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TRANSCRIPTOME ANALYSIS OF SUCROSE SIGNAL TRANSDUCTION PATHWAY IN CV. ZHOUMAI 18 OF *TRITICUM AESTIVUM* L.

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Abstract

Sucrose is the primary form of photosynthetic products in plants, not only constituting the carbon skeleton of plants, but also the energy source for sink organs. In addition, sucrose could function as a signaling molecule and participates in multiple biological processes. Palatinose, an isomer of sucrose, does not participate in sugar metabolism, but regulates sugar signaling in plants. The utilization of palatinose could overcome the problem of the conversion between hexose and sucrose, and is conducive to studying the perception and transmission pathways of sucrose signals. In this study, Illumina HiSeq platform was used to perform transcriptome and expression profiling on wheat leaves treated with sucrose and palatinose, respectively. Through comparative transcriptome analysis, we identified 4,174 common differentially expressed genes (DEGs). According to bioinformatics analysis including functional annotation and pathway enrichment, these DEGs were divided into four categories, namely signaling (587), plant hormone regulation (121), transcription factor (210) and metabolic pathways (890). These DEGs were most likely related genes of the sucrose signal transduction pathway. This study provides a new insight and theoretical foundation for the enrichment of the sucrose signal transduction pathway and the elucidation of its mechanism of action.

Key words: Sucrose; Palatinose; Expression profiling; Functional annotation; Signal transduction.

Introduction

Sucrose, which is composed of glucose and fructose, widely exists in the plant kingdom and could be considered as the representative of sugar (Zhang *et al*., 2013). As the main form of transportation of photosynthetic products in plants, sucrose not only consists of the carbon skeleton of plants, but also works as an energy source of fruits, seeds, roots and other sink organs. In addition, sucrose also functions as a signal molecule to participate in signal transduction in plants, which in turn affects growth, development and stress response, including germination (Barbier *et al*., 2015), root growth (Dümmer *et al*., 2016), fruit maturation (Lia *et al*., 2019) and stress resistance (Zhao *et al*., 2017a). However, whether sucrose is a real signaling molecule has been controversial for a long time. Sucrose is different from glucose which is a recognized signaling molecule, and the effect of sucrose has been questioned, especially in the hexokinase signaling pathway. The reason for this question is that sucrose can be rapidly degraded into glucose and fructose by invertase, and therefore, it is difficult to confirm whether sucrose itself or its degradation products are involved in the signal transduction process. However, Dümmer *et al*., (2016) used the *Arabidopsis ehb1* mutant, which exhibited abnormal geotropism and phototropism, to confirm the signal transduction function of sucrose. The upstream promoter region of *EHB1* had sucrose and light-responsive elements, so after exogenous addition of sucrose, the phenotype of the *ehd1* mutant was restored. Furthermore, sucrose is a crucial signaling molecule during plant growth and development. Exogenous addition of sucrose can upregulate the expression of auxin synthesis genes, which are quite critical for the growth of young shoots. The growth as well as differentiation of buds also depends on the concentration of sucrose (Barbier *et al*., 2015). Sucrose can also regulate fruit ripening. Exogenous application of sucrose could increase the glucose, fructose and sucrose contents in strawberry fruits and regulate the accumulation of anthocyanins (Lia *et al*., 2019). Although increasing number of studies have confirmed the signal transduction function of sucrose, its specific signal transduction pathway and mechanism are still unrevealed.

Palatinose (6-O-2-D-glucopyranosyl-D-fructose), also known as isomaltulose, is the isomers of sucrose and consists of glucose and fructose, which are combined with α-1, 6 glycosidic bond. Previous studies have confirmed that palatinose can neither be recognized by plant sucrose transporters (SUT), nor can be cleaved by enzymes in plants to participate in sugar metabolism. However, it can regulate sugar signaling pathway in plants (Atanassova *et al*., 2003; Huang *et al*., 2015). Fructokinase-like protein2 (FLN2) is a component of plastid-encoded plastid RNA polymerase (PEP) complex, whose activity is crucial for the green phenotype of *Arabidopsis*. *Arabidopsis fln2-4* mutant plants could not grow green true leaves and still showed albino leaves when grown on the nutrient medium containing palatinose without sucrose. This phenotype is the same as *fln2* grown on sugar-free MS medium. However, the medium containing sucrose shows a delayed green phenotype, indicating that palatinose cannot provide energy for the growth of the mutant (Huang *et al*., 2015). *Vitis vinifera Hexose Transporter*

1(*VvHT1*) encodes a hexose transporter during grape ripening which could be induced by both sucrose as well as palatinose during cell culture. Since palatinose can hardly be transported, its effect may be mediated by a sensor located in the plasma membrane (presumably a sucrose sensor). These results indicate that sucrose and its isomer palatinose may participate in the common sugar sensing mechanism, but the sugar signaling pathway is different, that is, the activity of *VvHT1* promoter is activated through two independent signaling pathways, i.e. hexose pathway and sucrose pathway (Barbier *et al*., 2015).

Sucrose and its degradation products are all involved in the carbon metabolism of plants. Therefore, it is not easy to study the perception of sucrose as a specific signal molecule, and the relationship between the perception of sugar signals and sugar metabolism has become the enormous obstacle to the research of sugar signaling pathways. After sucrose is degraded, glucose and fructose molecules can be detected by hexose sensors, and most plant tissues can easily synthesize sucrose after feeding hexose. The application of non-metabolizable or non-cleavable sugars would overcome the problem of mutual conversion between hexose and sucrose, in order to investigate the perception and transmission pathways of sucrose signals **in** this study, the Illumina HiSeq platform was employed to sequence the transcriptome and expression profiling on wheat leaves treated with sucrose and palatinose, respectively. A large number of related genes involved in the sucrose signal transduction pathway were identified via comparative transcriptome analysis, providing a theoretical basis for the improvement of the sucrose signal transduction pathway and the elucidation of its mechanism of action.

Materials and Methods

Plant materials, growth conditions and treatment: Wheat cultivar Zhoumai 18 was taken as the experimental material. Uniform and full-grained wheat grains were chosen and disinfected. After being soaked in sterile water for 12 h, the seeds were placed on the floating net, and germinated for 3 d in the artificial growth chamber under the constant temperature 25°C and in dark, and then transplanted after being illuminated for 1 d. The wheat seedlings with uniform growth were transplanted on the floating plate for hydroponic cultivation. The volume of hydroponic plastic box is 2 L, which could be transplanted with 40 wheat plants. The hydroponic nutrient solution was Hoagland culture solution (pH 6.6~6.8), which was replaced every 3 days. The nutrient solution needed to be ventilated for 30 mins every day. The culture photoperiod was 16h/8h (day/night), and the temperature was 25°C/20°C (day/night). The treatment began when the wheat seedlings grew to 2-leaves stage, including CK (Hoagland nutrient solution), sucrose (Hoagland nutrient solution + 1 mmol/L sucrose) and palatinose (Hoagland nutrient solution + 1mmol/L palatinose).Three days after treatment, samples were taken for RNA sequencing analysis.

RNA isolation and qualification: In terms of the description of Lia *et al*., (2019), the total RNA was isolated and reversely transcribed. RNA concentration was perceived by Nano Drop 2000 (Thermo Scientific). The integrity of RNA was assessed via the RNA Nano 6000 Assay Kit of the Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA).

Library preparation, clustering and sequencing: A total of 1 μg RNA from each sample was used as input material for the RNA sample preparations. Sequencing libraries were generated using NEB Next Ultra TM RNA Library Prep Kit for Illumina (NEB, USA) following manufacturer's specification and index codes were added to attribute sequences to each sample. The library quality was assessed through the Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA). Most raw data quality could reach or exceed Q30. Before conducting data analysis, the sufficient quality of these reads needed to be ensured to guarantee the accuracy of subsequent analysis. Biomarker Technologies Corporation performed strict quality control on the data, using the following filtering procedures. (1) Removed the reads containing connectors; (2) Removal of low-quality reads (including the removal of reads whose proportion of N is higher than 10%; the removal of bases with a quality value of Q≤10 accounts for more than 50% of the entire reads). The clustering of the index-coded samples was performed on a cBot Cluster Generation System by Tru Seq PE Cluster Kit v4-cBot-HS (Illumina) according to the manufacturer's instructions. After clustering, the library was sequenced on an Illumina Hiseq Xten platform and paired-end reads were generated.

Data processing: The Illumina sequencing data were processed further and analyzed by the Biomarker Technologies Corporation. Based on the reference genome, only these reads with a perfect match or one mismatch would be further analyzed and annotated. The software Tophat2 tool was used to map with reference genome.

Bioinformatics analysis: During the detection process of differentially expressed genes (DEGs), fold change ≥ 2 and false discovery rate (FDR) <0.01 were adopted as screening criteria. Fold change represented the ratio of expression between the two samples. The FDR was obtained by correcting the p-value of the significance of the difference. Since the differential expression analysis of transcriptome sequencing is an independent statistical hypothesis test for a large number of gene expressions, there might be false positive problems. Therefore, in the process of differential expression analysis, the accepted Benjamini-Hochberg correction method was used to correct the original hypothesis, and the significant p-value obtained through the test was corrected, and FDR was finally used as the key indicator for screening DEGs. Gene function was annotated based on the following databases: NCBI non-redundant protein sequences (Nr); NCBI non-redundant nucleotide sequences (Nt);

Protein family (Pfam)；Clusters of Orthologous Groups of Proteins (KOG/COG); The manually annotated and reviewed protein sequence database Swiss-Prot；KEGG Ortholog database (KO); Gene Ontology (GO). GO enrichment analysis of the genes with differential expression was implemented by the GO seq R packages based on Wallenius non-central hyper-geometric distribution, which can adjust gene length bias in DEGs. KEGG is a database resource for understanding high-level functions and utilities of the biological system, like the cell, the organism and the ecosystem, from molecular-level information, especially large-scale molecular datasets generated by genome sequencing and other high-throughput experimental technologies (http://www.genome.jp/kegg/). The KOBAS software was used to detect the statistical enrichment of DEGs in KEGG pathways.

qRT-PCR validation: Eight DEGs were selected to test the expression level through qRT-PCR. The reaction system and PCR procedures depend on the description of Feng *et al*., (2019). The fold variation was calculated by $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen 2001). Take β-actin as internal control.

Results

Transcriptome data acquisition, quality control and identification of DEGs: Based on sequencing by synthesis (SBS) technology, the Illumina HiSeq high-throughput sequencing platform sequenced cDNA libraries and produced a large amount of high-quality data. Biomarker Technologies Corporation performed strict quality control on the data. After the quality control, 46 Gb high-quality clean data were obtained. The indexes including clean reads and clean bases are shown in (Table 1). The Q30 base percentage of each sample was not lower than 87.66%. Gene expression has temporal and spatial specificity. In the two different conditions, genes or transcripts with significantly different expression levels are called DEGs. 10,152 and 10,441 DEGs were obtained from sucrose and palatinose treated samples, respectively. Under sucrose treatment, there were 1,160 and 8,992 DEGs up-regulated and down-regulated, respectively, while palatinose treatment had 3,846 and 6,595 DEGs up-regulated and down-regulated, separately (Fig. 1). After further comparison and analysis of the DEGs under the two treatments, 121 DEGs were up-regulated and 4,053 DEGs were down-regulated (a total of 4,174 common DEGs). The down-regulated genes were far more high in number than up-regulated genes under these two treatments, which might indicate that there were more negative regulatory patterns in the sucrose and palatinose signaling pathways. Because palatinose cannot participate in metabolism, it is more used as a signaling molecule (Atanassova *et al*., 2003; Huang *et al*., 2015), which could exclude some genes involved in metabolism under sucrose treatment. Therefore, the shared part of the two treatments is the focus of our subsequent analysis and research.

Table 1. Statistics of RNA-seq data.

Samples	Clean reads	Clean bases	GC content	%≥Q30
CK	82,939,265	24,773,709,458	56.81%	88.32%
Sucrose	46,208,811	13,769,352,624	56.63%	87.66%
Palatinose	31,177,051	9,320,856,688	55.58%	91.24%

Bioinformatics analysis of common DEGs: The GO database was a structured standard biological annotation system constructed by the GO Organization (Gene Ontology Consortium) in 2000. It aims to establish a standard vocabulary system for knowledge of genes and their products, which is applicable to various species. The GO annotation system is a directed acyclic graph, which contains three main branches, namely: Biological Process, Molecular Function and Cellular Component. The GO analysis and statistical results of the common DEGs under the two treatments are shown in (Fig. 2). These shared genes were mainly concentrated in catalytic activity and binding (Molecular Function), cell part and cell (Cellular Component), metabolic process, cellular process and signal-organism process (Biological Process). The cluster of orthologous groups of proteins (COG) database is constructed depending on the phylogenetic relationship of multiple organisms, like bacteria, algae, and eukaryotes. This database could be used to categorize the gene products according to orthology. The statistical results for COG classification of DEGs are shown in (Fig. 3). These common DEGs and their encoded products were mainly involved in processes such as chromatin structure and dynamics, carbohydrate transport and metabolism, and secondary metabolites biosynthesis. In organisms, different gene products coordinate with each other to perform biological functions. Annotation and analysis of pathways of DEGs helps to further interpret the function of genes. Kyoto encyclopedia of genes and genomes (KEGG) is a database for systematic analysis of gene function and genomic information. It helps researchers to study genes and expression information as a whole network. The annotation results of these DEGs were classified in terms of the type of pathway in KEGG (Fig. 4). Metabolic pathways, biosynthesis of secondary metabolites and phenylpropanoid biosynthesis were the three most concentrated categories of annotated genes. According to functional annotation and pathway enrichment analysis, common DGEs were mainly divided into four categories, namely signaling (587), hormone regulation (121), transcription factor (210) and metabolic pathways (890) (Table 2).

Fluorescence quantitative verification of transcriptome results: Eight genes (4 up-regulated and 4 down-regulated) were randomly selected from common DEGs for fluorescence quantitative verification. The expression results are shown in (Fig. 5). The gene expression patterns (up-regulation or down-regulation) were consistent with the transcriptome sequencing results, indicating that the transcriptome results were true and reliable and could be used in subsequent experiments.

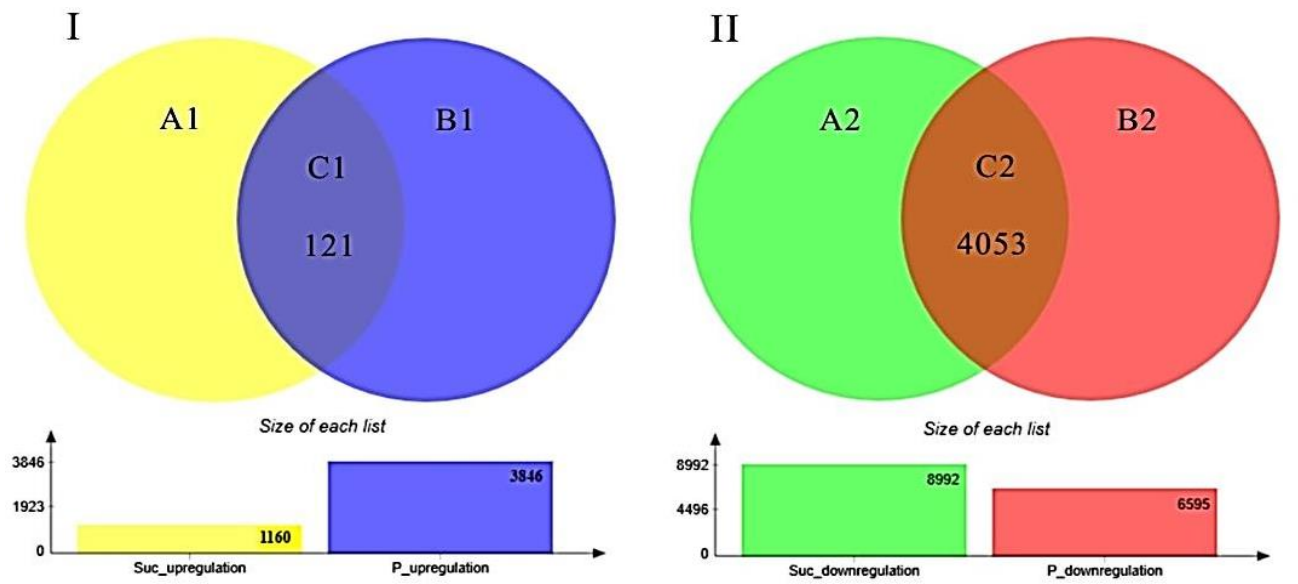

Fig. 1. Venn diagram shows number of DEGs under sucrose and palatinose treatments. (I) number of up-regulated genes in A1 (Suc) and B1 (P); (II) number of downregulated genes in A2 (Suc) and B2 (P). C1, the common DEGs of A1 and B1; C2, the common DEGs of A2 and B2. Suc, Sucrose; P, Palatinose.

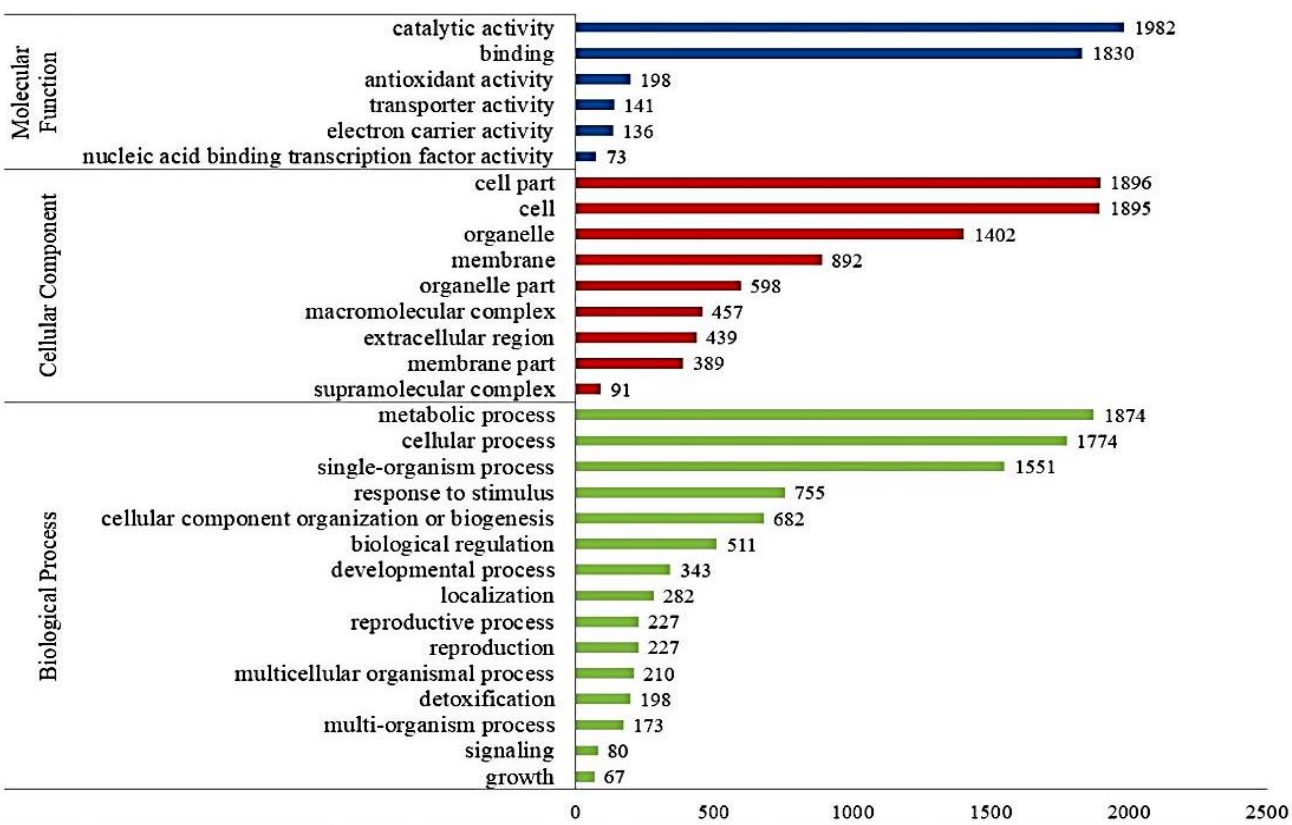

Fig. 2. GO classification of common DEGs under sucrose and palatinose treatments.

Discussion

Sucrose is not only the main form of assimilation products of photosynthesis between "source" and "sink", but also can be used as a signal molecule to participate in the growth as well as development of plants and respond to adversity through complex regulatory mechanisms. Currently known genes involved in sucrose signal transduction include *sucrose will eventually be exported transporter* (*SWEET*), *sucrose transporter* (*SUT*), *regulator of G protein signaling 1* (*RGS 1*), *hexokinase* (*HXK*), *sucrose non-fermentation 1 related protein kinase* (*SnRK1*) and some related transcription factors (TFs).

Sucrose is the primary carbohydrate transported in higher plants (Wind *et al*., 2010), and exerts a significant role in many physiological processes. The SWEETs protein is a family of sugar transporter discovered later, which can promote the diffusion of sucrose across the cell membrane

to the apoplast in a concentration gradient (Kristen & Braun 2012). When the expression of *IbSWEET10* was inhibited in sweet potatoes, the low sucrose concentration under tissue culture conditions would cause the decrease of fresh weight in RNAi plants. At high sucrose concentrations, the difference in fresh weight between RNAi plants and controls was not significant. One reason might be that the silenced expression of *IbSWEET10* resulted in the output of sucrose in the source tissue (leaf) to be restricted, which is not conducive to plant growth. However, when the supply of sucrose is sufficient, the silently expressed plants could obtain enough sucrose from the medium for growth. The activity of SWEET is regulated by the feedback of its substrate, thus, when the supply of exogenous sucrose is sufficient, its expression is down-regulated accordingly (Eom *et al*., 2015). In this study, three of the genes collectively expressed in sucrose and palatinose were annotated as *SWEET*, and their expressions were all down-regulated, consistent with the characteristics of SWEET protein, which further demonstrated that palatinose could participate in sucrose signaling pathway as a signaling molecule.

The SnRK1 in plants is homologous to yeast sucrose non-fermenting-1 (SNF1) and mammalian AMP-activated protein kinase (AMPK), which are energy receptors that can be activated by energy deficiency. SnRK1 could retard the growth as well as development in plants by inhibiting the biosynthetic enzymes, whose activities are related to carbon and nitrogen metabolism (Luo *et al*., 2018) and can also affect metabolism, signal transduction, transcription, and stress tolerance (Elena *et al*., 2007; Elena & Sheen 2008; Zhao *et al*., 2017b). In this experiment, the three genes annotated as SnRK1 were down-regulated under the treatment of exogenous sucrose and palatinose, and their activities were suppressed, which was beneficial to the growth of plants. G protein functions critically in regulating numerous signaling pathways in eukaryotes kingdom, whose core part is composed of a Gα, a Gβ and a Gγ protein. Studies on plant G protein adopting gene knockout, overexpression lines and mutants confirmed that G protein was involved in nearly every aspect of plant life activities, including regulating growth and development, cross-talk with environmental and hormone signals, and responding diverse stresses (Sona *et al*., 2006; Chakravorty & Botella 2007; Steffens & Sauter 2010; Li *et al*., 2012; Magdalena *et al*., 2012; Liu *et al*., 2013). The RGS proteins family has a vital role in signal transduction of G protein coupled receptor (GPCR) by activating Gα subunits as well as accelerating GTP hydrolysis. RGS proteins family is the central interaction factor and regulator of Gα protein, and the interaction between RGS and Gα proteins is evolutionarily conserved among different species, including plants, animals and humans (Hackenberg *et al*., 2016). G protein and its interaction effectors are widely involved in plant growth, development and stress response. Liu *et al*., **(2013)** cloned 6 genes encoding G protein signaling pathway components from *Morus atropurpurea*. The expression of G protein encoding genes could be induced by PEG and NaCl under low temperature, but it would be down-regulated by high temperature, resembling to the results in *Brassica napus* and rice (Gao *et al*., 2010; Gao *et al*., 2011; Yadav *et al*., 2013; Yadav *et al*., 2014; Xu *et al*., 2018). The expression of *MaRGS* was upregulated by NaCl, while it was suppressed by low temperature and high temperature. This contradicts the results of *Brassica napus* (Chen *et al*., 2014). In our transcriptome data, 6 G protein genes and 13 RGS protein genes were screened, and their expressions were all down-regulated. On one hand, the reason might be that the treatment of exogenous sucrose and palatinose did not only activate the stress response, but also provided enough energy supply for plant growth. On the other hand, it may be related to the functional complexity of G protein and RGS. A previous study also showed that salt stress could activate the G protein signaling pathway, which in turn increased the salt tolerance of plants (Colaneri *et al*., 2014). In addition, the G protein signaling pathway can also interact or cross with hormone signaling molecules such as ABA and some TFs like bZIP and WRKY to regulate development and stress response in plant (Wang *et al*., 2018b; Wu *et al*., 2020).

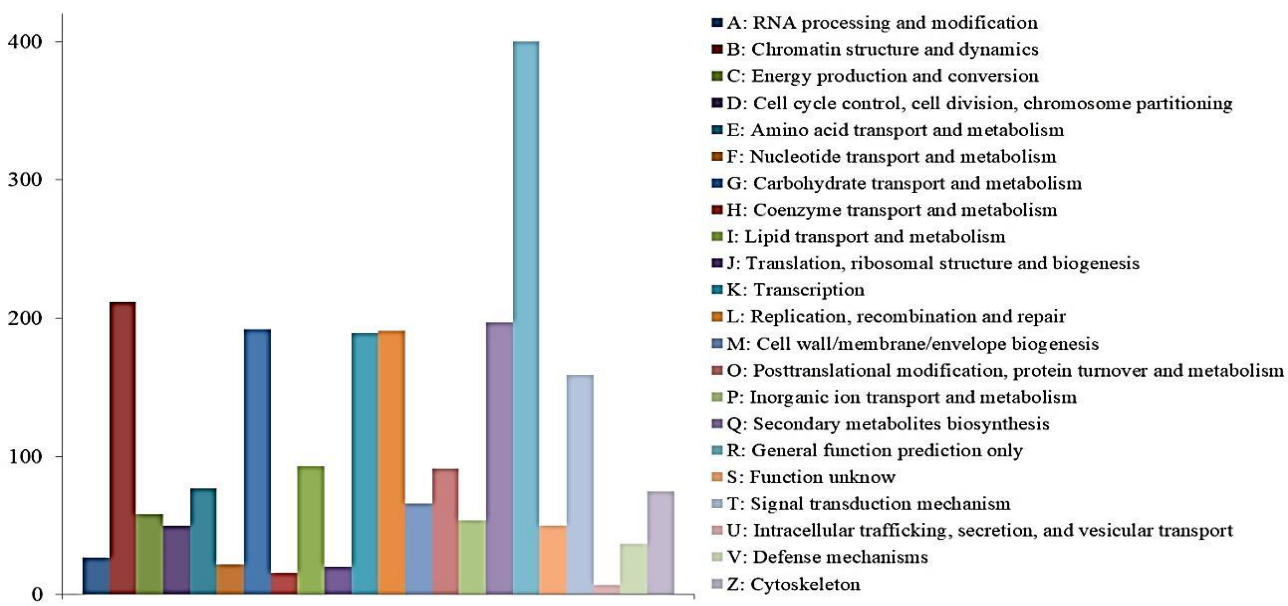

Fig. 3. COG classification of common DEGs under sucrose and palatinose treatments.

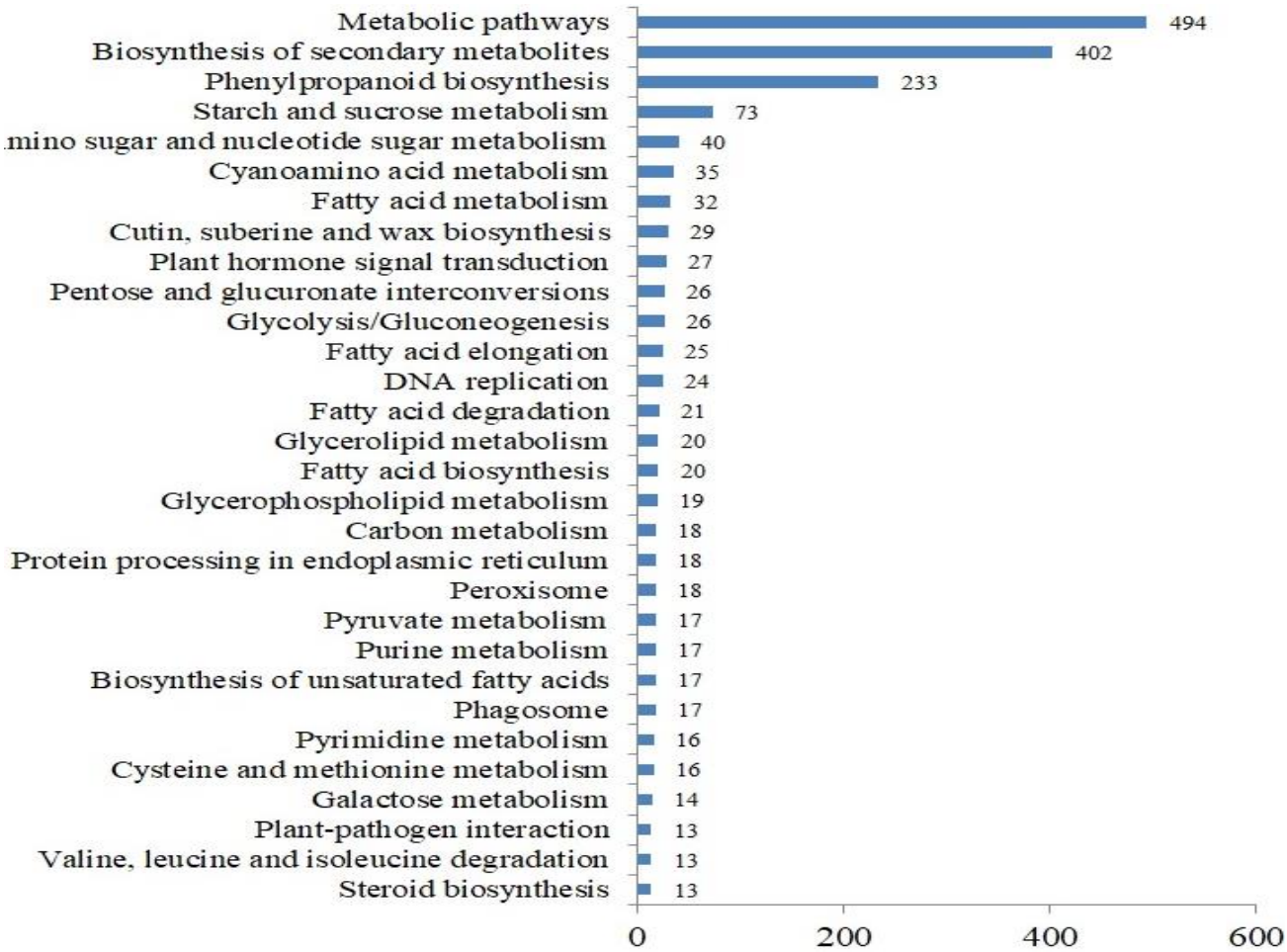

Fig. 4. KEGG function classification of common DEGs under sucrose and palatinose treatments.

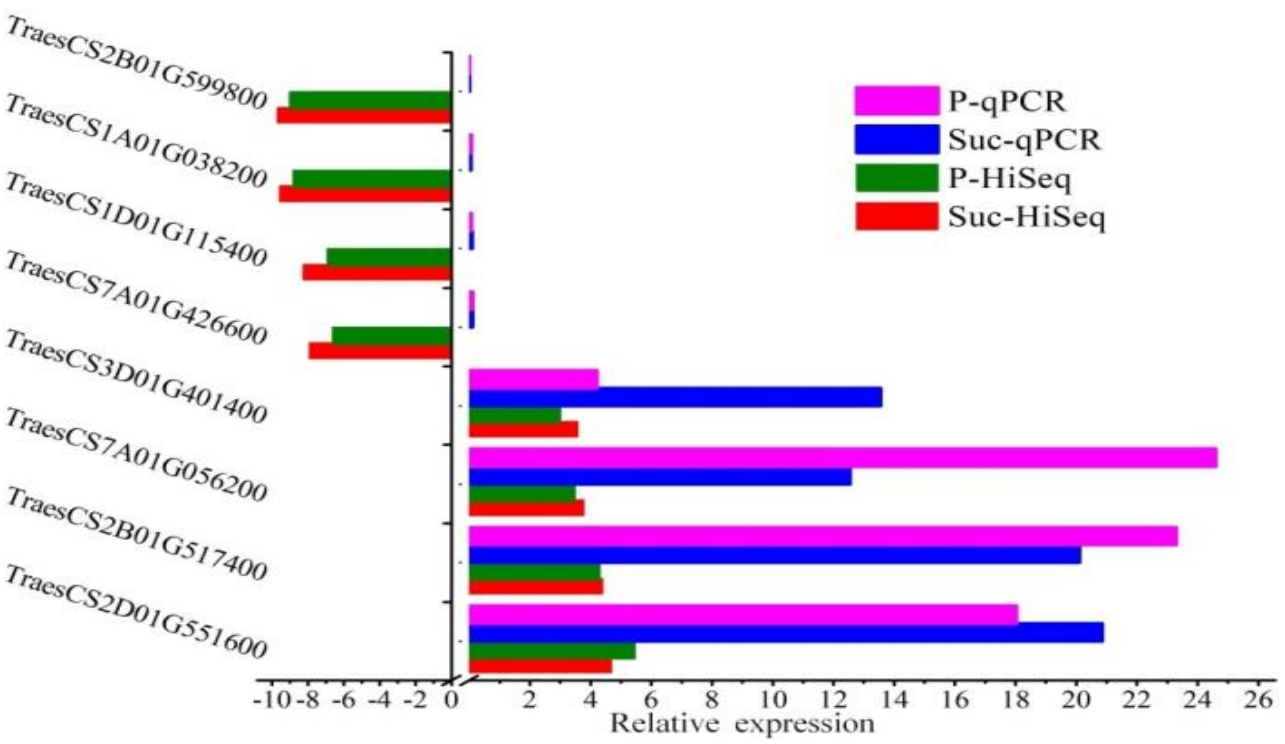

Fig. 5. Validation of the relative expression for 8 chosen common DEGs via qRT-PCR. Suc-qPCR, qPCR verification of sucrose treatment; P-qPCR, qPCR verification of palatinose treatment; Suc-HiSeq, transcriptional result of sucrose treatment; P-HiSeq, transcriptional result of palatinose treatment.

Table 2. Functional classification of major common DEGs (some representative genes).

Function categories	DEG ID	Expression level		Functional annotation/pathway enrichment
		Suc_log2FC	P_log2FC	
Signaling	TraesCS2D01G551600[a]	4.69	5.46	Signal transduction mechanisms
	TraesCS7D01G005200	2.89	2.26	Signal transduction mechanisms
	TraesCS3A01G237400	1.56	1.40	Signal transduction mechanisms
	TraesCS5B01G094600	-7.91	-6.60	Signal transduction mechanisms
	TraesCS1D01G115400	-8.28	-6.94	Signal transduction mechanisms
	TraesCS1D01G115400[a]	-8.28	-6.94	Signal transduction mechanisms
Hormone regulatin	TraesCS3D01G401400[a]	3.57	3.01	Gibberellin 20 oxidase 2 [Aegilops tauschii]
	TraesCS3A01G406200	2.62	1.97	Gibberellin 20 oxidase 2 [Aegilops tauschii]
	TraesCS4A01G398300	3.80	3.65	auxin polar transport
	TraesCS7A01G536900	3.11	2.39	cytokinin oxidase/dehydrogenase 3 [Triticum aestivum]
	TraesCS7A01G536900	3.11	2.39	cytokinin oxidase/dehydrogenase 3 [Triticum aestivum]
	TraesCS7A01G536900	3.11	2.39	cytokinin oxidase/dehydrogenase 3 [Triticum aestivum]
	TraesCS5D01G228400	2.65	2.02	Auxin-induced protein 5NG4 [Aegilops tauschii]
	TraesCS3B01G578400	-2.51	-2.06	ABA/WDS induced protein
	TraesCS3D01G038900	-2.66	-2.14	response to ethylene
	TraesCS6B01G274500	-3.10	-2.37	PREDICTED: ethylene-responsive transcription factor
	TraesCS1D01G369400	-3.14	-2.38	ABA-inducible protein PHV A1
	TraesCS6B01G355700	-3.17	-2.41	ACC oxidase [Triticum aestivum]
	TraesCS3B01G612000	-3.35	-2.50	response to ethylene
	TraesCS4D01G005400	-5.03	-3.81	Cytokinin-O-glucosyltransferase 1 [Aegilops tauschii]
	TraesCS7A01G056100	-5.21	-3.96	Cytokinin-O-glucosyltransferase 2 [Aegilops tauschii]
	TraesCS6A01G335900	-6.26	-4.76	response to gibberellin
	TraesCS7A01G190600	-6.44	-4.91	auxin transporter PIN1 [Triticum aestivum]
	TraesCS4D01G005300	-6.50	-4.95	Cytokinin-O-glucosyltransferase 1 [Aegilops tauschii]
	TraesCS7D01G191600	-7.47	-6.10	auxin transporter PIN1 [Triticum aestivum]
	TraesCS7A01G426600[a]	-7.93	-6.63	response to gibberellin
TF	TraesCS2B01G517400[a]	4.40	4.31	WRKY45-like transcription factor [Triticum aestivum]
	TraesCS2B01G517400	4.40	4.31	WRKY45-like transcription factor [Triticum aestivum]
	TraesCS7A01G305200	3.49	2.80	NAC transcription factor [Triticum aestivum]
	TraesCS1B01G374900	3.37	2.65	hypothetical protein F775_52480 [Aegilops tauschii]
	TraesCS2A01G489500	3.17	2.41	WRKY45-like transcription factor [Triticum aestivum]
	TraesCS2A01G489500	3.17	2.41	WRKY45-like transcription factor [Triticum aestivum]
	TraesCS7D01G345700	2.98	2.33	WRKY11 transcription factor, partial [Triticum aestivum]
	TraesCS7D01G345700	2.98	2.33	WRKY11 transcription factor, partial [Triticum aestivum]
	TraesCS2D01G274600	-3.83	-2.77	Transcription factor bHLH62 [Aegilops tauschii]
	TraesCSU01G075200	-4.74	-3.52	Transcription factor bHLH19 [Triticum urartu]
	TraesCS3D01G105100	-5.10	-3.85	Transcription factor bHLH51 [Triticum urartu]
	TraesCS4A01G404700	-6.24	-4.74	Transcription factor bHLH96-like [Oryza brachyantha]
	TraesCS3A01G293700	-6.91	-5.34	bZIP transcription factor
	TraesCS5A01G143300	-6.92	-5.37	bZIP transcription factor
	TraesCS5A01G143300	-6.92	-5.37	bZIP transcription factor
	TraesCS5A01G143300	-6.92	-5.37	bZIP transcription factor
	TraesCS4D01G302700	-7.53	-6.22	Transcription factor bHLH96-like [Oryza brachyantha]
	TraesCS4A01G016600	-7.75	-6.37	Transcription factor bHLH96
	TraesCS2B01G329300	-7.81	-6.45	Histone-like transcription factor (CBF/NF-Y)
	TraesCS1D01G039900	-7.93	-6.64	Histone-like transcription factor (CBF/NF-Y)
	TraesCS6B01G049000	-8.17	-6.86	Histone-like transcription factor (CBF/NF-Y)
	TraesCS1D01G039800	-8.38	-7.15	Histone-like transcription factor (CBF/NF-Y)
	TraesCS4B01G287600	-8.53	-7.35	sequence-specific DNA binding transcription factor activity
	TraesCS3A01G102400	-8.55	-7.39	Histone-like transcription factor (CBF/NF-Y)
	TraesCS1A01G038200[a]	-9.60	-8.85	Histone-like transcription factor (CBF/NF-Y)
Metabolic pathways	TraesCS7A01G056200[a]	3.77	3.48	Carbohydrate transport and metabolism
	TraesCS5A01G181500	3.25	2.55	Carbohydrate transport and metabolism
	TraesCS2A01G175400	3.17	2.41	Carbohydrate transport and metabolism
	TraesCS2B01G194200	2.64	1.99	Starch and sucrose metabolism
	TraesCS4B01G344800	2.28	1.76	Secondary metabolites biosynthesis, transport and catabolism
	TraesCS2B01G236200	2.01	1.64	Starch and sucrose metabolism
	TraesCS7D01G071200	1.94	1.61	Carbohydrate transport and metabolism
	TraesCS2B01G613500	-8.30	-6.98	Carbohydrate transport and metabolism
	TraesCS6A01G250300	-8.43	-7.17	Carbohydrate transport and metabolism
	TraesCS2A01G590600	-8.51	-7.28	Secondary metabolites biosynthesis, transport and catabolism
	TraesCS6A01G138000	-8.52	-7.29	Carbohydrate transport and metabolism
	TraesCS3D01G031800	-8.55	-7.36	Carbohydrate transport and metabolism
	TraesCSU01G036600	-8.60	-7.44	Carbohydrate transport and metabolism
	TraesCS6A01G185100	-8.65	-7.50	Secondary metabolites biosynthesis, transport and catabolism
	TraesCS6D01G232100	-9.30	-8.44	Carbohydrate transport and metabolism
	TraesCS2B01G599800[a]	-9.72	-9.03	Carbohydrate transport and metabolism

[a] genes validated by qRT-PCR

Wang *et al*., (2018a) analyzed the castor seeds treated with sucrose and mannose by transcriptome technology and found that in addition to metabolic pathways, sucrose treatment also specifically affected auxin, brassinolide, cytokinin, etc. hormone signaling pathway. It is believed that sucrose signaling pathway may influence the development of castor seed through multiple hormone signaling pathways such as auxin. Auxin has always been regarded as the core regulator of apical dominance. The accepted explanation is that the indole acetic acid (IAA) synthesized by the shoot tip is transported down the stem to the lateral bud and inhibits the latter's germination. Artificially increasing the plant sucrose level can suppress the expression of the TF BRANCHED1 (BRC1) that regulates apical dominance and causes rapid bud germination. In consequence, increasing sugar supply is necessary for the removal of lateral bud inhibition deriving from apical dominant. Research works have demonstrated that the interaction of sugar with hormones depended on its concentration. The antagonistic effect of sugar and ABA was observed in *Arabidopsis thaliana* seeds treated with 30 mM sucrose. On contrary to the promotion of low concentrations of sugar and ABA, high concentrations of sucrose (> 200 mM) would delay germination and the following seedling growth, but they could promote anthocyanin accumulation. It is speculated that the low concentration of exogenous sugar can alleviate the metabolic obstacles caused by ABA and promote germination by providing energy and nutrition. Exogenous application of gibberellin (20 μM) can inhibit the sucrose-induced anthocyanin synthesis pathway in Arabidopsis, while ABA (5 μM) interacts with sucrose (90 mM) synergistically, and the induction of sucrose is not only induced by ABA, indicating that there might be cross-talk or intersection between sucrose and phytohormone signaling pathway. Liu *et al*., (2017) found that during the maturation of soybean (*Glycine max*) seeds, the sucrose content in the seeds was inversely proportional to the ABA content. Sucrose also negatively regulates the ethylene content or signaling pathways in plants. A large amount of ethylene is released during the senescence of cut flowers, and the addition of sucrose to nutrition is a common method to delay the senescence of cut flowers (Shi *et al*., 2015). These above conclusions confirm the interaction effect between sucrose and phytohormone, and the specific methods (antagonism, synergy, cross-talk) are related to sucrose concentrations, hormone type, growth stage, tissues and organs. In this study, a total of 121 common DEGs were annotated as phytohormone response elements or pathways, and most of which them were down-regulated, which revealed that low concentrations of sucrose and palatinose could promote plant growth and reduce or alleviate ABA-induced growth disorders, as well as the apical dominance of auxin regulation.

In addition to the aforementioned SnRK1, basic leucine zipper (bZIP) TFs are also typical representatives of low energy responses in plants. The members of bZIP family (bZIP11 has been confirmed to participate in this process) control the growth of root meristems by directly activating IAA3/SHY2 transcription, which is a key negative regulator of root growth (Weiste *et al*., 2017). As a result, SnRK1 and bZIP, which were activated via low-energy, could balance the relationship between growth and cellular energy resources in plants. In our study, 28 genes annotated as bZIP were down-regulated in the similar way as SnRK1, indicating that sufficient energy supply inhibited the expression of these signaling molecules, which was conducive to plant growth. Huang *et al*., (2016) performed RNA and small RNA sequencing in corn endosperm treated with sucrose, and obtained 47 TFs (including WRKY, bHLH, and MYB, etc.) in response to sucrose induction and 214 differentially expressed miRNAs (24 known and 190 novel). Most target mRNAs of these miRNA are annotated as TFs. Therefore, in the process of sucrose signal transduction, there may be a mode of interaction among miRNA, TFs and sucrose. Furthermore, Sun *et al*., (2003) isolated the SUSIBA2 from barley, which was involved in the sugar signaling of barley and belonged to the WRKY TF family. *SUSIBA2* mainly expresses in endosperm, but it can also ectopically express in sucrose-treated leaves, demonstrating that its transcription could be induced by sugar. Sucrose could likewise induce high expression of *AtWRKY20* in Arabidopsis leaves, and then activate the transcriptional activity of the *ApL3* gene encoding sugar-induced AGPase large subunit(Nagata *et al*., 2012). Previous studies have shown that WRKY family has various members, which can act as both suppressors and activators in plant growth and development, stress response and other processes. TF family basic helix-loop-helix (bHLH) presents in almost all eukaryotes, and their diverse functions vary with species. Ectopic expression of different members of the *Pleurotus ostreatus* bHLH in *Arabidopsis thaliana* leads to different results. The growth and development could be inhibited by the ectopic expression of *PobHLH5* or *PobHLH8*, while the transgenic plants expressing *PobHLH1* and *PobHLH2* had almost no difference in morphology from wild type(Chen *et al*., 2017). Among the TFs, which responded to sucrose induction, and sequenced from the corn endosperm transcriptome mentioned above, most members of the bHLH family were highly induced by sucrose. However, the expression of *ZmbHLH171* was suppressed under the combination of sucrose and ABA (Huang *et al*., 2016). Moreover, leaf development transcriptome sequencing results of *Camellia sinensis* L. showed that the majority of *bHLH* genes were up-regulated during leaf development, whereas only two were down-regulated (Cui *et al*., 2018). The seven genes annotated as bHLH in this study were all down-regulated (bHLH19, 2, 49, 51, 62, 96, 135), which could be related to the concentration of exogenous sugar treatment and the sampling location. High concentrations of sucrose could cause osmotic stress to the plant, which in turn activates the stress response (Mishra & Singh 2016). Whereas, even the expression of the same gene in the source and sink tissues may be different. Consequently, these selected TFs may participate in sucrose signal transduction in diverse modes of action.

It is not easy to study the function of sucrose as a specific signal molecule, because sucrose is rapidly metabolized into glucose and fructose molecules in the organism. However, most plant tissues can easily synthesize sucrose after feeding hexose, so the signal transduction effect of hexose or sucrose cannot be directly clarified. The non-metabolizable palatinose can overcome the interference

and hindrance of sugar metabolism in the process of sugar signal transduction. Through transcriptome sequencing of wheat treated with sucrose and palatinose, a large number of DEGs involved in sugar signal transduction were obtained. Among the currently known components involved in sucrose signal transduction, SUT is a sucrose sensor, which could receive and transmit signals (Niu *et al*., 2019); G protein and RGS1 are the core of the G protein signal component (Hackenberg *et al*., 2016). In the midst of the differential genes collectively expressed in sucrose and palatinose, we only screened the G protein and RGS related genes, and did not find the related genes encoded or annotated as SUT. Hence, there may be another sensor that can sense sucrose and palatinose, and in turn initiates the transmission of sugar signals. This is also the inference that needs to be verified in the next stage.

Conclusion

After high-throughput sequencing and bioinformatics analysis, 10,152 and 10,441 DEGs were obtained in wheat leaves treated with sucrose and palatinose, respectively. A total of 4,174 shared DEGs were obtained from further comparison and analysis, of which 121 were up-regulated and 4,053 were down-regulated. According to functional annotation and pathway enrichment analysis, four major types of these common DEGs were involved in sucrose signal transduction pathways, which were signaling, plant hormone regulation, TF and metabolic pathways. These results provide a new insight and theoretical foundation for the enrichment of sucrose signal transduction pathway and the elucidation of its mechanism of action, but a comprehensive research is still needed in the future to determine the related sucrose signal pathway and molecular regulatory network.

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References

Atanassova, R., M. Leterrier, C. Gaillard, A. Agasse, E. Sagot, P. Coutos-Thévenot and S. Delrot. 2003. Sugar-regulated expression of a putative hexose transport gene in grape. *Plant Physiol.*, 131(1): 326-334.

Barbier, F., T. Péron, M. Lecerf, M.D. Perez-Garcia, Q. Barrière, J. Rolčík, S. Boutet-Mercey, S. Citerne, R. Lemoine, B. Porcheron, H. Roman, N. Leduc, J.L. Gourrierec, J. Bertheloot and S. Sakr. 2015. Sucrose is an early modulator of the key hormonal mechanisms controlling bud outgrowth in *Rosa hybrida*. *J. Exp. Bot.*, 66(9): 2569-2582.

Chakravorty, D. and J.R. Botella. 2007. Over-expression of a truncated Arabidopsis thaliana heterotrimeric G protein γ subunit results in a phenotype similar to α and β subunit knockouts. *Gene*, 393(1-2): 163-170.

Chen, S., W. Yang, Q. Jia, W. Wei and S. Wang. 2017. *Pleurotus ostreatus* bHLH transcription factors regulate plant growth and development when expressed in Arabidopsis. *J. Plant Interact.*, 12(1): 542-549.

Chen, Y., X. Zhu, X. Zhu, Y.U. Yi-Fan and J. Liang. 2014. Identification of the regulator of G-protein signaling protein responsive to plant hormones and abiotic stresses in *Brassica napus*. *J. Integr. Agr.*, 13(12): 2634-2644.

Colaneri, A.C., M. Tunc-Ozdemir, J.P. Huang and A.M. Jones. 2014. Growth attenuation under saline stress is mediated by the heterotrimeric G protein complex. *BMC Plant Biol.*, 14: 129.

Cui, X., Y. Wang, Z. Liu, W. Wang, H. Li and J. Zhuang. 2018. Transcriptome-wide identification and expression profile analysis of the bHLH family genes in *Camellia sinensis*. *Fun. Integr. Genom.*, 18(5): 489-503.

Dümmer, M., C. Michalski, C. Forreiter and P. Galland. 2016. Phenotypic reversal in *Arabidopsis thaliana*: sucrose as a signal molecule controlling the phenotype of gravi- and photo-tropism mutants. *J. Plant Growth Regul.*, 35: 430-439.

Elena, B.G., F. Rolland, T. Johan M and J. Sheen. 2007. A central integrator of transcription networks in plant stress and energy signalling. *Nature*, 448(7156): 938-942.

Elena, B.G. and J. Sheen. 2008. Convergent energy and stress signaling. *Trends Plant Sci.*, 13(9): 474-482.

Eom, J.S., L. Chen, D. Sosso, B.T. Julius, I. Lin, X. Qu, D.M. Braun and W.B. Frommer. 2015. SWEETs, transporters for intracellular and intercellular sugar translocation. *Curr. Opin. Plant Biol.*, 25: 53-62.

Feng, Y.L., Y.Y. Zhao, C. Ma, J. Zhang, Y. Xiong and J. Yin. 2019. Identification of photoperiod responsive genes in wheat cultivar Jing 841 by transcriptome sequencing. *Int. J. Agric. Biol.*, 22(5): 1093-1101.

Gao, Y., T. Li, Y. Liu, C. Ren, Y. Zhao and M. Wang. 2010. Isolation and characterization of gene encoding G protein α subunit protein responsive to plant hormones and abiotic stresses in *Brassica napus*. *Mol. Biol. Rep.*, 37(8): 3957-3965.

Gao, Y., T. Li, Y. Zhao, C. Ren, Y. Zhang and M. Wang. 2011. Isolation and characterization of a G protein γ subunit gene responsive to plant hormones and abiotic stresses inBrassica napusL. *Acta Physiol. Plant.*, 33(2): 391-399.

Hackenberg, D., M.R. Mckain, S.G. Lee, R.C. Swarup, M. Tyler, S. Spencer, H. Alex, C.J. Pires, G.K.S. Wong and J.M. Jez. 2016. Gα and regulator of G-protein signaling (RGS) protein pairs maintain functional compatibility and conserved interaction interfaces throughout evolution despite frequent loss of RGS proteins in plants. *New Phytol.*, 216: 562-575.

Huang, C., Q. Yu, X. Yuan, Z. Li, J. Wang, L. Ye, L. Xu and Z. Yang. 2015. Rubisco accumulation is important for the greening of the *fln2-4* mutant in *Arabidopsis*. *Plant Sci.*, 236: 185-194.

Huang, H., S. Xie, Q. Xiao, B. Wei, L. Zheng, Y. Wang, Y. Cao, X. Zhang, T. Long, Y. Li, Y. Hu, G. Yu, H. Liu, Y. Liu, Z. Huang, J. Zhang and Y. Huang. 2016. Sucrose and ABA regulate starch biosynthesis in maize through a novel transcription factor, ZmEREB156. *Sci. Rep.*, 6: 27590.

Kristen, R.F.B. and A.L.D.M. Braun. 2012. SWEET as sugar: new sucrose effluxers in plants. *Mol. Plant*, 5(4): 766-768.

Li, S., Y. Liu, L. Zheng, L. Chen, N. Li, F. Corke, Y. Lu, X. Fu, Z. Zhu and M.W. Bevan. 2012. The plant-specific G protein γ subunit AGG3 influences organ size and shape in *Arabidopsis thaliana*. *New Phytol.*, 194(10): 690-703.

Lia, D., X. Zhang, Y. Xua, L. Lia, M.S. Aghdamb and Z. Luo. 2019. Effect of exogenous sucrose on anthocyanin synthesis in postharvest strawberry fruit. *Food Chem.*, 289: 112-120.

Liu, C., B. Tu, Y. Li, B. Tian, Q. Zhang, X. Liu and S.J. Herbert. 2017. Potassium application affects key enzyme activities of sucrose metabolism during seed filling in vegetable soybean. *Crop Sci.*, 57(5): 2707-2717.

Liu, J., P. Ding, T. Sun, Y. Nitta, O. Dong, X. Huang, W. Yang, X. Li, J.R. Botella and Y. Zhang. 2013. Heterotrimeric G proteins serve as a converging point in plant defense signaling activated by multiple receptor-like kinases. *Plant Physiol.*, 161(4): 2146-2158.

Livak, K.J. and T.D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{(-\Delta\Delta CT)}$ method. *Methods*, 25: 402-408.

Luo, J., Y. Zhang, Y. Zhao, X. Chen, Y. Xiao and F. Peng. 2018. Effects of salicylic acid on the SnRK1 activity and growth of strawberry plant. *Plant Physiol. J.*, 54(1): 113-120.

Magdalena, D.C., C. Sánchez-Rodríguez, V. Escudero, E. Miedes, P.V. Fernández, L. Jordá, C. Hernández-Blanco, A. Sánchez-Vallet, P. Bednarek, P. Schulze-Lefert, S. Somerville, J.M. Estevez, S. Persson and A. Molina. 2012. Arabidopsis heterotrimeric G-protein regulates cell wall defense and resistance to necrotrophic fungi. *Mol. Plant*, 5(1): 98-114.

Mishra, V.K. and R.M. Singh. 2016. Sorbitol and sucrose-induced osmotic stress on growth of wheat callus and plantlet regeneration. *Curr. Trends Biotechnol. & Pharm.*, 10(1): 20-28.

Nagata, T., H. Hara, K. Saitou, A. Kobashi, K. Kojima, T. Yuasa and O. Ueno. 2012. Activation of ADP-glucose pyrophosphorylase gene promoters by a WRKY transcription factor, AtWRKY20, in *Arabidopsis thaliana* L. and sweet potato (*Ipomoea batatas* Lam.). *Plant Prod. Sci.*, 15(1): 10-18.

Niu, J., J. Huang, T.T. Phan, Y. Pan, L. Yang and Y. Li. 2019. Molecular cloning and expressional analysis of five sucrose transporter (SUT) genes in sugarcane. *Sugar Tech.*, 21(1): 47-54.

Shi, J., G.A. Shi and Z. Tian. 2015. Effect of exogenous hydrogen peroxide or ascorbic acid on senescence in cut flowers of tree peony (*Paeonia suffruticosa* Andr.). *J. Hort. Sci. Biotech.*, 90(6): 689-694.

Sona, P., J. Chen, A.M. Jones and S.M. Assmann. 2006. G-protein complex mutants are hypersensitive to abscisic acid regulation of germination and postgermination development. *Plant Physiol.*, 141(1): 243-256.

Steffens, B. and M. Sauter. 2010. G proteins as regulators in ethylene-mediated hypoxia signaling. *Plant Sign. & Behav.*, 5(4): 375-378.

Sun, C., S. Palmqvist, H. Olsson, M. Borén, S. Ahlandsberg and C. Jansson. 2003. A novel WRKY transcription factor, SUSIBA2, participates in sugar signaling in barley by binding to the sugar-responsive elements of the *iso1* promoter. *The Plant Cell*, 15(9): 2076-2092.

Wang, B., Y. Zhang, M.E. Haque, W. Xu, F. Li and A. Liu. 2018a. Transcriptomic analyses reveal complex and interconnected sucrose signaling cascades in developing seeds of castor bean. *J. Plant Physiol.*, 221: 1-10.

Wang, X.L., R.R. Qin, R.H. Sun, J.J. Wang and X.G. Hou. 2018b. No post-drought compensatory growth of corns with root cutting based on cytokinin induced by roots. *Agric. Water Manag.*, 205: 9-20.

Weiste, C., L. Pedrotti, J. Selvanayagam, P. Muralidhara, C. Fröschel, O. Novák, K. Ljung, J. Hanson and W. Dröge-Laser. 2017. The Arabidopsis bZIP11 transcription factor links low-energy signalling to auxin-mediated control of primary root growth. *PLoS Genet.*, 13 (2): e1006607.

Wind, J., S. Smeekens and J. Hanson. 2010. Sucrose: metabolite and signaling molecule. *Phytochem.*, 71(14-15): 1610-1614.

Wu, T., S. Krishnamoorthi, H. Goh, R. Leong, A.C. Sanson and D. Urano. 2020. Crosstalk between heterotrimeric G protein-coupled signaling pathways and WRKY transcription factors modulating plant responses to suboptimal micronutrient conditions. *J. Exp. Bot.*, 71(10): 3227-3239.

Xu, G., D. Lu, H. Wang and Y. Li. 2018. Morphological and physiological traits of rice roots and their relationships to yield and nitrogen utilization as influenced by irrigation regime and nitrogen rate. *Agric. Water Manag.*, 203: 385-394.

Yadav, D.K., D. Shukla and N. Tuteja. 2014. Isolation, *in silico* characterization, localization and expression analysis of abiotic stress-responsive rice G-protein β subunit (RGB1). *Plant Sign. & Behav.*, 9(5): e28890.

Yadav, D.K., D. Shukla and N. Tuteja. 2013. Rice heterotrimeric G-protein alpha subunit (RGA1): In silico analysis of the gene and promoter and its upregulation under abiotic stress. *Plant Physiol. & Biochem.*, 63: 262-271.

Zhang, X.Y., Q.Y. Tong, W.X. Zhu and F. Ren. 2013. Pasting, rheological properties and gelatinization kinetics of tapioca starch with sucrose or glucose. *J. Food Eng.*, 144(2): 255-261.

Zhao, X.P., B. Hui, L.X. Hu, Q.Z. Cheng and B.K. Via. 2017a. Potential of near infrared spectroscopy to monitor variations in soluble sugars in *Loblolly pine* seedlings after cold acclimation. *Agr. Forest Meteorol.*, 232: 536-542.

Zhao, Y., X. Chen, F. Peng, J. Luo and Y. Xiao. 2017b. Over expression of a peach *PpSnRK1βγ1* gene improving oxidative stress tolerance in *Arabidopsis thaliana*. *Plant Physiol. J.*, 53(7): 1251-1258.

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