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SWEET transporters in *Dendrobium* species: molecular insights into the regulation of polysaccharide biosynthesis

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Abstract

Dendrobium, a large genus in the orchid family, is globally valued for its medicinal and ornamental properties. However, *Dendrobium* species exhibit significant variations in their phenotypic traits, chemical compositions, and contents of bioactive compounds, particularly polysaccharides, which are a key determinant of quality. This study employed comparative genomics to investigate the evolutionary trajectories of four *Dendrobium* species with distinct polysaccharide profiles and phenotypic characteristics to identify putative genomic loci controlling polysaccharide biosynthesis. The analysis revealed differential evolutionary patterns potentially associated with polysaccharide accumulation. The *SWEET* gene family was systematically characterized, encoding sugar transporters implicated in polysaccharide metabolism, across *Dendrobium* species. Promoter analysis demonstrated that *SWEET* genes predominantly contain abiotic stress-responsive *cis*-acting elements. Using *D. officinale* as a model, *SWEET* gene expression dynamics were investigated through transcriptomic profiling and RT-qPCR validation. Heatmap analysis revealed tissue-specific expression patterns and differential responses to environmental stress factors (high light, cold, and salinity) and phytohormones. Integrated expression profiling identified six candidate genes (*SWEET1*, *8*, *13*, *15*, *17*, and *24*) as potential key regulators of the quality formation mechanism of *D. officinale*. These six genes were silenced via virus-induced gene silencing. Polysaccharide accumulation was significantly reduced in all silenced lines, with a 14%–22% decrease observed across lines. Silencing *DoSWEET1*, *8*, *13*, *17*, and *24* had the most pronounced effects on polysaccharide contents. This study enhances the understanding of the mechanisms underlying quality formation in medicinal plants and provides critical genetic resources for the improvement and breeding of *Dendrobium* species.

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Introduction

Dendrobium is a genus of perennial herbaceous plants in the orchid family (Orchidaceae). These plants are widely distributed across tropical and subtropical regions of Asia, particularly in China, India, and Southeast Asia[1]. *Dendrobium* plants are edible, medicinal, and ornamental, with effects such as nourishing yin energy, moistening the lungs, promoting the production of bodily fluids, and quenching thirst[2,3]. Modern pharmacological research has shown that *Dendrobium* contains large numbers of polysaccharides, alkaloids, phenanthrenes, bibenzyls, sesquiterpenoids, and other chemical constituents with pharmacological effects, including enhancing immunity, lowering blood sugar, and exhibiting antitumor, antioxidant, and anti-inflammatory effects[4,5].

The phenotypes, compositions, and contents of these components vary greatly among *Dendrobium* species, and the pharmacological activities and medicinal effects of these species are not the same[6]. For example, the stems of the common *Dendrobium* species *D. officinale*, *D. huoshanense*, *D. nobile*, and *D. chrysotoxum* are of different sizes and shapes, and their polysaccharide, alkaloid, and bibenzyl contents also differ. *D. officinale* stems are long, slender, and cylindrical, and their main active ingredients include polysaccharides and flavonoids[7]. *D. nobile* stems are erect, fleshy and thick, slightly flattened, and cylindrical. Their major effective chemical components include alkaloids and sesquiterpenes[8]. *D. chrysotoxum* stems are partially fleshy and fusiform, with more rounded and blunt strips of ribs, and their primary active compounds are in the bibenzyl and phenanthrene classes[9]. *D. huoshanense* has shorter stems, which are thicker above the base and thinner in the upper part; the predominant compounds in its stems are polysaccharides and flavonoids[10]. In-depth research has revealed that the polysaccharide and flavonoid contents are higher in *D. officinale* and *D. huoshanense* than in other *Dendrobium* species, making these two species expensive and of excellent quality. In recent years, increasing numbers of studies have investigated *Dendrobium* polysaccharides, with the majority of studies focusing on *D. officinale*. However, while significant attention has been devoted to developing quality evaluation methodologies for *D. officinale*, few studies have explored the genetic mechanisms underlying polysaccharide biosynthesis in this medicinal plant.

The declining cost of sequencing and the development of bioinformatics technologies have made it possible to obtain the genome sequences of many plant species and to carry out evolutionary studies at the genome-wide level. At the same time, the large amount of sequencing data has enabled comparative analyses of genomes across species[11]. Through comparative genomics,

researchers can identify conserved coding/non-coding regulatory elements and lineage-specific sequences, elucidate both conserved and divergent genomic features through orthologous sequence alignment, trace phylogenetic relationships and ancestral origins via homology analysis, facilitate structural annotation and functional prediction of protein-coding genes, and reconstruct phylogenetic relationships while deciphering molecular adaptation mechanisms underlying species evolution[12,13]. Comparative genomics has been widely used to study plants such as *Arabidopsis thaliana* and rice (*Oryza sativa*)[14].

Sugar serves as the primary energy source for plants and plays critical roles in plant growth, reproduction, development, and adaptation to stress[15]. In plants, carbon flux is coordinated by sugar transporters[16], including SUT (sucrose transporter), MST (monosaccharide transporter), and SWEET (sugar will eventually be exported transporter) proteins[17]. SWEET proteins are a conserved class of bidirectional sugar transporters that function in fundamental physiological processes by mediating the transmembrane translocation and intercellular allocation of saccharides. These membrane-spanning proteins function as bidirectional facilitators of sucrose efflux, orchestrating source-to-sink carbohydrate partitioning to ensure proper growth, developmental transition, and stress adaptation in plants[18]. SWEET proteins play major roles in fructose transport within vesicles[19] and affect sugar transport in the phloem, leaves, and seeds, while also regulating sugar accumulation in fruits[20]. Furthermore, SWEET proteins transport monosaccharides or disaccharides across the plasma membrane or intracellular membranes[21], thereby affecting sugar accumulation and metabolism in plants. *SWEET* genes play multiple roles in plant growth and development and resistance to biotic and abiotic stresses[22]. Plants under biological stress usually regulate sugar levels *in vivo* by upregulating or downregulating the expression of *SWEET* genes. Plants reduce osmotic pressure under stress by increasing sugar content in the plant body or by regulating sugar transfer and redistribution to maintain the balance of osmotic pressure and thus ensure their survival[23].

During the domestication of *Dendrobium* species from wild to cultivated, drastic changes in their growth environment led to variations in physiological phenotypes and genotypes and the formation of a rich variety of *Dendrobium* species, such as *D. officinale* and *D. huoshanense* (with high polysaccharide contents) and *D. nobile* (with high alkaloid contents)[24]. However, little systematic research has focused on the underlying gene expression patterns and the basis of genome evolution in this genus owing to the lack of coverage and functional gene mining of the whole-genome sequences of *Dendrobium* species.

In this study, comparative genomics was used to analyze four *Dendrobium* species with high-quality genome information (*D. officinale*, *D. huoshanense*, *D. nobile*, and *D. chrysotoxum*) and to elucidate the mechanism underlying the high polysaccharide contents of *D. officinale* from an evolutionary perspective with a focus on the *SWEET* gene family. These findings shed light on the evolution of this important genus and the roles of key DoSWEET proteins in polysaccharide accumulation. In addition, they lay the foundation for further exploring the molecular mechanism of quality formation in *Dendrobium*.

Materials and methods

Plant materials and growth conditions

Soil-grown *Dendrobium* plants were used in this study. The seedlings were grown under a 12/12 h light/dark cycle at 22–25 °C. The plants were maintained under normal growth light (GL) conditions using white LEDs (100 μmol·m^{-2}·s^{-1}).

Genomic comparison of four *Dendrobium* species

The homologous chromosomes of *D. officinale* from *D. nobile*, *D. huoshanense*, and *D. chrysotoxum* were identified using Minimap2 (https://github.com/lh3/minimap2/releases?after=v2.21), and genome phasing and analysis of the specific enrichment of long terminal repeat (LTR) retrotransposons (LTR-RTs) among chromosomes were performed using SubPhaser. OrthoVenn3 was employed to identify orthologous gene clusters, followed by Gene Ontology (GO) enrichment analysis to investigate the functional relevance of these clusters. The analysis focused on identifying genes associated with biological processes related to carbohydrate metabolism and transport.

Identification of the *SWEET* gene family in *Dendrobium*

The PFAM profile hidden Markov model (HMM) for *SWEET* domain MtN3_slv (PF03083) was downloaded from the PFAM database (http://pfam.xfam.org/), and the *SWEET* protein sequences of *D. officinale*, *D. nobile*, *D. huoshanense*, and *D. chrysotoxum* were searched with a threshold value of 1e^{-5}. The protein sequence files of the *SWEET* gene family of *A. thaliana* were compared with those of *D. officinale*, *D. nobile*, *D. huoshanense*, and *D. chrysotoxum* by BLAST analysis with a threshold of 1e^{-10}. After merging the HMM search results with the results of this comparison, the National Center for Biotechnology Information (NCBI; www.ncbi.nlm.nih.gov) CD-Search tool was used to conduct domain searches and to screen candidate *SWEET* gene family members.

Phylogenetic analysis of *SWEET* gene family members

OrthoVenn3 (https://orthovenn3.bioinfotoolkits.net/) was used to compare and annotate the orthologous gene clusters among *D. nobile*, *D. chrysotoxum*, *D. huoshanense*, and *D. officinale*. Using the gene sequences and intergenic regions of all samples, gene/region-specific alignments were performed using MAFFT (v7.299b). The sequences of low quality were automatically removed using trimAl v1.4 software with default values. The approximate maximum likelihood tree was generated by FastTree v2.1.7 software (www.microbesonline.org/fasttree). Support values were computed by the Shimodaira-Hasegawa test with 1,000 resamples[25–27].

Analysis of conserved structures and *cis*-regulatory elements

The deduced amino acid sequences of *Dendrobium* SWEET proteins were uploaded to the MEME Suite (https://meme-suite.org/meme/doc/meme.html) and to the NCBI Conserved Domain Database (www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi) to search and obtain the conserved motif model and conserved structural domains; the 2,000 bp upstream sequences of the coding regions of the genes were submitted to PlantCARE (https://bioinformatics.psb.ugent.be/webtools/plantcare/html/) to predict *cis*-regulatory elements in the promoter regions.

RNA-Seq data acquisition and analysis

The original transcriptome data for different *D. officinale* organs (PRJNA715099), *D. officinale* hormone treatment (PRJNA763165), *D. officinale* salt stress treatment (PRJNA715099), and *D. officinale* cold stress (PRJNA949802) were downloaded from NCBI SRA. The transcriptional data of *D. officinale* varieties under high-light treatment were acquired by the research team. Fastp software was used for the quality control of the original data, and hisat2 software was used for genome comparison. The feature counts were used to calculate the count of reads aligned to each gene. Transcripts per million (TPM)

were calculated by DESeq2, and log-transformed TPM [$\log_2$(TPM + 1)], referred to as log-TPM, was used for the downstream analysis. The TPM values were used the $\log_2$(TPM + 1) to construct the heatmap by TBtools.

Expression analysis of *DoSWEET* genes in different tissues and under abiotic stress treatment

RNA-seq data for *SWEET* genes in *D. officinale* in different tissues and under abiotic stress were downloaded from NCBI (www.ncbi.nlm.nih.gov) under the following accession numbers: cold stress (PRJNA949802), hormone treatment (PRJNA763165), salt stress (PRJNA715099), and different tissues (PRJNA715099). The data for *SWEET* genes under high-light treatment included in this study are available upon request from the corresponding author. All fragment per kilobase million mapped reads values were $\log_2$ transformed and used to construct gene expression heatmaps with TBtools software.

Reverse-transcription quantitative PCR (RT-qPCR)

Total RNA was extracted from the samples with RNA Plant Plus Reagent (Magen). RNA concentrations were measured with a NanoDrop 2000 spectrophotometer, and an Evo M-MLV Tracking Kit (AG11734; Accurate Biotechnology Co., Ltd, China) was used to produce complementary DNA (cDNA). qPCR was conducted using the LightCycler 480 Real-Time PCR System (Roche, Basel, Switzerland). All reactions were performed with SYBR Green Premix Pro Taq HS qPCR according to the manufacturer's protocol (AG11735; Accurate Biotechnology Co., Ltd, China). The *Actin* genes from *D. officinale*, *D. nobile*, *D. huoshanense*, and *D. chrysotoxum* were used as internal controls, and relative transcript levels were determined using the ΔΔCt method and normalized. All transcript-level data were obtained by RT-qPCR; three biological samples and three replicates were performed per sample (Supplementary Table S1).

Vector construction for virus-induced gene silencing

Specific primers targeting the *DoSWEET* genes were designed using https://crm.vazyme.com/cetool/singlefragment.html (Supplementary Table S1). The *DoSWEET* fragments were PCR amplified and cloned into pTRV2, which was digested with KpnI and EcoRI. The recombinant plasmids were introduced into *Agrobacterium tumefaciens* strain GV3101. The volume of TRV1 culture inoculated was equal to the sum of all pTRV2 cultures, and the Agrobacterium cells were grown at 28 °C in LB medium with 50 μg·mL^{-1} kanamycin and 25 μg·mL^{-1} rifampicin for 24 h. Cultures were pelleted at 3,000× g for 15 min and resuspended by gentle vortexing in one volume of infiltration buffer (1 mM MES, 1 mM $MgCl_2$, 0.1 μM acetosyringone, and 0.002% Triton). The pelleting was repeated, the cells were resuspended again in one-half volume of infiltration buffer, and the OD_{600} was measured. Suspensions were diluted to an OD_{600} = 1.0 with infiltration buffer. Equal volumes of TRV1 and each separate TRV2 suspension were mixed by gentle inversion and incubated in the dark for 3 h at 22 °C to induce viral gene expression. Forty-day-old seedlings of *D. officinale* were used to conduct the virus-induced gene silencing (VIGS) experiment. To facilitate the vacuum infiltration, when the vacuum (generated by a vacuum pump) reached roughly 0.02 mbar, the vacuum pressure was maintained for 60 s, the vacuum pump was turned off, the system was left alone for 3 min, and the pressure was slowly released. After that, the plants were transplanted to soil and grown under a 12 h light/12 h dark cycle at 22 °C with a relative humidity of 65% for 30 d (Supplementary Fig. S1).

Measurement of polysaccharide contents

Total polysaccharide contents in the samples were measured using the phenol-sulfuric acid method with a Plant Polysaccharide Test Kit (Sangon Biotech, Shanghai, China) following the manufacturer's protocol.

Results

Four *Dendrobium* species show high homology

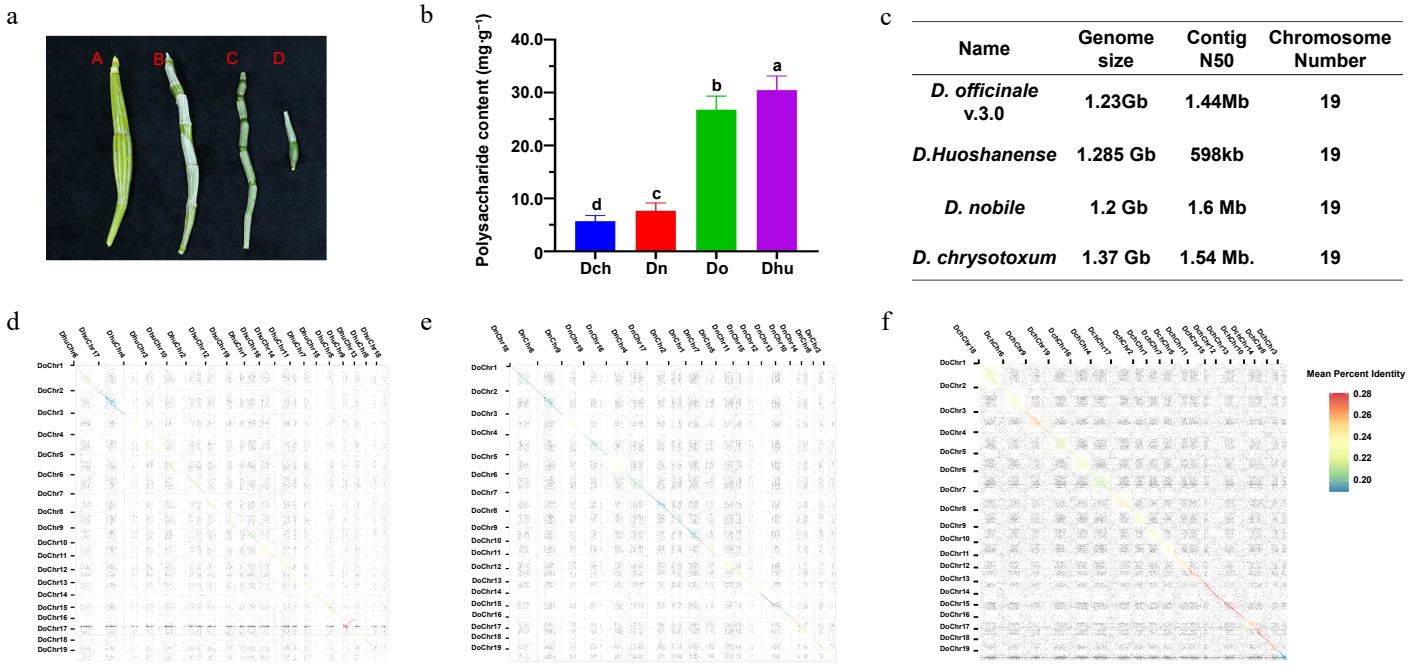
Dendrobium has attracted much attention owing to its important medicinal and ornamental value. To date, high-quality genomes of *D. officinale*, *D. nobile*, *D. huoshanense*, and *D. chrysotoxum* with different polysaccharide contents, alkaloid contents, and phenotypes (Fig. 1a) have been published. However, no studies have explored the different *Dendrobium* species in depth. MCscan was used to search for collinearity among the *D. nobile*, *D. chrysotoxum*, *D. huoshanense*, and *D. officinale* genomes. Ultraviolet (UV) spectrophotometry was then employed to determine the polysaccharide contents in the stems of these four species. *D. huoshanense* exhibited the highest polysaccharide contents, followed by *D. officinale*, while *D. chrysotoxum* showed the lowest polysaccharide contents (Fig. 1b).

To investigate whether the polysaccharide contents in these four *Dendrobium* species (*D. officinale*, *D. nobile*, *D. huoshanense*, and *D. chrysotoxum*) are associated with their genomic characteristics (Fig. 1c), a comprehensive analysis of their genome sizes and assembly status was conducted. All four species exhibited genome sizes exceeding 1 Gb, with a diploid chromosome number of $2n = 38$ (19 chromosome pairs). The focus was on *D. officinale*, a widely used medicinal species, as the reference genome. Minimap2 (v2.24) was employed for comparative genomic analysis to detect homologous regions across *D. officinale*, *D. nobile*, *D. chrysotoxum*, and *D. huoshanense* and visualized the alignment results with dotPlotly to assess macro-synteny and evolutionary conservation.

The *D. officinale* and *D. huoshanense* genomes share a substantial number of syntenic genes, indicating a close evolutionary relationship between them (Fig. 1d). Chromosomes *DoChr3*, *DoChr13*, *DoChr14*, *DoChr15*, *DoChr16*, *DoChr17*, and *DoChr18* of *D. officinale* share strong homology with those of *D. nobile* (Fig. 1e), and chromosome *DoChr14* is highly homologous to that of *D. chrysotoxum* (Fig. 1f). The chromosomal homology between the genomes of *D. officinale* and *D. huoshanense* is low, but homology occurs at the top and bottom positions of chromosomes *DoChr4* and *DhuChr4*, *DoChr5* and *DhuChr3*, *DoChr7* and *DhuChr2*, and *DoChr8* and *DhuChr12*, thus preserving a large proportion of syntenic blocks. The variation between *D. officinale* and *D. huoshanense* during the evolutionary process is thought to be mainly due to the changes in chromosome positions, leading to species differentiation. The plant phenotypes and polysaccharide contents of *D. officinale* and *D. huoshanense* were similar, indicating that they did not change significantly after species differentiation. These results suggest that the genes controlling the polysaccharide contents and phenotypes of *Dendrobium* are primarily distributed in the top and bottom ends of chromosomes *DoChr4* and *DhuChr4*, *DoChr5* and *DhuChr3*, *DoChr7* and *DhuChr2*, and *DoChr8* and *DhuChr12*.

Genome characteristics and analysis of LTR-RT insertion times in four *Dendrobium* species

To gain an in-depth understanding of the genomic characteristics of the four *Dendrobium* plants, SubPhaser was used to phase their genomes (Fig. 2a, b). *kmer* heatmap clustering and principal component analysis revealed similarities among the homologous chromosomes in these species (Fig. 2c, d), suggesting that each genome shares specific features, as expected, and that inter-genome-specific features are present.



Name	Genome size	Contig N50	Chromosome Number
D. officinale v.3.0	1.23Gb	1.44Mb	19
D.Huoshanense	1.285 Gb	598kb	19
D. nobile	1.2 Gb	1.6 Mb	19
D. chrysotoxum	1.37 Gb	1.54 Mb.	19

Fig. 1 Characterization of four *Dendrobium* species. (a) Phenotypes of stems of the four *Dendrobium* species (A *D. chrysotoxum* [Dch], B *D. nobile* [Dn], C *D. officinale* [Do], D *D. huoshanense* [Dhu]. Scale bar is 1 cm). (b) Polysaccharide contents of the four *Dendrobium* species. Different letters indicate significant differences among groups, as determined by one-way ANOVA with Tukey's multiple-comparisons test ($p < 0.05$). (c) Summary of sequencing data for the four *Dendrobium* genome assemblies. (d) Syntenic relationship of *D. officinale* and *D. huoshanense*. (e) Syntenic relationship of *D. officinale* and *D. nobile*. (f) Syntenic relationship of *D. officinale* and *D. chrysotoxum*.

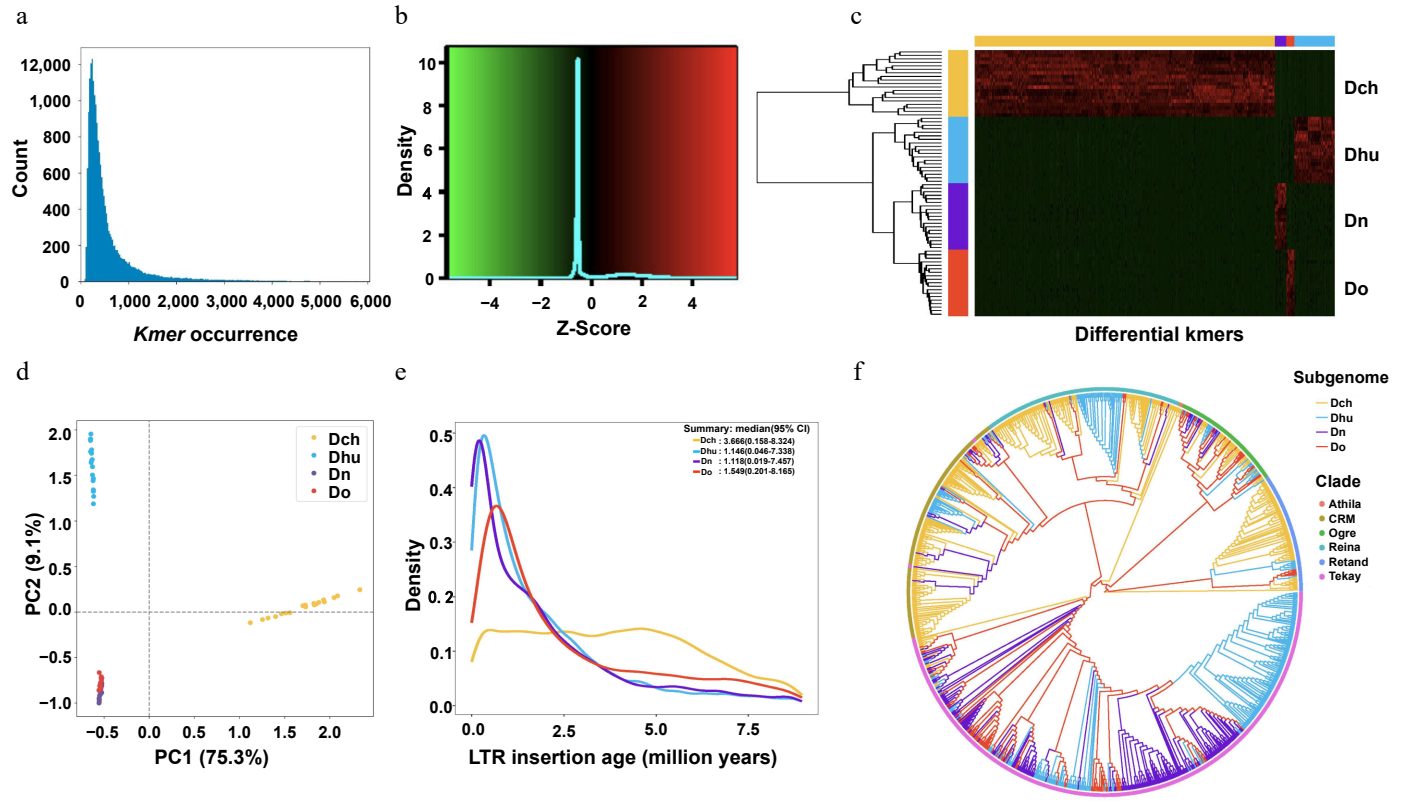


Fig. 2 Genome phasing of four *Dendrobium* species (Dch, Dn, Do, and Dhu) using SubPhaser. (a) Distribution frequency of different 15-mers in homologous chromosomes of the four species. (b) Heatmap showing the Z-scale relative abundance of *kmers*. (c) Unsupervised hierarchical clustering heatmap; horizontal color bar at the top indicates *kmer* specificity to the genome, and vertical color bar on the left indicates chromosomes. (d) Principal component analysis of different 15-mers. (e) Genome-specific long terminal repeat (LTR) retrotransposon (LTR-RT) insertion times (95% confidence interval). (f) Phylogenetic tree of 1,000 Gypsy LTR-RTs from random data resampling.

By analyzing the insertion times of long terminal repeat (LTR) retrotransposons (LTR-RTs), it was determined that, with the exception of *D. nobile*, the LTR-RT insertion events in *Dendrobium* species were predominantly concentrated between 1.395 and 1.863 million years ago (MYA). By contrast, the LTR-RT insertion events in *D. nobile* occurred approximately 5.222 MYA. This distinctive timing may be associated with a significant increase in global carbon dioxide concentrations during that period (Fig. 2e). *D. officinale* is thought to have originated in the Yunnan-Guizhou Plateau, adjacent to the Xizang Plateau, where continuous plateau uplift occurred between 0 and 8 MYA[24]. Approximately 2.4 MYA, LTR-RT insertion events also appeared in *D. officinale*, suggesting that these events during this period might be an important cause of intraspecific differentiation within *D. officinale*. Many LTR-RTs in the genomes of the four *Dendrobium* species occurred during the 1–2.5 MYA period, far from the time when species differentiation occurred (22 MYA), but the global climate and environment changed greatly during this time. The insertion events during this period might have been caused by natural selection during plant responses to environmental changes and might have been an important factor leading to changes in plant gene function. For this study, 1,000 Gypsy LTR sequences were randomly sampled for phylogenetic analysis. The LTR sequences of the Athila-Gypsy family were highly prevalent, dominating the genomes of *D. nobile*, *D. huoshanense*, and *D. officinale* (Fig. 2f), indicating that the LTR sequences of this family underwent a large-scale expansion during evolution that promoted the differentiation of these three species.

Whole-genome comparisons reveal evolutionary divergence in four *Dendrobium* species

Climate change directly or indirectly affects the adaptation of plants to the environment, plant diversity, and species migration. According to predictions about the four *Dendrobium* species using the TimeTree database, the light intensity gradually increased approximately 22 MYA and the four *Dendrobium* species differentiated, suggesting that light intensity was an important factor causing the species differentiation of *Dendrobium* (Fig. 3a). To investigate potential gene-exchange events between homologous chromosomal segments across distinct *Dendrobium* genomes, molecular typing and cluster analysis of chromosomal sequences was systematically conducted using an integrative approach combining whole-chromosome sequencing with *kmer* frequency profiling (Fig. 3b). Comparative Circos mapping of the four *Dendrobium* genomes revealed distinct patterns of intergenomic chromosomal exchanges. Notably, chromosomes *DoChr4* and *DoChr6* in *D. officinale* exhibited pronounced kmer enrichment patterns corresponding to *DnChr11* and *DnChr17* in *D. nobile*, suggesting substantial chromosomal rearrangements between these species. By contrast, the exchange patterns observed between *DoChr16* of *D. officinale* and *DnChr16/DnChr6* of *D. nobile* demonstrated a singular genomic interaction event exclusively with *D. huoshanense*. However, *DoChr16* of *D. officinale* and *DnChr16* and *DnChr6* of *D. nobile* only experienced one genome exchange with *D. huoshanense*, showing different exchange patterns, suggesting that *D. nobile* and *D. officinale* might come from the same ancestor.

To explore the functions of the genes in the four genomes in detail, OrthoVenn3 was used to identify the orthologous genes between the genomes and to cluster the genes (Fig. 3c). The four *Dendrobium* species shared 8,855 common gene clusters. *D. nobile* contained the most gene clusters (17,498), followed by *D. officinale* (15,438), and *D. huoshanense* contained the fewest gene clusters (13,162; Fig. 3c, d). Gene Ontology (GO) analysis revealed that 23.94% of the genes in the ten most abundant gene clusters were enriched in biological process-related GO terms, and 14.51% were enriched in metabolic process–related terms. Moreover, genes responsible for transporter activity and membrane components accounted for 8.59% of molecular function-related terms and 22.55% of cell component–related terms, respectively (Fig. 3e–g). Among the enriched GO terms associated with biological activity, 283 genes were involved in the metabolism and transport of carbohydrates. A significant number of *SWEET* genes are annotated in the AmiGO2 database as key genes responsible for intercellular carbohydrate-directed transport. *SWEET* genes might have undergone subfunctionalization (e.g., specializing in transporting different types of sugars) or neofunctionalization (e.g., participating in stress responses) during evolution.

Identification and distribution of *cis*-promoter elements in *SWEET* genes in *Dendrobium*

Sugars account for over 50% of the total organic matter in plants and play critical roles in plant growth and development. *SWEET* genes play key roles in sugar transport, participating in fructose transport in vacuoles; sugar transport in the phloem, seeds, and leaves; and the regulation of sugar accumulation in fruits. Numerous *SWEET* genes that were annotated as key genes in the targeted transport of carbohydrates were identified between cells using the AmiGO2 database. To explore the evolutionary relationships and classification of the *Dendrobium SWEET* genes, OrthoVenn3 (https://orthovenn3.bioinfotoolkits.net/) was used to compare and annotate the orthologous gene clusters among *D. nobile*, *D. chrysotoxum*, *D. huoshanense*, and *D. officinale*. Using the gene sequences and intergenic regions of all samples, gene/region-specific alignments were performed using MAFFT (v7.299b). The protein sequence files of the *SWEET* gene family in *A. thaliana* were then compared with those of *D. officinale*, *D. nobile*, *D. chrysotoxum*, and *D. huoshanense* by BLAST analysis with a threshold of $1e^{-10}$ (Supplementary Table S2). The sequences of low quality were automatically removed using trimAl v1.4 software with default values. The approximate maximum likelihood tree was generated by FastTree v2.1.7 software (www.microbesonline.org/fasttree). Support values were computed by the Shimodaira-Hasegawa test with 1,000 resamples. Following this, 105 *SWEET* gene family members were identified, including 24 in *D. officinale* (*DoSWEET*s), 21 in *D. huoshanense* (*DhuSWEET*s), 25 in *D. chrysotoxum* (*DchSWEET*s), and 35 in *D. nobile* (*DnSWEET*s) (Supplementary Table S3). A phylogenetic tree containing these 105 *Dendrobium SWEET* genes and *A. thaliana SWEET* genes was constructed using FastTree software (Fig. 4a). The *SWEET* genes in the five species were divided into four clades (I, II, III, and IV) according to evolutionary distance. Clade II is the largest, containing 54 *SWEET* genes, including seven *DhuSWEET*, 16 *DnSWEET*, 13 *DoSWEET*, 13 *DchSWEET*, and five *AtSWEET* genes. The second largest clade (Clade III) contains six *DhuSWEET*, nine *DnSWEET*, eight *DoSWEET*, six *DchSWEET*, and seven *AtSWEET* genes. Clade I contains five *DhuSWEET*, five *DnSWEET*, two *DoSWEET*, three *DchSWEET*, and three *AtSWEET* genes, while Clade IV includes two *DhuSWEET*, one *DnSWEET*, one *DoSWEET*, two *DchSWEET*, and two *AtSWEET* genes. A previous study showed that Clade II SWEET proteins have a preference for hexose transport[28]. Hexoses are monosaccharides containing six carbon atoms. Common hexoses include glucose, mannose, fructose, and galactose, and the major polysaccharides in *Dendrobium* are composed mainly of mannose and glucose. The different polysaccharide contents and monosaccharide compositions of *Dendrobium* stems might be caused by the differences in the activity of sugar transporters.

To further investigate the syntenic relationships of *SWEET* genes in the genomes of the four *Dendrobium* species, collinearity analysis

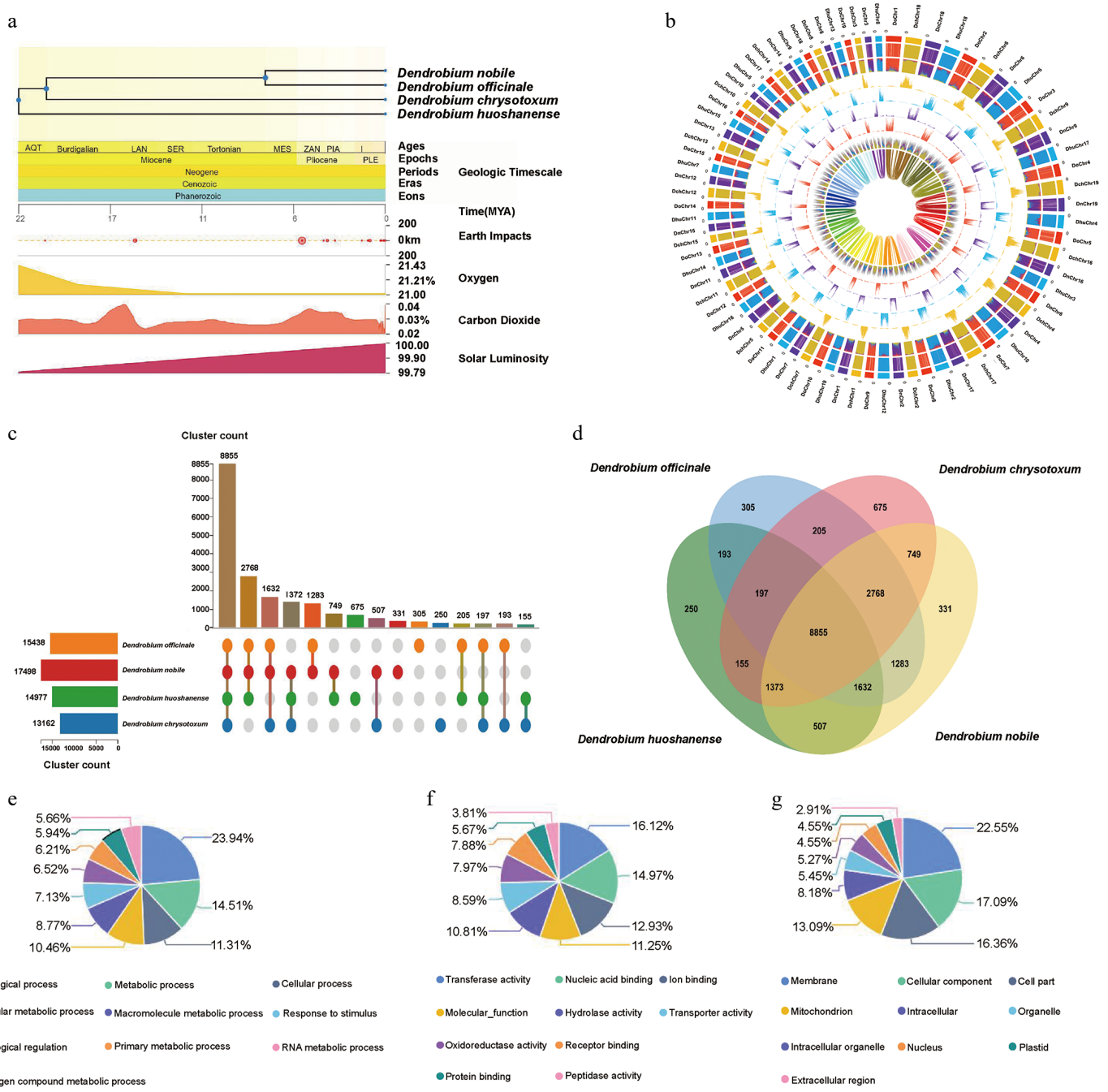


Fig. 3 Genomic comparisons and evolutionary divergence among four *Dendrobium* species. (a) Speciation of the four *Dendrobium* species and the timing of geo-environmental change events. (b) Chromosome characterization of the four *Dendrobium* species. From the inner to outer circles (1–8): (1) genome types assigned based on the K-means algorithm; (2) enriched genome-specific *kmers*; (3) genome-specific *kmers* normalized against each other; (3–6) absolute values of the pooled counts of each genome-specific *kmer*; and (8) the density of long terminal repeat (LTR) retrotransposons (LTR-RTs); a color consistent with the genome class indicates that LTR-RTs are significantly enriched in these genome-specific *kmers*, and gray indicates non-specific LTR-RTs. (c) The number of orthologous clusters in each species. (d) Venn diagram of intersecting direct homologous gene clusters. (e) GO enrichment analysis of biological processes. (f) GO enrichment analysis of molecular functions. (g) GO enrichment analysis of cellular components.

was conducted using MCScanX. Limited chromosomal homology was detected between *D. officinale* and *D. huoshanense*. However, synteny was detected in four chromosome pairs, *DoChr4–DhuChr4*, *DoChr5–DhuChr3*, *DoChr7–DhuChr2*, and *DoChr8–DhuChr12*, which have retained a significant proportion of homologous sequences (Fig. 4b). Phylogenetic analysis revealed that chromosomal rearrangements, rather than whole-genome duplication, were the dominant drivers of speciation between these two medicinal *Dendrobium* species. The plant phenotypes and polysaccharide contents of *D. officinale* and *D. huoshanense* were similar, indicating that the phenotypes and compound contents did not change significantly following species differentiation, suggesting that the genes controlling the polysaccharide contents and phenotypes of *Dendrobium* might be mainly distributed in the top and bottom ends of chromosomes *DoChr4* and *DhuChr4*, *DoChr5* and *DhuChr3*, *DoChr7* and *DhuChr2*, and *DoChr8* and *DhuChr12*. These findings suggest that the *SWEET* gene family might be responsible for the differences in polysaccharide contents in stems among *Dendrobium* species.

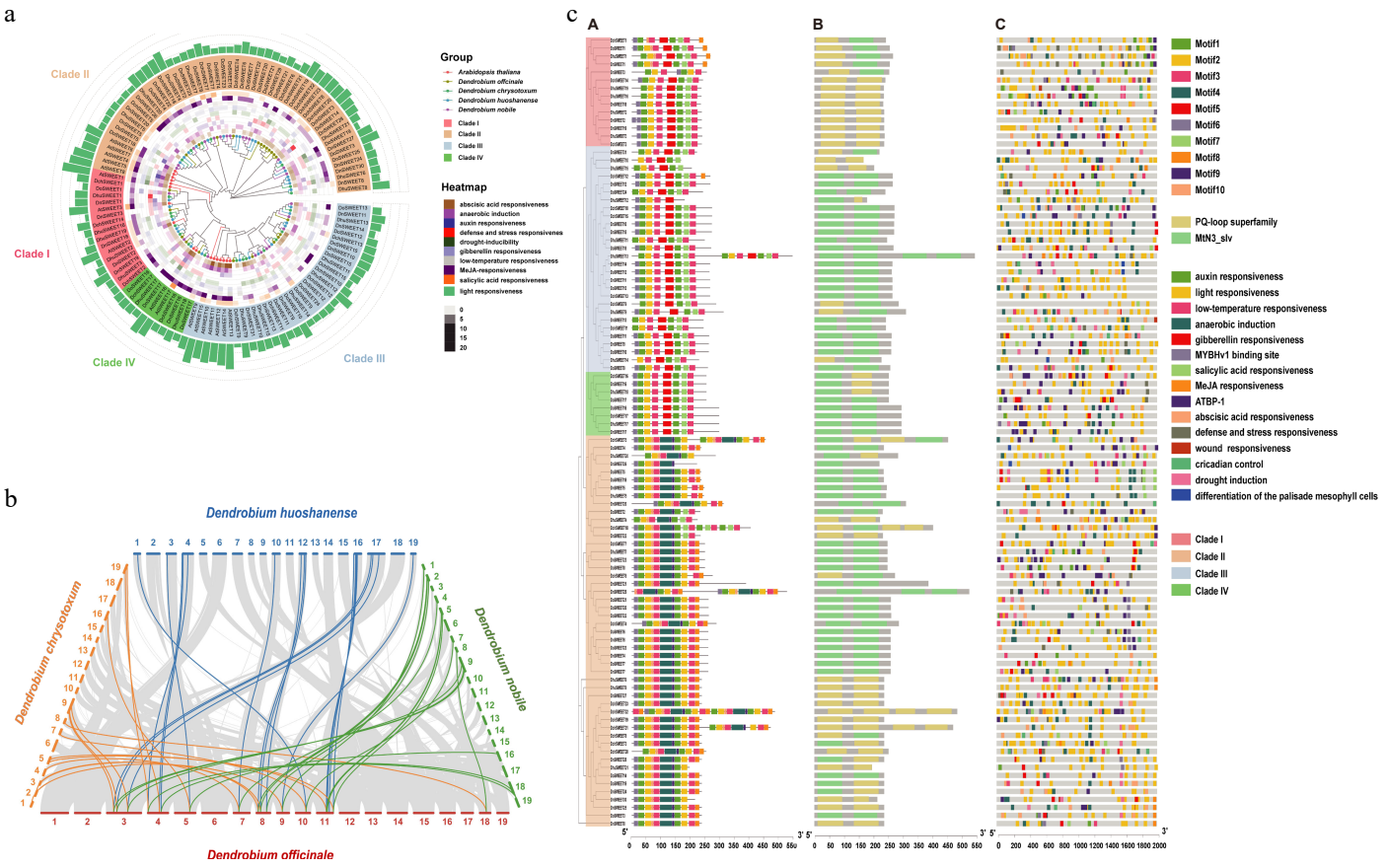


Fig. 4 Genome-wide identification and functional analysis of the *SWEET* gene family in *Dendrobium*. (a) Phylogenetic relationships of SWEET proteins in *Arabidopsis thaliana* (*At*), *D. officinale* (*Do*), *D. chrysotoxum* (*Dch*), *D. huoshanense* (*Dhu*), and *D. nobile* (*Dn*). The trees were constructed based on protein sequence alignment using the maximum likelihood method. The four clades and different *cis*-elements in the heatmap are marked with different colors. (b) Syntenic relationships of *D. officinale*, *D. chrysotoxum*, *D. huoshanense*, and *D. nobile SWEET* are genes shown on the chromosome maps. (c) Analysis of conserved motifs and conserved domains in SWEETs. ([A] Conserved motifs and gene exon-intron structures in the SWEETs. [B] Conserved domains in SWEET proteins identified by a search using NCBI (www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). [C] Predicted *cis*-acting elements in the upstream regions of *SWEET* genes).

To further explore the structural characteristics and conservation of the *SWEET* gene family in *Dendrobium*, MEME Suite was used to identify conserved motifs in SWEET proteins (Fig. 4c). Most of these proteins contain two MTN3 motifs. However, DhuSWEET13 and DchSWEET5, 18, 21, and 22 contain four such motifs, perhaps resulting from gene duplication and replicative transposition. The distribution patterns of ten conserved motifs were also analyzed, finding that Motif1, Motif3, and Motif4 were the most widely distributed and highly conserved in the MtN3_slv domain. Notably, Motif4 and Motif9 are exclusively found in Clade II. This restricted distribution may reflect gene replacement or insertion events that occurred during the evolution of the *SWEET* gene family.

To study the response of *SWEET* genes in *Dendrobium* to various abiotic stresses, their promoters (2 kb upstream of the transcription start site) were submitted to PlantCARE to predict the *cis*-acting elements in each promoter. Fifteen *cis*-acting elements related to plant hormones, stress, and development, were identified, including five plant hormone response elements (auxin response, MeJA response, abscisic acid response, gibberellin response, and salicylic acid response) and six stress response elements (light induction, abscission induction, drought induction, low-temperature induction, defense and stress induction, and trauma induction). The distribution of the 15 major *cis*-acting elements in the *Dendrobium SWEET* gene family is shown in Fig. 4c, with a large number of MeJA- and salicylic acid-responsive elements widely distributed in each species in Clade II. Numerous abscisic acid response elements are present in each species of Clade III. The variable number of light-responsive binding elements in the promoters of *SWEET* genes of various *Dendrobium* species is notable, suggesting that light might be one of the main factors driving the evolutionary differentiation of *Dendrobium* species. These results suggest that the SWEET gene family in each *Dendrobium* species is functionally differentiated and is involved in multiple biological processes, as suggested by the variation in transcriptional levels and responsiveness to environmental stress of the *SWEET* genes.

Expression patterns of *SWEET* genes in different organs under abiotic stress

Expression patterns of SWEET genes in different organs of D. officinale

To examine the expression patterns of the *SWEET* genes, the commonly studied species *D. officinale* was chosen. The expression levels of *SWEET* genes in different organs of *D. officinale* were analyzed (Fig. 5a). Of the 24 *DoSWEET* genes, 22 exhibited significant differential expression across the organs. Six *DoSWEET* genes (*DoSWEET1*, *2*, *9*, *15*, *16*, and *24*) were highly expressed in leaves and flowers, and ten (*DoSWEET3*, *DoSWEET5*, *DoSWEET6*, *DoSWEET13*, *DoSWEET14*, *DoSWEET15*, *DoSWEET17*, *DoSWEET19*, *DoSWEET20*, and *DoSWEET24*) were highly expressed in flowers. Four *DoSWEET* genes (*DoSWEET4*, *12*, *21*, and *22*) were highly expressed in roots, and six

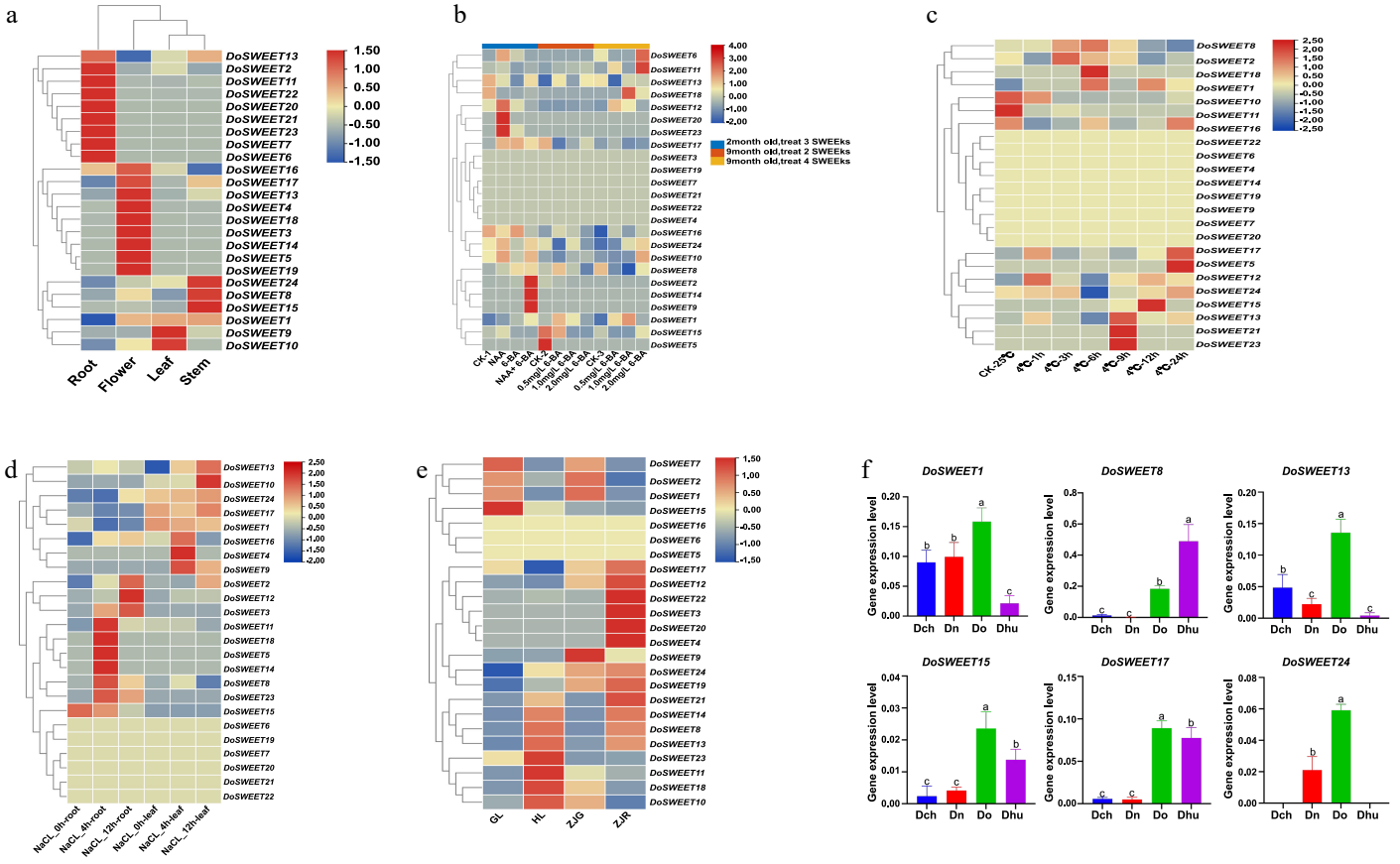


Fig. 5 Expression patterns of *SWEET* genes in *D. officinale*. (a) Heatmap of the differential expression of *SWEET* genes in four *D. officinale* organs. (b)–(d) Heatmaps of the differential expression of *DoSWEET* genes in response to different (b) phytohormones, (c) Cold, (d) salt, and (e) differences in light conditions and ecotype. ZJG and ZJR indicate ecotypes with green stems and red stems, respectively, from Zhejiang Province, China, that were maintained under normal growth light. GL and HL indicate normal growth light and high-light conditions, respectively, applied to the YNG ecotype. (a)–(e) The expression levels of *SWEET* genes are represented by $\log_2$-transformed transcripts per million (TPM) values acquired from RNA-seq data. (f) Expression analysis of *SWEET* genes in the four *Dendrobium* species (Do, Dhu, Dn, and Dch), as determined by RT-qPCR. The data are representative of three independent experiments. The error bars indicate SD, and different lowercase letters indicate significant differences in expression levels at the 95% confidence level.

(*DoSWEET2*, *7*, *8*, *9*, *11*, and *18*) were highly expressed in stems. *DoSWEET7*, *8*, *11*, and *18* were more highly expressed in stems than in leaves, flowers, and roots, suggesting that the higher polysaccharide contents in stems than in roots, leaves, and flowers are mainly due to the differential activities of these *DoSWEET* genes in different organs.

Expression patterns of SWEET genes in D. officinale treated with plant hormones

Analysis of *cis*-elements in the *SWEET* gene promoters revealed many phytohormone-responsive elements. To explore the potential roles of *DoSWEET*s in plant hormonal regulation, the *SWEET* gene expression profiles of *D. officinale* treated with different concentrations of 6-BA and NAA were analyzed (Fig. 5b). The expression levels of *DoSWEET*s in *D. officinale* were generally not high in the 2-month-old control (CK) group, but some *SWEET* genes were significantly induced after three weeks of phytohormone treatment. *DoSWEET12*, *20*, and *23* were highly induced by NAA treatment, while *DoSWEET2*, *9*, and *14* were highly induced by the combination of NAA and 6-BA. In 9-month-old plants, *DoSWEET5* and *15* were highly expressed in the CK group, but their expression decreased in response to 6-BA treatment. However, after four weeks of treatment, *DoSWEET6* and *11* were significantly induced by 2.0 mg·L^{-1} 6-BA treatment, and *DoSWEET18* was highly induced by 1.0 mg·L^{-1} 6-BA treatment. These results suggest that the expression levels of *DoSWEET*s are related to the phytohormones NAA and 6-BA.

Expression patterns of SWEET genes in D. officinale under cold stress

Analysis of *cis*-elements in the promoters of *SWEET* genes revealed the presence of many low-temperature-responsive elements. To explore the potential functions of *DoSWEET*s in the plant response to cold stress, the gene expression profiles of *D. officinale* seedlings were analyzed at different temperatures (Fig. 5c). *DoSWEET 10*, *11*, and *16* were highly expressed under normal conditions, while *DoSWEET1*, *17*, and *12* were highly inhibited by this treatment. *DoSWEET10*, *12*, and *17* were highly expressed under 4 °C-1 h; *DoSWEET8* and *12* were highly expressed under 4 °C-3 h; *DoSWEET1*, *8*, and *18* were highly expressed under 4 °C-6 h; *DoSWEET13*, *21*, and *23* were highly expressed under 4 °C-9 h; *DoSWEET1* and *15* were highly expressed under 4 °C-12 h. *DoSWEET5*, *16*, and *17* were highly induced by cold treatment (4 °C-24 h). These results suggest that the expression patterns of *DoSWEET*s were affected by cold temperature treatment.

Expression patterns of SWEET genes in D. officinale under salt stress

Analysis of *cis*-elements in the *SWEET* gene promoters revealed numerous stress-responsive elements. To explore the potential functions of *DoSWEET* genes in plant responses to salt stress, their

expression profiles were analyzed in leaves and roots after 4 and 12 h of salt stress (Fig. 5d). *DoSWEET15* was highly expressed in roots under normal conditions, but its expression decreased gradually with increasing salt stress treatment, while *DoSWEET11*, *18*, *5*, *14*, *8*, and *23* expression was induced by 4 h of salt stress treatment. After 12 h of salt stress treatment, *DoSWEET2*, *12*, and *3* were highly induced in roots. *DoSWEET13* was expressed at low levels in leaves under normal conditions but was highly upregulated in response to salt stress. After 4 h of salt stress, *DoSWEET16*, *4*, and *9* were highly expressed; this effect decreased after 12 h of salt stress treatment, while *DoSWEET8* expression was significantly reduced by this treatment. Therefore, the expression patterns of *DoSWEET*s under salt stress were affected by the duration of salt stress and the plant organ.

Expression patterns of SWEET genes in D. officinale under high-light stress

Climate change directly or indirectly affects plant adaptation to the environment, plant diversity, and species migration. According to the predictions by the TimeTree database, the light intensity gradually increased before 22 MYA, and the four *Dendrobium* plants differentiated, suggesting that light intensity is an important factor causing the species differentiation. Analysis of the *cis*-elements in the *SWEET* gene promoters revealed numerous light-responsive elements, providing additional evidence that light might be a driver of *Dendrobium* evolution. This is in agreement with the hypothesis stated earlier (Fig. 3a). In a previous study in which *D. officinale* was divided into green-stem, purple-stem, and red-stem ecotypes, anthocyanin content increased with the deepening of stem color. The depth of stem color in *D. officinale* is mainly determined by the anthocyanin content, and the formation of anthocyanin is dependent on light[29]. Therefore, in this study, the effects of normal growth light (GL; control) treatment were examined on *D. officinale* ecotypes ZJR (with red stems) and ZJG (with green stems), both from Zhejiang Province, China. A third ecotype, *D. officinale* YNG (green-stemmed *D. officinale* varieties from Yunnan), was exposed to both GL and high-light (HL) treatment. Under GL conditions, the expression levels of *DoSWEET4*, *20*, *3*, *22*, and *12* were higher in ZJR than in ZJG, while the expression levels of *DoSWEET9*, *2*, and *1* were higher in ZJG than in ZJR, as revealed by transcriptome analysis. Notably, the expression levels of *DoSWEET11* in ZJG and *DoSWEET8*, *13*, and *14* in ZJR under GL conditions were close to those of the HL group. However, in *D. officinale* YNG, *DoSWEET14*, *8*, *13*, *23*, *11*, *18*, and *10* were highly induced under HL treatment. Finally, in *D. officinale* YNG, *DoSWEET7*, *2*, *1*, and *15* were highly expressed under GL conditions and were significantly inhibited under HL conditions. These results suggest that *DoSWEET*s are critical for the HL response and that *DoSWEET8*, *13*, and *14* might be key genes in the response of *D. officinale* to high light (Fig. 5e).

RT-qPCR analysis of candidate genes associated with polysaccharide accumulation

Among the tissues of *Dendrobium*, the stems contain the highest polysaccharide levels, followed by the flowers and leaves, while the roots contain the lowest polysaccharide levels. To validate the correlation between differentially expressed genes identified from the RNA-seq data and polysaccharide accumulation, six genes that are highly expressed specifically in stems—*SWEET1*, *8*, *13*, *15*, *17*, and *24*—were selcted and their expression patterns were analyzed across the four *Dendrobium* species using RT-qPCR. *SWEET8*, *15*, and *17* were expressed at significantly higher levels in *D. officinale* and *D. huoshanense* than in *D. nobile* and *D. chrysotoxum*. *SWEET1* and *13* were more highly expressed in *D. nobile*, *D. chrysotoxum*, and *D. officinale* than in *D. huoshanense*, whereas *SWEET24* was more highly expressed in *D. nobile* and *D. officinale* than in *D. huoshanense* and *D. chrysotoxum* (Fig. 5f). *D. officinale* and *D. huoshanense* are the two species with the highest polysaccharide contents among the four species studied, suggesting that *SWEET8*, *15*, and *17* are closely associated with polysaccharide accumulation and serve as key genes determining polysaccharide levels in *Dendrobium* species. The expression patterns of these genes align strongly with the distribution of polysaccharides, further supporting their potential roles in polysaccharide metabolism.

Functional validation of *SWEET* genes in *D. officinale*

Finally, to investigate the roles of *DoSWEET1*, *8*, *13*, *15*, *17*, and *24* in polysaccharide biosynthesis during *D. officinale* growth and metabolism, virus-induced gene silencing (VIGS) was performed. Prior to initiating formal VIGS experiments, *D. officinale* seedlings were pre-infiltrated with the TRV2-GFP vector to confirm the successful establishment of the VIGS system. Observation using in vivo imaging on day 3 post-infiltration revealed the following results: Plants infiltrated with pTRV2-GFP emitted strong green fluorescence (Supplementary Fig. S2a); specific GFP fluorescence signals were also clearly visible under UV light illumination (Supplementary Fig. S2b). Further examination using the THUNDER wide-field high-definition imaging system showed that GFP fluorescence intensity in the vascular bundles and surrounding parenchyma cells of the stems was significantly higher in the pTRV2-GFP-infiltrated group compared to the wild-type(WT) control (Supplementary Fig. S2c). Collectively, these results successfully tracked the dynamic changes of the virus using the fluorescence encoded by EGFP tagged on the TRV2 vector, further confirming that TRV can infect *D. officinale*. This preliminarily demonstrates the successful construction of the VIGS system. Building upon this successfully established VIGS system, the *DoSWEET1*, *8*, *13*, *15*, *17*, and *24* genes were targeted for silencing. At 30 d post-infiltration, relative gene expression levels were measured in the plants using a UV-VIS spectrophotometer (Thermo Fisher Scientific Inc., USA), and RT-qPCR, and polysaccharide contents were examined via a phenol-sulfuric acid assay. Polysaccharide accumulation was markedly reduced across all silenced lines (14.3%–22.2% reduction) compared to the pTRV2-GFP control, with *DoSWEET1*, *8*, *13*, *17*, and *24* showing the most pronounced effects (> 10% reduction; Fig. 6a). As shown in Fig. 6b, targeted silencing of *DoSWEET1*, *8*, *13*, *15*, *17*, and *24* resulted in their significant downregulation compared to the pTRV2-GFP control (Original data are in Supplementary Table S4). These results suggest that SWEET-mediated monosaccharide transport is critical for polysaccharide biosynthesis in *D. officinale*, with specific SWEET proteins (*DoSWEET1*, *8*, *13*, *17*, and *24*) serving as key regulators of carbohydrate partitioning and storage.

Discussion

The SWEET protein family in plants represents a novel type of transporter capable of transporting sugars, sugar alcohols, and hormones[30]. By regulating sugar translocation and distribution, this protein family plays crucial roles in plant growth and development[20]. In recent years, *SWEET* genes have been shown to affect polysaccharide accumulation in other species. In *Polygonatum cyrtonema* Hua, polysaccharide accumulation is highly correlated with the expression levels of *SUS*, *INV*, *SWEET*, and *PLST* genes, and SWEETs affect polysaccharide biosynthesis via the transport of large amounts of sucrose and several monosaccharides[31]. From a chromosome-scale assembly of the *D. chrysotoxum* genome, *SWEET* genes were identified, and phylogenetic analysis showed that 17 *SWEET* genes might be associated with fleshy stems that are

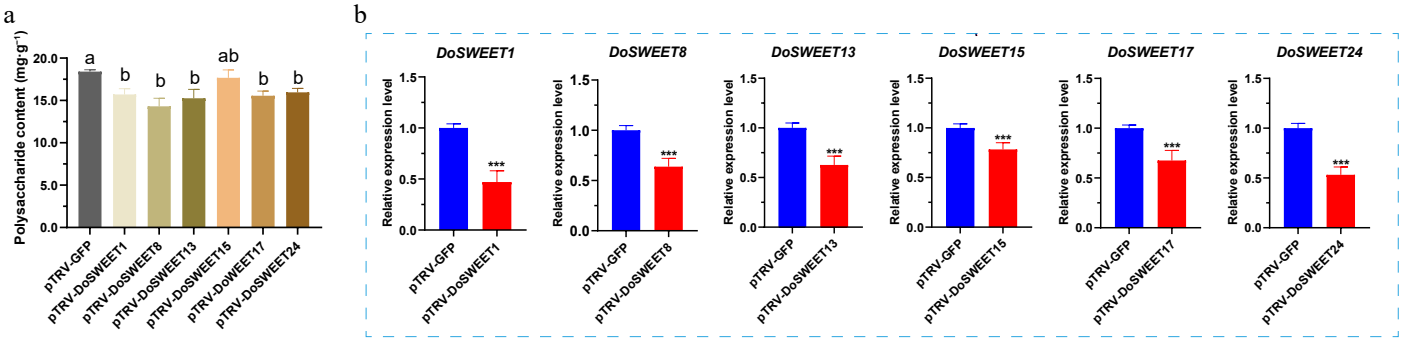


Fig. 6 The *SWEET* gene family plays a critical role in polysaccharide accumulation in *D. officinale*. (a) Total polysaccharide contents in *D. officinale* after virus-induced gene silencing (VIGS). Different lowercase letters indicate significant differences at the 95% confidence level. (b) Expression patterns of *DoSWEET1*, *8*, *13*, *15*, *17*, and *24* in *D. officinale* stems after VIGS. Error bars indicate the standard deviation of three biologically independent repeats. Student's *t*-test: *** $p < 0.001$.

abundant in polysaccharides and other medicinal compounds[32]. In a similar study in apple, analysis of sugar accumulation and the underlying mechanisms in the F2 progenies of a hybridization between the high-sugar apple (*Malus* × *domestica*) variety 'Gala' and the high-flavonoid apple germplasm 'CSR6R6', *MdSWEET9b* was shown to help mediate sugar accumulation in fruits. Moreover, MdWRKY9 binds to the *MdSWEET9b* promoter and regulates its activity in response to abscisic acid signaling, which enhances its regulation of *MdSWEET9b* expression[33]. Here, multiple *SWEET* genes were identified in four *Dendrobium* species, with numbers comparable to those in model species such as *Arabidopsis*, *rice*, and *soybean*. Phylogenetic analysis classified these genes into four distinct clades (Fig.4a),consistentwithpreviousstudies.Notably,allofttheseSWEETproteins contained the conserved mtN3/saliva domain. Similar features were observed in studies of the medicinal plant *Bletilla striata* and *Euphorbiaceae plants*, indicating that *SWEET* gene family members are highly conserved.

The main bioactive substances of *Dendrobium* contain polysaccharides, dendrobine, phenanthrenes, amino acids, and trace mineral elements[34,35]. Polysaccharides, which make up a substantial proportion of *D. officinale* plant biomass, are the predominant bioactive compounds in *Dendrobium* and are used as a quality index for this plant in the Chinese pharmacopoeia. In this study, it was found that the content of *Dendrobium* polysaccharides was correlated with the expression of *SWEET* genes. Specifically, in the varipolysaccharide contents of *D. officinale* and *D. huoshanense* with higher (Fig. 1b), *DoSWEET8, DoSWEET15,* and *DoSWEET17* exhibit a significantly higher expression (Fig. 5f). It is hypothesized that SWEET-mediated sugar transport promotes polysaccharide accumulation. Among the three *DoSWEET* genes in D. *officinale*, *DoSWEET8* belongs to Clade II, similar to *Arabidopsis SWEET13*, which transports sugars and supports pollen grain development[36]. In *Citrus sinensis*, *CsSWEET15* plays a crucial role in sucrose accumulation in the juice sac[37]. *DoSWEET17*, on the other hand, is assigned to Clade IV, similarly to *Arabidopsis SWEET17*, which is involved in sugar transport in vacuoles[38]. In the tuber of the medicinal plant *Bletilla striata*, as polysaccharides continuously accumulate, *BsSWEET1* participates in the transport and accumulation of sugars. Similarly, in the medicinal plant *Potentilla anserina*, *PaSWEET7*, *PaSWEET9*, and *PaSWEET12* promote the formation and development of taproots. In this study, the *DoSWEET* genes in *D. officinale* were analyzed and it was found that the expression levels of *DoSWEET1*, *DoSWEET8*, *DoSWEET13*, *DoSWEET15*, *DoSWEET17*, and *DoSWEET24* in the stem are higher than those of other *DoSWEETs* (Fig. 5a). Given that the stem has the highest polysaccharide contents among different tissues of *D. officinale*, *DoSWEET1*, *DoSWEET8*, *DoSWEET13*, *DoSWEET15*, *DoSWEET17*, and *DoSWEET24* may play important roles in the transport and accumulation of polysaccharides in the stem of *D. officinale*, thereby influencing its medicinal value and quality formation.

Environmental factors strongly influence polysaccharide dynamics in *Dendrobium*. Temperature-dependent polysaccharide degradation patterns were previously observed in this genus, with low-temperature storage effectively preserving carbohydrate content, likely through enhanced osmotic adjustment via polysaccharides and compatible solutes during cold-induced declines in water potential[39]. Sugars play multifaceted roles in plant physiology, functioning not only as energy sources and structural components for biopolymers such as starch and cellulose but also as osmoregulators that mitigate abiotic stress-induced cellular damage. The *SWEET* genes in plants widely respond to abiotic stresses, such as low temperature, drought, and salt stress[40–42]. In this study, the expression levels of many of the *SWEET* genes (such as *DoSWEET1,* Do*SWEET8,* Do*SWEET13,* Do*SWEET15,* Do*SWEET17*, and Do*SWEET24)* showed significant differences during the cold, NaCl, and high-light treatments (Fig. 5d, e). This result is consistent with many studies on the *SWEET* gene family; *SWEET1* has been recently reported to play an important role in cold resistance in longan[41], and homologous genes of *CsSWEET13* show increased expression levels under salt stress[43].

This study on *D. chrysotoxum*, *D. nobile*, *D. officinale*, and *D. huoshanense* revealed a noteworthy phenomenon: no significant positive correlation was observed between stem robustness and polysaccharide contents. Specifically, compared to *D. officinale* and D. *huoshanense*, which have relatively slender stems, the polysaccharide contents in *D. chrysotoxum* and *D. nobile*, which have stouter stems, were significantly lower (Fig. 1a, b). This finding suggests that the accumulation mechanism of polysaccharides—a key active component in *Dendrobium* medicinal materials—may operate independently of intuitive morphological traits (such as stem robustness). It appears that the biosynthesis and accumulation of polysaccharides are primarily regulated by intrinsic genetic programs, rather than being driven by morphogenesis. Sugar transport efficiency is a crucial link connecting primary photosynthetic products with the synthesis of secondary metabolites[44,45]. The analysis of the *SWEET* gene family in these four *Dendrobium* species demonstrates that the expression and regulation of genes in this family play key roles in polysaccharide accumulation. Therefore, targeting *SWEET* genes represents a promising avenue for enhancing the medicinal quality of authentic *Dendrobium* materials through molecular-assisted breeding strategies.

Conclusions

In summary, this study provides comprehensive insights into the genomic similarity of *D. nobile*, *D. chrysotoxum*, *D. huoshanense*, and *D. officinale*. Through comparative genomic analysis, it was determined that genes regulating polysaccharide contents and phenotypic traits in *Dendrobium* species are predominantly localized at the terminal regions of chromosomes *DoChr4* and *DhuChr4*, *DoChr5* and *DhuChr3*, *DoChr7* and *DhuChr2*, and *DoChr8* and *DhuChr12*. Additionally, an analysis of LTR-RT insertion times revealed that the amplification of the Athila-Gypsy family LTR-RT sequences contributed substantially to the divergence of these species. Analysis of chromosomal characteristics suggested that *D. officinale* and *D. nobile* may share a common ancestor. Moreover, comparative genomics combined with functional annotation using the AmiGO2 database highlighted SWEET family proteins as critical regulators of carbohydrate transport between cells.

A total of 105 *SWEET* genes were identified across the four *Dendrobium* species, including 24 *DoSWEET*s, 21 *DhuSWEET*s, 25 *DchSWEET*s, and 35 *DnSWEET*s. The distribution of *SWEET* gene domains and *cis*-promoter elements revealed functional divergence within this family, indicating that they function in diverse biological processes. Expression profiling of *DoSWEET*s demonstrated their differential expression across various organs, environmental conditions (e.g., high light and low temperature), developmental stages (seedling age), abiotic stress (salt stress), and phytohormone treatments. Notably, VIGS identified *SWEET1*, *SWEET8*, *SWEET13*, *SWEET17*, and *SWEET24* as key contributors to polysaccharide accumulation in *Dendrobium*.

Overall, the evolutionary dynamics of the SWEET gene family were elucidated in *D. nobile*, *D. chrysotoxum*, *D. huoshanense*, and *D. officinale*, as well as its role in plant responses to abiotic stress. Collectively, the evolutionary dynamics of the *SWEET* gene family (such as duplication, selection, and expression regulation) and the evolutionary history of *Dendrobium* species (such as divergence times and habitat adaptation) have shaped the diversity of polysaccharide biosynthesis capacity in this genus. These findings provide a foundation for further exploring the molecular mechanisms underlying polysaccharide biosynthesis in *D. officinale* and hold significant implications for its functional genomics and biotechnological applications.

Author contributions

The authors confirm contributions to the paper as follows: study conception and design: Jin HL, Wang HB; data collection: Li D, Lu P, Li F, Qi Q, Ren Z, Lin Y, Zeng J, Mai H; analysis and interpretation of results: Lai Z, Li J, Wang Z, Hong Z, Tian J, Lu P; draft manuscript preparation: Qi Q, Li D; revision of the manuscript: Li D, Lu P, Jin H. All authors reviewed the results and approved the final version of the manuscript.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Conflict of interest

The authors declare that they have no conflict of interest.

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References

1. Takamiya T, Wongsawad P, Sathapattayanon A, Tajima N, Suzuki S, et al. 2014. Molecular phylogenetics and character evolution of morphologically diverse groups, *Dendrobium* section *Dendrobium* and allies. *AoB Plants* 6:plu045
2. Teixeira da Silva JA, Ng TB. 2017. The medicinal and pharmaceutical importance of *Dendrobium* species. *Applied Microbiology and Biotechnology* 101:2227–39
3. Ng TB, Liu J, Wong JH, Ye X, Sze SCW, Yao T, et al. 2012. Review of research on *Dendrobium*, a prized folk medicine. *Applied Microbiology and Biotechnology* 93:1795–803
4. Meng LZ, Lv GP, Hu DJ, Cheong KL, Xie J, et al. 2013. Effects of polysaccharides from different species of *Dendrobium* (Shihu) on macrophage function. *Molecules* 18:5779–91
5. Ye G, Li J, Zhang J, Liu H, Ye Q, et al. 2021. Structural characterization and antitumor activity of a polysaccharide from *Dendrobium wardianum*. *Carbohydrate Polymers* 269:118253
6. Lam Y, Ng TB, Yao RM, Shi J, Xu K, et al. 2015. Evaluation of chemical constituents and important mechanism of pharmacological biology in *Dendrobium* Plants. *Evidence-Based Complementary and Alternative Medicine* 2015:841752
7. Chen H, Li X, Xu Y, Lo K, Zheng H, et al. 2018. Study on the polar extracts of *Dendrobium nobile*, *D. officinale*, *D. loddigesii*, and *Flickingeria fimbriata*: metabolite identification, content evaluation, and bioactivity assay. *Molecules* 23:1185
8. Duan H, Er-bu A, Dongzhi Z, Xie H, Ye B, et al. 2022. Alkaloids from *Dendrobium* and their biosynthetic pathway, biological activity and total synthesis. *Phytomedicine* 102:154132
9. Zhang T, Yang X, Wang F, Liu P, Xie M, et al. 2023. Comparison of the metabolomics of different *Dendrobium* species by UPLC-QTOF-MS. *International Journal of Molecular Sciences* 24:17148
10. Han B, Jing Y, Dai J, Zheng T, Gu F, et al. 2020. A chromosome-level genome assembly of *Dendrobium Huoshanense* using long reads and Hi-C data. *Genome Biology and Evolution* 12:2486–90
11. Yang X, Liu D, Tschaplinski TJ, Tuskan GA. 2019. Comparative genomics can provide new insights into the evolutionary mechanisms and gene function in CAM plants. *Journal of Experimental Botany* 70:6539–47
12. Alföldi J, Lindblad-Toh K. 2013. Comparative genomics as a tool to understand evolution and disease. *Genome Research* 23:1063–68
13. Xiang KL, Wu SD, Lian L, He WC, Peng D, et al. 2024. Genomic data and ecological niche modeling reveal an unusually slow rate of molecular evolution in the Cretaceous Eupteleaceae. *Science China Life Sciences* 67:803–16
14. Gill SS, Chahar P, Macovei A, Yadav S, Ansari AA, et al. 2021. Comparative genomic analysis reveals evolutionary and structural attributes of

MCM gene family in *Arabidopsis thaliana* and *Oryza sativa*. *Journal of Biotechnology* 327:117–32
15. Braun DM. 2012. SWEET! The pathway is complete. *Science* 335(6065):173–74
16. Lemoine R, La Camera S, Atanassova R, Dédaldéchamp F, Allario T, et al. 2013. Source-to-sink transport of sugar and regulation by environmental factors. *Frontiers in Plant Science* 4:272
17. Chen LQ, Lin IW, Qu XQ, Sosso D, McFarlane HE, et al. 2015. A cascade of sequentially expressed sucrose transporters in the seed coat and endosperm provides nutrition for the *Arabidopsis* Embryo. *The Plant Cell* 27:607–19
18. Chardon F, Bedu M, Calenge F, Klemens PAW, Spinner L, et al. 2013. Leaf fructose content is controlled by the vacuolar transporter SWEET17 in *Arabidopsis*. *Current Biology* 23:697–702
19. Wen S, Neuhaus HE, Cheng J, Bie Z. 2022. Contributions of sugar transporters to crop yield and fruit quality. *Journal of Experimental Botany* 73:2275–89
20. Ji J, Yang L, Fang Z, Zhang Y, Zhuang M, et al. 2022. Plant SWEET family of sugar transporters: structure, evolution and biological functions. *Biomolecules* 12:205
21. Tao Y, Cheung LS, Li S, Eom JS, Chen LQ, et al. 2015. Structure of a eukaryotic SWEET transporter in a homotrimeric complex. *Nature* 527:259–63
22. Breia R, Conde A, Badim H, Fortes AM, Gerós H, et al. 2021. Plant SWEETs: from sugar transport to plant–pathogen interaction and more unexpected physiological roles. *Plant Physiology* 186:836–52
23. Gautam T, Dutta M, Jaiswal V, Zinta G, Gahlaut V, et al. 2022. Emerging roles of SWEET sugar transporters in plant development and abiotic stress responses. *Cells* 11:1303
24. Hou B, Luo J, Zhang Y, Niu Z, Xue Q, et al. 2017. Iteration expansion and regional evolution: phylogeography of *Dendrobium officinale* and four related taxa in Southern China. *Scientific Reports* 7:43525
25. Sielemann K, Pucker B, Schmidt N, Viehöver P, Weisshaar B, et al. 2022. Complete pan-plastome sequences enable high resolution phylogenetic classification of sugar beet and closely related crop wild relatives. *BMC Genomics* 23:113
26. Huo J, Zhong S, Du X, Cao Y, Wang W, et al. 2020. Whole-genome sequence of *Phellinus gilvus* (mulberry Sanghuang) reveals its unique medicinal values. *Journal of Advanced Research* 24:325–35
27. Cao Y, Feng X, Ding B, Huo H, Abdullah M, et al. 2025. Gap-free genome assemblies of two *Pyrus bretschneideri* cultivars and GWAS analyses identify a CCCH zinc finger protein as a key regulator of stone cell formation in pear fruit. *Plant Communications* 6:101238
28. Chen LQ, Qu XQ, Hou BH, Sosso D, Osorio S, et al. 2012. Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. *Science* 335:207–11
29. Li D, Ye G, Li J, Lai Z, Ruan S, et al. 2023. High light triggers flavonoid and polysaccharide synthesis through *DoHY5*-dependent signaling in *Dendrobium officinale*. *The Plant Journal* 115:1114–33
30. Zhu L, Lan J, Zhao T, Li M, Ruan YL. 2025. How vacuolar sugar transporters evolve and control cellular sugar homeostasis, organ development and crop yield. *Nature Plants* 11:1102–15
31. Chen L, Xu S, Liu Y, Zu Y, Zhang F, et al. 2022. Identification of key gene networks controlling polysaccharide accumulation in different tissues of *Polygonatum cyrtonema* Hua by integrating metabolic phenotypes and gene expression profiles. *Frontiers in Plant Science* 13:1012231
32. Zhang Y, Zhang GQ, Zhang D, Liu XD, Xu XY, et al. 2021. Chromosome-scale assembly of the *Dendrobium chrysotoxum* genome enhances the understanding of orchid evolution. *Horticulture Research* 8:183
33. Zhang S, Wang H, Wang T, Zhang J, Liu W, et al. 2023. Abscisic acid and regulation of the sugar transporter gene *MdSWEET9b* promote apple sugar accumulation. *Plant Physiology* 192:2081–101
34. Yoo SR, Jeong SJ, Lee NR, Shin HK, Seo CS. 2017. Simultaneous determination and anti-inflammatory effects of four phenolic compounds in Dendrobii Herba. *Natural Product Research* 31:2923–26
35. Cao YY, Li K, Li Y, Tian XT, Ba HX, et al. 2020. *Dendrobium candidum* aqueous extract attenuates isoproterenol-induced cardiac hypertrophy through the ERK signalling pathway. *Pharmaceutical Biology* 58:176–83
36. Zhu J, Zhou L, Li T, Ruan Y, Zhang A, et al. 2022. Genome-wide investigation and characterization of SWEET gene family with focus on their evolution and expression during hormone and abiotic stress response in maize. *Genes* 13:1682
37. Feng G, Wu J, Xu Y, Lu L, Yi H. 2021. High-spatiotemporal-resolution transcriptomes provide insights into fruit development and ripening in *Citrus sinensis*. *Plant Biotechnology Journal* 19:1337–53
38. Guo WJ, Nagy R, Chen HY, Pfrunder S, Yu YC, et al. 2014. SWEET17, a facilitative transporter, mediates fructose transport across the tonoplast of Arabidopsis roots and leaves. *Plant Physiology* 164:777–89
39. Yu Z, Yang Z, Teixeira da Silva JA, Luo J, Duan J. 2019. Influence of low temperature on physiology and bioactivity of postharvest *Dendrobium officinale* stems. *Postharvest Biology and Technology* 148:97–106
40. Cao L, Wang J, Wang L, Liu H, Wu W, et al. 2024. Genome-wide analysis of the *SWEET* gene family in *Hemerocallis citrina* and functional characterization of HcSWEET4a in response to salt stress. *BMC Plant Biology* 24:661
41. Fang T, Rao Y, Wang M, Li Y, Liu Y, et al. 2022. Characterization of the *SWEET* gene family in Longan (*Dimocarpus longan*) and the role of *DlSWEET1* in cold tolerance. *International Journal of Molecular Sciences* 23:8914
42. Li P, Lin P, Zhao Z, Li Z, Liu Y, et al. 2022. Gene co-expression analysis reveals transcriptome divergence between wild and cultivated sugarcane under drought stress. *International Journal of Molecular Sciences* 23:569
43. Abuslima E, Kanbar A, Ismail A, Raorane ML, Eiche E, et al. 2025. Salt stress-induced remodeling of sugar transport: a role for promoter alleles of *SWEET13*. *Scientific Reports* 15:7580
44. Colle M, Leisner CP, Wai CM, Ou S, Bird KA, et al. 2019. Haplotype-phased genome and evolution of phytonutrient pathways of tetraploid blueberry. *GigaScience* 8:giz012
45. Ren R, Yue X, Li J, Xie S, Guo S, et al. 2020. Coexpression of sucrose synthase and the SWEET transporter, which are associated with sugar hydrolysis and transport, respectively, increases the hexose content in *Vitis vinifera* L. grape berries. *Frontiers in Plant Science* 11:321