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TRANSCRIPTOME ANALYSIS PROVIDES INSIGHTS INTO THE SUCROSE SIGNAL TRANSDUCTION IN WHEAT (*TRITICUM AESTIVUM* L.)

CHAO MA[1], YALAN FENG[2*], BINBIN GUO[1], JUN ZHANG[1], SHUANG ZHOU[1], KANG DU[1], KE XV[3] AND XUELI QI[4*]

[1]College of Agriculture, Henan University of Science and Technology, Luoyang 471023, Henan province, P.R. China
[2]College of Life Science, Wuchang University of Technology, Wuhan 430223, Hubei province, P.R. China
[3]Department of Agriculture and Rural Affairs of Shanzhou District, Sanmenxia 472100, Henan province, P.R. China
[4]Wheat Research Institute, Henan Academy of Agricultural Sciences, Zhengzhou 450002, Henan province, P.R. China
**Corresponding author's email: fengyalan2004@163.com; xueliqi888@163.com*

Abstract

Sucrose is not only the main photosynthetic product and energy substance in plants, but also an important signaling molecule that regulates plant growth and development. Because sucrose in plants is easily hydrolyzed into glucose and fructose, whose signal transduction pathways are not completely the same as those of sucrose. In this study, Illumina high-throughput sequencing platform was used to perform transcriptome and expression profile sequencing in wheat leaves treated with sucrose, glucose and fructose, respectively. A total of 1327 (711 up-regulated and 616 down-regulated) differentially expressed genes (DEGs) that specifically responded to sucrose were screened by comparative transcriptome analysis. Based on functional annotation and pathway enrichment analysis, these differentially expressed genes were divided into four major categories, namely, transcription factors, plant hormone-related, signal-related and metabolism related. Four of the differentially expressed genes belong to three categories (transcription factors, phytohormone-related, and signal-related), and 16 of them belong to two categories: signal transduction and phytohormone. These differentially expressed genes are relevant genes independent of glucose and fructose signaling pathways, and participate in sucrose-specific signal transduction pathways. At the same time, the conclusion of this research will provide a theoretical basis for the improvement of sucrose signal transduction pathways and the illustration of their mechanism of action.

Key words: Wheat, Sucrose, Expression profiling, Functional annotation, Signal transduction.

Introduction

Sucrose is not only the major form of long-distance transport of photosynthetic products from source tissues, such as leaves, to sink tissues, like roots, flowers, fruits, etc., but also an important component of the carbon skeleton in plants. Additionally, it also provides energy for growth and development. As a signaling molecule, sucrose likewise plays a role at different stages of growth and development throughout the life cycle of a plant (Horacioa & Martinez-Noel, 2013). However, unlike glucose, a recognized signaling molecule, whether sucrose is a real signaling molecule has been widely disputed. Especially in the hexokinase signaling pathway, the role of sucrose has been questioned. The reason for this is that sucrose can be rapidly degraded into glucose and fructose under the action of invertase, so it is difficult to determine which is/are involved in the signal transduction process, sucrose itself or its degradation products (Meng *et al.,* 2020). It was not until the study of *Arabidopsis enhanced bending 1* (*ehb1*) mutants by Dümmer *et al*., confirmed the signal transduction function of sucrose. The *ehb1* mutant showed abnormal geotropism and phototropism in the absence of sucrose. Since the promoter region upstream of EHB1 has sucrose and light-responsive elements, the phenotype of *ehd1* mutant was restored after the addition of exogenous sucrose (Esparza-Reynoso *et al.,* 2021). In addition, sucrose is a critical signaling molecule that regulates plant growth and development. Sucrose can be used as a signaling molecule to participate in the coordination of cell division in stem apex meristem (SAM) (Wang *et al.,* 2020). Sucrose as a signal molecule regulating the expression of flowering genes in plants has been confirmed. Exogenous application of sucrose can accelerate the flower in late flower mutations *fve* (*flowering locus ve*), *fca* (*flowering locus ca*), *co* (*constans*) and *gi* (*gigantea*) in *Arabidopsis*, but cannot affect the two late flower mutations *ft* (*flowering locus t*) and *fwa* (*flowering wageningen*). It can be speculated that the sites where the *FVE*, *FCA*, *CO* and *GI* genes play a role are upstream of the site of sucrose, while FT and FWA play a role downstream of the sucrose control pathway (Roldán *et al.,* 2000; Funck *et al.,* 2012). Although numerous studies have confirmed the signal transduction function of sucrose, its specific signal transduction pathway and mechanism of action still need to be confirmed by further studies.

Sucrose can be degraded into glucose and fructose, which are also important hexoses and signaling molecules in plants. While hexose is considered to be an effective signaling molecule for plants, the signal transduction pathways of the two are not completely the same as those of sucrose, and sucrose-specific signaling pathways also affect gene transcription and translation. As signaling molecules, glucose and fructose are more effective in inhibiting the expression of photosynthesis-related genes in maize protoplasts than sucrose (Pommerrenig *et al.,* 2018). Tauzin & Giardina (2014) first demonstrated that sucrose can specifically regulate the transcription and translation of the sucrose symporter, while other sugars such as hexose do not have this regulatory effect. Barbier *et al.,* (2015) found that glucose is related to the early growth of organs, and plays an important role in maintaining the osmotic potential to enlarge the newly divided cells, whereas sucrose is mainly related to the maturity of plant organs, such as the development of phloem. In *Paspalum vaginatum* and some other grasses, sucrose can promote the occurrence of stolons, while glucose and fructose do

not have similar effects (Willemoës *et al.,* 1988). The expression of leucine zipper gene *ATB2* (*Arabidopsis thaliana bZIP*) in *Arabidopsis* is equally regulated by sucrose, which could be inhibited by 50-100mmol/L sucrose, while other sugars have no such effect, indicating that there is a special sucrose signaling system in plants (Smeekens & Rook, 1997; Rook *et al.,* 2010). Sucrose signaling pathway can regulate the distribution of assimilation products at the level of phloem operation, and hexokinase inhibitors cannot affect this signaling pathway, further indicating that hexokinase is not a receptor for this signaling (Tauzin & Giardina, 2014).

Plants can not only sense sucrose, but also hexose and other disaccharides (Liu *et al.,* 2018). The existence of multiple sugar sensing mechanisms and sugar signaling pathways increases the complexity of plant sugar signaling (Wang *et al.,* 2020; Chen *et al.,* 2022). Since sucrose in plants is easily hydrolyzed into glucose and fructose, so the interpretation of sucrose-specific signaling pathways is more difficult. Therefore, in this study, Illumina high-throughput sequencing platform was used to perform transcriptome and expression sequencing on wheat leaves treated with sucrose, glucose and fructose, respectively. Through comparative transcriptome analysis, the different genes specifically expressed under sucrose were screened to identify related genes involved in sucrose-specific signal transduction pathways, which would provide a theoretical basis for the improvement of sucrose signal transduction pathways and the elucidation of its mechanism of action.

Material and Methods

Plant materials and treatments: The experimental material was wheat cultivar Zhoumai 18. The wheat seeds were sterilized with 2% sodium hypochlorite solution and 75% ethanol in sequence and placed on a floating plate. These seeds were germinated in a dark climate chamber at 25°C for 3 days, then illuminated for 1 day and transplanted to the hydroponic chamber. Each hydroponic chamber contained 40 wheat plants. The nutrient solution was Hoagland culture solution (pH 6.6~6.8), which was ventilated for 30 min every day and replaced every 3 days. The culture conditions: 16h/8h (light/dark) and 25°C/20°C (light/dark). The experimental treatments began when these seedlings grow to 2-leave stage. Treatments included: CK (Hogeland Nutrient Solution), sucrose (Hogeland Nutrient Solution+1mmol/L sucrose), glucose (Hogeland Nutrient Solution+1mmol/L glucose) and fructose (Hogeland Nutrient Solution+1mmol/L fructose). The exogenous sugar treatments have been carried out when wheat seedlings grew to the two-leaf stage. Samples were taken after three days of treatment. 5 seedlings from each treatment were chosen, and their whole leaves of the second fully expanded leaf were quickly frozen in liquid nitrogen, and then store them in a refrigerator at -80°C.

RNA isolation and qualification: The methods of isolation and reverse transcription for total RNA based on the description of Ma *et al.,* (2019). Nano Drop 2000 (Thermo) was employed to detect the concentration and purity of RNA. The integrity of RNA was assessed by the Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA), including RIN value, 28S/18S and 5S peak.

Library preparation and quality control of transcriptome sequencing data: After the RNA samples were qualified, the library was constructed. The main process and method refer to Feng *et al.,* (2019). The library quality was checked after it was constructed, and the sequencing can be carried out if the result could meet the requirements. Biomarker Technologies Corporation strictly controls the quality of sequencing data and filters them. A total of 66.7 Gb of high-quality clean data was obtained. The indexes including clean reads and clean bases are shown in (Table 1), and the Q30 base percentage of each sample was not less than 87.36%.

Table 1. Statistics of RNA-seq data.

Samples	Clean reads	Clean bases	GC content	%≥Q30
CK	82939265	24773709458	56.81%	88.32%
Fructose	45715856	13664653824	55.50%	87.36%
Glucose	48785320	14570284210	56.09%	87.77%
Sucrose	46208811	13769352624	56.63%	87.66%

Data processing and bioinformatics analysis: Biomarker Technologies Corporation further processed and analyzed the Illumina sequencing data. The specific steps depended on Feng *et al.,* (2022). According to the reference genome, only these reads with a perfect match or one mismatch would be further analyzed and annotated. The software Tophat2 tool was adopted to map with reference genome. In this project, the assigned genome was used as a reference for sequence alignment and subsequent analysis. The download address of reference genome: https://urgi.versailles.inra.fr/download/iwgsc/IWGSC_RefSeq_Assemblies/v1.0/. The expression levels of transcripts and genes were quantified by the position information of Mapped Reads on genes using the Cuffquant and Cuffnorm components of the Cufflinks software. The Fold Change≥1.5 and FDR<0.01 were used as screening criteria to acquire unique genes. Bioinformatics analysis included the differential expression analysis, gene functional annotation, Gene Ontology (GO) annotation and enrichment analysis, Cluster of Orthologous Groups (COG) of proteins classification and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, which could make reference to the description of Feng *et al.,* (2022).

Quantification real-time fluorescence PCR (qRT-PCR) validation: 8 DEGs (4 up-regulated and 4 down-regulated) were selected to detect the expression level via qRT-PCR. The PCR procedures and reaction system refer to the description of Feng *et al.,* (2015). The fold variation was calculated by $2^{-\triangle\triangle Ct}$ method (Livak & Schmittgen, 2001).

Results

Morphological changes and growth parameters of wheat seedlings under different treatments: The difference of wheat phenotypes was significant after adding three sugars to the culture medium. All three sugars can promote the growth of wheat seedling leaves and roots (Fig. 1A). Compared with the control, after adding fructose, glucose and sucrose, the plant height increased by 2.0%, 15.6% and 8.6%, respectively (Fig. 1B), the root length increased by 3.1%, 17.2% and 9.2%, respectively (Fig. 1C), and the biomass increased by 5.6%, 31.3% and 21.2%, respectively (Fig. 1D). The promotion effect was glucose>sucrose>fructose.

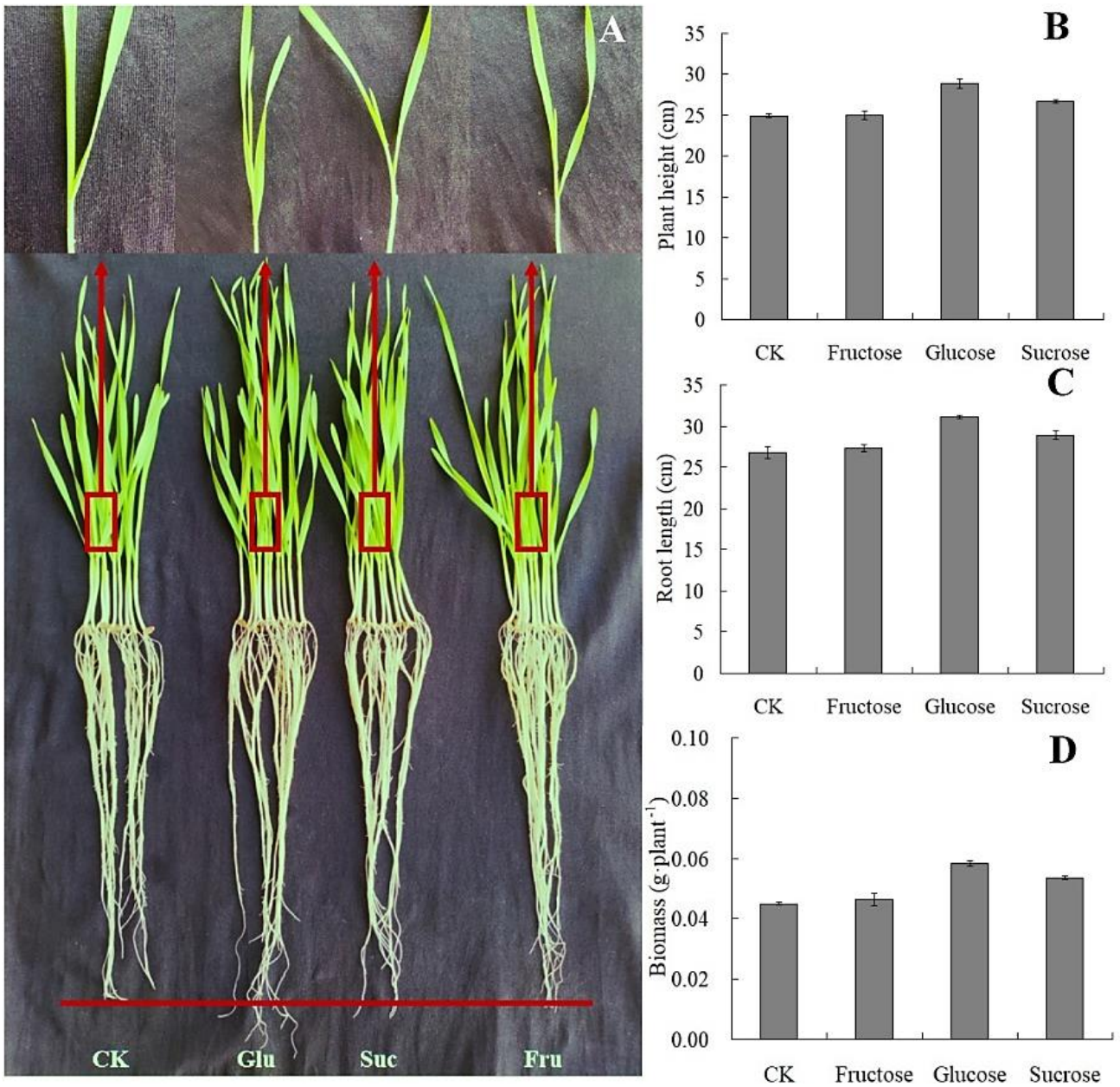

Fig. 1. Physiological changes of wheat seedling in response to different sugars. (A) Morphological changes; (B) Plant height; (D) Root length; (D) Biomass. Glu, Glucose; Fru, Fructose; Suc, Sucrose.

Identification of DEGs: Gene expression has spatiotemporal specificity. Under different treatment conditions, genes or transcripts with significantly different expression levels are called DEGs. 10152, 9489 and 9171 DEGs were obtained from sucrose, glucose and fructose-treated samples (Fig. 2). 1160 and 8992 DEGs were up-regulated and down-regulated respectively under sucrose treatment, 457 and 9032 DEGs were up-regulated and down-regulated respectively under glucose treatment, and 589 and 8583 DEGs were up-regulated and down-regulated under fructose treatment, respectively.

Hierarchical cluster analysis (HCA) of the expression of key candidate genes in transcriptome based on three exogenous sugar treatments showed that most of the candidate genes changed significantly under three different exogenous sugar treatments. According to the expression patterns of candidate target genes under different exogenous sugar treatments, they can be clearly divided into four main clusters (Fig. 3). The candidate gene in group I expressed the highest amount under sucrose treatment. In group II, most of the candidate genes were expressed at the highest level under sucrose treatment, and individual genes were also up-regulated in glucose and sucrose treatment. In group III, all candidate genes were down-regulated under sucrose treatment. For group IV, all candidate genes were down-regulated under three sugar treatments (Fig. 3).

Further comparative analysis of the DEGs under the three treatments revealed that since sucrose can be hydrolyzed into glucose and fructose, and all three sugars can be used as signal molecules, the common expression part of the three and the common expression part between the two can be excluded. The remaining DEGs specifically expressed in sucrose are the focus of our subsequent analysis and research.

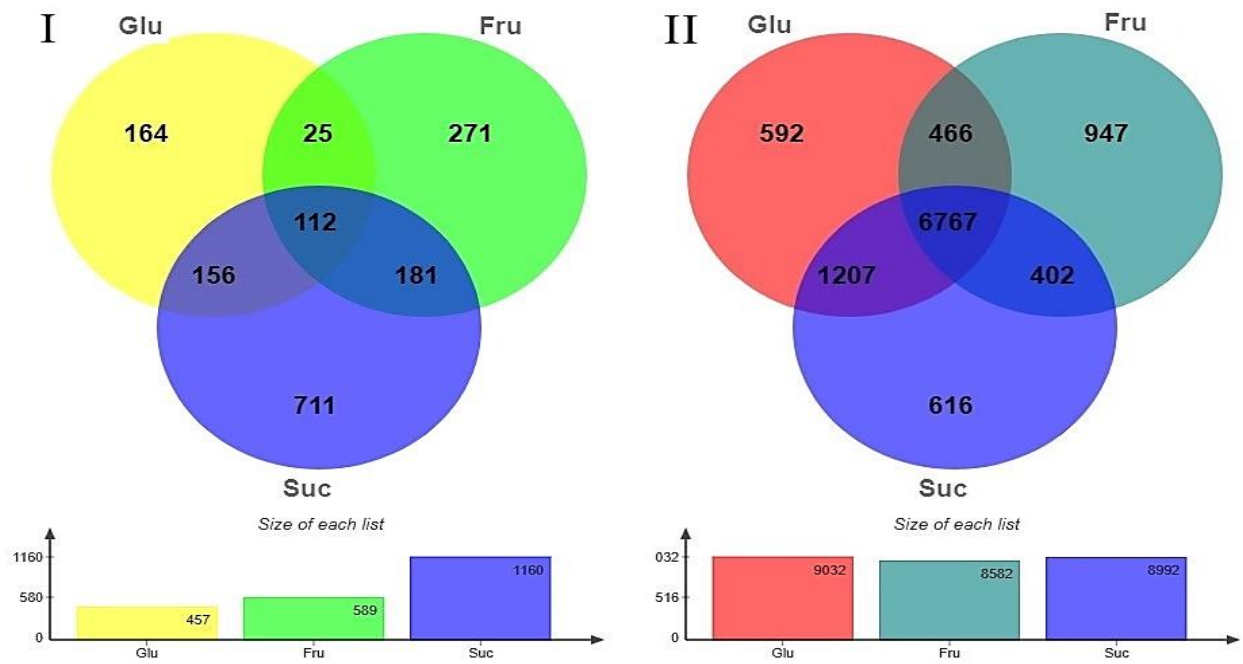

Fig. 2. Venn diagram shows number of DEGs in glucose, fructose and sucrose treatments.
(I) number of up-regulated genes; (II) number of downregulated genes. Glu, Glucose; Fru, Fructose; Suc, Sucrose.

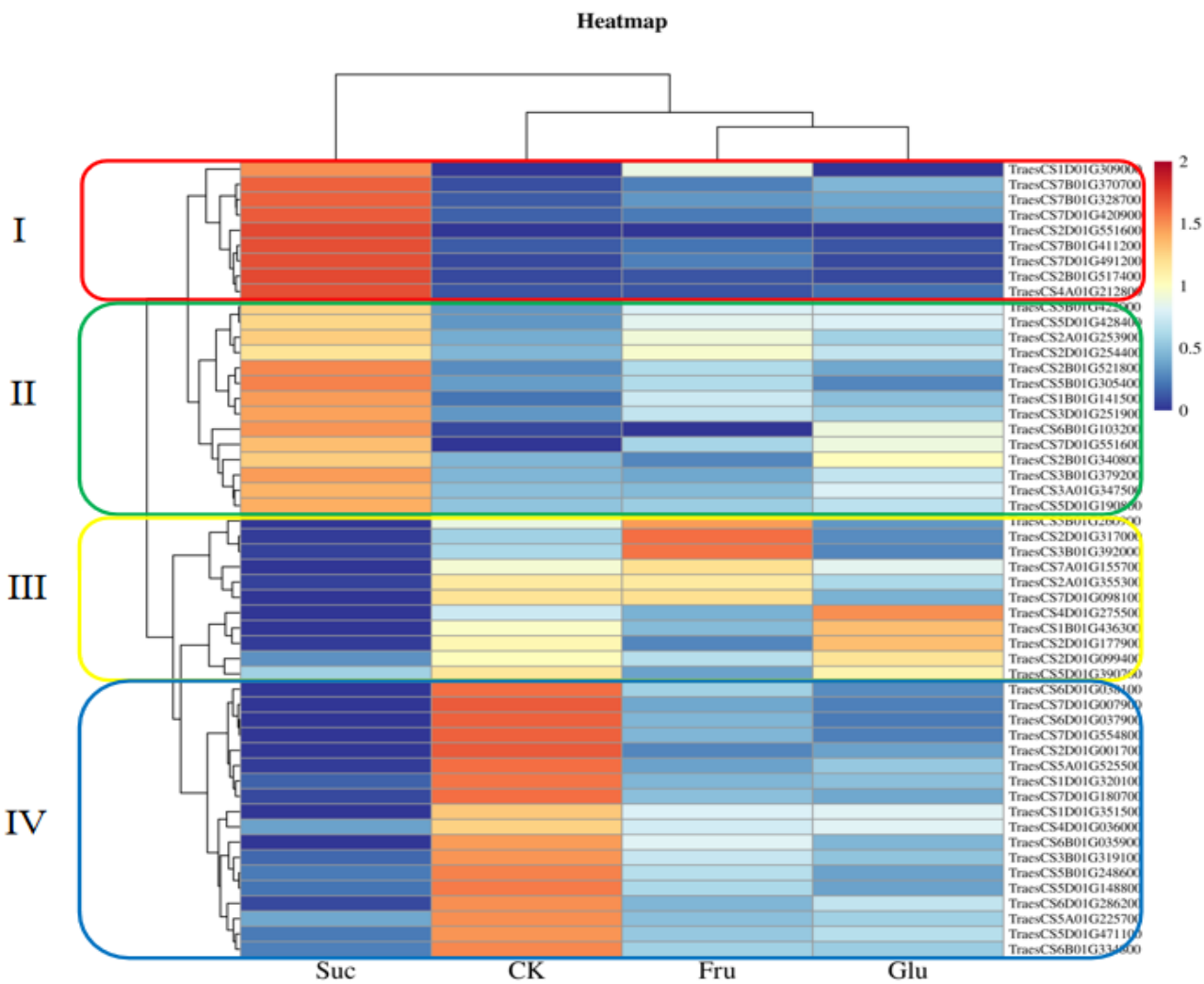

Fig. 3. Heat map of gene expression value in four samples. CK, Control; Glu, Glucose; Fru, Fructose; Suc, Sucrose. I, II, III, and IV represent different groups of candidate genes.

Table 2. Functional classification of DEGs specifically expressed in sucrose (some representative genes).

	DEG ID	Expression level	Functional annotation/pathway enrichment
		Suc_log2FC	
Signal related	TraesCS2D01G551600*	4.69	Signal transduction mechanisms/Phytosulfokine receptor 1 [*Triticum urartu*]
	TraesCS1D01G309000	4.55	Signal transduction mechanisms/Putative disease resistance RPP13-like protein 1 [*Aegilops tauschii*]
	TraesCS5D01G428400	1.92	Transcription factor PIF3[*Arabidopsis thaliana*]
	TraesCS5B01G422000	1.90	Transcription factor PIF3[*Arabidopsis thaliana*]
	TraesCS2A01G253900	1.85	Transcription factor PIF4[*Brachypodium distachyon*]
	TraesCS2D01G254400	1.70	Transcription factor PIF4[*Brachypodium distachyon*]
	TraesCS7D01G554800	-4.21	Signal transduction mechanisms/hypothetical protein F775_23689 [*Aegilops tauschii*]
	TraesCS2D01G001700	-4.26	Signal transduction mechanisms/Cysteine-rich receptor-like protein kinase 10 [*Triticum urartu*]
	TraesCS7D01G007900	-4.30	Signal transduction mechanisms/F-box/kelch-repeat protein SKIP11 [*Triticum urartu*]
	TraesCS6B01G035900	-4.39	Signal transduction mechanisms/Disease resistance protein RPM1 [*Triticum urartu*]
	TraesCS7A01G155700*	-4.80	Signal transduction mechanisms/hypothetical protein F775_07750 [*Aegilops tauschii*]
Hormone related	TraesCS6B01G103200*	3.81	Auxin-activated signaling pathway/predicted protein [*Hordeum vulgare subsp. vulgare*]
	TraesCS1B01G141500	2.54	Plant hormone signal transduction/Histidine-containing phosphotransfer protein 4 [*Aegilops tauschii*]
	TraesCS2B01G521800	2.43	Abscisic acid-inducible protein kinase [*Triticum urartu*]
	TraesCS5B01G305400	2.06	Plant hormone signal transduction/predicted protein [*Hordeum vulgare subsp. vulgare*]
	TraesCS3D01G251900	2.05	Plant hormone signal transduction/predicted protein [*Hordeum vulgare subsp. vulgare*]
	TraesCS5D01G428400	1.92	Plant hormone signal transduction/predicted protein [*Hordeum vulgare subsp. vulgare*]
	TraesCS5B01G422000	1.90	Plant hormone signal transduction/predicted protein [*Hordeum vulgare subsp. vulgare*]
	TraesCS2A01G253900	1.85	Plant hormone signal transduction/Transcription factor PIF4
	TraesCS2D01G254400	1.70	Plant hormone signal transduction/Transcription factor PIF4
	TraesCS2D01G099400	-1.65	ETHYLENE INSENSITIVE 3-like 1 protein/Os07g0685700 [*Oryza sativa Japonica Group*]
	TraesCS4D01G036000	-1.73	Ethylene-insensitive protein 2/Nramp domain-containing protein [*Triticum aestivum*]
	TraesCS5D01G471100	-2.50	Plant hormone signal transduction: adenylate isopentenyltransferase 5, chloroplastic-like [*Setaria italica*]
	TraesCS6B01G334800	-2.50	Plant hormone signal transduction/HVA22-like protein [*Triticum aestivum*]
	TraesCS3B01G319100*	-2.99	Plant hormone signal transduction/predicted protein [*Hordeum vulgare subsp*]

Table 2. (Cont'd.).

	DEG ID	Expression level	Functional annotation/pathway enrichment
		Suc_log2FC	
TF	TraesCS2B01G517400*	4.40	WRKY45-like transcription factor [*Triticum aestivum*]
	TraesCS3B01G379200	1.71	putative WRKY transcription factor 33 [*Triticum urartu*]
	TraesCS5D01G428400	1.92	Plant hormone signal transduction/Transcription factor PIF3 [*Arabidopsis thaliana*]
	TraesCS5B01G422000	1.90	Plant hormone signal transduction/Transcription factor PIF3 [*Arabidopsis thaliana*]
	TraesCS2A01G253900	1.85	Plant hormone signal transduction/Transcription factor PIF4 [*Brachypodium distachyon*]
	TraesCS2D01G254400	1.70	Plant hormone signal transduction/Transcription factor PIF4 [*Brachypodium distachyon*]
	TraesCS3A01G347500	1.58	putative WRKY transcription factor 33 [*Triticum urartu*]
	TraesCS2B01G340800	1.55	Probable WRKY transcription factor 19 [*Aegilops tauschii*]
	TraesCS5D01G190800	1.51	WRKY transcription factor [*Triticum aestivum*]
	TraesCS5D01G390700	-1.87	Transcription factor bHLH92 [*Triticum urartu*]
	TraesCS5A01G225700	-1.89	Transcription factor bHLH69 [*Brachypodium distachyon*]
	TraesCS5B01G248600	-2.21	Transcription factor bHLH49 [*Aegilops tauschii*]
	TraesCS5D01G148800	-2.79	NAC domain-containing protein 29 [*Aegilops tauschii*]
	TraesCS1D01G320100	-2.99	Transcription factor bHLH112 [*Arabidopsis thaliana*]
	TraesCS4D01G275500*	-4.92	Transcription factor bHLH13 [*Aegilops tauschii*]
Metabolism related	TraesCS7D01G551600*	5.01	Putative 4-hydroxy-4-methyl-2-oxoglutarate aldolase 2 [*Arabidopsis thaliana*]
	TraesCS7D01G491200	3.98	Lipid transport and metabolism/Patatin group A-3 [*Aegilops tauschii*]
	TraesCS4A01G212800	3.89	Glycerolipid metabolism/Probable monogalactosyldiacylglycerol synthase 2 [*Oryza sativa subsp*]
	TraesCS7B01G370700	3.84	Carbohydrate transport and metabolism/Chitinase 8 [*Aegilops tauschii*]
	TraesCS6B01G103200	3.81	Carbohydrate transport and metabolism/predicted protein [*Hordeum vulgare subsp*]
	TraesCS7B01G328700	3.68	Nitrogen metabolism/probable high-affinity nitrate transporter 2.4 [*Brachypodium distachyon*]
	TraesCS7B01G411200	3.57	Lipid transport and metabolism/Patatin group A-3 [*Aegilops tauschii*]
	TraesCS7D01G420900	3.51	Nitrogen metabolism/probable high-affinity nitrate transporter 2.4 [*Brachypodium distachyon*]
	TraesCS3B01G392000	-3.54	Carbohydrate transport and metabolism/Triosephosphate isomerase [*Oryza sativa subsp*]
	TraesCS2D01G317000	-3.55	Carbohydrate transport and metabolism/7-deoxyloganetin glucosyltransferase [*Gardenia jasminoides*]
	TraesCS5A01G525500	-3.63	Carbohydrate transport and metabolism/Aquaporin NIP3-3 [*Aegilops tauschii*]
	TraesCS5B01G260900	-3.72	Lipid transport and metabolism/jmjC domain-containing protein 7 [*Brachypodium distachyon*]
	TraesCS7D01G180700	-3.77	Carbohydrate transport and metabolism/UDP-glycosyltransferase [*Arabidopsis thaliana*]
	TraesCS2D01G177900	-3.79	Carbohydrate transport and metabolism/Adipocyte plasma membrane-associated protein [*Aegilops tauschii*]
	TraesCS6D01G286200	-3.88	Carbohydrate transport and metabolism/Probable galacturonosyltransferase 9 [*Arabidopsis thaliana*]
	TraesCS2A01G355300	-3.93	Carbohydrate transport and metabolism/Peroxidase 18 [*Arabidopsis thaliana*]
	TraesCS6D01G038100	-4.17	Nitrogen metabolism/Nitrate transporter [*Aegilops tauschii*]
	TraesCS6D01G037900	-4.21	Nitrogen metabolism/Nitrate transporter [*Aegilops tauschii*]
	TraesCS1B01G436300	-5.05	Lipid transport and metabolism/ 3-ketoacyl-CoA synthase 4 [*Triticum urartu*]
	TraesCS7D01G098100	-5.29	Inorganic ion transport and metabolism/ fe^{2+} transport protein 1-like [*Brachypodium distachyon*]
	TraesCS1D01G351500*	-5.41	Energy production and conversion/ Malate dehydrogenase, chloroplastic [*Aegilops tauschii*]

DEGs= Differentially expressed genes, Suc= Sucrose, TF= Transcription factor and *= Genes selected for detection by qRT-PCR

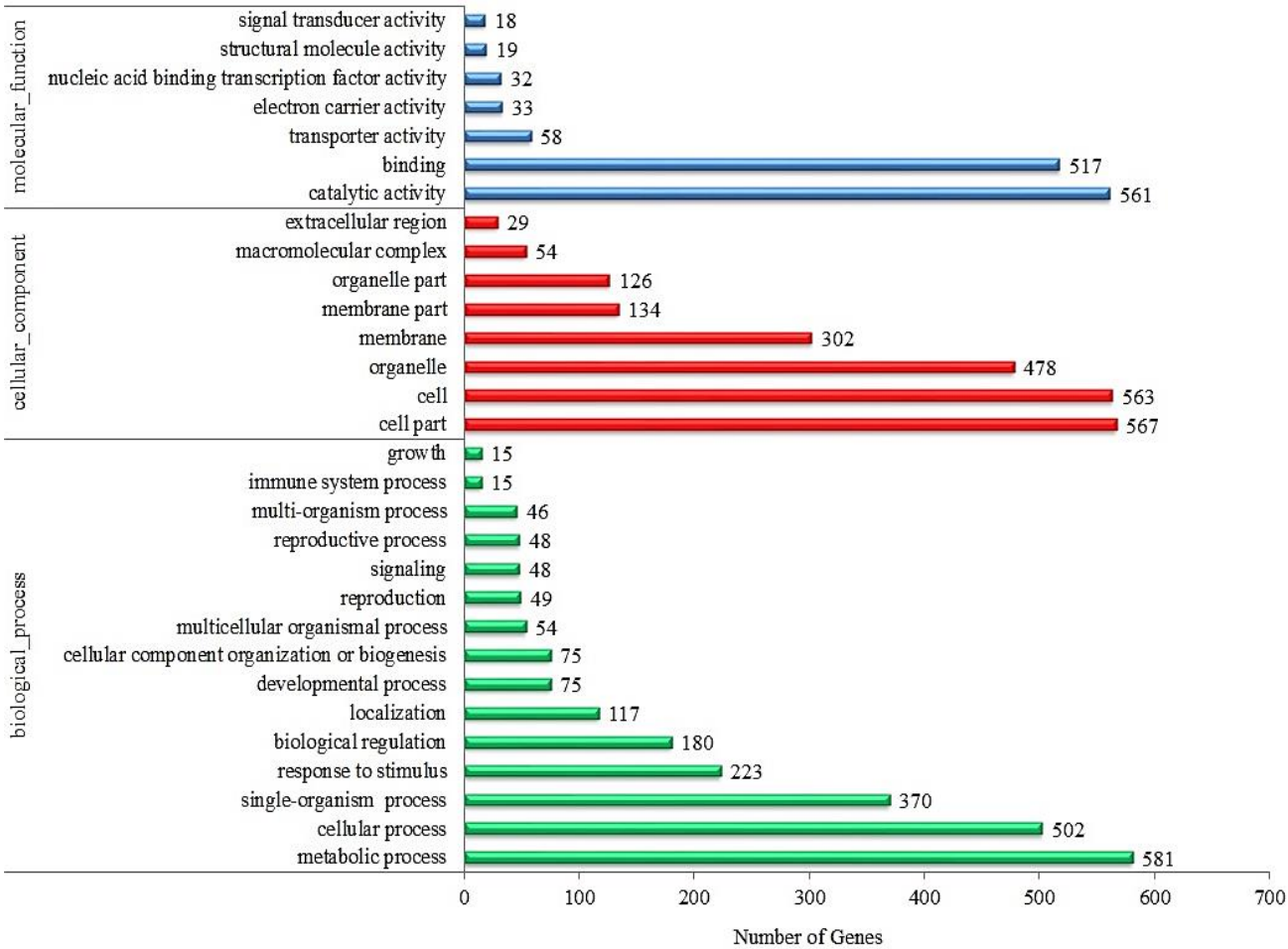

Fig. 4. GO classification of genes specifically expressed in sucrose.

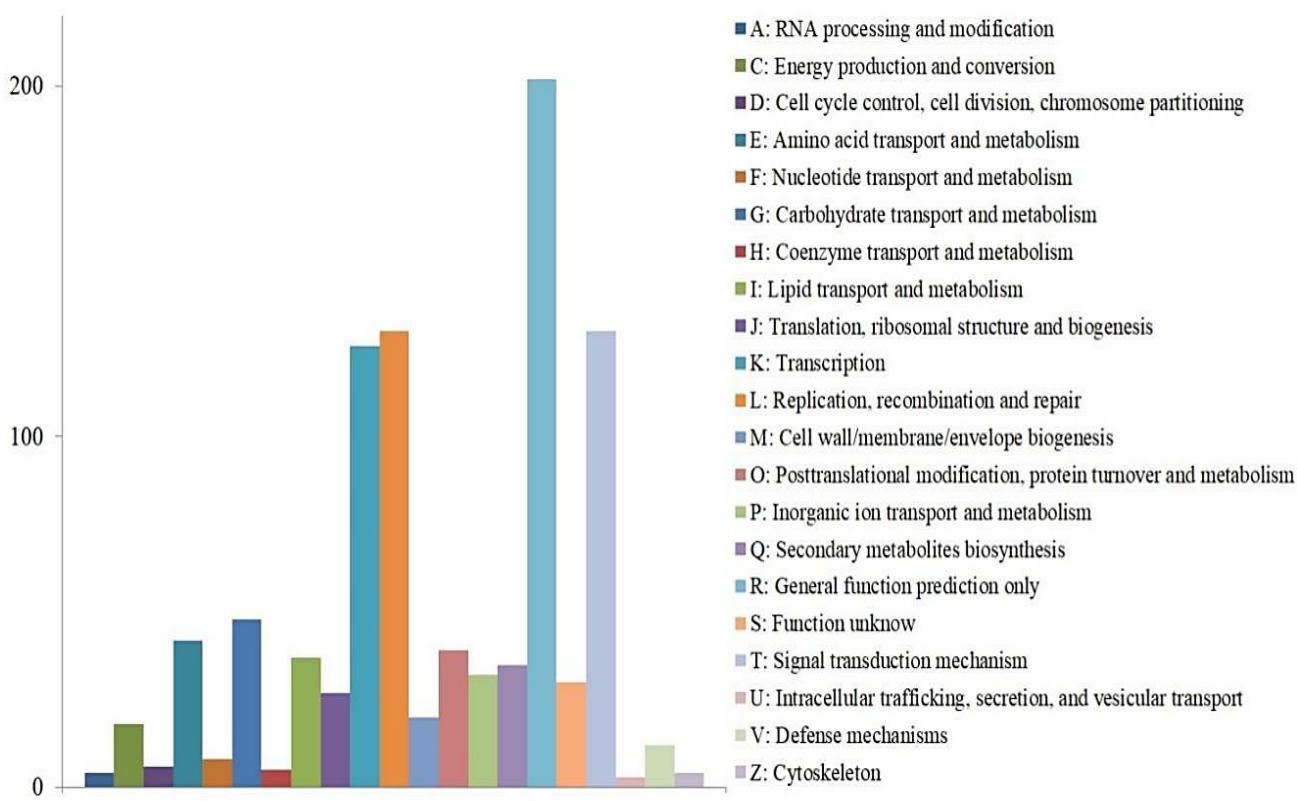

Fig. 5. COG classification of genes specifically expressed in sucrose.

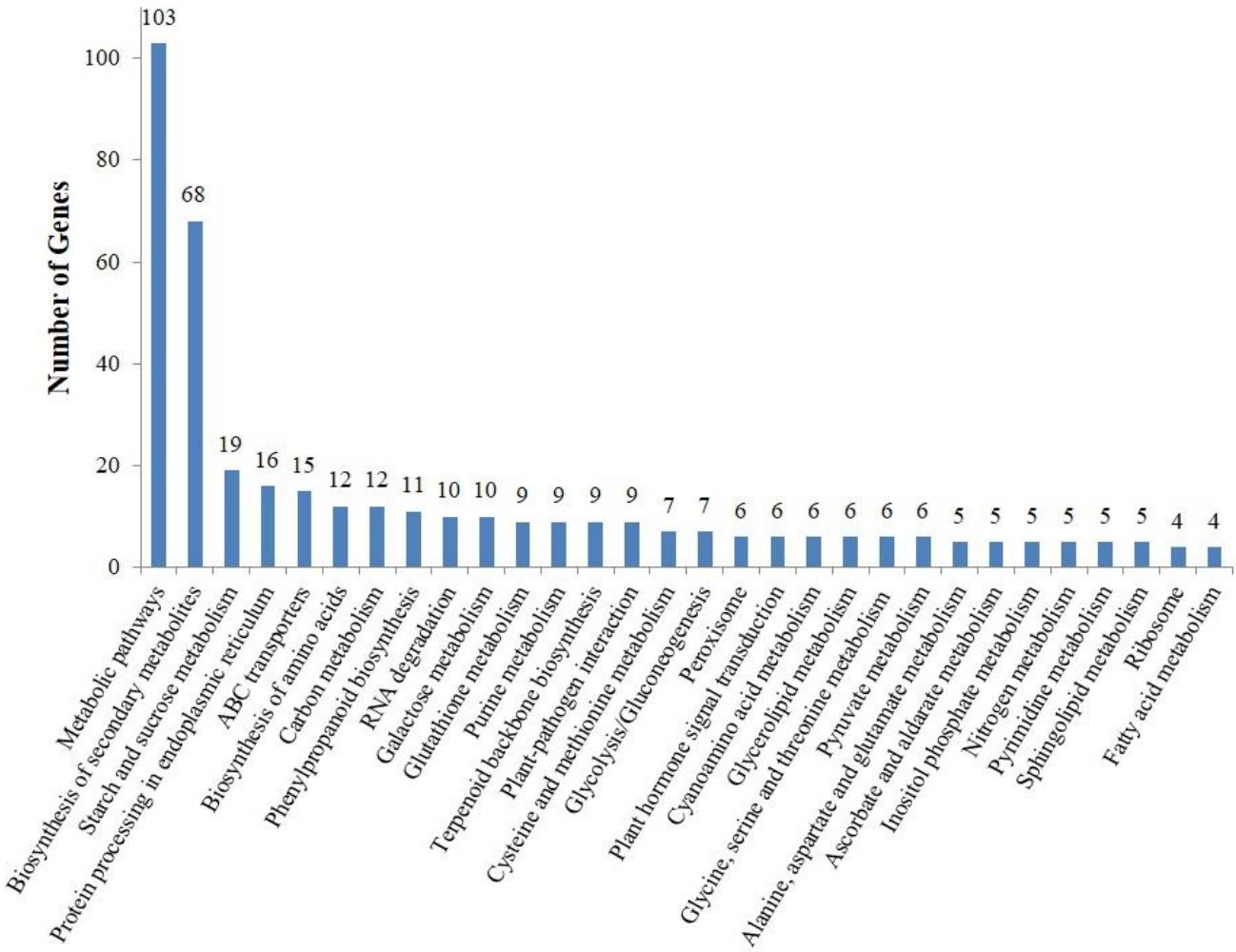

Fig. 6. KEGG function classification of genes specifically expressed in sucrose.

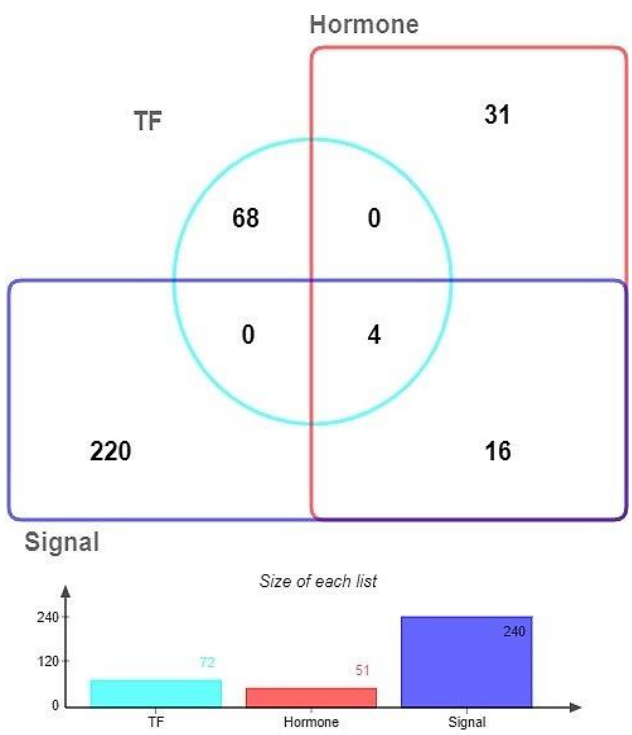

Fig. 7. Venn diagram of functional classification for differentially expressed specific genes in sucrose.

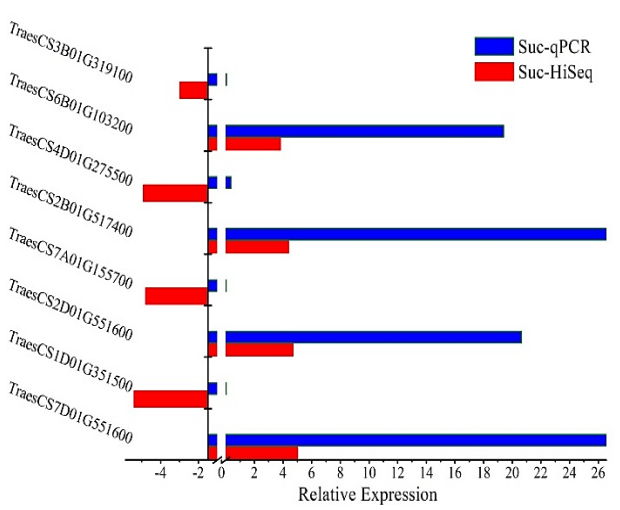

Fig. 8. Relative expression of 8 selected specially DEGs by qRT-PCR. Suc-qPCR, qPCR verification of sucrose treatment; Suc-HiSeq, transcriptional result of sucrose treatment.

Bioinformatics analysis of sucrose-specific DEGs: The GO database is a structured standard biological annotation system constructed by the Gene Ontology Consortium (GO) Organization, which is suitable for various species. The GO annotation system is a directed acyclic graph, which contains

three primary branches, namely: Biological Process, Molecular Function and Cellular Component. The GO analysis and statistical results of specific DEGs under sucrose treatment are shown in (Fig. 4). These specific genes are chief concentrated in three branches of catalytic activity and binding (Molecular Function), cell part and cell (Cellular Component), metabolic process and cellular process (Biological Process). The COG database constructed based on the phylogenetic relationship of bacteria, algae and eukaryotes can orthologously classify gene products. The statistical results of COG classification of DEGs are shown in (Fig. 5). The products encoded by these DEGs in sucrose are mostly involved in the processes of Replication, recombination and repair, Signal transduction mechanism and Transcription. KEGG is a database that systematically analyzes gene function and genomic information. Through this database, it is possible to annotate and analyze the pathways of DEGs, which contributes to further interpret the function of genes. As the central public database of pathway, the integrated metabolic pathway notes provided by KEGG, including the metabolism of carbohydrates, nucleosides, amino acids, etc. and the biodegradation of organic matter, not only provides all possible metabolic pathways, but also comprehensively annotates the enzymes catalyzing each step of the reaction, which is a powerful tool for *In vivo* metabolic analysis and metabolic network research. The KEGG annotation results of DEGs are classified according to the types of pathways in KEGG. The classification diagram is shown in (Fig. 6). Metabolic pathways and Biosynthesis of secondary metabolites are the two most concentrated classifications among annotated genes. After bioinformatics analysis, based on functional annotation and pathway enrichment analysis, the specific DGEs in sucrose are mainly divided into four categories, including signal transduction related (240), hormone response or regulation (47), transcription factor (72) and metabolism related (295) (Table 2). The four categories of DEGs were further compared and analyzed and the results are shown in (Fig. 7). There were four DEGs (TraesCS5D01G428400, TraesCS5B01G422000, TraesCS2A01G253900, and TraesCS2D01G254400), whose functional annotations involved three major categories (signal transduction-related, hormone response or regulation, transcription factor), and their expressions were up-regulated, annotating as members of the transcription factor Phytochrome-Interacting Factor (PIF) family in the Swiss-Prot and Nr databases. Another 196 DEGs were involved in both signal transduction and hormone response regulation.

Real-time quantitative PCR to verify transcriptome results: 8 DEGs were randomly selected from DEGs specifically expressed in sucrose for verification by real-time quantitative PCR. The expression results are shown in (Fig. 8). The expression trends (up or down) of the 8 DEGs are consistent with the transcriptome sequencing results, indicating that the transcriptome results are accurate and credible and can be used for subsequent analysis.

Discussion

In this study, four DEGs specifically responding to sucrose were obtained from the high-throughput sequencing data after the second comparative analysis, and annotated as phytochrome-interacting factors 3 (PIF3) (TraesCS5D01G428400 and TraesCS5B01G422000) and PIF4 (TraesCS2A01G253900 and TraesCS2D01G254400), respectively. Their expressions were up-regulated after sucrose treatment, consistent with the conclusion of Shor *et al.,* (2018). Sucrose affects the activity and level of PIFs, which in turn influences PIF-mediated signal transduction of the circadian system, and the effect is sucrose-dependent (Shor *et al.,* 2018). The increase in circadian rhythm caused by the inhibition of photosynthesis can be reversed by the addition of sucrose (Haydon *et al.,* 2013). Therefore, PIFs are directly involved in the sucrose signal transduction pathway. The PIF transcription factor family in *Arabidopsis* consists of seven members (PIF1, PIF3-8) and contains a common basic helix-loop-helix (bHLH) domain, which is the binding site for PIF protein dimerization, DNA binding, and photoactivated plant pigments (Leivar & Quail, 2011). Earlier studies defined PIF as a bHLH transcription factor that interacts with the light-activated red/far red photocytochrome (Shor *et al.,* 2017). Increasing evidence demonstrated that the PIF family is widely involved in multiple signal transduction pathways in plants, including light, temperature (Shor *et al.,* 2018) and hormone response (Wei *et al.,* 2017), circadian rhythm (Shor *et al.,* 2017), and sucrose signal transduction (Shor *et al.,* 2018). Monte et al. reported that PIF3 is a negative regulator of light morphogenesis under red light and is involved in chloroplast development (Monte *et al.,* 2004). PIF4 can not only respond to external signals, such as light and temperature (Shor *et al.,* 2018), but also participate in the transduction of internal signals, including auxin (Franklina *et al.,* 2011) and circadian rhythm (Shor *et al.,* 2018). PIFs act as hubs for multiple signaling pathways in plants, and in addition to environmental signals, PIFs are also involved in the metabolism and regulation of hormones, including brassinosteroid (BR), auxin, and gibberellin (Lucas *et al.,* 2008; Barbier *et al.,* 2015; Wei *et al.,* 2017). BR biosynthesis is also regulated by PIF4 and PIF5, which combine with the promoter region of BR biosynthesis genes to directly promote their expressions (Wei *et al.,* 2017). PIF4 and PIF5 are also negative regulators of auxin signal transduction, and play a role through the introduction of abscisic acid (ABA) signaling to activate the expression of many senescence-related genes (Sakuraba *et al.,* 2014; Shi *et al.,* 2015). Moreover, PIF4 can also bind DNA in a temperature-dependent manner, triggering an increase in auxin biosynthesis (Kumar *et al.,* 2012). After bioinformatics analysis and functional classification, TraesCS5D01G428400, TraesCS5B01G422000, TraesCS2A01G253900, and TraesCS2D01G254400 were not only identified as TF, but also participated in signal transduction-related and hormone-related pathways (Fig. 7). DGEs annotated as auxin, ABA and ethylene or DEGs involved in the phytohormone signal transduction pathway were both up-regulated and down-regulated after sucrose treatment, indicating that the function of PIFs in the hormone signal transduction pathway is diverse, and this transcription factor family is the bridge between sucrose signal transduction and hormone signal pathway, which might be

even a key core hub. However, numerous researches are still required to further confirm its specific function and mechanism in sucrose signal transduction.

In addition to PIF, there are several transcription factor (TFs) involved in sucrose response, including WRKY, bHLH, and MYB. The WRKY transcription factor family is widely distributed in the plant kingdom, and the multitudinous members have different divisions of function. They can act as activators or enhancers, or as inhibitors in plant growth and stress response processes (Wang *et al.,* 2019). bHLH transcription factor family exists in almost all eukaryotes, and its functions are diversified due to different species, such as the above-mentioned PIFs. Furthermore, there are 16 DEGs that belong to the two major categories of signal transduction and phytohormones, which also confirm that the interaction between the environment and the organism is achieved by the regulation of hormones in many cases. For example, the rapid accumulation of ABA in plants can induce increased expression of stress tolerance genes to cope with various abiotic stresses (Sah *et al.,* 2016). The relationship among plant hormone pathways has also been elucidated by interfering with the transcription of genes involved in hormone metabolism (Li *et al.,* 2016; Yang *et al.,* 2018).

In plant, various signaling pathways do not exist in isolation, but form some complicated network systems with each other (Xu *et al.,* 2019). In particular, there is a very broad interaction between sugar signals and hormone signals (Wang *et al.,* 2018). For example, sugar signals in plants can induce some hormone synthase (Rook *et al.,* 2001). The bridge or core factor connecting the two becomes the key to perfect the control signal network. The 20 DEGs selected in this study can be regarded as a bridge between sucrose and hormone signals at the functional classification level, but more evidences are still necessary to support this inference.

Conclusion

A total of 1327 DEGs (711 up-regulated, 616 down-regulated) with specific responses to sucrose were selected from wheat leaves treated with sucrose, glucose, and fructose through high throughput sequencing and comparative transcriptome analysis by Illumina. Bioinformatics analysis divides them into four major categories, namely transcription factors, plant hormone related, signal related, and metabolic related. Four differentially expressed genes belong to three categories (transcription factors, plant hormone related, and signal related), and 16 of them belong to signal transduction and plant hormones. These DEGs are related genes that are independent of glucose and fructose signaling pathways and participate in sucrose specific signal transduction pathways. The conclusions of this study will provide theoretical basis for the improvement of sucrose signal transduction pathways and the elucidation of their mechanisms, but further research is still needed to determine the relevant sucrose signal pathways and molecular regulatory networks in the future.

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