

The expression of transcription factor WRINKLED1 of *Dunaliella parva* promotes the accumulation of lipids in *Auxenochlorella protothecoides*

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Abstract

WRINKLED1 (WRI1) is a member of the APETALA2 (AP2)/EREBP (ethylene response element binding protein) transcription factor family and has been shown to be one of the important transcription regulators involved in the oil biosynthesis pathway. To investigate the role of WRI1 in the regulation of oil synthesis in *Auxenochlorella protothecoides* (*A. protothecoides*), the *wri1* gene, cloned from *Dunaliella parva* (*D. parva*), was introduced and expressed in *A. protothecoides*. We discovered that the oil content of the genetically modified algae strain (*A. protothecoides*-*Dpwri1*) increased by 38.3% compared to the wild strain. The transcriptome analysis revealed that the expression of *Dpwri1* in *A. protothecoides* up-regulated the expression of glucose-6-phosphate isomerase (GPI), 3-phosphoglycerinaldehyde dehydrogenase (GAPDH), phosphoenolpyruvate carboxykinase (PEPCK), diacylglycerol acyltransferase (DGAT), which involved in glycolytic pathway and triglyceride synthesis pathway respectively, resulting in the flow of carbon to the synthesis of lipids. In addition, the expression of acyl-CoA oxidase (ACOX) and acetyl-CoA acetyltransferase 1 (ACAT1) were down-regulated, which is beneficial to the accumulation of lipids in cells. This study reveals the important role of the exogenous WRI1 transcription factor in regulating the accumulation of lipids in *A. protothecoides*, which is of guiding significance for the construction of high-yield oil-producing strains.

INTRODUCTION

Rapid global economic growth has increased the demand for energy, highlighting the need for clean energy sources due to environmental pollution caused by traditional fossil fuels. As one of the third-generation biofuels, microalgae biodiesel plays a pivotal role in global bioenergy production (Leung et al. 2010, Gaurav et al. 2024). *A. protothecoides*, a single-celled organism, is prized for its high oil content, rapid growth cycle, remarkable photosynthetic efficiency, and non-competition with arable land resources. It has emerged as a significant source of biodiesel production (Çakır et al. 2024).

Studies have shown that numerous transcription factors, such as MYB (Shi et al. 2022), Dof (Salas-Montantes et al. 2018), bZIP (Kwon et al. 2018), LEC1 (Liu et al. 2021), LEC2 (Liu et al. 2022), WRI1 (Shang et al. 2022), FUS3 (Elahi et al. 2016) are involved in the regulation of gene expression for oil synthesis. WRI1, as a member of the AP2/EREBP family, is characterized by an N-terminal region abundant in threonine and serine, and a C-terminal region rich in acidic amino acids, comprising two signature AP2/EREBP protein domains (Cernac and Benning 2004). It serves as the primary transcription factor regulating the biosynthetic pathway of lipids. Studies have revealed that mutations in *wri1* gene can lead to an 80% reduction in Arabidopsis oil content (Focks and Benning 1998). The expression of *wri1* can significantly increase the oil content of soybeans (Chen et al. 2018), oil palms (Dussert et al. 2013), rapeseed (Wu et al. 2014), maize (Pouvreau et al. 2011). The overexpression of the *wri1* in *jatropha* not only boosted the oil content of seeds but also significantly enhanced oleic acid levels, substantially ameliorating the quality of biodiesel produced (Ye et al. 2018). There is a conserved coding exon of 9 bases in *wri1* homolog genes in diverse species, and the typical variant strain of Arabidopsis with the "VYL" mutation cannot recover the full oil content of seeds, indicating that "VYL" plays a crucial

role in Arabidopsis oil formation (Ma et al. 2013). However, the "VYL" is absent in WRI1 of castor bean but does not affect its ability to increase oil content when expressed in tobacco. In rice, "VYL" is replaced by "GCL", without affecting the activity of WRI1. The "VYL" motif seems to play a critical role in certain plants, but in others, it can be replaced by different amino acid residues without impacting the WRI1 transcription factor's activity (Ji et al. 2018, Mano et al. 2019). This suggests that "VYL" is not always essential for all WRI1 orthologs.

WRI1 orthologs have been identified in multiple plant species, including green algae species (Tang et al. 2019). Shang et al. identified a WRI1 transcription factor in *D. parva* that can significantly express under nitrogen-limiting conditions and can promote sugar conversion into lipids by regulating target genes associated with carbohydrate metabolism in photosynthesis, glycolysis, the tricarboxylic acid cycle, and starch metabolism, thereby enhancing lipid biosynthesis in *D. parva* (Shang et al. 2022). Lipid synthesis involves multiple subcellular pathways including glycolysis, fatty acid synthesis, triglyceride synthesis, etc., forming a complex metabolic network. Enhancing lipid accumulation necessitates not just augmenting the carbon flux towards lipid synthesis but also curtailing lipid consumption (Hui Chen 2021, Sánchez et al. 2022). The WRI1 transcription factor can not only regulate the synthesis of enzymes within in the pathway but also interact with other transcription factors to regulate lipid synthesis (Shen et al. 2010, Yeap et al. 2017). As significant microalgae for oil production, no reports on the presence or function of WRI1 in *A. protothecoides* have been documented to date. In this study, the *wri1* from *D. parva* was expressed in *A. protothecoides* to study its regulation mechanism of lipid accumulation in *A. protothecoides*, laying a foundation for further study of WRI1 in microalgae.

MATERIALS AND METHODS

Materials

A. protothecoides and *D. parva* were acquired from the Freshwater Algae Culture Collection at the Institute of Hydrobiology. The expression vector pCAMBIA1300-35S-EGFP and *Agrobacterium tumefaciens* EHA105 receptor cells were purchased from the Biotransduction Lab Co. Ltd in Wuhan.

Culture of *A. protothecoides* and *D. parva*

A. protothecoides and *D. parva* cultured in BG11 and DM media (solid medium with 1.8% agarose) at 25°C and 40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ under a 12:12 h d⁻¹ cycle.

Antibiotic Sensitivity Assessment for *A. protothecoides*

The BG11 medium containing various concentrations of hygromycin B and cephalosporin were utilized to cultivate *A. protothecoides*. The optimal antibiotic concentrations for selecting transgenic *A. protothecoides* were determined by monitoring growth for 14 days.

Construction of the Dpwri1 plant expression vector

The full-length cDNA of *wri1* (GenBank No: KR185335.1) was amplified from *D. parva* using the following primers: Dpwri1-F, 5'-TACGAACAAACACACCTCTG-3', and Dpwri1-R, 5'-GCTCTGCTAGTTACTCTCCG-3'. The amplicons were then cloned into the PMD19-T vector and sequenced. Finally, *Dpwri1* was digested using restriction enzymes *Kpn I* and *Xba I*, and inserted into the vector pCAMBIA1300-35S-EGFP between *Kpn I* and *Xba I*.

Genetic transformation of *A. protothecoides*

The recombinant plasmid pCAMBIA1300-*Dpwri1* was transformed into *Agrobacterium tumefaciens* EHA105 using the freeze-thaw method, with successful transformants selected for subsequent *A. protothecoides* infection. *A. protothecoides* was precultured on BG11 solid medium supplemented with 100 µmol/L acetosyringone. The *Agrobacterium tumefaciens* containing the pCAMBIA1300- *Dpwri1* plasmid was cultured in LB liquid medium at 28 °C overnight. The cells were collected by centrifugation and resuspended in BG11 liquid medium supplemented with acetosyringone. The resuspended bacteria were evenly spread onto the culture dish with *A. protothecoides* and incubated 48 h at 28 °C in the dark. *A. protothecoides* was scraped off and washed with BG11 liquid medium containing 500 µg/mL cefotaxime. Then, 200 µL of the algal suspension was spread onto the BG11 solid medium containing hygromycin B for selection. Positive algal colonies were isolated, subcultured three times, and subsequently confirmed by PCR, RT-PCR and Western blot. Western blot was conducted using Tubulin as the housekeeping protein, with Goat Anti-Mouse IgG-HRP (Bio-Transduction Lab, Wuhan) serving as the secondary antibody, to indirectly characterize the expression of *Dpwri1* by assessing the expression of the fusion protein GFP.

Growth analysis

The wild type and transgenic strain (*A. protothecoides*-*Dpwri1*) were cultured in fresh BG11 liquid medium, and samples were taken every other day to measure cell density (OD680), which was used to characterize their growth. The data were then analyzed and growth curves were plotted using Origin software(2021).

Lipid content analysis

Following the methods of Zheng (Zheng et al. 2022) and Rumin (Judith et al. 2015), the relative lipid content within algae cells was assessed via Nile red staining method. The lipid droplets in algal cells were observed using an Olympus IX73 inverted microscope. Fluorescence intensity was quantified using a spectrophotometer with excitation and emission wavelengths set at 534 nm and 578 nm, respectively. The ratio of fluorescence intensity to cell density (OD680) was employed to determine the relative oil content of algal cells.

The total oil content of algal cells was determined following the method described by Liu (Liu et al. 2022). Cells were harvested and dried to constant weight at 80°C, and then extracted with chloroform–methanol mixture (2:1, v/v) for 4 h. After ultrasonic extraction for 1 hour, the residue was collected by

centrifugation at 8000 rpm for 8 min, and then dried at 60 °C until a constant weight was achieved. The oil content was calculated using the following equation: $Y (\%) = (W_p - W_r) / W_p$, where W_p and W_r were the weights of algal powder and residue, respectively.

RNA Sequencing Analysis

At the culmination of the logarithmic phase, *A. protothecoides* cells were harvested by centrifugation (4°C, 10 min, 8000 g). Transcriptome analysis was conducted by Bgi Genomics Co., Ltd. The raw data was filtered with SOAPnuke (v1.5.2) (Chen et al. 2017) by 1) Removing reads containing adapters (adapter contamination); 2) Removing reads whose unknown base ('N' base) ratio is more than 10%; 3) Removing reads whose low-quality base ratio (Base quality less than or equal to 15) is more than 50%, afterwards clean reads were obtained and stored in FASTQ format. The clean data were mapped to the reference genome by HISAT (v2.1.0) (Kim et al. 2015). The clean data were mapped to the assembled unique gene by Bowtie2 (v2.2.5) (Langmead and Salzberg 2012). The expression level of genes was calculated by RSEM (v1.2.8) (Li and Dewey 2011). Annotating assembled Unigene with seven major functional databases (KEGG, GO, NR, NT, SwissProt, Pfam, and KOG). Within-group differential gene analysis was performed using DESeq (Wang et al. 2010) under the conditions of Fold Change ≥ 2 and Adjusted P value ≤ 0.001 . PossionDis was performed between-group differential gene analysis under the conditions of Fold Change ≥ 2 and FDR ≤ 0.001 . Using the pheatmap function on the differential gene set to draw a heatmap of differential gene clusters. According to the GO and KEGG annotation results and classifications, the differentially expressed genes (DEGs) were functionally classified, the phyper in R software was used for KEGG enrichment analysis, and the TermFinder package was used for GO Enrichment analysis (<https://metacpan.org/pod/GO::TermFinder>). With a Q value of ≤ 0.05 as the threshold, candidate genes that met this condition were defined as significantly enriched.

DEGs Validation Study

Confirmatory analysis of expression levels of filtered DEGs involved in lipid accumulation from RNAseq experiments was performed using Real-Time PCR (qPCR), according to the $\Delta\Delta C_q$ relative quantification method applied for proper transcriptional biomarker validation.

RESULTS

Antibiotic sensitivity of *A. protothecoides*

Antibiotic sensitivity analysis of *A. protothecoides* revealed that the growth of this algal species on BG11 solid culture was entirely inhibited at a hygromycin B concentration of 40 µg/mL, while 200 µg/mL was required to achieve the same effect in liquid culture. However, varying concentrations of cefotaxime had no significant inhibitory impact on the growth of *A. protothecoides*. Therefore, hygromycin B can serve as a selection marker for transgenic algal strains, while cefotaxime can be employed to eliminate *Agrobacterium*.

Obtain of *wri1* transgenic strains

The *Dpwri1* gene was cloned from *D. parva* and introduced into *A. protothecoides* via *Agrobacterium tumefaciens*-mediated transformation. Subsequent to transformation, positive clones were selected and their integration of the transgene was confirmed through PCR, RT-PCR, and Western blot analysis. Agarose gel electrophoresis was utilized to detect PCR and RT-PCR amplification products. The results are presented in Fig. 1. A and B. The transgenic algae strains produced a single, distinct fragment approximately 1000 bp in size. In contrast, no bands were observed in the wild type strains. Likewise, Western blot showed that the specific bands for DPWRI1 were obtained from *A. protothecoides-Dpwri1*, whereas no bands were detected in the wild type strains.

Effect of Dpwri1 expression on the growth of *A. protothecoides-Dpwri1*

Growth curves were established to investigate the growth of *A. protothecoides-Dpwri1* and the wild type. Both strains showed initial slow growth in the first two days, followed by a rapid proliferation between the 3rd and 8th days, and a subsequent slowdown on the 10th day (Fig. 2). The growth curves of *A. protothecoides-Dpwri1* and wildtype (WT) were found to be similar, indicating that the expression of *Dpwri1* did not significantly affect the growth of *A. protothecoides*.

The expression of Dpwri1 increases the lipid content of *A. protothecoides-Dpwri1*

The wild and transgenic algae were collected at the stationary phase of cultivation to determine their relative lipid content. The relative lipid content of *A. protothecoides-Dpwri1* was 140.34, significantly higher than that of the wild type (107.93). Subsequently, the cells were stained with Nile red and observed under an inverted microscope. The observations revealed that the transgenic algae displayed a greater concentration of larger orange lipid droplets within the cells as opposed to the wild type (Fig. 3), which reveals that the *A. protothecoides-Dpwri1* had a higher accumulation of lipid droplets.

To accurately assess the oil content of algal cells, the total oil content was analyzed, which revealed that the oil content of the transgenic algae was 26.7% (Fig. 4), 38.3% higher than that of the wild type. All these suggests that the expression of *Dpwri1* promoted the accumulation of oil in *A. protothecoides-Dpwri1*.

Transcriptome analysis

To better elaborate the regulation mechanism of DpWRI for oil accumulation in *A. protothecoides-Dpwri1*, transcriptome analysis was investigated. In total, 29626 transcripts were identified, of which 28164 transcripts were quantitated. Compared with wild type, 268 transcripts were regulated, including 125 up-regulated and 143 down-regulated transcripts (Fig. 5a). Based on the GO terms level 1 of biological process, approximately 36% of regulated transcripts were involved in the metabolic process, suggesting that higher metabolic differences occurred in *A. protothecoides-Dpwri1* (Fig. 5b).

Table 1 displays the DEGs involved in lipid metabolism. Among these, several key enzymes were up-regulated, including glucose-6-phosphate isomerase (GPI), glyceraldehyde-3-phosphate dehydrogenase

(GAPDH), phosphoenolpyruvate carboxykinase (PEPCK), enoyl-acyl carrier protein reductase (ER), and diacylglycerol acyltransferase (DGAT). The up-regulation of these genes is beneficial to the flow of carbon to the synthesis of lipids. While transcriptomic expression levels of acyl-CoA oxidase (ACOX) and acetyl-CoA acetyltransferase (ACAT1) were down regulated significantly. The downregulated genes reduced the rate of fatty acid degradation, thus enhancing oil accumulation in *A. protothecoides-Dpwri1*.

Table 1
Genes with significant differences in expression related to oil accumulation.

Gene ID	log2FoldChange	KEGG Pathway	Gene Definition
CL694.Contig2_All	8.3	Glycolytic	GPI
CL383.Contig11_All	8.6	Glycolytic	GAPDH
CL1489.Contig4_All	20.8	Glycolytic	PEPCK
CL257.Contig14_All	19.9	Fatty acid biosynthetic process	ER
CL1287.Contig11_All	8.6	Triglyceride synthesis	DGAT
CL1290.Contig6_All	-22.6	Fatty acid β -oxidation	ACOX
CL928.Contig6_All	-22	Fatty acid β -oxidation	ACAT1

The protein–protein interaction (PPI) networks of DEGs

To gain insight into the potential interactions among all the differentially expressed genes, we retrieved the protein-coding information of 67 genes from the STRING platform and conducted a thorough analysis of their relationships. This analysis revealed a PPI enrichment p-value of 4.99E-04, indicating biological relevance. To visualize the PPI network, we utilized Cytoscape 3.10.1 software, which enabled a topological analysis of the input results (Fig. 6). In Fig. 6, Nodes represent genes, with the size and color intensity reflecting their degree score. The width and color of Edges reflecting their combined score. ER, DGAT and ACAT exhibit the highest degree and combined. Notably, the genes ER, DGAT, and ACAT emerged as the most highly correlated genes, exhibiting strong biological relevance. In fact, ER and DGAT are crucial enzymes involved in the triglyceride synthesis pathway, while ACAT plays a role in the β -oxidation of fatty acids. Enhanced expression of ER and DGAT, along with reduced expression of ACAT, facilitates the accumulation of oil in algal cells.

Real-Time PCR Validation

Real-Time PCR was performed to verify the substantial changes in gene expression levels as indicated in Table 1. Primers sequence are reported in Supplementary Material S1. The results demonstrated significant upregulation of genes such as GPI, PEPCK, DGAT, and ER, as well as a notable downregulation of CAAT1 and ACOX genes, as depicted in Fig. 7. It suggests that the *Dpwri1* expression in *A. protothecoides* exerts a stable regulatory effect on genes involved in lipid accumulation.

DISCUSSION

A. protothecoides has high economic value, and the regulation study of its oil synthesis is of great significance. The transcription activator WRI1 plays a crucial role in regulating oil synthesis. Previous studies in *Arabidopsis thaliana* have demonstrated that WRI1 can activate the expression of genes associated with glycolysis and fatty acid synthesis, thereby promoting oil synthesis. In this study, we focused on the expression of enzymes involved in glycolysis, oil synthesis, and fatty acid metabolism pathways.

Enzymes involved in the glycolysis pathway, such as GPI, GAPDH, and PEPCK, were significantly upregulated. GPI can catalyze the conversion of G6P to fructose-6-phosphate (F6P). F6P is subsequently converted into fructose-1,6-bisphosphate (F-1,6-BP) and then into pyruvate through a series of enzyme reactions, with pyruvate further participating in lipid synthesis. GAPDH catalyzes the conversion of glyceraldehyde-3-phosphate (GAP) into 1,3-bisphosphoglycerate (1,3-BPG). Yao et al. (Yao et al. 2014) found that the overexpression of GAPDH in *Phaeodactylum tricornutum* increased the neutral lipid content of algal cells by about 60%, indicating that upregulation of GAPDH may promote lipid accumulation in cells by enhancing the synthesis of 3-phosphoglycerate. Additionally, GAP inhibits the activity of PEPCK. In the *A. protothecoides-Dpwri1* strain, the upregulation of GAPDH promotes the conversion of GAP to 1,3-BPG, increasing the expression of PEPCK indirectly. In algae, PEPCK mainly plays a decarboxylation role, catalyzing the decarboxylation of oxaloacetic acid (OAA) to produce phosphoenolpyruvate (PEP) (Torresi et al. 2023). The upregulation of PEPCK reduces the flow of acetyl-CoA to the TCA cycle. On one hand, PEP can be converted into dihydroxyacetone phosphate, which is then converted into 3-phosphoglyceric acid by 3-phosphoglycerate dehydrogenase, entering the triglyceride synthesis pathway; on the other hand, PEP can be converted into pyruvate by pyruvate kinase, which is then catalyzed by pyruvate dehydrogenase to produce acetyl-CoA, entering the fatty acid synthesis process. Therefore, the expression of *Dpwri1* in *A. protothecoides-Dpwri1* allows more carbon to flow into fatty acid synthesis via upregulating the expression of GPI, GAPDH, PEPCK and ER, as illustrated in Fig. 8.

DGAT, the ultimate enzyme in the triglyceride synthesis pathway, is a rate-limiting factor that catalyzes the formation of triacylglycerols from diacylglycerols and acyl-CoA (Guo et al. 2017). Studies have shown that the expression of DGAT is strongly correlated with lipid accumulation in *Chlorella*. Under nutrient stress conditions, the expression of DGAT is significantly up-regulated, and the oil content of algal cells increases. The heightened expression of DGAT is beneficial for the accumulation of lipids in *Chlorella* (Liufu et al. 2023, Rincon et al. 2019, Fan et al. 2014). According to the research of Shang et al. (Shang et al. 2022), *dgat* is a target gene of WRI1-like in *D. parva*. In this study, DpWRI1 significantly up-regulated the expression of *dgat* in the *A. protothecoides-Dpwri1*, promoting the accumulation of triacylglycerols (Fig. 8).

In *A. protothecoides-Dpwri1*, the expression of ACOX and ACAT1, which are involved in the β oxidation pathway of fatty acid, was significantly down-regulated. ACOX catalyzes the conversion of acyl-CoA to

Δ^2 -trans-enoyl-CoA, and is a rate-limiting enzyme in β oxidation. The down-regulation of ACOX reduced the degradation of fatty acids (DuPlessis et al. 1998). ACAT1, also known as peroxisomal 3-ketoacyl-CoA thiolase, is part of the thiolase family and primarily participates in the final step of β oxidation. It catalyzes the formation of acetyl-CoA and acyl-CoA (less 2C) from β -ketoacyl-CoA, which is an important enzyme in regulating fatty acid β oxidation (Sharma et al. 2023). The downregulation of ACOX and ACAT1 inhibits the β oxidation of fatty acids in *A. protothecoides-Dpwri1*, promoting the accumulation of fatty acids in algal cells (Fig. 8).

In recent years, there has been a significant increase in research focusing on the regulation of oil accumulation mediated by the transcription factor WRI1. Overexpression of GmWRI1a has demonstrated the ability to enhance the total oil and fatty acid content in soybean seeds (Chen et al. 2018), and transient expression of HaWRI1 from sunflower in *Nictiana benthamiana* leaves was reported by Lim et al (Lim et al. 2022). to promote TAG biosynthesis. The expression of HA-EgWRI1 from the oil palm mesocarp has successfully reversed the low oil content observed in *wri1* mutant (Ma et al. 2013). Li et al. (Li et al. 2022) overexpressed LuWRI1a from flax in Arabidopsis, leading to a significant increase in oil content in seeds, reaching up to 23.3%, which is comparable to with our study (26.7%). However, the expression of Arabidopsis WRINKLED1 in potato tubers resulted in a 20-fold increase in TAG content. Despite variations in the magnitude of WRI1's regulation of oil accumulation across different crops, all studies consistently demonstrate the beneficial effect of WRI1 expression on oil accumulation in the host. Kuczynski et al. (Kuczynski et al. 2022) conducted a comprehensive scan of the DNA binding sites of WRI1 and developed a conserved regulatory blueprint, which predicts potential targets involved in glycolysis and fatty acid biosynthesis regulated by WRI1. Among the predicted targets, GPI, DGAT, and ER are identified as target genes of WRI1, which establishes a theoretical foundation for the upregulation of GPI, DGAT, and ER expression in *A. protothecoides-Dpwri1*.

CONCLUSION

WRI1 is a crucial transcription factor regulating oil accumulation in plants. In this study, the *Dpwri1* gene of *D. parva* was transferred into *A. protothecoides* using Agrobacterium mediated transformation. An analysis of oil content revealed that the oil content of the transgenic algal strain *A. protothecoides-Dpwri1* increased by 38.3%. The transcriptomes analysis demonstrated that the expression of GPI, GAPDH, PEPCK, DGAT, and GDPD involved in glycolytic pathway, triglyceride synthesis, and GPLs metabolism pathway were up-regulated respectively, allowing carbon to flow towards the synthesis of lipids. Additionally, the expression of fatty acid β -oxidation rate-limiting enzymes ACOX and ACAT1 were down-regulated, which is beneficial for the accumulation of lipids in cells.

Declarations

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical Approval

This research was exempt from the requirement of ethics committee review or approval because it did not entail the utilization of patient information or the involvement of experimental animals.

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Author Contribution

Conceptualization, Weiwei Xue and Yongchang Xue; methodology, Yibo Zhang and Xiaoyun Feng; writing—original draft preparation, Yanhong Qiu; writing review and editing, Changbin Liu; funding acquisition, Han Wu.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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Figures

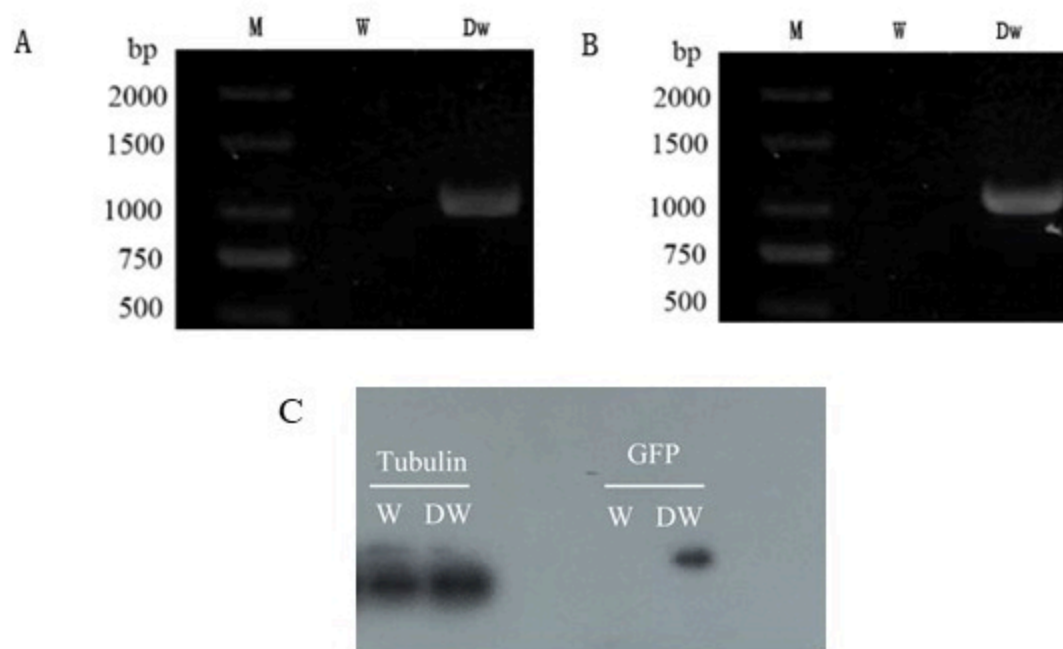


Figure 1

The identify of transgenic strain. A: PCR, B: RT-PCR, C: Western blot; M: DL2000 DNA Marker, W: The wild strain, Dw: *A. protothecoides-Dpwri1*.

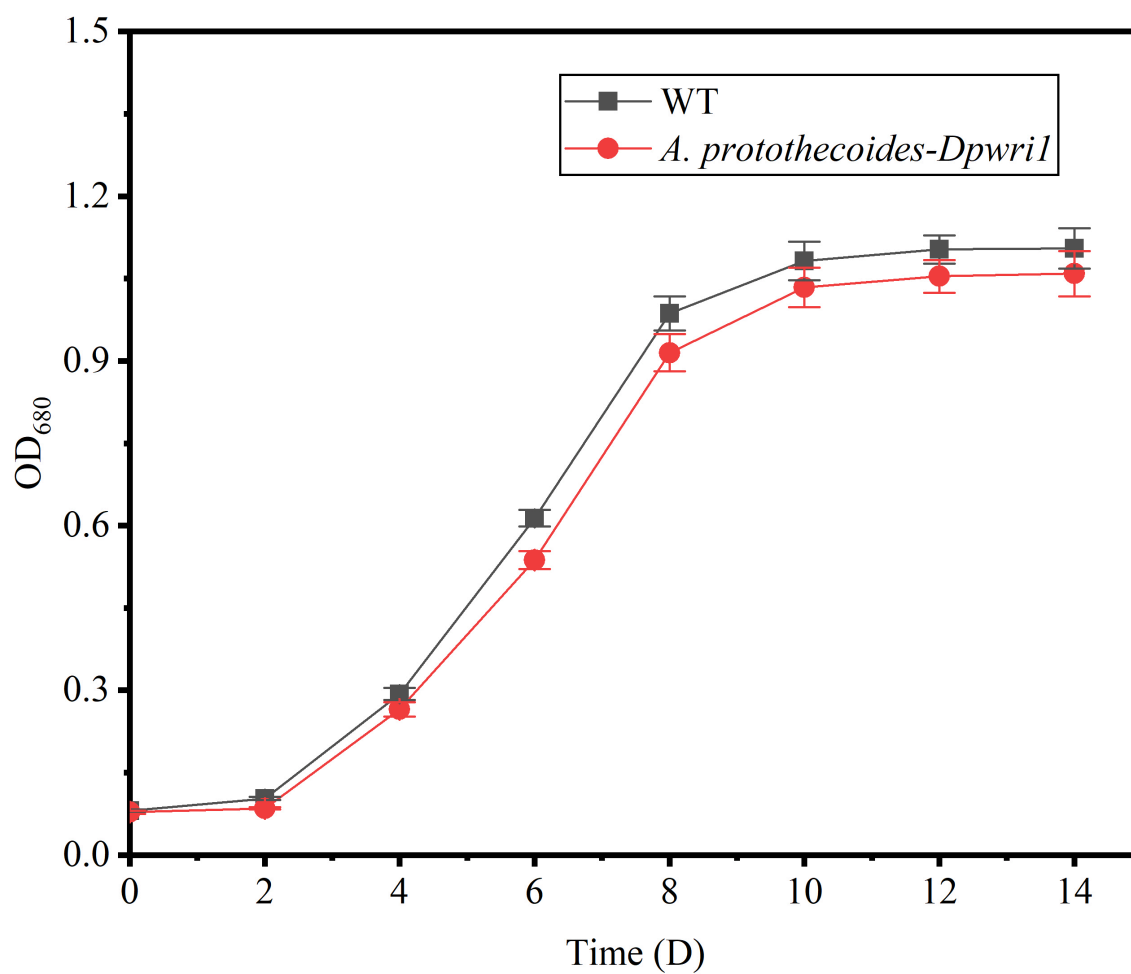


Figure 2

The growth curve of *A. protothecoides-Dpwri1* and wild strain.

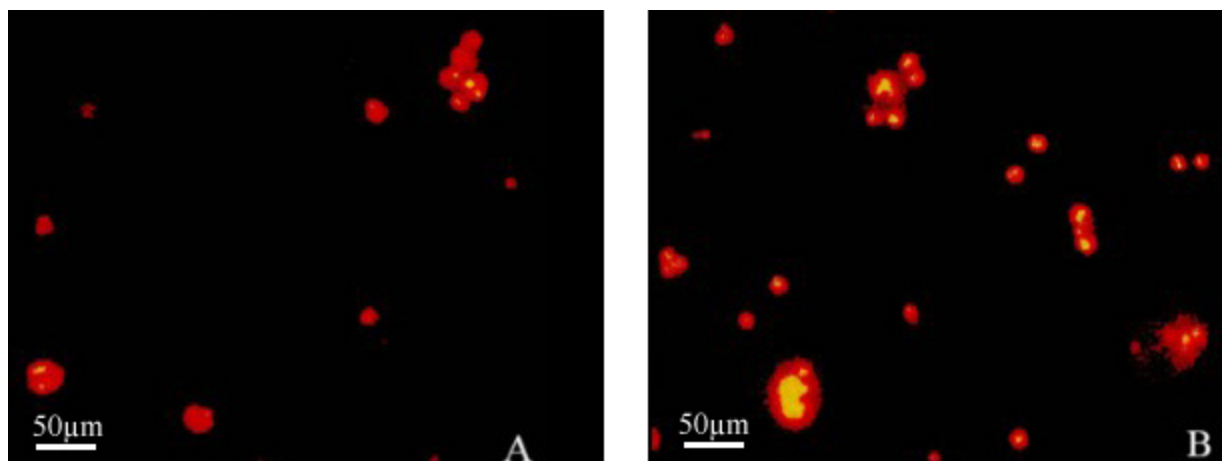


Figure 3

The Olympus IX73 inverted microscope observation image. A: Wild strain, B: *A. protothecoides-Dpwri1*

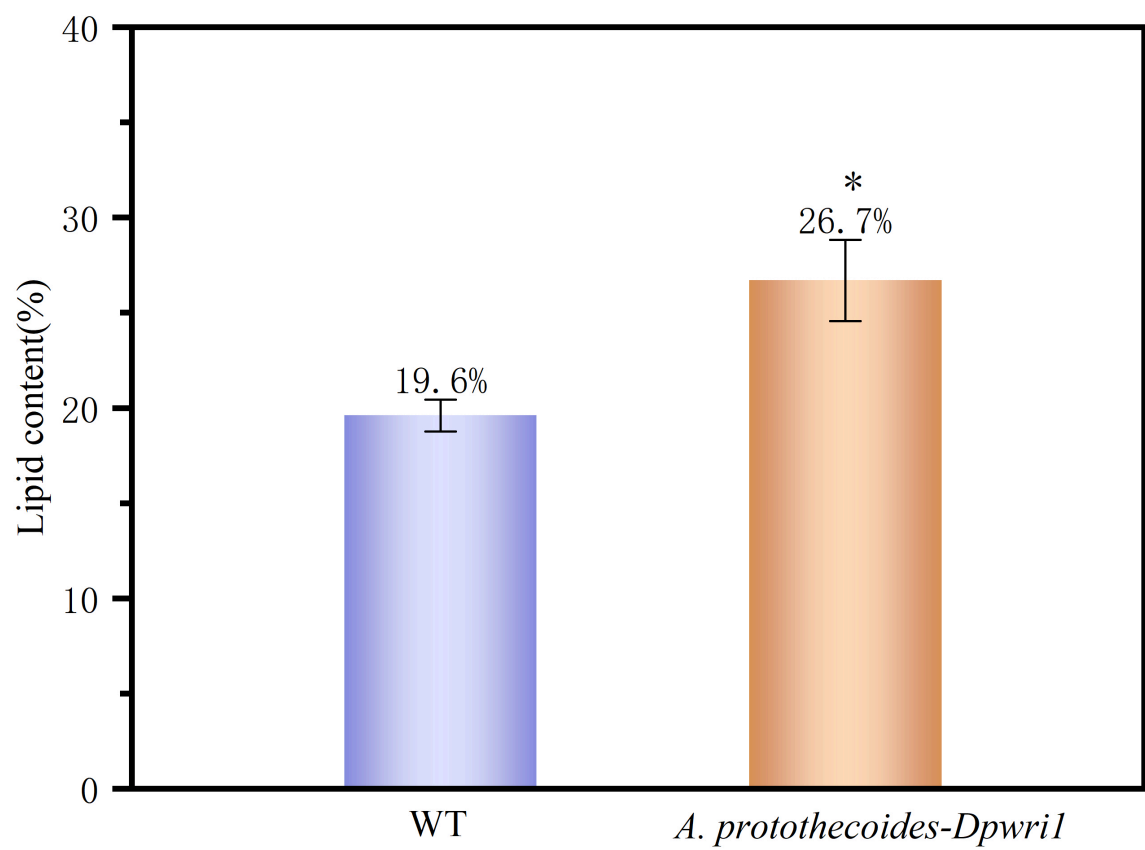


Figure 4

The oil accumulation of Wild and transgenic strain.

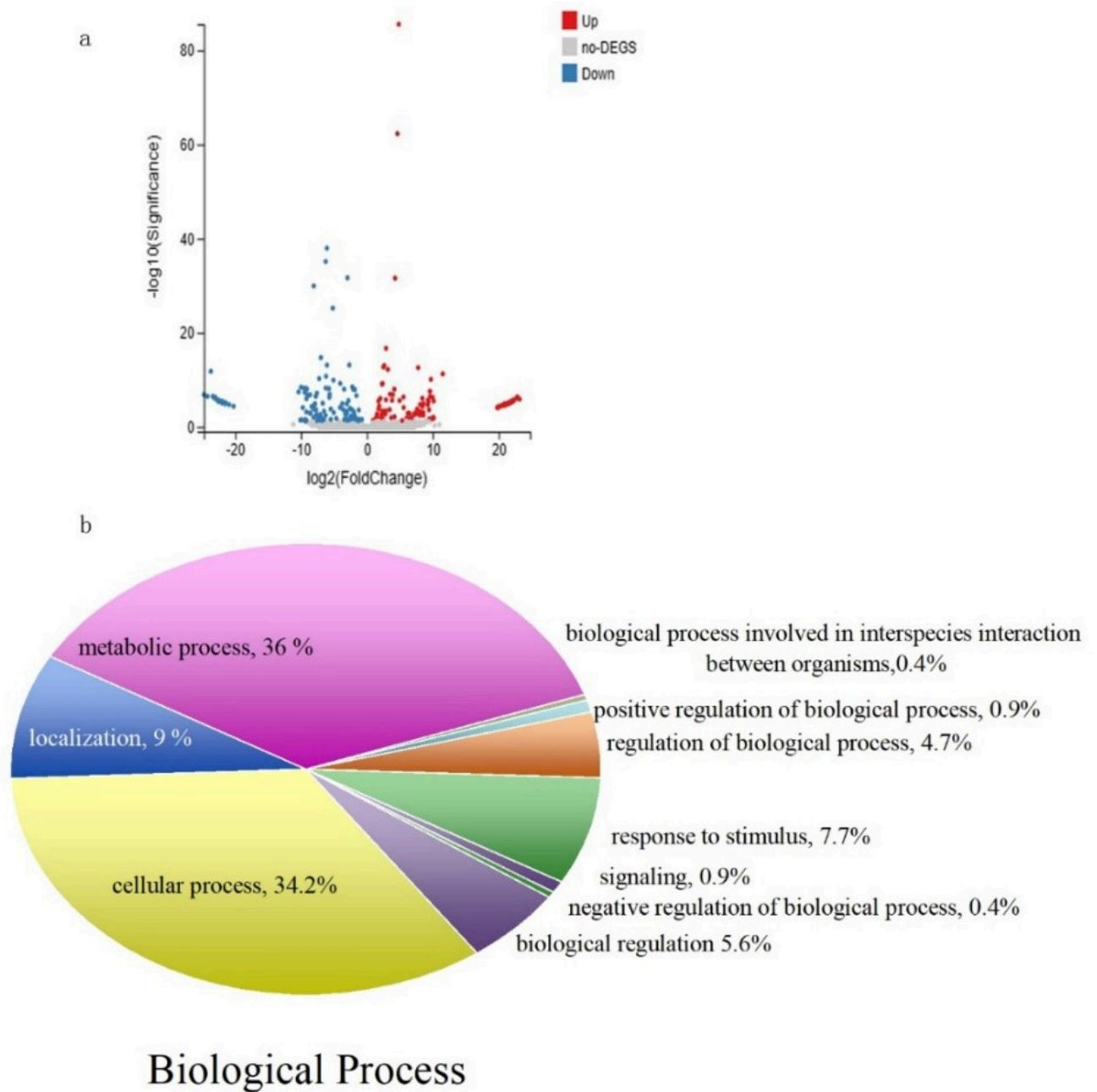


Figure 5

The comparison of genes expression changes through the transcriptome experiment. a: Volcano map of DEGs. b: Proteins related with biological process detected through the transcriptome experiment which were classified using the Gene Ontology (GO) classification method.

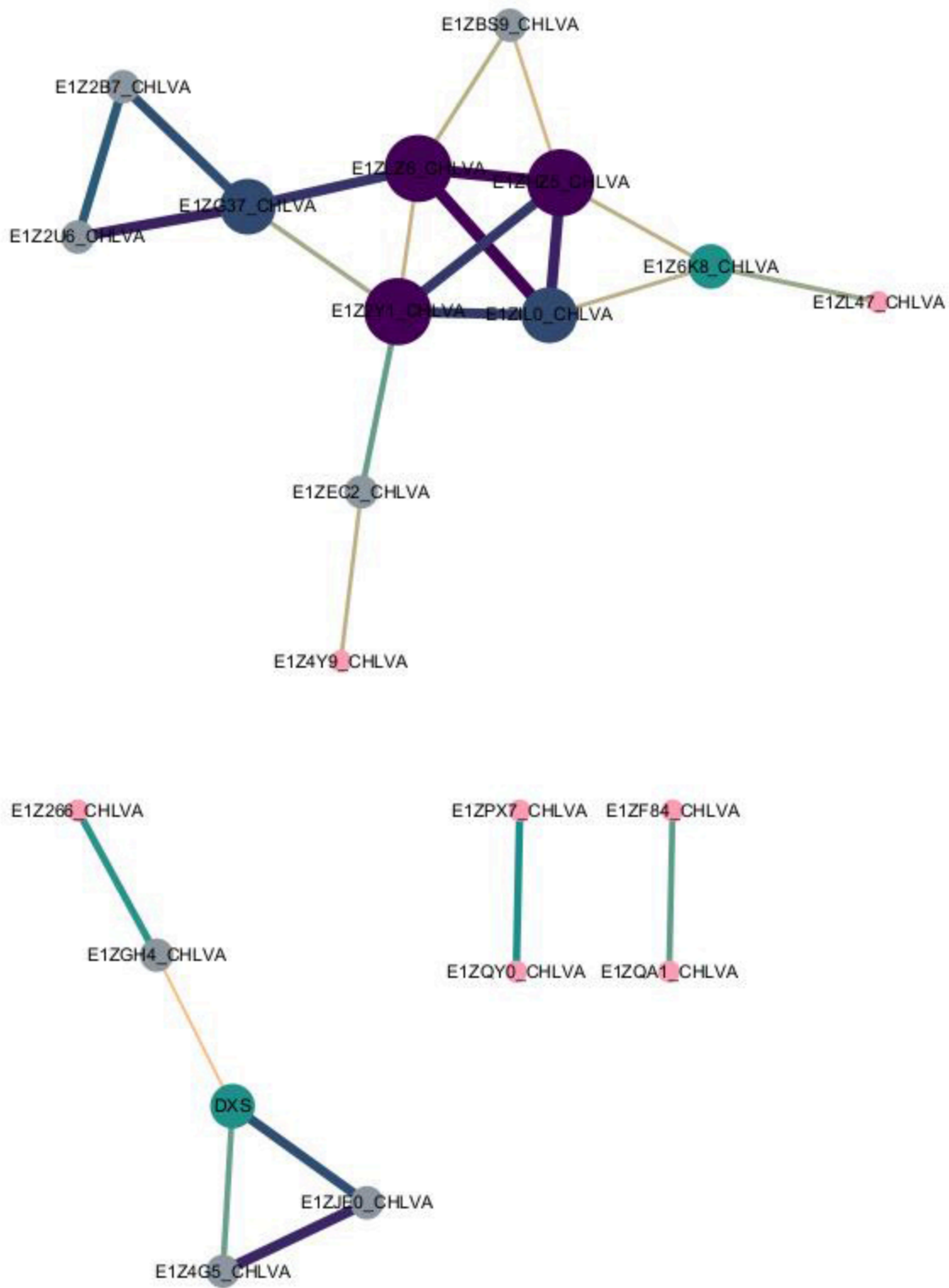


Figure 6

The PPI of DEGs in *A. protothecoides-Dpwri1*.

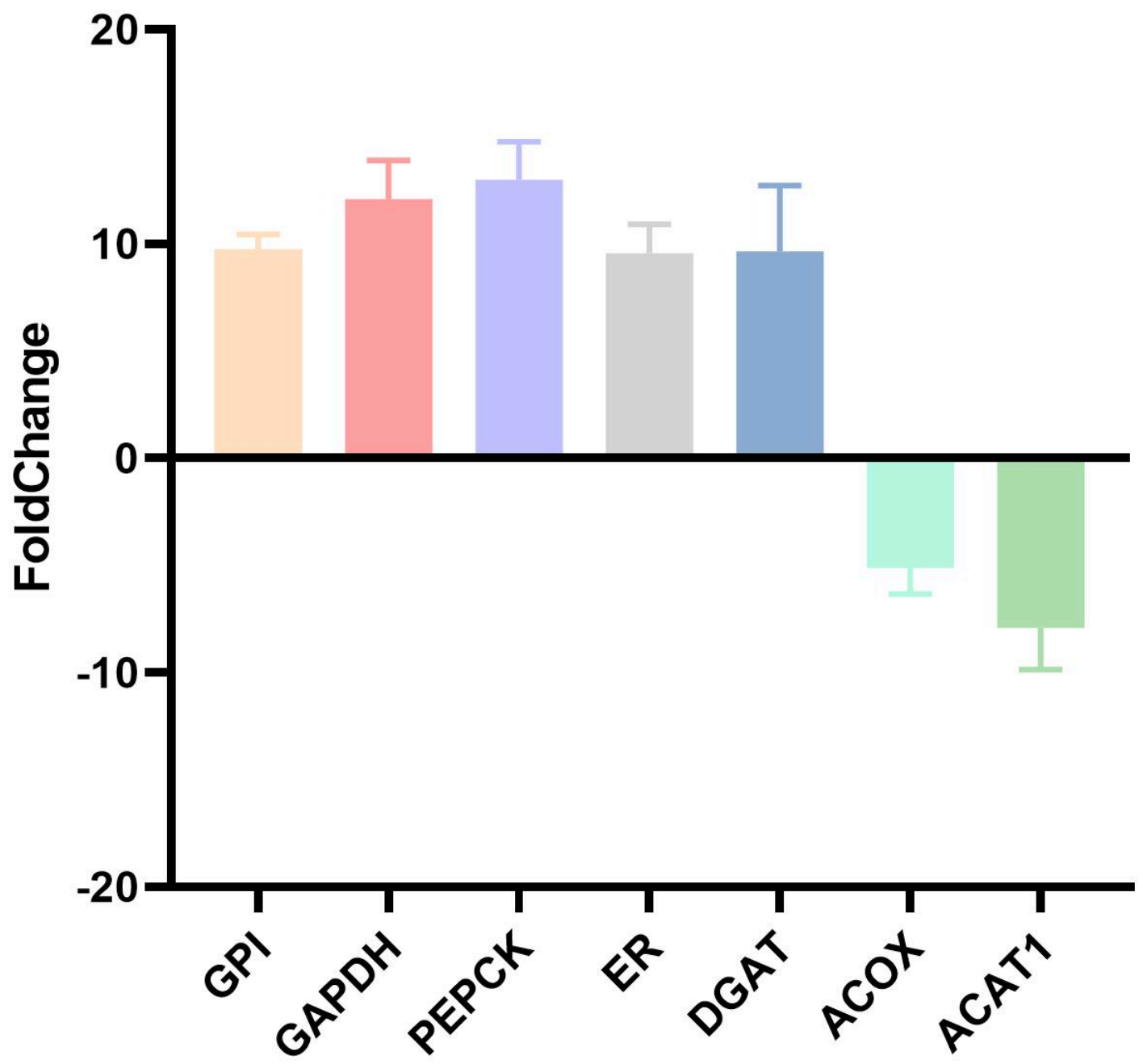


Figure 7

The Real-Time PCR Validation of DEGs involved in lipid accumulation

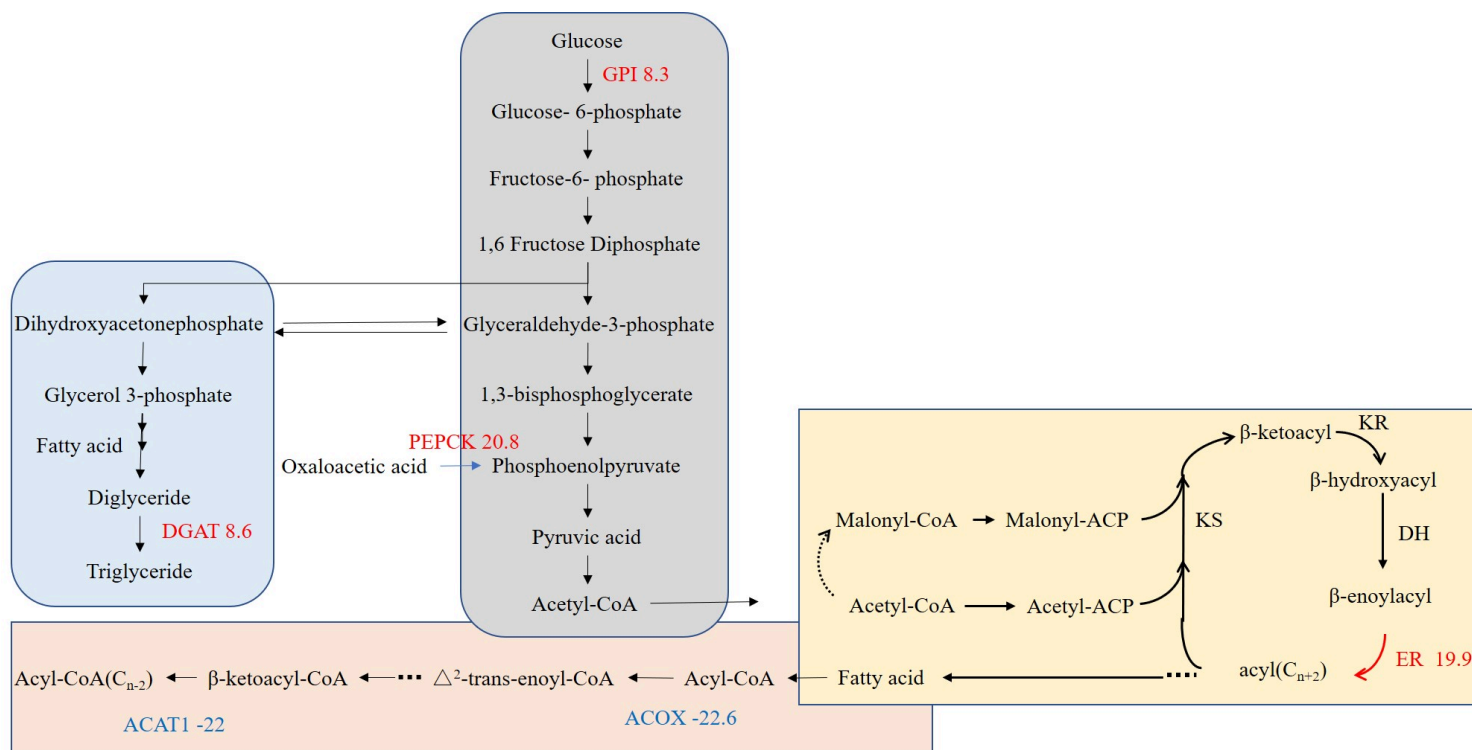


Figure 8

DpWRI1 regulation network on oil accumulation in *A. protothecoides*-*Dpwri1*. The upregulated proteins are shown in red, the downregulated in blue. The numbers are log₂FoldChange of DEGs.

Supplementary Files

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- [SupplementaryMaterialS1.xlsx](#)