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Comprehensive characterization and expression profiling of sucrose phosphate synthase (SPS) and sucrose synthase (SUS) family in *Cucumis melo* under the application of nitrogen and potassium

Iftikhar Hussain Shah[1†], Muhammad Aamir Manzoor[1†], Muhammad Azam[1], Wu Jinhui[1], Xuyang Li[1], Asad Rehman[1], Pengli Li[1], Yidong Zhang[1], Qingliang Niu[1] and Liying Chang[1*]

Abstract

Background Sugars are not only important biomacromolecules that play vital roles in plant growth, development and environmental stress tolerance, but they also provide carbon skeletons for the synthesis of other macromolecules, such as proteins and nucleic acids. Sugar-related proteins play key roles in the movement of sugars from source tissues (such as leaves) to sink tissues (such as fruits), ultimately influencing fruit development. However, the evolutionary dynamics of this important sugar-related gene family in the *Cucumis melo* (*C.melo*) crop are still unknown, and the functional differentiation of melon genes remains unclear.

Results To understand the sucrose metabolism in *C. melo* we identified the sugar base protein by bioinformatics tools and their expression changes under nitrogen and potassium fertilization. Sucrose phosphate synthase (SPS) and sucrose synthase (SUS) are key sugar-based transfer enzymes that play a vital role in sugar accumulation. However, to date, the evolutionary history and functional characteristics of sugar-related protein in *C. melo* remain unknown. Therefore, in this work, we investigated six SPS genes and four SUS genes from *C. melo*, along with the conserved domain of SUS proteins of *Arabidopsis thaliana*. Phylogeny and structural features demonstrated that SPS and SUS genes were categorized into four subfamilies (I to IV) and had non-uniform form distribution across the seven melon chromosomes. Moreover, the functional divergence between clades was shown by gene structure and conserved motifs. In *C.melo*, transposed duplication events have been essential to the growth and development of the sugar gene family. Analysis of the upstream regions showed growth-promoting elements that could be targeted to manage various stress conditions through a variety of trans-acting factors involving sugar metabolism. Moreover, the target of

†Iftikhar Hussain Shah and Muhammad Aamir Manzoor contributed equally to this work and share first authorship.

*Correspondence:
Liying Chang
changly@sjtu.edu.cn

Full list of author information is available at the end of the article

microRNAs revealed that miRNAs have a role in the development and control of sugar genes. Furthermore, expression profiling revealed the differential expression of these genes during fruit developmental stages.

Conclusion This work established the foundational knowledge to investigate the function and mechanism of sucrose accumulation in fruit.

Clinical trial number Not applicable.

Keywords Genome-wide, Sugar metabolism, Nitrogen and potassium, qRT-PCR, *Cucumis melo*

Introduction

Soluble sugars, the primary byproducts of photosynthesis, serve as temporary energy storage and provide carbon skeletons for the synthesis of various cellular compounds, signaling molecules, osmolytes, and transport molecules. These sugars are essential for plant growth and development [1, 2]. They serve as molecules that both supply carbon and act as signaling molecules that regulate many gene networks [3]. Sugars are produced in photosynthetic leaves (the source) in the form of sucrose, raffinose, and stachyose and translocated to non-photosynthetic tissues (the sink), including those in roots and fruits, to serve as a carbon and energy source for vegetative plant growth and fruit development. Fructose (Fru), glucose (Glu), and sucrose (Suc) are involved in metabolic pathways and vital sources for improving the quality of fruit because they are both essential nutrients and sweetening agents [4]. Contribution of two crucial enzymes involved in sucrose storage in fruit is sucrose phosphate synthase (SPS, EC 2.4.1.14) and sucrose synthase (SUS, EC 2.4.1.13). SPS must first catalyze fructose-6-phosphoric acid (F6P), and uridine diphosphate glucose (UDPG) before sucrose phosphatase (SPP) can permanently convert sucrose-6-phosphoric acid (S6P) to sucrose [5]. On the other hand, sucrose may carry out a variety of metabolic procedures, taking to the reversible enzyme SUS to stimulate various metabolic processes [6]. Several investigations demonstrated the strong connection between sucrose accumulation and increased SUS activity during fruit development [7, 8]. Right now in literature, three families of eukaryotic sugar transporters, sucrose transporters (SUTs), monosaccharide transporters (MSTs), and SWEETs, are thought to play important roles in the process of transferring sugars from one organism to another ultimately [9, 10]. These sugar transporters are now generally acknowledged to have important roles in the entire plant sugar translocation and allocation process. Moreover, several SUS and SPS genes have been transferred from numerous species [6, 11]. To date, there are several reports of a comprehensive analysis of sugar-related genes, and their functional analysis in different plants has been reported, such as strawberry [12], carrot [13], Arabidopsis [14], sugarcane [15], citrus [16], Corn [17], apples [18] rice [19], cassava [20], and cucumber [1]. However, the number of SUS genes varies significantly amongst different species. For instance, 17 SUS and 8 SPS genes were found in pears [21], oranges have 4 SPS genes [22], peaches have 6 SUS genes [23], grapes have 5 SUS genes [24] and oranges have 4 SUS genes [22]. Members of the gene family share distinct roles and features of expression. However, SPS1 was found to be expressed preferentially, whereas three *SPS2*,*SPS6*, and *SPS8* were detected in rice and showed distinct patterns during fruit development [6].

Cucumis melo L. is a diverse and prominent horticultural crop that is grown all over the world in temperate climates, and it yielded over 27 million tons in 2019 [25, 26]. Genetics determines fruit shape and size even before the floral meristem develops. This has led to a partial understanding of the underlying genes, their interactions, and how they influence the variation displayed using genomic tools, transcriptome sequencing, and functional analysis. There are various comprehensive studies on sucrose and photosynthetic by-product accumulation in cucurbit crops that have been carried out to date [27, 28]. The majority of these studies have concentrated on a small number of enzyme activities and functional analysis of sugar transporters [29, 30]. However, Comprehensive analysis and expression analysis of the SUS and SPS genes in *C. melo* is still unclear. A comprehensive study and their regulatory role of sugar accumulation were carried out to comprehend the traits of the SUS and SPS family members of *C. melo* and their potential role in sugar accumulation in fruit.

In this study, the impact of exogenous potassium and nitrogen on the movement and accumulation of sugar in *C. melo* fruit was investigated. Numerous reports have shown that, as fruit developed, higher application of K^+ up-regulated the expression genes involved in sugar metabolism in the leaves, including SPS1, SUS, S6PDH and SDH3. This upregulation leads to increased accumulation of soluble carbohydrates, such as sucrose, glucose, sorbitol, and fructose, in the leaves. At the fruit maturation stage, fruit accumulates more sucrose due to the upregulation of SUT expression in the leaves and SPS1, SUS, and SUT in the fruit, all of which are involved in sucrose metabolism [31, 32]. Based on previous studies of the sugar biosynthesis pathway in plants, a comprehensive model of the effects of K^+ on the expression of key genes involved in sucrose and sorbitol metabolism

in leaf and fruit in Asian pear from the young fruit stage to maturity [33]. In this regard, candidate genes for novel *C. melo* sucrose synthase (*CmSUS*) and sucrose phosphate synthase (*CmSPS*) were identified, with their roles in response to nitrogen and potassium further explored. The third goal of this study is to examine the functional analysis of *A. thaliana* genes involved in sugar metabolism as a basis for future studies on genetic modifications aimed at enhancing melon fruit quality.

Materials and methods

Identification, collection, and physiochemical properties

To identify the SPS and SUS genes, the melon genome database (DHL92 available at http://cucurbitgenomics.org/organism/18) was employed to download the FASTAS sequences related to sugar transporters (SPS and SUS) genes [34]. Candidate sequences were then analyzed by the NCBI conserved database using https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi research result. Prot ein sequences were first aligned using PF00862 (Sucrose synthase), PF00534 (Glycosyl transferases group 1), and PF05116 (Sucrose-6 F-phosphate phosphohydrolase). All potential genes were identified using the Hmmsearch tool against the whole-genome database. The proteins were confirmed by NCBI CDD (http://www.ncbi.nlm.nih.gov/cdd) and duplicates were excluded. The SUS and SPS genes were then further validated by applying tools such as SMART (http://smart.emblheidelberg.de), Pfam database (http://pfam.xfam.org), and InterProScan (http://www.ebi.ac.uk/interpro/sea) [35, 36]. The physiochem ical properties and structural features of selective genes, including molecular weight, number of amino acids, and theoretical point [IP]) of molecules, as well as their subcellular localization, were predicted using the online ExPASY service (http://web.expasy.org/protparam) [37, 38].

Phylogenic tree, intron, and exon analysis of sweet genes family

The phylogenetic tree was constructed using protein sequences from the SPS and SUS gene families of *C. melo* and *A. thaliana* to analyze their evolutionary relationship. Protein sequence alignment was performed using the maximum likelihood (ML) method to generate a phylogenetic tree with 1,000 bootstrap values. The analysis was conducted using the software available online at http://iqtree.cibiv.univie.ac.at [39]. Finally, the phylogenetic tree was visualized using iTOL software (http://itol.embl.de) [40, 41]. Additionally, structural analysis of genes of SPS and SUS genes, including their intron-exon organization was conducted using the GFF file and the results were displayed with TBtools software.

Chromosomal distribution, collinearity analysis, and gene duplication events

The melon genome database (DHL92) was used to determine the size of each melon chromosome and identify the location of SPS and SUS genes. Collinearity analysis of putative gene family members was performed using (MCScanX) software [39]. MCscanX software was used to evaluate duplication events in SPS and SUS genes [42]. Additionally, Circos software was utilized to identify the tandem duplication, using the chromosomal locations of both SPS and SUS gene family members [43].

Analysis of cis acting regulatory elements and MiRNA target site

The distribution pattern of the cis-acting regulatory elements of selected genes was examined using online PLANTCARE tools (http://bioinformatics.psb.ugent.be/webtools/plantcare/). The upstream region of all SPS and SUS musk melon genes were analyzed, starting from the start codon (ATG) and upstream regions of promoter sequences. To investigate the post-transcriptional regulatory role of SUS and SPS genes, miRNA target sequences of *C. melo* were downloaded from the online pmiren database (https://www.pmiren.com). The psRNA Target method predicted potential miRNA-targeted sites on the SUS and SPS genes (http://plantgrn.noble.org/psRNATarget/analysis/) [44].

Hydroponic systems for the growth of melon seedlings

This study was conducted in the greenhouse of the Chongming District Academy of Agricultural Sciences in Shanghai from February 2023 to July 2023. Greenhouse No. 1 can accommodate 720 plants. Kula melon seedlings were in their seedling stage from February to March, and From March to June, the melon was treated with nitrogen and potassium during vegetative and fruiting stages. The study aimed to gain a comprehensive understanding of the fruit development. Fruits were collected at three stages: premature, mid-stage, and fully repined stage. At each stage, three fruits were harvested from three different plants. Fresh mesocarp tissue was collected from the center-equatorial portion of the fruit and immediately flash-frozen in liquid nitrogen before being stored at -80 °C.

qRT-PCR analysis of genes

To validate the sugar metabolism in fruit development, six randomly selected gene expressions by evaluated at different developmental stages by qRT-PCR. Total RNA was extracted from fruits 1–5 days after flowering (DAF) using an RNA isolation kit (TIANGEN, Aidlab Biotech, Beijing, China). RNA integrity and degradation were assessed using 1.5% agarose gels. For the synthesis of first-strand cDNA, Takara's (Japan) PrimerScriptTM RT

Reagent was utilized. The reaction was carried out with a SYBR qPCR mix (Takara, Shiga, Japan) and a Bio-Rad/CFX ConnectTM Real-Time PCR Detection System (Bio-Rad, San Diego, CA, USA). Three technical and three biological replicates were used for each reaction. Actin was used as an internal reference gene, and the relative gene expression levels were calculated using the $2^{-\triangle\triangle Ct}$ method [28]. Specific primers were designed through primer premier software 5.0, and the sequences are listed in Table S1.

Results

Identification and classification, chromosomal distribution of SUS and SPS genes in *Cucumis Melo*

A comprehensive genome-wide analysis was conducted to identify the SPS and SUS gene members from the *C. melo* genome. The sequenced genome of *A. thaliana* was downloaded from the TAIR website (https://www.arabidopsis.org/). The SMART database, Pfam database, and Interpro tool were used to verify the presence of the relevant domains in each sugar transporter gene. After the elimination of duplicate entries, 10 SPS and SUS genes with a typical sugar synthetase domain were identified in the melon genome database. These genes were numbered based on the sequence in which they were discovered in the melon database. Table 1 provides detailed information on these genes, including their domains, polypeptide sequences, molecular weights, and theoretical isoelectric points (pI). The predicted polypeptide sequence lengths ranged from 98 to 1062 amino acids. Domain analysis revealed that seven out of the 10 SPS and SUS genes contained two domains, while three *CmSPS* genes contained three domains (Table 1).

Further, the MEGA-7 software program was used to construct a phylogeny after the protein sequences were aligned using ClusterX. Additionally, we constructed phylogenetic trees using two inference techniques, neighbor-joining (N-J) with a 1000-times bootstrap for support. The N-J tree was selected for further investigation because it exhibited better bootstrap values than the ML tree, although both trees displayed similar topologies (Fig. 1). Based on sequence similarities, topography, and bootstrap values, four subfamilies (I–IV) were identified for each detected SUS and SPS protein. As shown Fig. 1, subfamily I contains the maximum number of these proteins, followed by other subfamilies. Subfamily II and IV had two protein members. All protein sequences were clustered in each of the subfamilies. These findings suggest that gene gain or loss events during evolution may have contributed to the functional divergence observed.

Subsequently, chromosomal distribution in the *C. melo* genes was analyzed, revealing that 10 SUS and SPS genes were dispersed across the scaffolds. The distribution of sugar genes across the twelve chromosomes of *C. melo* was uneven (Fig. 2). Nevertheless, no correlation was observed between the phylogenetic relationships and the chromosomal distribution of these genes. Gene family expansion and the maintenance of gene copy numbers may be facilitated by tandem duplication. As a result, it was found that five genes underwent tandem duplication. Two tandemly duplicated genes were located on chromosomes 2, 4, and 7, while one tandemly duplicated gene was found on chromosomes 3, 5, 9, and 12.

Conserved motif and gene structure analysis of SUS and SPS genes in *Cucumis Melo*

The evolutionary relationship and classification of genes were investigated by conserved motif analysis. Remarkably, the majority of SUS and SPS that are closely related phylogenetically have comparable structures and motifs (Fig. 3). It was found that the SUS and SPS protein sequences contained predicted motifs. All examined proteins contained the motif sets 1, 2, 3, 4, 5, 11, and 13. The motifs varied in length from 15 to 92, with different motifs predicting a portion of the putative sugar transporter domain.

Further, intron and exon investigations were conducted to observe the comprehensive structural diversity and systematic diversity among gene families. The gene features (exon-intron) organization and encoded conserved motifs were compared to obtain insight into possible functions and structural diversification among all genes.

Table 1 Physiochemical properties of SPS and SUS genes in *Cucumis melo*

Gene ID	Gene name	Domain	Amino acids	Molecular weight	Theoretical pI:	Sub-cellular localization
MELO3C020357.2.1	*CmSPS1*	3	1062	119013.38	5.95	Nucleus
MELO3C010300.2.1	*CmSPS2*	3	1054	117968.75	6.09	Nucleus
MELO3C008101.2.1	*CmSPS3*	3	1028	115867.38	6.44	Nucleus
MELO3C025101.2.1	*CmSUS4*	2	834	94611.68	6.1	Nucleus
MELO3C001956.2.1	*CmSUS5*	2	849	96011.91	8.13	Nucleus
MELO3C017942.2.1	*CmSPS6*	2	811	92676.06	6.01	Nucleus
MELO3C015552.2.1	*CmSUS7*	2	806	92588.98	5.83	Nucleus
MELO3C025824.2.1	*CmSPS8*	3	109	12457.42	9.05	Nucleus
MELO3C003715.2.1	*CmSPS9*	2	98	10769.02	4.49	Nucleus
MELO3C016129.2.1	*CmSUS8*	2	400	44749.03	8.45	Nucleus

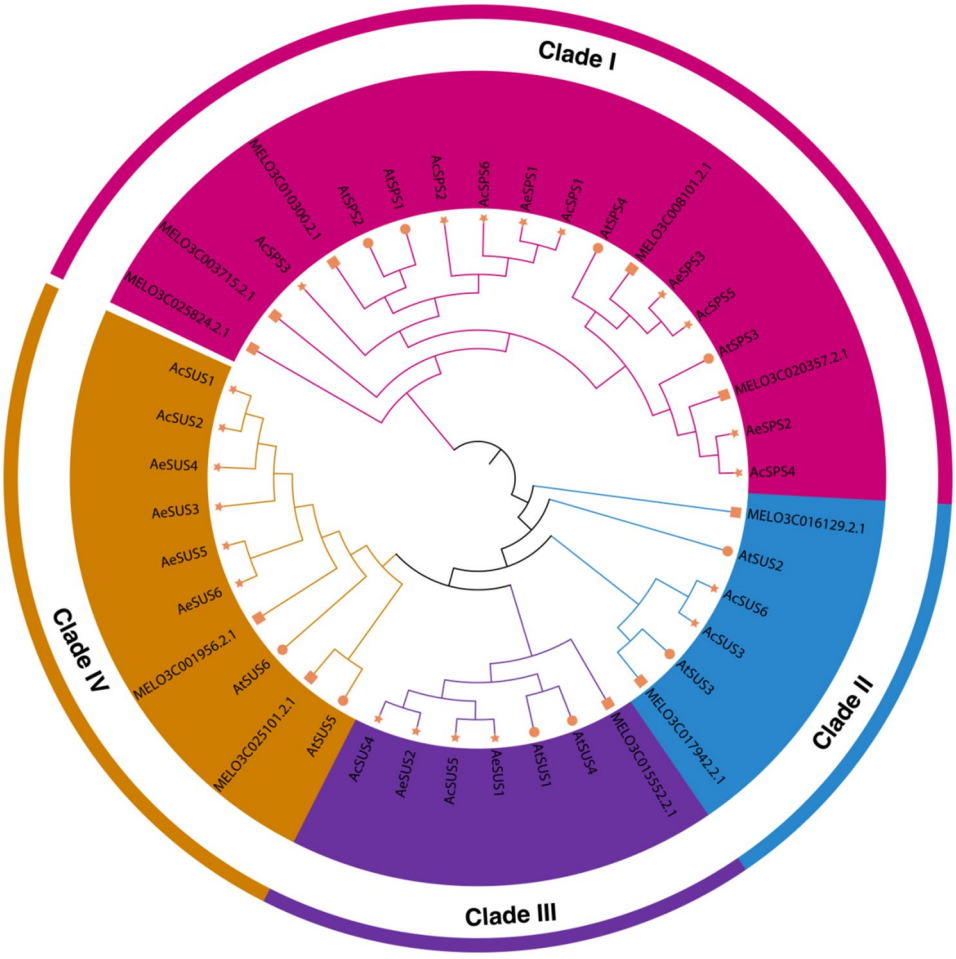

Fig. 1 Phylogenetic relationship of SUS and SPS genes family in *C. Melo*; colors of each clade representing a subfamily

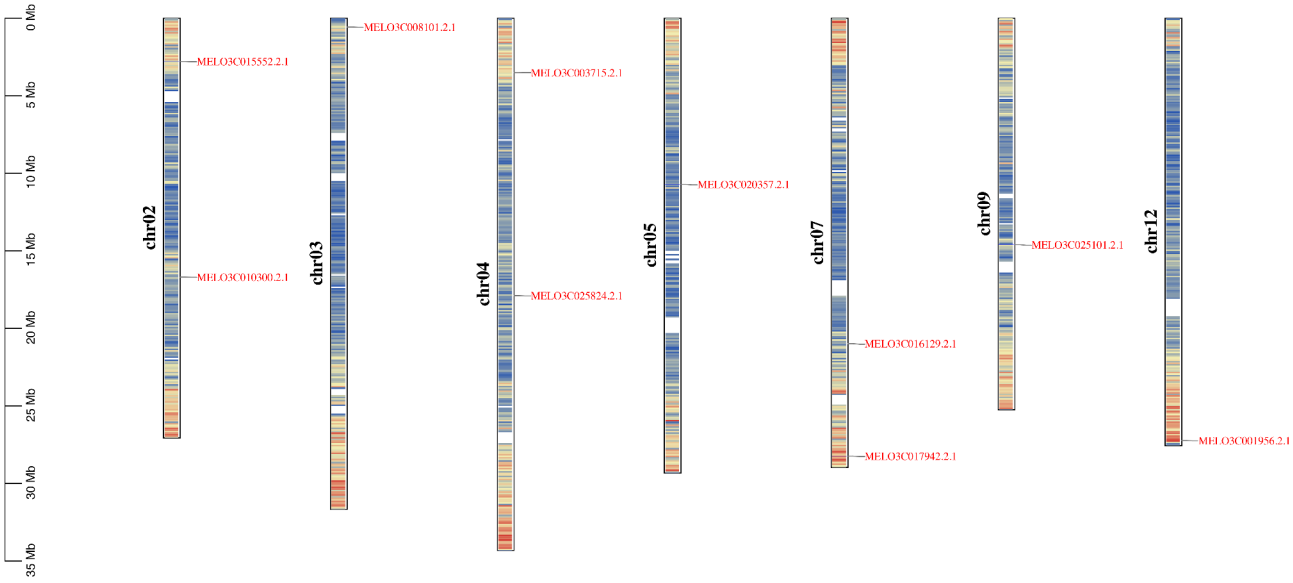

Fig. 2 Chromosomal distribution and localization of SUS and SPS genes in melon

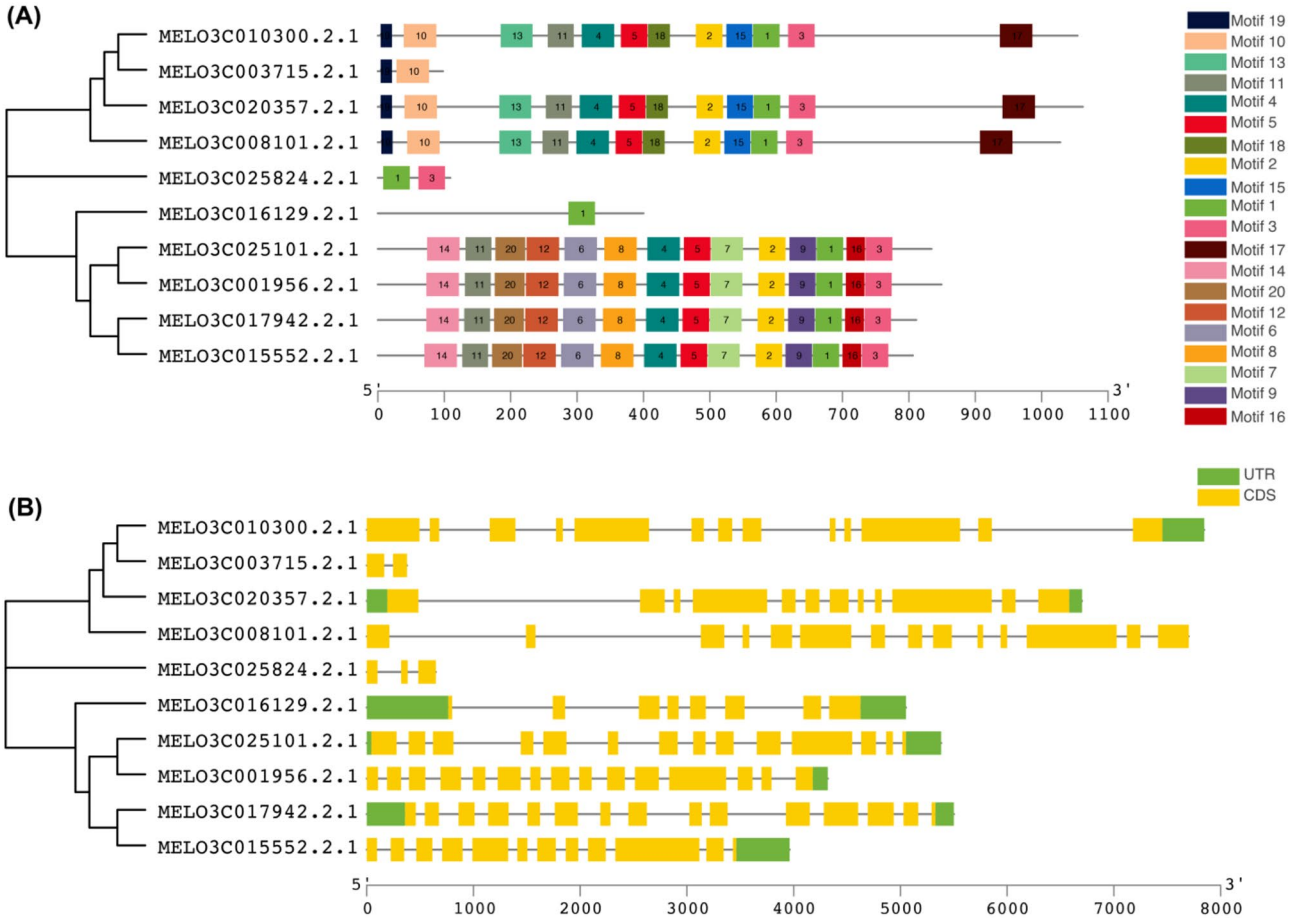

Fig. 3 Conserved motif distribution and structural analysis (intron/exon). (**A**) Phylogenetic relationship (left): Conserved motifs were done using the MEME suite (motif-based sequence analysis online tool); each motif is shown as a different colored box. (**B**) Here is a representation of the intron-exon arrangement in SUS and SPS: boxes with yellow color indicate exons (CDS), thin sharp black lines specify the introns, and green boxes at the start and end region indicate the un-translated (UTR) region (right)

The number of introns and exons varied between 2 and 14 and 3 and 15, respectively. The gene *MELO3C010300* had the most introns and exons, with a composition of 12/13, while *MELO3C003715* contained only 1–2 introns/exons. Most genes within the same subfamily share a similar number of exons and introns. These findings suggest that the motif compositions and exon/intron structures are conserved, indicating that the genes have evolved to support diverse functions both within and between subfamilies. Moreover, a close evolutionary relationship and group classification were confirmed by the remarkably similar exon/intron structures of the SPS and SUS genes that were grouped in the same subfamily. It was also established that each subfamily had different gene lengths. The predicted evolutionary relationship of the *SUS and SPS* gene family caused functional differences across the melon genome.

Gene duplication and synteny analysis of SUS and SPS genes in *Cucumis Melo*

To examine the features of the SUS and SPS genes family, we analyzed gene duplications across chromosomes. For this analysis, five types of gene duplications were carried out to explain the evolutionary connections among these genes: whole-genome duplication (WGD), tandem repeat duplication (TRD), proximal duplication (PD), tandem duplication (TD), and distal duplication (DSD) (Fig. 4). The current study revealed that all members of SUS and SPS gene families were located on 2, 3,4,5,7, and 9 chromosomes of *C.melo*. Chromosome 2 had the highest number of SUS and SPS genes. Chromosome 9 only contained 1 gene member. Further, we investigated genes tandem and syntenic relationships between these genes. In SUS and SPS genes, 1 pair of DSD, 1 pair of WGD, and 3 pairs of TRD were identified in our evaluation (Fig. 4 Table S2). However, tandem duplication (TD) was not detected in melon sugar-related genes. The presence of TRD suggests that transposed duplication

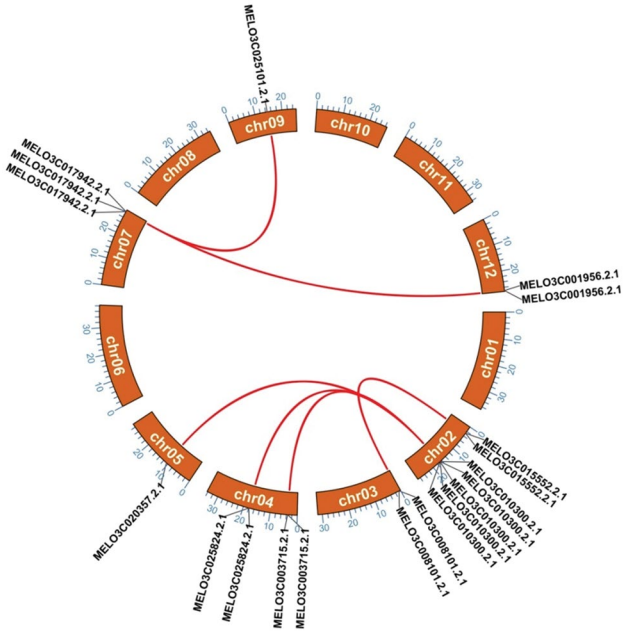

Fig. 4 Chromosomal distribution and gene duplication analysis: Background gray lines indicate the synteny blocks and visible red lines imply duplicated SPS and SUS gens pairs

plays a significant role in the growth and evolution of the SPS and SUS gene families, more so than dispersed and whole-genome duplications.

To investigate the evolution of SUS and SPS gene families, we conducted an interspecies synteny analysis on *A. thaliana*, *C. pepo*, and *C. lanatus*. Based on our results, 28 orthologous gene pairs were comprised between *C. melo*, *A. thaliana* and *C. lanatus.* In current findings, 8 syntenic gene pairs were observed between *C. lanatus* and *C. melo*, and 5 genes showed a syntenic relationship between *A. thaliana* and *C. melo*. These analyses suggest that both species share strong evolutionary relationships. Among the 28 gene collinearity relationships, 20 genes exhibited a syntenic relationship between *C. pepo* and *C. melo* (Fig. 5 Table S3). The syntenic relationship between *C. melo* and other species provides significant insights into the evolution of sugar genes.

MiRNA target and gene ontology (GO) analysis of SUS and SPS genes in *Cucumis Melo*

Natural short RNAs (20-24nt), single standard, and non-coding microRNAs (miRNAs) are essential for plant growth and act as post-transcriptional regulators in stress responses (Fig. 6). In this study, the whole miRNA target candidates for *C. melo* were downloaded using the pmiren website (https://www.pmiren.com) to compreh end the regulatory mechanism of miRNAs. To demonstrate the posttranscriptional regulation of SUS and SPS genes, we identified different miRNA target sites on the genomic regions of 10 sugar genes using a small-RNA target analysis server (psRNATarget). In our current investigation, six novel miRNAs from various miRNA families were found to regulate gene expression broadly (Fig. 7). The analysis revealed that several annotated miRNAs targeted six of the ten *CmSUS* and *CmSPS* genes. Our most recent findings indicate that multiple miRNAs

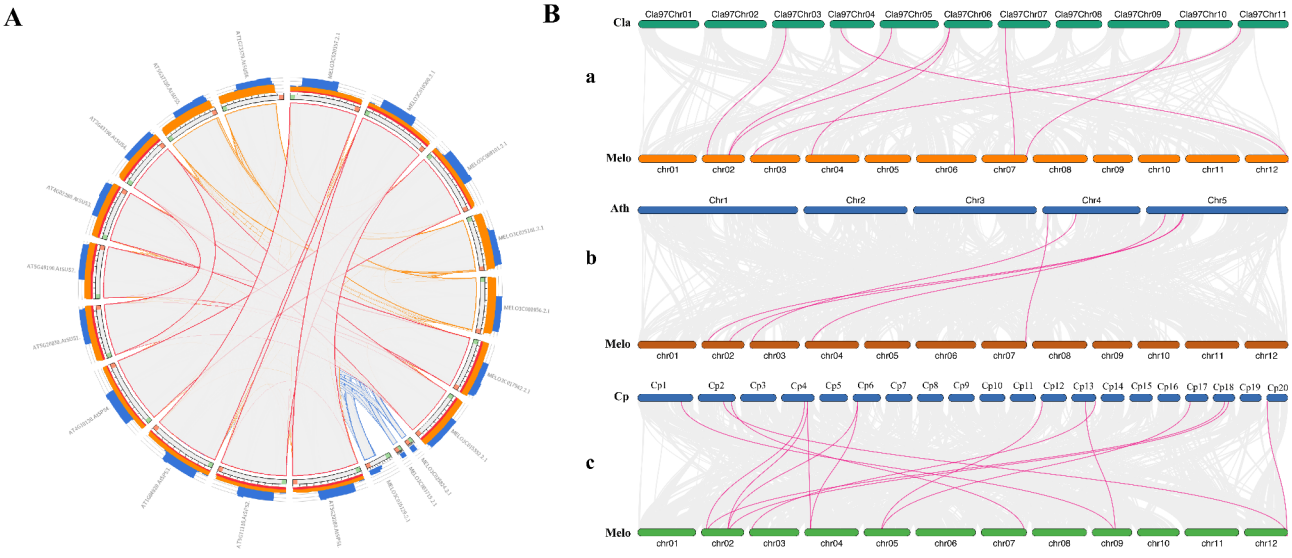

Fig. 5 Collinearity relationships of SUS and SPS in *C. melo*. (**A**) Chromosomal localization (**B**) Collinearity analyses of SUS and SPS genes among *C. melo*, *A. thaliana*, and *C. lanatus*. Three color graphics (a, b and c)) present the collinear relationship In the background, grey lines exposed the collinear relationship across the whole genome, while the clear red lines presented the collinear gene pairs

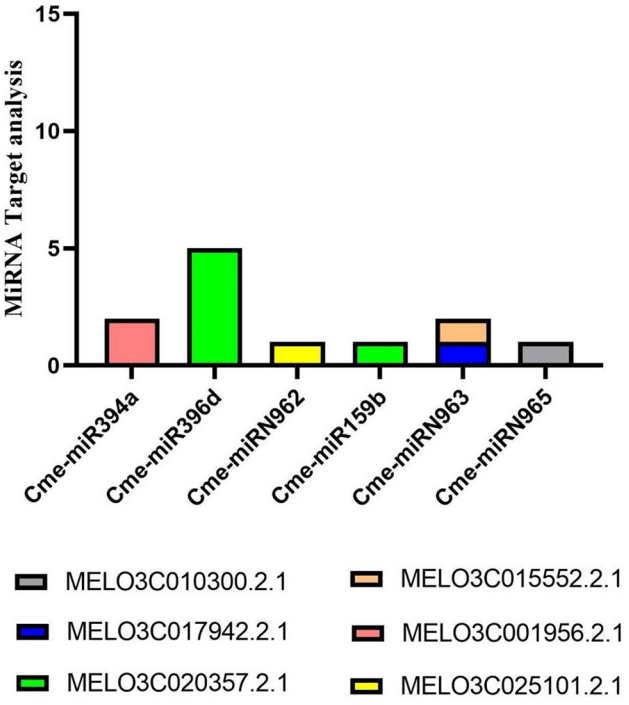

Fig. 6 SUS and SPS MicroRNAs (miRNAs) Target analysis of *C. melo*. The endogenous regulatory mechanism and critical role in sugar metabolism. The predicted miRNAs show that one miRNA can target more than one SUS and SPS genes

can target the same gene and play a crucial role in plant growth.

To demonstrate the biological functions, subcellular localization, cellular components and molecular function in SPS and SUS genes. Consequently, a GO enrichment analysis of sugar synthase genes was analyzed using the CELLO2GO tool (Fig. 7 and Table S4). Six functional groups were discovered to be associated with molecular and cellular components; fourteen of these groups were traced and important for biological processes. Further, molecular functions, including functions of ion binding, sucrose synthase, sucrose–phosphate synthase, transferase, and clathrin binding activities were found in 90.0, 50.0, 40.0 and 30.0% of SUS and SPS, respectively (Fig. 7). Conversely, the cellular processes, GO term indicated cell wall, integral components and plasma membrane (20.0%), cytosol, Golgi complex and plasmodesmata (10.0%). Additionally, GO analysis revealed that the biological components associated with these genes include regulation of meristem growth, nodulation, mannosylation, nectar secretion, pollen wall assembly, seed maturation, and protein glycosylation, with each process showing an involvement of 10.0%.

Prediction of cis acting elements of the promoter region

Cis-regulatory elements are genomic regions recognized by various transcription factors that regulate gene expression. The PlantCARE database was used to analyze the transcriptional control of SUS and SPS genes (Table S5). The 1500 bp upstream promoter region of nucleotide sequences of the 10 SUS and SPS genes were analyzed for cis-acting regulatory elements. The P-Box, CAT box, ABRE, ABA, TCA-elements TGACG-motif, CGTCA-motif, ARE, MBS, and LTR are among the cis-acting elements found in most genes (Fig. 8). ABRE (28%), ERE (3%), P-box (5%), TGACG motif (17%), TCA-element (8%), GARE motif (7%), and CGTCA-motif (13%), which are linked to ABA, SA, ethylene, and MeJA responses, was also found to be phytohormone response related cis-elements, suggesting that these are involved in fruit development. Furthermore, we found promoter cis-acting elements SPS and SUS are highly related to gene function. According to their specific function, we arrange them into different categories. Specifically, 11% include the 02-site, which is linked to zein metabolic responsiveness; Box-4 contains 12%, which is related to light response; 5% contain circadian, which is related to circadian control; and 10% contain CAT-box, which is related to meristem expression. Finally, several stress-responsive elements were identified in the promoter region of these genes, such as LTR (9%), MBS (15%), and ARE (19%), which are connected to the cold, stress, and light stress responses, respectively (Fig. 8 Table S4).

Tissue specific expression pattern of SUS and SPS genes

By utilizing publicly available transcriptome data (PRGNA383830), we analyzed the expression patterns of the SUS and SPS genes in different tissues, including the root, leaf, female flower, and male flower [45]. Similar expression patterns were observed in specific genes associated with SUS and SPS, highlighting the functional importance of these genes at different developmental stages. Several genes exhibited similar expression levels in various tissues. *MELO3C016129.2.1*, *MELO3C001956.2.1*, *MELO3C01552.2.1*, *MELO3C010300.2.1*, *MELO3C025101.2.1*, *MELO3C017942.2.1*, and *MELO3C020357.2.1* exhibited elevated expression levels in all tissues. The genes *MELO3C008101.2.1*, *MELO3C003715.2.1*, and *MELO3C025824.2.1* exhibited decreased expression levels in some tissues (Fig. 9).

Expression profiling of SUS and SPS genes

QRT-PCR was performed to assess the biological functions of candidate genes involved in sugar metabolism. Six genes associated with sugar metabolism, selected randomly from the phylogenetic analysis, were chosen for functional validation. These candidate genes were successfully compared with previous literature, and their expression was confirmed. In the current study, these genes exhibited prominent expression under nitrogen (N)

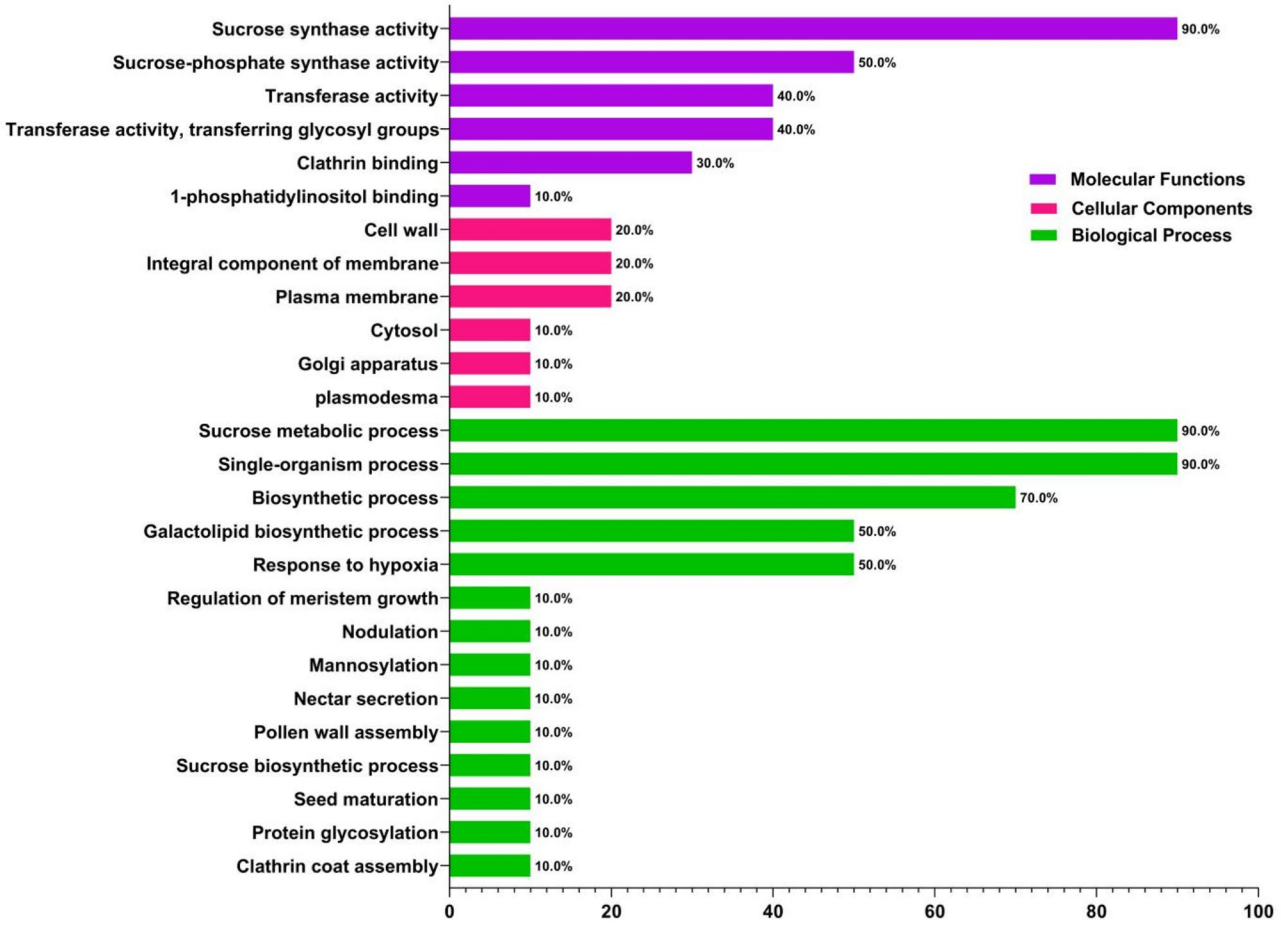

Fig. 7 Go ontology (GO) annotation of SUS and SPS proteins

and phosphorus (P) applications. The *MELO3C020357* gene showed a notable increase during fruit maturation, peaking in the early fruit development stage (Fig. 10). During fruit development and ripening, three sucrose synthases genes (*CmSUS4*, *CmSUS2*, and *CmSUS1*) displayed significant expression patterns. The expression level of *CmSUS4* was slightly reduced from the young fruit stage to the expanding stage, then rapidly increased at the premature stage, followed by a slight decrease in the mature fruit (Fig. 10B). Furthermore, the sugar-related genes *MELO3C001956* and *MELO3C008101*, which were not detected in previous reports, were analyzed in the present study. These genes showed moderate expression at the initial stage, reached high expression at 12DAF, and began to decrease at the repining fruit stage. *CmSUS2* exhibited higher expression levels when the fruit was still young. As the fruit developed, this expression level gradually declined. In contrast, the transcript expression level of *CmSPS1* was relatively low in young fruits and steadily decreased as the fruits matured. In the current study, the application of phosphorus (P) and nitrogen (N) significantly enhanced sugar accumulation in melon. Consequently, we compared the relative gene expression of these six sugar transporter genes. Most of the genes showed a positive correlation with previous findings. These genes were also associated with sugar accumulation during the maturation and ripening stages of the Kula cultivar.

Discussion

Sugar content and organic acid buildup have a major impact on the flavor and quality of melon fruit. To create dessert cultivars, researchers combine the high sugar and high acidity features of melons [46, 47]. Melon fruits do not contain polysaccharides; instead, they accumulate high concentrations of sucrose during the late stages of development. The process of sucrose accumulation in melon fruit is more intricate than that of sucrose transport in plants. The fruit sink's metabolism of carbohydrates controls and determines the developmental process [29]. Understanding the possible molecular mechanism and transportation linked to total sugar and organic acid in melon fruit, there is a dire need to investigate the comprehensive analysis of the expression pattern of sugar key proteins. Recent studies on fruit ripening in species like loquats, *A. thaliana*, oranges, citrus, apples,

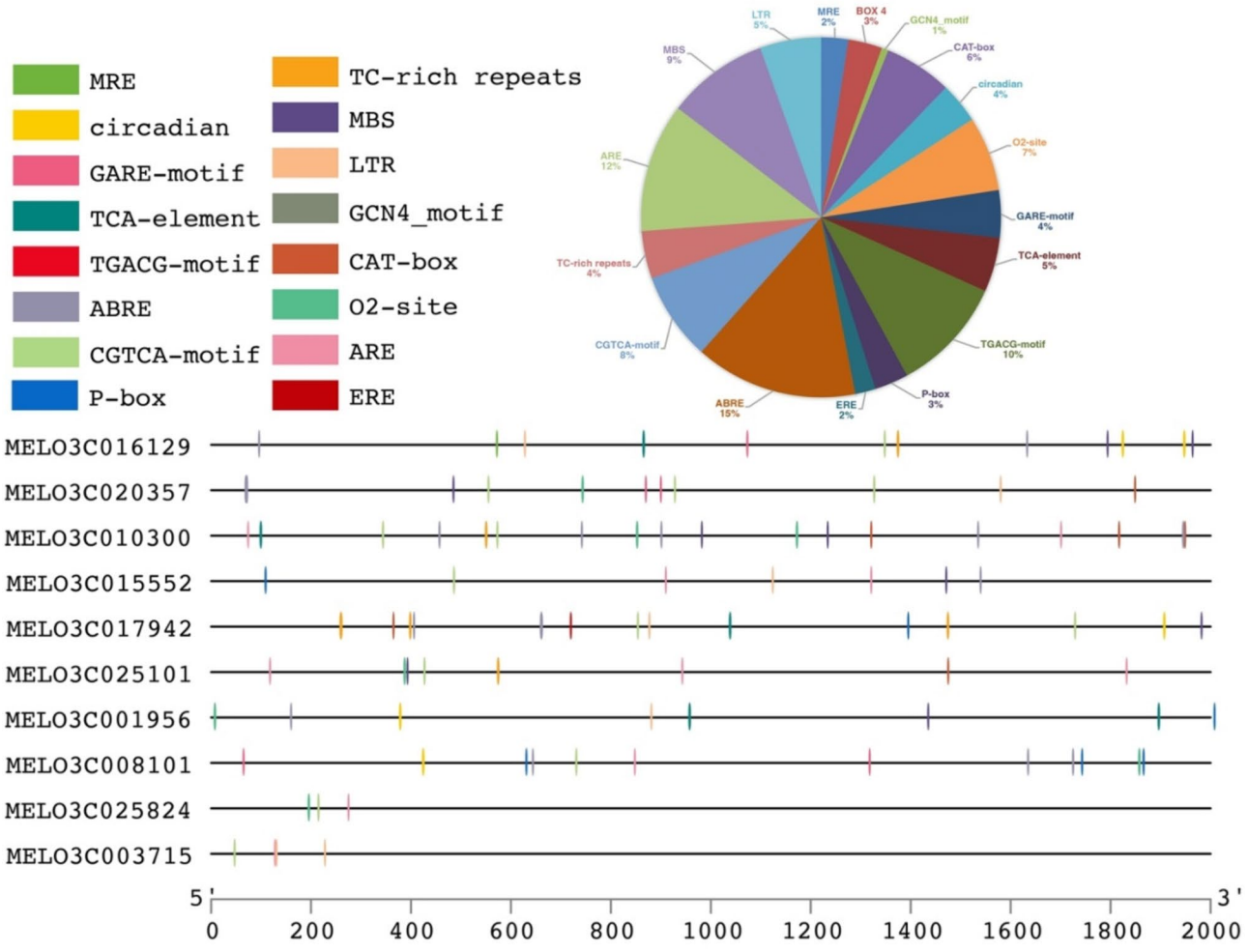

Fig. 8 Promoters cis-acting elements of SPS and SUS genes in *Cucumis melo*. Different color boxes represent different cis-acting elements

and actinidia have focused on the genomic analysis of sucrose accumulation and metabolism [6, 48].

To date, there is no Genome wide analysis and expression pattern of these gene families in *C. melo* has been reported. The phylogenetic relationship divided the putative SPS and SUS gene families' members into four subgroups. Gene acquisition or loss may have had an impact during the evolutionary transition. When several SPS and SUS members were added or removed, functional divergence emerged. Furthermore, 3 SPS genes had 3 domains, while other genes, including SUS, have 2 domains, according to our analysis. Furthermore, previous research suggested that exon/intron diversity, motif structure, and variation of different clades might have unique roles in evolution history [49, 50]. Gene structure analysis, particularly intron-exon analysis, significantly influences gene evolutionary relationships and phylogeny. It provides valuable insights into gene family origins and functions, emphasizing the importance of intron/exon sequencing properties [39, 51, 52]. Our investigation focused on the genetic makeup and conserved motifs of SPS and SUS, showing similarities with plants. We found high conservation throughout evolution, with constant coding sequences and introns in both families. Conserved motif analysis showed similar motifs clustering together in the evolutionary tree (19, 20, 36).

Gene duplication sheds light on the emergence of novel functions and the evolution of genes. Within the SUS and SPS gene family, the current findings investigated five different types of duplications: TRD, PD, WGD, TD and DSD. Through a variety of mechanisms, these duplications aid in the proliferation of particular genes in plants [20]. GO ontology and Cis regulatory elements of the upstream region of gene promoter analyses are two significant analyses in silico techniques that are frequently used to forecast the potential biological roles and regulatory patterns of gene sets of interest in organisms. We investigated the Go enrichment analysis of SUS and SPS genes for melon [25]. A review of gene promoters showed that all members of the SPS and SUS families share several hormone regulatory, light-response and stress regulatory components. This implies that

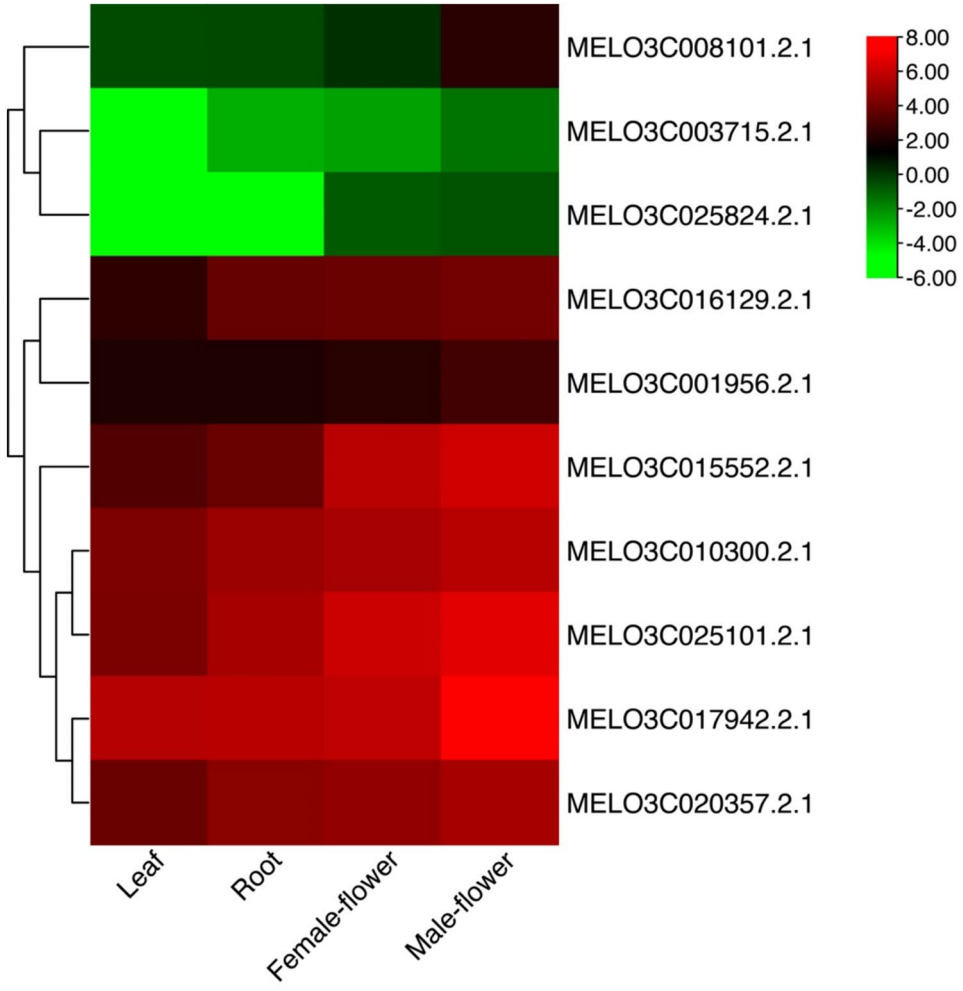

Fig. 9 Transcriptomic evaluation of selected SPS and SUS genes in different parts of melon. Different colors represent the expression intensity

homologous genes gradually arise during plant development, avoiding growth obstructions brought on by the missing of a single gene function caused by mutation. The genes SPS and SUS are involved in the processes of growth and development as well as stress management [53, 54]. The single-stranded, non-coding, small RNAs known as micro-RNAs (miRNAs) have a nucleotide length of 20–23 and play a vital role in the transcription of several genes in various organisms. Numerous studies have revealed the extensive involvement of miRNAs [55, 56]. Moreover, 26 conserved miRNA families have been discovered in musk melon related to plant growth and development [57]. In the present work, *Cm-miR396* was involved in the potential targets of *CmSUS* and *CmSPS* genes, which play a possible role in plant growth and development (Fig. 6) [58]. It is well-recognized that an expression analysis is used to estimate the molecular roles of genes in a range of physiological processes under different treatments. The expression patterns of SUS genes have been thoroughly illustrated for several species, including cotton [59] rubber tree [60], grape [24], rice [61] and Arabidopsis [62]. Customers attract melons for their sweet fruit, which is defined as late-stage fruit development and sweetness. This study reveals that sucrose in Kula melon fruit accumulates rapidly during developmental stages, increasing significantly from fruit expansion to fully repined stage, reaching its highest level in ripe fruit Fig. 11. The majority of SPS genes showed an inclination towards increased expression during fruit ripening, according to the results of gene expression profiling; the genes *CmSPS1*, *CmSPS2*, *CmSPS4*, *CmSPS5*, and *CmSPS3* showed the strongest correlation coefficients. These genes may contribute to the highest sucrose accumulation trait found in kiwifruit, which is consistent with findings from research on melon and apple [27]. The K^+ application rates increased sucrose accumulation in fruit and reduced it in leaves, promoting decomposition of sucrose in leaves and synthesis of sucrose in fruit, and up-regulating SUT gene expression. Interestingly, at harvest, several SPS genes, including *CmSPS5*, showed notable expression, suggesting their role in sucrose buildup in the final stages of fruit development. On the other hand,

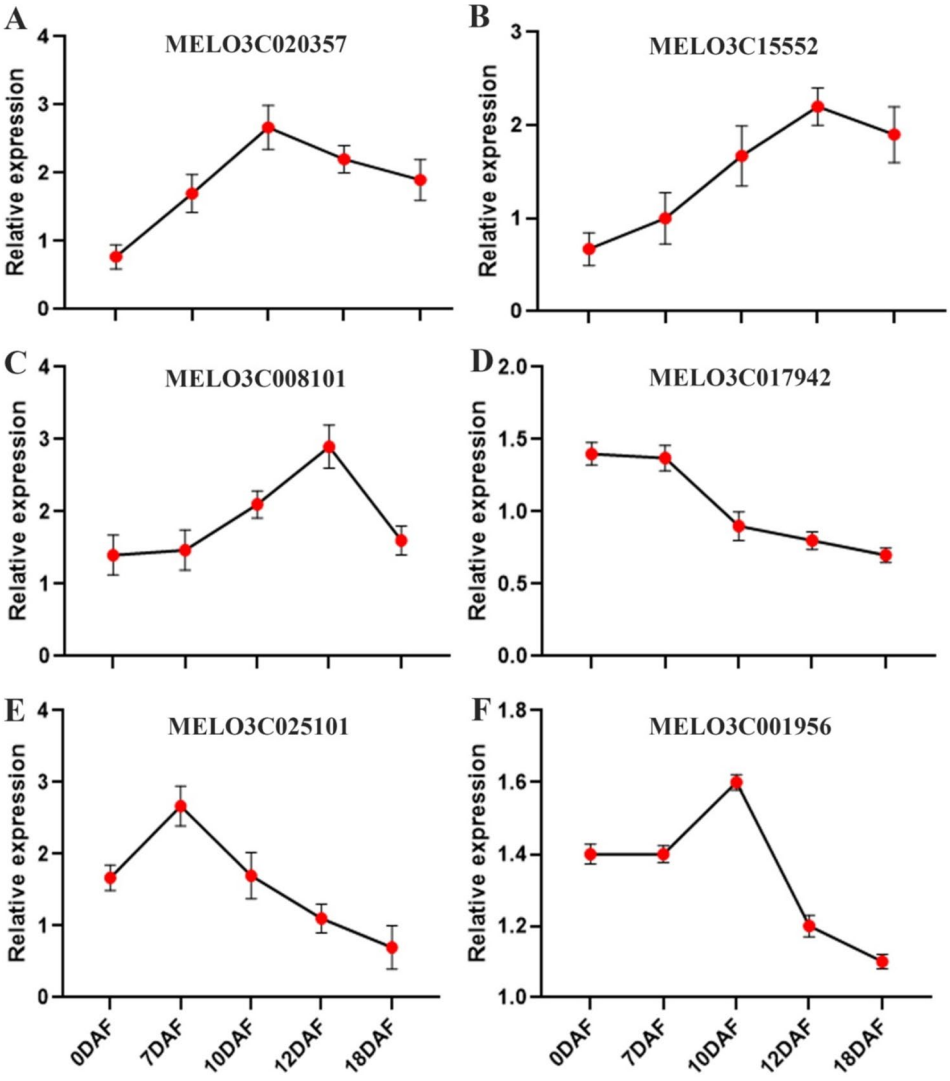

Fig. 10 Relative expression analyses of putative SPS and SUS genes. Melon seedlings were supplemented with N and P at 0 DAF (CK) and 7DAF, 10DAF, 12DAF, and 18DAF. The Error bars represent the Mean ± SD of the triplicate experiment

early expression of *CmSPS6*, *CmSPS1*, and *CmSPS2* was prevented from building up in the fruit by invertase-mediated hydrolysis of produced sucrose, which was caused by high invertase activity during this time. The mutation in *AtSPS4* has been studied in Arabidopsis studies [27].

Conclusion

The present study did a comprehensive genome-wide analysis of the sucrose phosphate synthase (SPS) and sucrose synthase (SUS) gene families in Cucumis melo, emphasizing their evolutionary history, functional divergence, and possible roles in sugar metabolism and fruit development. The identified SUS and SPS proteins were divided into four subgroups (1–1 V). Phylogenetic tree, physiochemical properties, gene structure (intron/exon), gene duplication, syntenic relationship, conserved motif domain, and cis-regulatory elements of promoter regions illustrate the adaptability of these genes to diverse developmental and stress conditions. Additionally, miRNA target analysis, and GO enrichment annotation offer significant insights into the regulatory networks governing sugar related genes in melon. Expression profiling and qRT-PCR analysis under nitrogen and potassium treatments demonstrate a significant correlation between these genes and sucrose metabolism throughout several fruit developmental stages, underscoring their critical role in enhancing fruit quality. Taken together, Current findings provide new insight for further investigation into the molecular mechanism of sucrose transportation in other cucurbit crops.

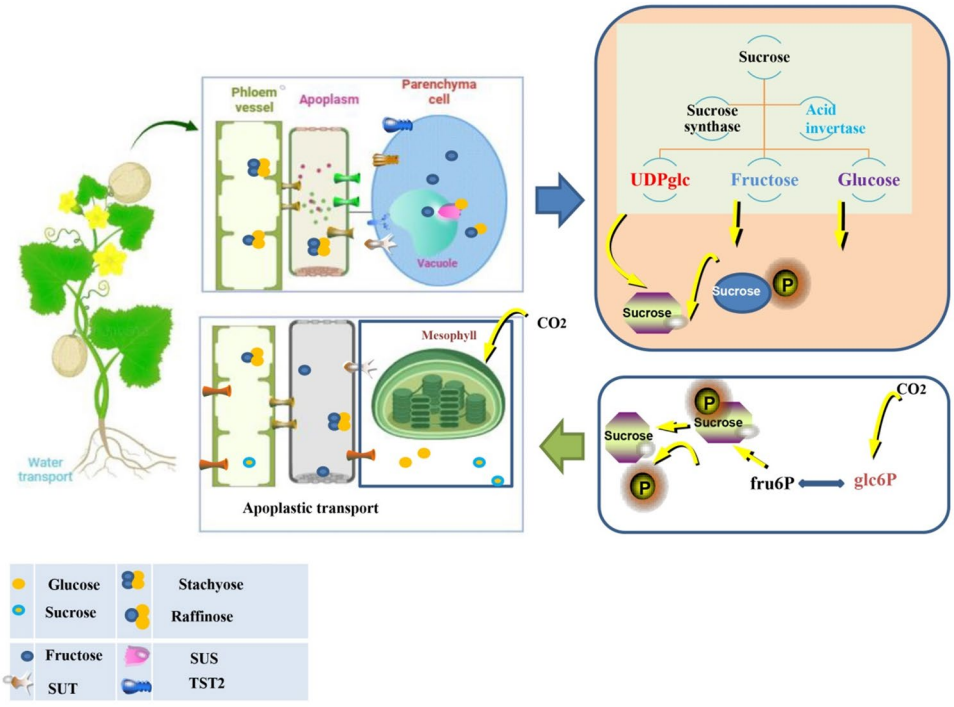

Fig. 11 A schematic illustration of the impacts of sugar transport. The impact of sugar loading and unloading in melon fruits through the phloem and the role of sugar transporter genes are the main topics of current research. A model based on information from the transcriptome and genome databases of melon, as well as previously published research, is being created to comprehend sugar unloading and accumulation in melon. It has been suggested that sucrose entry into fruit parenchyma cells may be facilitated by transportation through SUT/SUC channels in the apoplasmic space. A particular sugar transporter gene that is highly expressed in melon fruit, *CmSUT*, is probably responsible for the build-up of sucrose. Furthermore, it seems that the activity of *CmTST2*, a tonoplast sugar transporter found in the vacuoles of mesocarp cells, is directly related to the ultimate accumulation of sucrose in melon fruits

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06308-0.

Supplementary Material 1

Acknowledgement
Not applicable.

Author contributions
LC supervised, and contributed to the review conception, literature analysis, and writing. IHS and MAM performed experiments, write the original draft and data evaluation using tools. MAM and MA contributed to the review editing, writing, and data evaluation. WJ, XL, and AR were involved in reviewing data, and LP participated in Table, YZ, and QN, supervision, and resources.

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Data availability
The melon Sucrose Phosphate synthase (SPS) and Sucrose Synthase (SUS) protein sequences were collected from the melon genome database (http://cucurbitgenomics.org/organism/18). The Arabidopsis SPS and SUS protein sequences were downloaded from the genome sequences of the Arabidopsis information source (TAIR) database (http://www.arabidopsis.org). All RNA-Seq data (root, leaf, female flower, and male flower) were downloaded from CuGenDBv2 database (http://cucurbitgenomics.org/rnaseq/home) under the following accession number PRGNA383830.

Declarations

Ethics approval and consent to participate
The plant materials used in this study were grown in the greenhouse of the Chongming District Academy of Agricultural Sciences in Shanghai. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai 200240, PR China

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