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# Phytohormone-triggered Transcriptional Changes Revealed *β-Glucosidase* as a Key Player for Polysaccharide Metabolism in *Dendrobium officinale**

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**Abstract** **Objective** *Dendrobium officinale* has long been used as an important medicinal herb in oriental medicine. Polysaccharide, flavonoid, and alkaloid are the major active ingredients, the production and accumulation of which are frequently affected by numerous environmental cues. Phytohormone supplemented in culture medium has facilitated the mass production of orchids. However, their mechanism of action on the production of active components in *Dendrobium officinale* is far from clear. **Methods** Here, major medicinal metabolites were comparatively analyzed in *Dendrobium officinale* seedlings exposed to the most commonly used phytohormones (NAA and/or 6-BA), and transcriptomes corresponding to the treatments were generated. **Results** Results showed that phytohormones greatly affected the accumulation of polysaccharide, alkaloid, and flavonoid, and triggered tremendous transcriptional changes. It was demonstrated that 6-BA induced more transcripts than NAA and that *β-glucosidase* (*BGLU*) expression was closely related to polysaccharide production. Further functional analysis revealed that factors including phytohormone category, concentration, treating duration, and seedling growth stages, can drastically affect the *BGLU* expression and the corresponding polysaccharide production, thus partially answered the key question at molecular level why the medicinal constituents are unstable in tissue culture derived *Dendrobium* plants. **Conclusion** Altogether, the present study clearly demonstrated that *BGLU* is a key regulator for polysaccharide production in *Dendrobium officinale* in response to phytohormone treatments.

**Key words** *Dendrobium officinale*, transcriptomic analysis, polysaccharide, β-glucosidase
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The *Dendrobium* is one of the largest genera in the Orchidaceae that contains more than 1 500 species around the world with high economic and scientific value[1]. *Dendrobium officinale* (*D. officinale*), an endangered perennial epiphytic herb widely distributed in subtropical Asia[2], has been ranked as one of the most prized *Dendrobium* species[3] exhibiting diverse therapeutic effects such as stomach nourishing, body fluids secretion, immunity enhancement, and cancer inhibition[4-5]. The stem contains a variety of active compounds including polysaccharides, alkaloids, flavonoids, phenols, *etc*[6-7]. *D. officinale* is widely used both for medicine and dietary supplement in the form of juice, powder, capsules, and functional wine or tea according to the consumer's preferences[8], leading to intensified agricultural production of this species. Currently, more than 6 000 ha of agricultural land is used for producing more than 20 000 tons of fresh *D. officinale* in southern China[9]. Thus, the quality of *D. officinale* production is crucial, which can be influenced by diverse genetic and environmental factors[10]. Therefore, high-efficient, stable and non-toxic propagation methods were necessary for commercial production of *Dendrobium* plants. Even though the improved technologies on tissue culture and micro-propagation had been performed[11], current widely used large scale propagation by hybridization and medium induced germination resulted in homozygous individual deficiency, unstable phenotypical traits and medicinal quality of progenies[12-13]. Yet, the underlining mechanism is far from clear.

We previously analyzed the functional mechanisms of active ingredients from *Dendrobium* species[5, 14-15], and the putative biosynthesis pathways for the active metabolites[10]. Here, comprehensive changes in the seedlings of *D. officinale* in response to phytohormones (NAA and/or 6-BA) treatments were compared using two RNA-seq analyses based on an Illumina NovaSeq 6000 platform, and the content of polysaccharide, flavonoid and alkaloid was determined accordingly. It was found that the *BGLU* (*β-glucosidase*) family members were frequently isolated. Particularly, *BGLU2L* in the *BGLU* family is associated with the accumulation of polysaccharide. Short period of 6-BA application induced soluble polysaccharide production, while knockdown of *BGLU2L* decreased the levels. This work provides new insights into the molecular mechanism of *D. officinale* seedlings under phytohormone treatments, which should be a useful resource to enhance the biosynthesis of active ingredients in *D. officinale* and other orchids.

## 1 Materials and methods

### 1.1 Plant materials and treatments

*D. officinale* seeds were germinated on half-strength Murashige and Skoog (MS) medium with 3.0% sucrose, 0.4% agar, and 20.0% potato (pH 5.8). Materials were grown in a culture room at (25±2)℃ with a 12 h/12 h light/dark cycle (40 μmol/$m^2$/S) and 60%~70% relative humidity. Two months later, protocorms were emerged from the seeds and then formed plantlets. For phytohormone feeding experiments in the first RNA sequencing, 2-month-old uniform plantlets were sub-cultured on a medium described above, supplemented with distilled water, 0.1 mg/L NAA, 1.0 mg/L 6-BA, and 0.1 mg/L NAA + 1.0 mg/L 6-BA, two biological replicates for each treatment. The plantlets were sampled after 3 weeks (w) of cultivation. For the second RNA sequencing, 9-month-old uniform plantlets were transferred onto mediums supplemented with varied concentrations of 6-BA (0, 0.5, 1.0, and 2.0 mg/L) and sampled at different time points (2 w and 4 w).

### 1.2 Measurement of the content of polysaccharide, flavonoid, and alkaloid

Water-soluble polysaccharide was determined using plant soluble polysaccharide assay kit (Solarbio, Beijing, China). Flavonoid was measured using flavonoid assay kit (Solarbio, Beijing, China). Reducing polysaccharide was determined according to Avwioroko *et al*. [16]. Alkaloid was estimated following the method by Wang *et al*. [17]. Phytohormone treated seedlings (0.5 g) were harvested, ground in liquid nitrogen, and extracted by extraction buffer. The isolated compounds were determined by using a spectrophotometer (BeckmanCoulter DU730).

### 1.3 cDNA library preparation and transcriptome sequencing

Total RNA was isolated from phytohormone treated *D. officinale* plantlets using the plant RNA isolation mini kit (BioTeke, Beijing, China) according to the manufacturer's recommendation. DNA contamination was removed with recombinant RNase-free DNase I (TaKaRa, Tokyo, Japan) and then mRNA

was enriched with Oligo(dT) magnetic beads (Thermo Fisher Scientific, MA, USA). The purified mRNA was cut into small pieces using fragmentation buffer and reverse-transcribed to produce the cDNA library using mRNA-seq sample preparation kit (Illumina, San Diego, CA, USA) as instructed by the manufacturer. The prepared libraries were sequenced in the 150-nt paired-end mode on an Illumina NovaSeq 6000 platform (BerryGenomics, Beijing, China).

### 1.4 Transcriptome data processing and analysis

The raw fastq format reads obtained from the sequencing were purified by filtering out low-quality reads. Next, the clean reads were mapped against the primary CDS from the *D. officinale* genome database[18] for quantification of the gene abundance as read counts using Hisat2 (version 2.0.6) software. The expression levels of genes were presented as the fragments per kilobase of transcript per million map reads (FPKM) using Cufflinks (version 2.2.2) software. EdgeR was used to compare differently expressed genes (DEGs) between groups. Genes were identified significantly differentially expressed with the *P* adjusted (*padj*) < 0.05 and $|\log_2$ fold change| >2 as the thresholds. KEGG (Kyoto encyclopedia of genes and genomes) enrichment analysis of DEGs were performed using KOBAS v2.0 software. Heatmap was used to generate heatmaps of DEGs.

### 1.5 Quantitative real−time PCR (qRT−PCR) validation

Eight genes of interest involved in glycolysis/gluconeogenesis (*PDC* and *GALM*), starch and sucrose metabolism (*TPS*, *OTSB*, and *BGLU2L*), and flavonoid biosynthesis (*CHS*, *DFR*, and *CYP75B1*) were randomly selected for further differential expression confirmation of the RNA-seq data by qRT-PCR. Total RNA was extracted from *D. officinale* plantlets after 3 w of phytohormone treatment using TRIzol reagent (Thermo Fisher Scientific). Total RNA (1.0 μg) was reverse-transcribed into cDNA using PrimeScript™ RT reagent kit with gDNA Eraser (Takara, Japan). qRT-PCR analysis was performed with SYBR green premix kit (Toyobo, Japan) in the ABI PRISM 7500 Fluorescent Quantitative PCR System (Thermo Fisher Scientific). *Actin* was used as the internal control for normalization of expression levels; other genes and the primers were listed in Table S1. $2^{-\Delta\Delta C_t}$ method was used to calculate relative gene expression.

### 1.6 RNAi−induced gene silencing of *BGLU2L* in *D. officinale* leaves

The expression of *BGLU2L* was suppressed by RNA interference (RNAi) method. Briefly, a 468-bp trigger fragment was PCR amplified from cDNA template of *D. officinale* seedlings by primers, 5' CTTACGCTTGATGCTTTTAG 3' (forward), 5' CATCCTCCATACTCATCTTG 3' (reverse), and cloned into pCambia1300 plasmid in both the sense and antisense orientations[19]. Agrobacterium carrying the plasmid was infiltrated into *D. officinale* leaves treated with or without 1.0 mg/L 6-BA for 3 w. Polysaccharide was measured after 72 h of infiltration.

## 2 Results

### 2.1 Phytohormones affected bioactive compounds production

Considering that polysaccharides are the main medicinal components in *D. officinale*, and that flavonoids are the second most abundant metabolites, we measured the content of them after 0.1 mg/L NAA and/or 1.0 mg/L 6-BA treatment of 2-month-old seedlings for 3 w. The results showed that NAA and 6-BA had no observable effects on the growth of the plantlets (Figure S1a), but significantly increased the water-soluble polysaccharides content from 130.176 mg/g (water-treated control) to 184.158 mg/g and 171.379 mg/g, respectively (Figure 1a). About two-fold increase was further observed when combining the two phytohormones, indicating of their synergistic effects on soluble polysaccharides production. Likewise, the reducing polysaccharides content at control was 35.500 mg/g, which was much lower than NAA and 6-BA treated plantlets, with 48.167 mg/g and 46.167 mg/g, respectively (Figure 1b). In a similar manner, NAA and 6-BA synergistically enhanced the accumulation of reducing polysaccharides (58.500 mg/g, Figure 1b). Moreover, the total flavonoid content in the samples treated separately with NAA or 6-BA was comparable with that of the non-treated control (16.172 mg/g), but markedly increased when the two phytohormones applied together (22.964 mg/g, Figure 1c). The results clearly demonstrated that the relative long-term treatment of NAA and 6-BA promoted polysaccharide and flavonoid production.

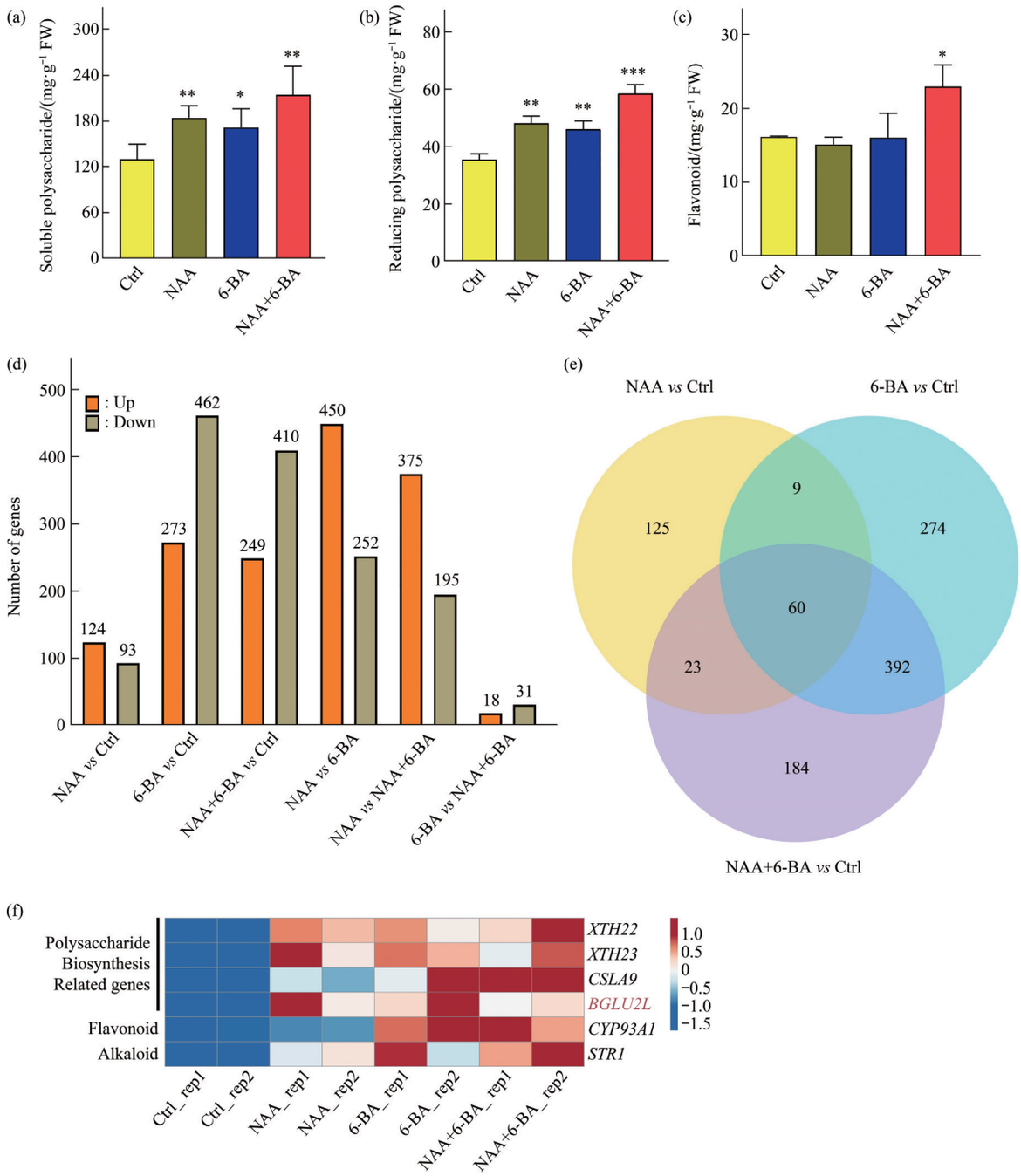


**Fig. 1　Determination of metabolites accumulation and associated gene expression in response to long−term phytohormone treatments in *D. officinale* seedlings**

(a−c) Content determination for soluble polysaccharide (a), reducing polysaccharide (b), and flavonoid (c). FW: fresh weight. The data were shown as means±SD ($n$=3), the statistically significant differences compared with water-treated plantlets were considered at *$P$<0.05, **$P$< 0.01, ***$P$< 0.001. (d) The number of DEGs in different comparisons. (e) Venn diagram of the DEGs under varied combinations of phytohormone treatments. DEGs and overlapping genes from varied treatments were analyzed. (f) Changes in transcripts involved in bioactive metabolite synthesis. Green/blue color-coded heat map represents relative transcript levels of different gene family members (red, up-regulated; blue, down-regulated). Abbreviations for enzyme names are given in Table S2.

### 2.2 Phytohormones triggered transcriptional changes

To further investigate the mechanisms underlining the differential accumulation of bioactive ingredients upon phytohormone application, 8 cDNA libraries using materials mentioned above were prepared from two independent biological replicates and sequenced. The raw- and processed-data were deposited in GEO (accession: GSE184090), and the differentially expressed genes were listed in Table S3. The quality score at the Q30 levels (sequence error rate <1‰) were from 91.72% to 95.29% and the average GC content was ranged from 45.45% to 46.23% (Table S4). These results suggest that the sequencing is of high quality. In total, 243 133 073 Illumina pared-end clean reads (about 30.4 million reads per sample) were obtained, 90% of which were uniquely mapped to the *D. officinale* reference genome (23 068 genes) (Table S4). Of these genes, 13 961 were annotated in the GO database, and 8 536 were annotated in the KEGG database by using topGO and KOBAS, respectively.

A total of 1 363 DEGs were identified; 6-BA stimulated more transcript changes than NAA (Figure 1d, Table S5). Because that KEGG assignments provide alternative functional annotations for biochemical pathway related genes, the DEGs were further mapped to terms in KEGG pathway database to identify enriched genes associated with metabolic pathways under phytohormone treatments. The top 20 pathways with $P<0.05$ were significantly enriched. Notably, the most enriched DEGs were observed in the pathway of plant hormone transduction, flavonoid biosynthesis, biosynthesis of secondary metabolites, starch and sucrose metabolism, and alkaloid biosynthesis (Figure S1b). Data sets from different comparison groups were analyzed by a Venn diagram, resulting in 60 overlapping DEGs compared with the water-treated control (Figure 1e). Among these, genes possibly involved in polysaccharide metabolism including *BGLU2L*, *XTH22*, *XTH23*, and *CSLA9* were up-regulated by phytohormone treatment (Figure 1f). Consistent with the increased accumulation of flavonoid, one cytochrome P450 family member *CYP93A1* was significantly upregulated by phytohormone application (Figure 1f). Additionally, the expression of genes associated with alkaloid biosynthesis including *STR1* were stimulated in response to phytohormones (Figure 1f).

To experimentally validate the RNA-seq data, eight genes from active ingredient metabolism pathway (*PDC* and *GALM* from glycolysis/gluconeogenesis; *TPS*, *OTSB*, and *BGLU2L* from starch and sucrose metabolism; *CHS*, *DFR*, and *CYP75B1* from flavonoid biosynthesis) were randomly selected for qRT-PCR analysis. Results showed that expression of the eight genes was basically consistent with the expression profile in the RNA-seq datasets (Figure 2), which further confirmed the reliability of the sequencing data.

### 2.3 Transcriptome profiling revealed *BGLU*s as key players for polysaccharide production

It appeared that 6-BA has stimulated more transcript changes than NAA as revealed previously, 6-BA application was thus further investigated. To verify if the responses is less dependent on developmental stages, 9-month-old plantlets were subjected to varied concentrations of 6-BA treatments (0, 0.5, 1.0, and 2.0 mg/L) for short-term (2 w) or long-term (4 w) periods. Consistent with our previous results, application of 6-BA had nonobservable effects on the growth of seedlings (Figure S2a, b), but significantly increased the alkaloid levels (Figure 3a). The polysaccharide content was slightly decreased under short period of 6-BA treatments, but somehow increased with prolonged application (Figure 3b). Consensus results also showed that the content of flavonoid was increased with an ascended concentrations of 6-BA, especially when comparing the levels of 2-w against that of 4-w treatments (Figure S2c). To reconfirm the RNA-seq data as well as to gain more detailed information about different developmental stages of *D. officinale* seedlings in response to varied concentrations of phytohormone, the 6-BA-treated samples mentioned above were subsequently applied to a second RNA-sequencing analysis (Table S6). As expected this time, more DEGs were obtained under varied concentrations of 6-BA treatments (Figure 3c). The overlapping DEGs were further analyzed by Venn diagrams (Figure 3d, e), revealing genes associated with biosynthesis of alkaloid (*CYP76B6* and *TR1*), polysaccharide

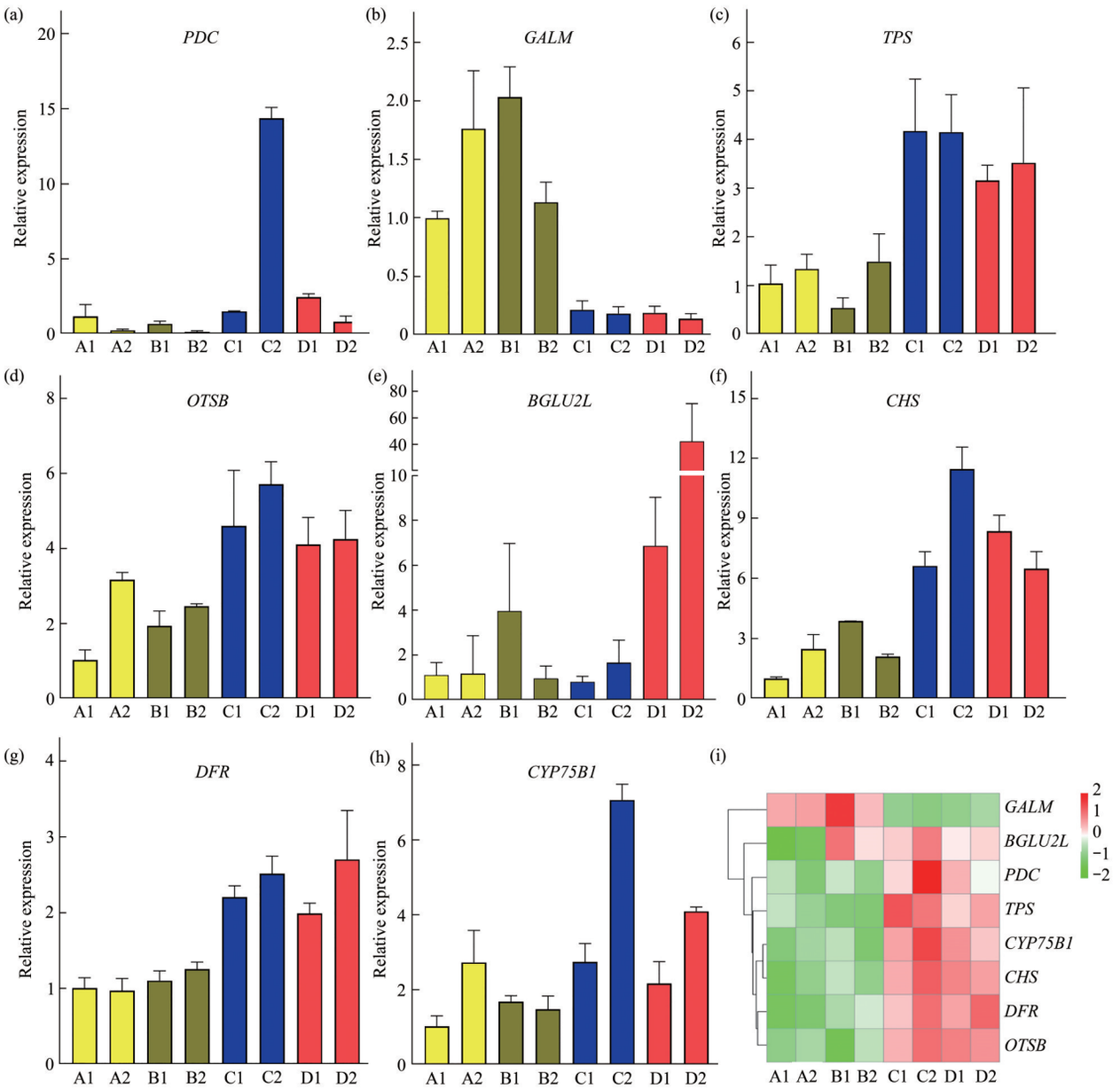


**Fig. 2　qRT−PCR validated the randomly selected genes involved in metabolites production in response to phytohormone treatments**

Expression of genes associated with glycolysis and gluconeogenesis (a, b), starch and sucrose metabolism (c−e), and flavonoid biosynthesis (f−h). (i) The expression patterns of the 8 DEGs in the transcriptome data. A1: water treated control, repeat 1; A2: water treated control, repeat 2; B1: 0.1 mg/L NAA treatment, repeat 1; B2: 0.1 mg/L NAA treatment, repeat 2; C1: 1.0 mg/L 6-BA treatment, repeat 1; C2: 1.0 mg/L 6-BA treatment, repeat 2; D1: 0.1 mg/L NAA+1.0 mg/L 6-BA treatment, repeat 1; D2: 0.1 mg/L NAA+1.0 mg/L 6-BA treatment, repeat 2.

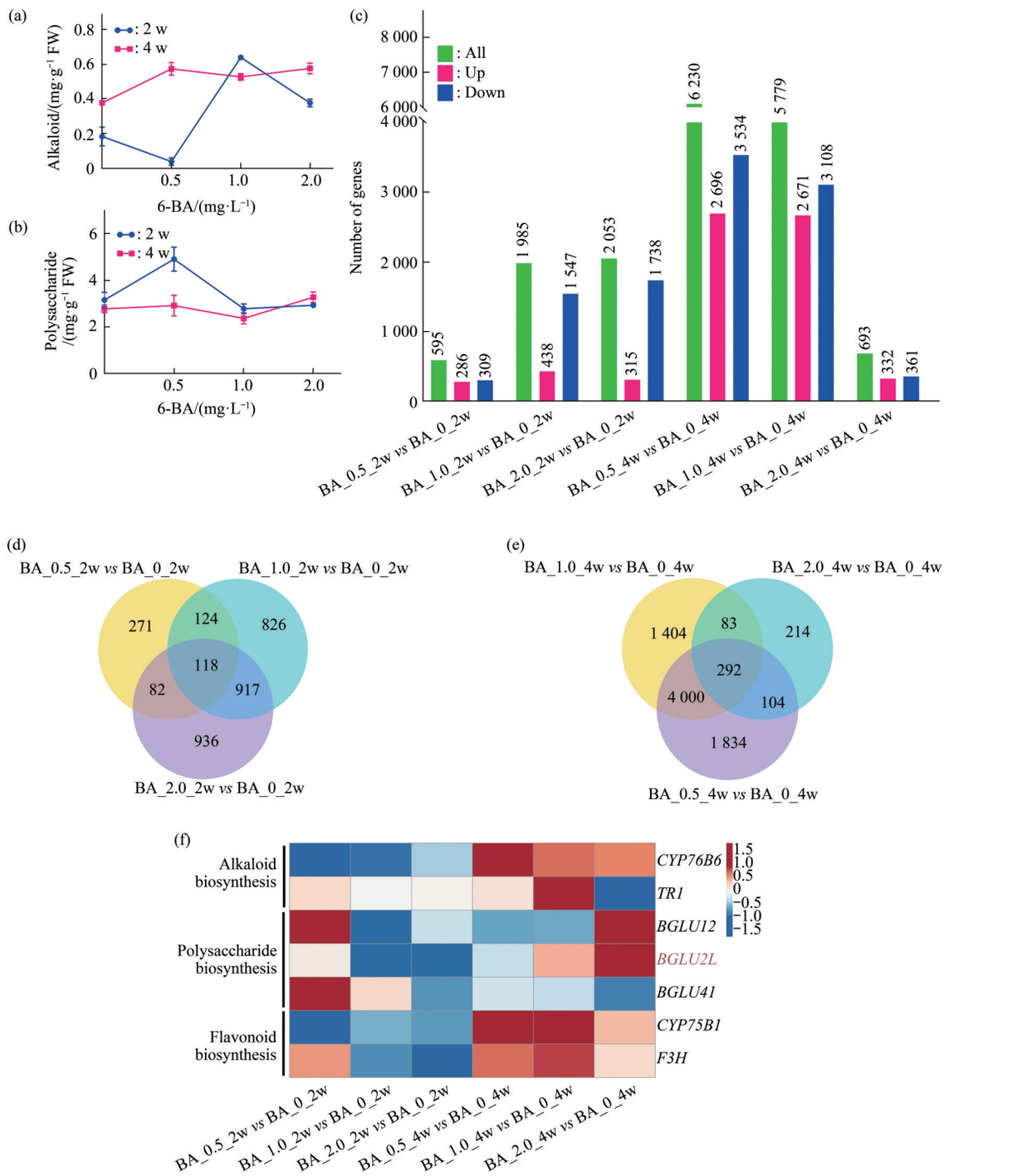


**Fig. 3 Determination of metabolites accumulation and associated gene expression in response to varied concentrations of 6−BA treatments in *D. officinale* seedlings**

(a−b) Contents determination for alkaloid (a) and polysaccharide (b). FW: fresh weight. The data were shown as means±SD ($n$=3). (c) The number of DEGs in different comparisons. (d−e) DEGs and overlapping genes from varied treatments were analyzed. Venn diagram of the DEGs under varied concentrations of 6-BA treatments for 2 w (d) or 4 w (e). (f) Changes in transcripts involved in bioactive metabolite synthesis. Green/blue color-coded heat map represents relative transcript levels of different gene family members (red, up-regulated; blue, down-regulated). Abbreviations for enzyme names are given in Table S2.

(*BGLU2L*, *BGLU12*, and *BGLU41*), and flavonoid (*CYP75B1* and *F3H*) in response to different 6-BA treatments (Figure 3f). It is worthy noting that the *BGLU* family members including *BGLU2L* were frequently isolated in the data sets, suggesting of its importance in polysaccharide metabolism. What's more fascinating is that the expression pattern of *BGLU*s, down-regulated first and upregulated as prolonged treating, was in good agreement with the polysaccharide levels.

### 2.4 *BGLU2L* in *BGLU* family modulated polysaccharide production in *D. officinale*

The consensus pattern of *BGLU*s expression with the polysaccharide level prompted us to further investigate their tight association. Firstly, a phylogenetic tree for BGLUs of *D. officinale* (DcBGLUs) was constructed based on protein sequences that available online (accession numbers were detailed in Table S7). The DcBGLUs were grouped into 4 sub-clusters (Figure S3a). For clarity, the 14 DcBGLUs were combined with 47 BGLUs from *Arabidopsis thaliana* (AtBGLUs) for phylogenetic analysis. Most of the DcBGLUs were grouped into cluster III and V, without any of them assigned to cluster I (Figure 4). Considering that *BGLU2L* was repetitively isolated as DEGs in the two RNA-seq data, it was selected as a representative for further functional confirmation. The expression of *BGLU2L* was silenced (*BGLU2L-kd*) *via* RNAi (Figure 5a), leading to decreased accumulation of reducing polysaccharide (Figure 5b) and soluble polysaccharide (Figure 5c) in transiently transformed

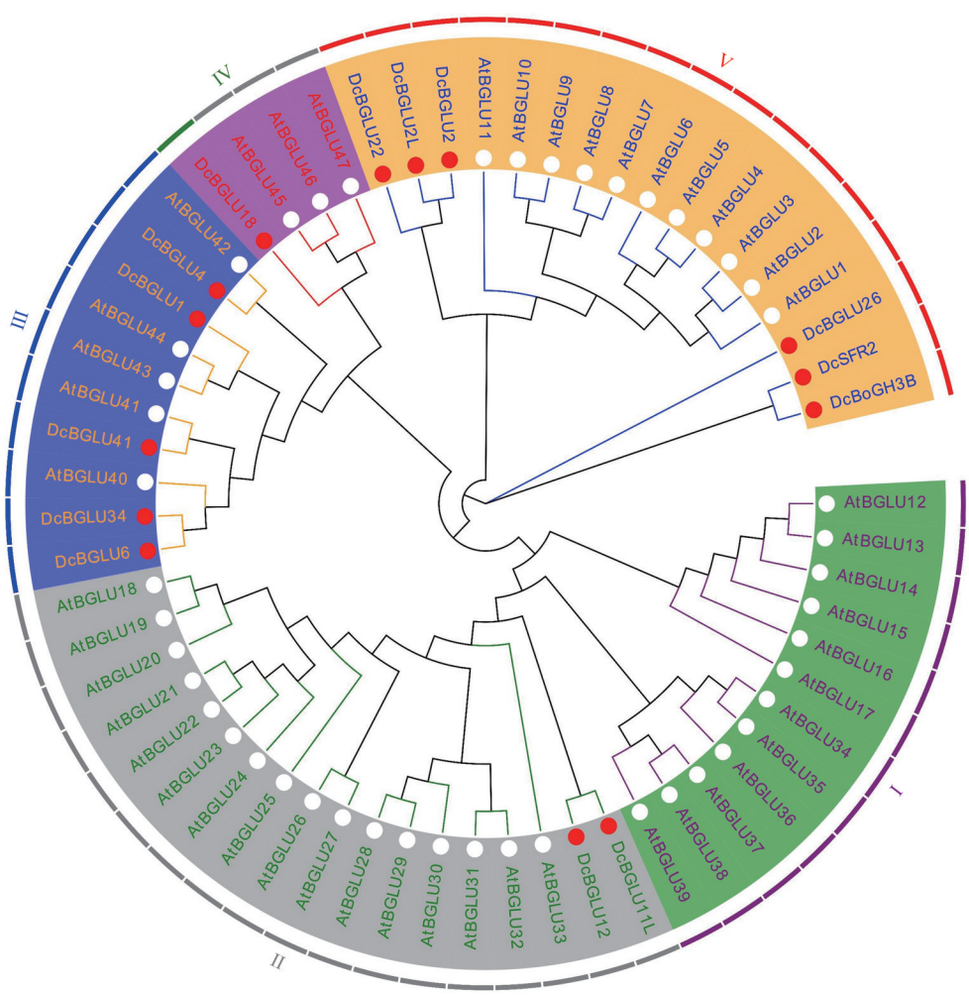


**Fig. 4 A molecular phylogenetic tree representing relationships among BGLU proteins from *D. officinale* and *Arabidopsis thaliana***

The GeneBank IDs of the proteins used in the alignment were described in Table S7. The phylogenetic tree was established using MEGA-X based on the Neighbor (NJ) method. Bootstrap values were performed with 1 000 replications. The different-colored arcs indicate varied groups of BGLU proteins. The red solid circles and hollow circles represent BGLU proteins from *D. officinale* and *Arabidopsis thaliana*, respectively.

2-year-old *D. officinale* leaves. In order to reassure the responsiveness of *BGLU2L* expression to phytohormone as mentioned above, 9-month-old seedlings were treated with or without 1.0 mg/L 6-BA for 3 w before RNAi transient transformation. Results showed that knock-down of *BGLU2L* (Figure 5d) suppressed the accumulation of reducing polysaccharide (Figure 5e) and the soluble polysaccharide (Figure 5f), which was well corroborated with the previous results obtained by the second RNAseq. Application of 6-BA did not lead to observable phenotypic changes (Figure S3b), but did inhibit reducing polysaccharide production. A slight increase of soluble polysaccharide upon 6-BA application was also noticed (Figure 5f), which might due to compensatory effects between the reducing and soluble polysaccharide because of their inter-convertible nature. These results reconsolidated the vital role of *BGLU*s in polysaccharide metabolism.

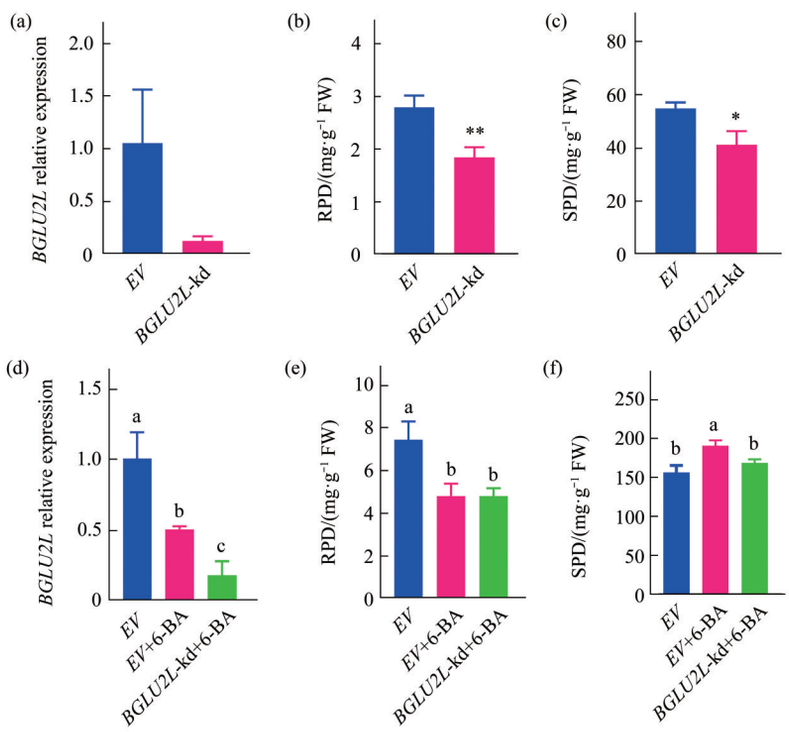


**Fig. 5 Verification of *BGLU2L* as a key player in polysaccharide production**

(a−c) Knock-down (kd) of *BGLU2L* (a) conferred compromised production of reducing polysaccharide (RPD) (b), and soluble polysaccharide (SPD) (c) in RNAi plasmid transiently transformed 2-year old *D. officinale* leaves. FW: fresh weight. The data were shown as means±SD ($n$=3). The statistically significant differences compared with agrobacterium carrying the empty vector (*EV*) infiltrated leaves were considered at *$P$<0.05, **$P$<0.01. (d-f) Knock-down of *BGLU2L* (d) inhibited accumulation of reducing polysaccharide (RPD) (e) and soluble polysaccharide (SPD) (f). Nine-month-old *D. officinale* plantlets treated with or without 1.0 mg/L of 6-BA for 3 w were transiently infiltrated with agrobacterium carrying *EV* or *BGLU2L-kd* vectors. Three days later, the infiltrated leaves were collected and polysaccharides were measured. The lowercase letters in (d−f) indicate the significance ($P$<0.05) according to one-way ANOVA tests.

## 3 Discussion

*D. officinale* is an extremely important medicinal orchid with polysaccharides, flavonoids, and alkaloids as the major effective components[18]. However, wild resources of *D. officinale* are rare and endangered because of low rate of propagation in nature, habitat destruction, and overexploitation[20]. Therefore, alternative methods for mass production of *D. officinale* plants are urgently needed. To this end, extensively exploited tissue culture techniques for the rapid and large scale micropropagation of orchids through phytohormone-induced protocorm-like bodies (PLB) have facilitated the successful culture of

*D. officinale in vitro*[21]. Even though phytohormones in the culture medium have been demonstrated to stimulate a variety of biological processes in various plants, an in-depth assessment of drug yielding potential and the underling mechanism is sporadic. The present study investigated the impact of NAA and 6-BA, two of the most frequently used plant growth regulators for orchid tissue culture, on the bioactive metabolite biosynthetic capacity in *D. officinale* plantlets micropropagated *in vitro*.

Cytochrome P450 superfamily members are associated with metabolism of alkaloid. The present study detected up-regulation of *CYP76B6* upon increased concentrations of 6-BA application, which is in good agreement with the accumulation of alkaloid in the corresponding samples. On the other hand, flavonoids are synthesized mainly through phenylpropanoid and polyketide pathways, using malonyl-CoA and p-coumaroyl-CoA as the starting materials[22]. CHS condensation and DFR catalysis are involved in most of flavonoids biosynthesis[23]. This study revealed that NAA and 6-BA increased accumulation of flavonoid and up-regulated *CHS* and *DFR* expression as well in 2-month-old *D. officinale* sedlings.

Glucosylation could increase the solubility and stability of compounds produced in plant metabolism, thus more suitable for storage in the vacuole or other organelles[24]. Plant *BGLUs* exhibit diverse biological functions in response to developmental and environmental cues. We and others previously sequenced the genome of *D. officinale*[18, 25], and the gene expression atlas is publicly available, which facilitated the functional genomic studies. However, members of the *BGLU* family in *D. officinale* have hardly been investigated. In the present study, a group of *BGLUs* including *BGLU2L*, *BGLU12*, and *BGLU41* were stimulated by 6-BA in 9-month-old *D. officinale* seedlings. Phylogenetic analysis revealed a close relatedness between *D. officinale* and *Arabidopsis thaliana*[18]. In this study, DcBGLU18 was grouped into cluster IV with AtBGLU45 and AtBGLU46, both of which function in lignification[26], indicating of its possible role in lignin precursor hydrolysis in *D. officinale*. DcBGLU2, DcBGLU2L, and DcBGLU22 were assigned into cluster V with AtBGLU6 and AtBGLU10, which act as glucosyltransferases in *Arabidopsis*[27], implying of their similar functions in *D. officinale*. It is noticed that even though the expression was closely associated with polysaccharide production, *BGLU* is extremely susceptible to environmental cues including phytohormone category, treatment duration, concentration, and seedling developmental stages, thus partially unraveled the molecular mechanism under the unstable production of medicinal compounds in tissue-culture derived *Dendrobium* plants. Altogether, our results presented clear evidences defining *BGLU* as a key player in polysaccharide metabolism possibly through its glucosyltransferase activity, yet the detailed chemical catalytic mechanism and other functions are still to be explored.

## 4 Conclusion

DEGs associated with the putative polysaccharide, flavonoid and alkaloid biosynthetic pathway in *D. officinale* seedlings in response to phytohormones were analyzed. The expression of DEGs was in good agreement with the content of corresponding active ingredients, especially *BGLU2L* in DEGs was involved in the accumulation of polysaccharide, thus unraveled the underlying mechanisms of altered compound accumulation under phytohormone treatments. The dataset presented paves the way for manipulating bioactive ingredients production through phytohormones in the tissue culture system of orchids.

**Supplementary** Available online (http://www.pibb.ac.cn or http://www.cnki.net).
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PIBB_20210352_Table S6.xlsx
PIBB_20210352_Table S7.pdf

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# 植物激素处理诱导的转录组改变揭示铁皮石斛β葡糖苷酶在多糖合成中的关键作用*

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**摘要　目的**　铁皮石斛是一种名贵的中草药，药用历史悠久，其药用活性成分主要包括多糖、类黄酮和生物碱。这些成分的种类和含量通常受到诸多环境因素的影响。组织培养中植物激素的广泛使用促进了许多兰科植物的大量快繁，然而植物激素对活性成分积累的影响及其作用机制尚待进一步深入研究。**方法**　本文利用铁皮石斛组织培养常用的两种植物激素NAA和（/或）6-BA处理铁皮石斛幼苗，测定活性化合物含量并对相应材料进行转录组测序分析。**结果**　激素处理影响多糖、生物碱和类黄酮等活性化合物的积累水平，同时引起较多的差异基因表达。统计分析表明，6-BA较NAA处理引起更多的转录本改变，β葡糖苷酶编码基因*BGLU*表达与多糖积累密切相关。进一步功能研究表明，包括激素种类、处理浓度、处理时间和处理材料生长时期等因素均能明显影响*BGLU*表达及相应多糖积累水平，从而在分子层面部分回答了一个长期困扰人们的关键问题，即为什么药材经组织培养后活性成分相对不稳定。**结论**　本研究明确提出了*BGLU*在激素处理后多糖的积累过程中有重要调控作用。

**关键词**　铁皮石斛，转录组分析，多糖，β-葡萄糖苷酶
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