre-processing or mapped to a single. Mapping rate: mapped rate

GO category analysis among comparison groups. To understand and classify the functions of the common DEGs across the three comparisons, gene ontology (GO) analysis was conducted. The enriched genes in the three comparisons were annotated in three main GO categories, including biological process (BP), cellular component (CC), and molecular function (MF). The top 20 GO enrichment terms were almost completely different among the three comparisons (Figure 5). In the comparison between S1 and S2, the DEGs had 7 enriched GO terms in the CC category, 11 in the BP category,

and 2 in the MF category (Figure 5a). In the comparison between S1 and S3, the DEGs had 10 enriched GO terms in the BP category, 6 in the CC category, and 4 in the MF category (Figure 5b). In the comparison between S1 and S4, no GO terms were detected in the MF category, but 5 in the CC category and 15 in the BP category were identified (Figure 5c). GO terms related to cell walls in the MF and CC categories were common across all three comparisons.

DEGs related to sugar metabolism. DEGs related to sugar metabolism including sucrose metabolism-related enzymes and sugar transport-

related proteins were screened from the entire DEG pool (Figure 6a). Cluster heat map analysis divided 48 DEGs into two groups according to their expression levels. Class I had DEGs downregulated compared to S1 and included three genes encoding sucrose synthase, four genes

encoding sucrose transporter proteins, six fructokinases, and three hexokinases. Class II had DEGs upregulated compared to S1 and included six genes encoding sucrose transporter proteins, one sucrose-phosphate phosphatase, one sucrose-phosphate synthase, one fructokinase, and two hexokinases.

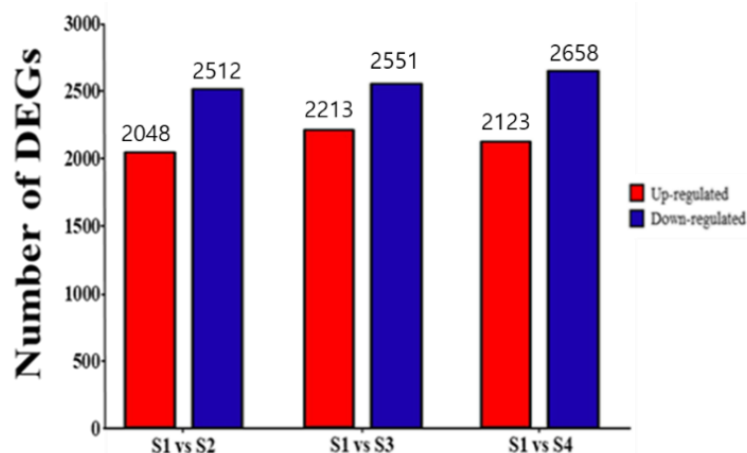


Figure 3. Number of DEGs identified in comparison different growth period for sugar beet.

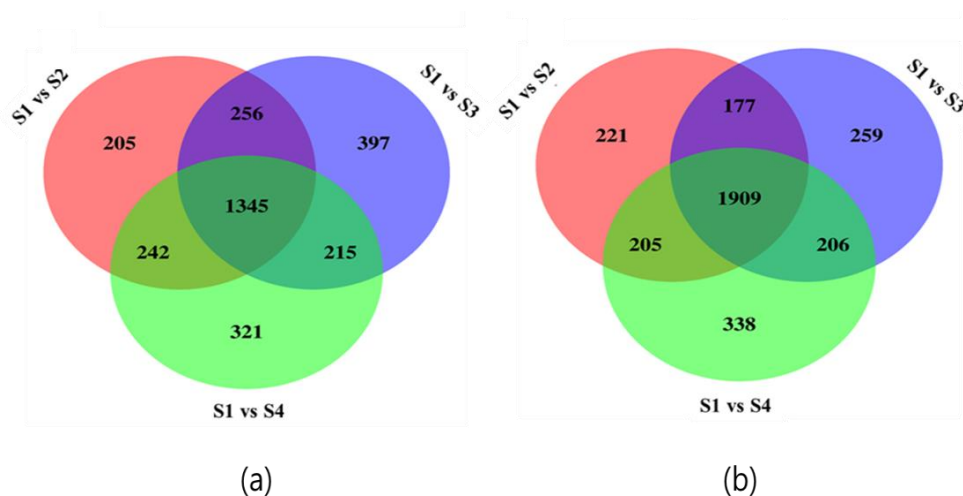


Figure 4. Venn diagrams displaying the number of common DEGs identified in sugar beet with different growth period: (a) Up-regulated DEGs, (b) Down-regulated DEGs.

DISCUSSION

In this study, comprehensive transcriptomic analyses were performed to identify genes involved in the sucrose biosynthesis pathway in sugar beet (*Beta vulgaris* L.) across four developmental stages: 50, 90, 160, and 330 DAS. The sugar content analysis revealed a significant increase from 50 to 160 DAS, followed by a decrease at 330 DAS. This pattern is consistent

with the known sucrose accumulation dynamics in sugar beet, where sucrose content increases during early development and may decline as the plant matures (Kenter *et al.*, 2006; Draycott, 2008). The decrease at 330 DAS may be attributed to physiological changes associated with plant maturation or remobilization of stored carbohydrates for reproductive growth (Milford *et al.*, 2000; Kenter *et al.*, 2006). Concurrently, the taproot's weight, length, and width

consistently increased throughout all growth periods. This indicates that physical growth and sucrose accumulation are not strictly correlated

in the later stages of development, suggesting a potential shift in the plant's metabolic priorities (Bellin *et al.*, 2007).

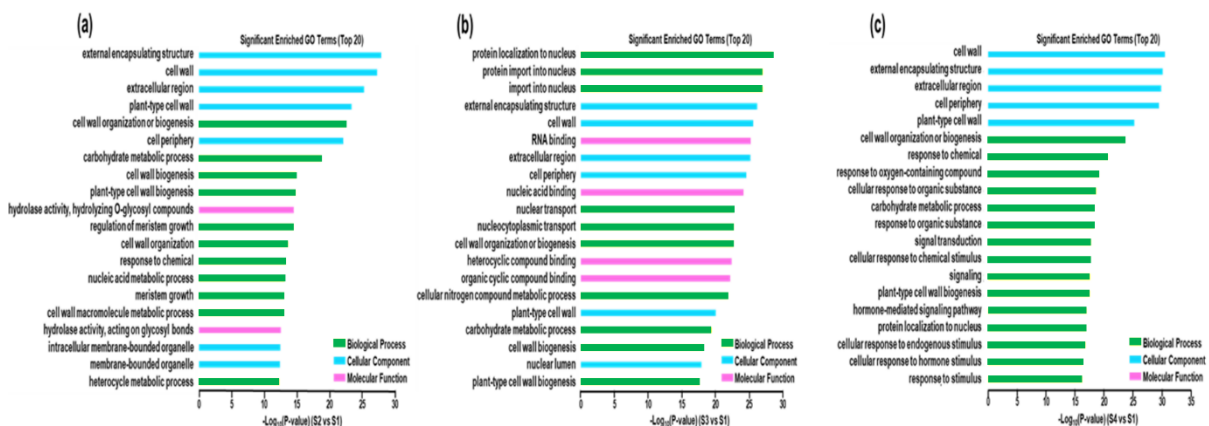


Figure 5. Top 20 GO terms for DEGs among each of the three comparison groups. (a) to (c) significantly enriched GO terms in the S1 vs. S2, S1 vs. S3, and S1 vs. S4 comparisons at the $p \leq 0.01$ level.

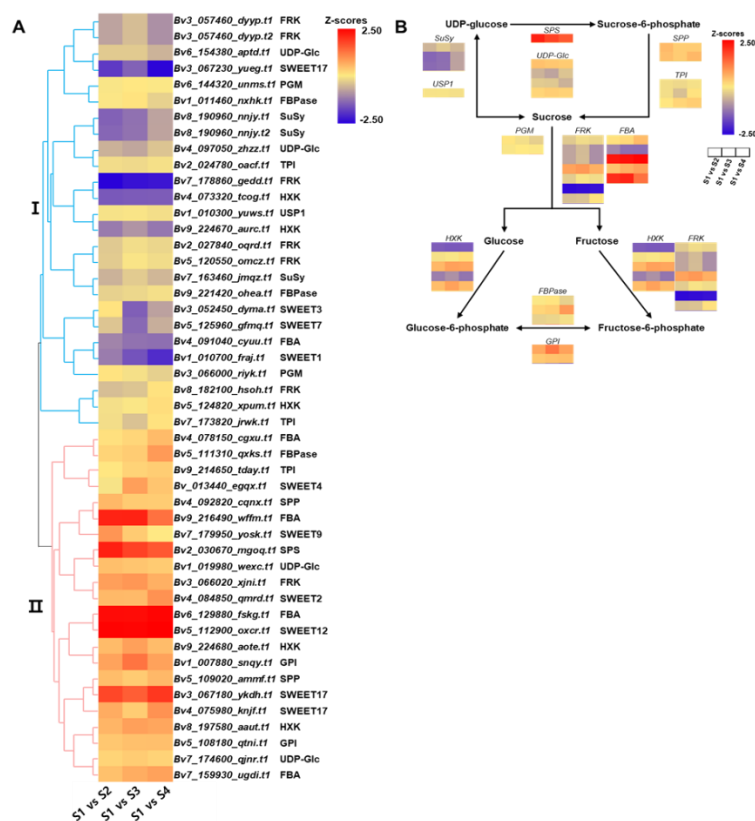


Figure 6. Heat map of DEGs related to sucrose transporters and sucrose metabolic enzymes. (A) The heat map was observed by TBtools based on RNA-seq FPKM. The right column shows the corresponding gene IDs and gene names, respectively. The color bar represents the expression level of each gene, where blue indicates downregulation and red indicates upregulation. (B) A simplified sucrose biosynthesis pathway. Heat map of columns and rows represents comparison groups and genes, respectively.

The identification of 4,991 DEGs across the three developmental comparisons highlights significant transcriptional changes associated with taproot development and sucrose metabolism. GO enrichment analysis revealed that the top enriched terms were consistently related to the external encapsulating structure, cell wall, and extracellular regions. This suggests that modifications in cell wall composition and structure are integral to taproot development and sucrose accumulation (Cosgrove, 2005). The cell wall plays a crucial role in cell expansion and storage tissue development, which may influence the capacity for sucrose storage in the taproot (Burton and Fincher, 2014).

Among the DEGs, forty-eight genes related to sugar metabolism were identified, including key enzymes and transporters involved in sucrose synthesis and allocation, such as SuSy, SPS, FRK, hexokinase (HXK), SUT/SUC, and SWEET proteins (Lunn and MacRae, 2003; Koch, 2004) (Figure 6b). Especially, SPS functions as a key regulatory enzyme catalyzing sucrose synthesis, and its increased expression has been directly correlated with enhanced sucrose accumulation in multiple crops such as sugarcane and maize (Lunn and MacRae, 2003). Similarly, SuSy plays dual roles in both sucrose cleavages for metabolic needs and sucrose biosynthesis, thereby modulating carbon partitioning (Amor *et al.*, 1995). SWEET transporters facilitate sucrose efflux and phloem loading, influencing the distribution of sucrose from source to sink tissues, as demonstrated in Arabidopsis and rice (Chen *et al.*, 2012). The observed differential expression of these genes in sugar beet aligns with findings in other species where shifts in their transcription correspond closely with sucrose content variations during development (Khan *et al.*, 2023). This indicates that modulation of SPS, SuSy, and SWEET expression likely governs sucrose biosynthesis and allocation dynamics in sugar

beet taproots. For example, increased SuSy activity has been linked to sucrose metabolism adjustment during developmental transitions in maize (Amor *et al.*, 1995). Our data showing upregulation of SPS and certain SWEET genes at mid to late stages correspond well with the sucrose accumulation peak at 160 DAS, while downregulation of some SuSy genes at 330 DAS supports the observed decrease in sucrose content. These patterns strongly support a direct mechanistic link between gene expression profiles and sucrose accumulation dynamics. Cluster analysis divided these DEGs into two groups based on their expression patterns: Class I genes were downregulated compared to the earliest stage (S1), and Class II genes were upregulated. The downregulation of Class I genes, which include some SuSy and sucrose transporter genes, during later stages suggests a decreased capacity for sucrose synthesis and transport, potentially contributing to the decline in sugar content at 330 DAS. Conversely, the upregulation of Class II genes, such as SPS and additional sucrose transporter genes, may indicate a compensatory mechanism or a shift in sucrose metabolism pathways.

These differential expression patterns highlight the complex regulatory networks governing sucrose metabolism in sugar beet taproots. Understanding these networks is essential for developing strategies to enhance sucrose accumulation. The identification of key DEGs provides valuable targets for genetic manipulation aimed at improving sugar yield, which is significant for both the sugar industry and the production of bio-based materials like PLA (Ruan, 2014). In future studies, the identified genes involved in sucrose biosynthesis will be validated using RT-qPCR to confirm their expression profiles. Also, the discrepancy between continuous taproot growth and declining sugar content at later stages suggests that factors other than physical growth influence sucrose

accumulation. This underscores the importance of integrating molecular approaches with traditional breeding practices to optimize both tap-root size and sugar content. Environmental factors, such as temperature and nutrient availability, may also impact sucrose metabolism and should be considered in future studies (Hoffmann *et al.*, 2009).

Understanding the expression dynamics of these genes provides valuable markers for breeding programs aimed at enhancing sucrose content. By selecting for variants with favorable expression profiles of key genes like SPS and SuSy, breeders can potentially develop cultivars that maintain higher sucrose content even at later developmental stages (Mitchell, 2010). Additionally, insights from this study could guide agronomic decisions related to optimal harvest times to maximize sucrose yield based on the developmental expression profiles of these critical genes (Kenter *et al.*, 2006). Further functional characterization of these genes could elucidate the molecular mechanisms underlying sucrose biosynthesis and identify targets for genetic improvement. Functional studies, including gene overexpression or silencing, have been effective in altering sugar content in various crops. By targeting these genes, it may be possible to develop sugar beet varieties with improved sucrose yields, benefiting the sugar production industry.

CONCLUSIONS

This study revealed the complex interplay between gene expression, taproot development, and sucrose metabolism in sugar beet. Sugar metabolism-related genes showed two major expression patterns, which may contribute to reduced sucrose accumulation or shifts in metabolic pathways during later growth stages. These patterns reflect intricate regulatory networks and offer promising molecular targets for improving

sucrose yield through genetic or biotechnological approaches. By integrating transcriptomic data with physiological traits, this study enhances our understanding of the temporal regulation of sucrose biosynthesis and allocation. The identified candidate genes serve as useful molecular markers for breeding programs and provide a basis for optimizing harvest timing, ultimately supporting increased productivity and broader industrial applications of sugar beet.

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