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Proteomics Research Reveals the Molecular Mechanism by Which Grape Seed Oil Inhibits Tuber Sprouting in Potato

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Abstract: Potato tubers are rich in starch, vitamins, protein, minerals, and other nutrients. However, tuber sprouting produces solanine and reduces the commodity value of potatoes during storage. At present, it is known that some plant essential oils can inhibit tuber sprouting. It has been reported that grape seed oil (GSO) has antioxidant, anti-inflammatory, and anticancer characteristics, reducing blood lipids and delaying aging. In this study, we found for the first time that GSO delayed tuber sprouting, and the soluble sugar content and peroxidase activity changed after 60 days of GSO treatment. Furthermore, a comparative proteomic analysis of tuber bud eyes showed that after 30 days of GSO treatment, there were 206 and 129 differentially abundant proteins (DAPs) with increased and decreased abundance levels, respectively. After analysis, we found that 15 ROS-related proteins and 14 proteins involved in energy metabolism were DAPs. Among them, gamma aminobutyrate transaminase 1 had decreased abundance after GSO treatment. Meanwhile, the transcription level of genes related to GABA synthesis increased significantly according to qRT-PCR analysis. Our results provide new approaches to the proteomic mechanism of potato sprouting after GSO treatment and provide a theoretical basis for the application of GSO in inhibiting potato seed sprouts.

Keywords: potato; tuber; grape seed oil; sprouting; proteomics

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1. Introduction

The potato (*Solanum tuberosum* L.) is an annual solanaceous plant with high yield and rich nutrition features. It is an important staple food in the world. Using physical, chemical, or genetic methods to study potato storage dormancy is critical for potato storage [1,2]. Low temperature (2–5 °C) conditions and ultraviolet-C irradiation can extend tuber dormancy [3,4]. Chlorpropham (isopropyl N-3-chlorophenyl carbamate; CIPC) is employed worldwide to stop stored commodity potatoes from sprouting. However, its use results in pesticide residues in potatoes, and the market tolerance of pesticide residues in food is declining [5,6].

A number of bioactive compounds, plant essential oils, and derivative products have been reported to have an effect on keeping agricultural products fresh [7,8]. Some researchers have found that essential oils inhibit potato sprouting. In addition, the essential oils of citronella, caraway, peppermint, coriander, eucalyptus, and garlic are also effective in suppressing potato sprouting and prolonging the storage life of potato tubers [9–11]. Further studies have found that fumigation with garlic essential oil can decrease α-amylase activity to suppress potato tuber sprout growth [11].

Grape seed oil (GSO) has been shown in many studies to have anti-inflammatory, antioxidant, antimicrobial properties, and antiapoptotic activities [12]. GSO is capable of scavenging reactive oxygen species (ROS), inhibiting protein oxidation, and inhibiting lipid

oxidation [13]. ROS are involved in plant growth and stress responses as vital signaling molecules [14], and other studies have reported that superoxide anions and H_2O_2 in buds are increased during the release of potato tubers from dormancy [15]. A recent study suggested GSO as a natural fumigant agent to protect corn during storage [16].

γ-Aminobutyric acid (GABA) is indeed an amino acid that is widely distributed in vertebrates, plants, and microorganisms [17]. In plants, GABA is metabolized through a pathway called the GABA shunt, which serves as a bypass or diversion of the tricarboxylic acid (TCA) cycle [18]. In plants, GABA is synthesized from glutamate in a reaction catalyzed by the enzyme glutamate decarboxylase (GAD) located in the cytoplasm and subsequently catabolized to succinate through two consecutive reactions catalyzed by GABA transaminase and succinic semialdehyde dehydrogenase [19,20]. GABA is primarily a metabolite in plants, involved in various metabolic processes. However, recent research has also uncovered its role as a signaling molecule in plants, participating in plant responses to modulating carbon and nitrogen metabolic fluxes [21], stomatal opening [22], root growth [23,24], fruit ripening [25,26] and seed germination [27,28]. In potatoes and pears, GABA inhibits the browning process by regulating antioxidant enzyme activities [29,30]. Baranzelli et al. reported that during seed germination, the endogenous GABA content increases [31]. Exogenous GABA can affect the germination process of barley seeds [27].

However, the impact of GSO on potato tuber growth and development is rarely reported. This study utilized proteomics to analyze the bud eye regions of tubers treated with GSO for 30 days, aiming to identify the important proteins involved in this process. After GSO treatment, changes in ROS-related proteins and proteins involved in the energy metabolism pathway were detected in the tubers. Furthermore, we focus on GABA, a protein involved in both energy metabolism and ROS pathways. Further research on GABA regulation of potato sprouting. Discovered that GSO treatment inhibits potato sprouting by regulating the synthesis of GABA. These results will further lay the foundation for studying the mechanism of potato sprouting regulation

2. Materials and Methods

2.1. Materials

Short-term dormancy potato variety "Favorita" original potato tubers, short-term dormancy potato variety "Chuanyu 5 "original potato tubers, and long-term dormancy potato variety "Qingshu 9" original potato tubers were provided by the College of Agronomy, Sichuan Agricultural University. GSO was purchased from Shanghai Pinwu Food Company, China.

2.2. Treatment of Potato Tuber

Three different varieties of potatoes were used for the experiment. First, two hundred well-developed tubers weighing approximately 80–120 g of each variety were selected and stored at 25 ± 2 °C for two weeks in the dark period to conduct the experiments. Then, twenty tubers were placed in 12 L boxes at approximately 23 °C in the dark for 150 days (4 treatments × 3 replications). The open centrifuge tubes were fixed with GSO (total 2.5 mL, 5 mL, and 10 mL) average in the four corners of the container, enabling the treatment of tubers through the effects of volatiles released by GSO (stored at 25 ± 2 °C). The control group was set up the same as the treated groups except that it was not treated with GSO treatment. After treatment for 30 d and 60 d, the bud eye regions with a 5 mm diameter × 7 mm height were picked, and the samples were frozen for subsequent research. The sprouting length of the tubers were measured on the 60th day, 120th day and 150th day.

Fifty potato tubers were immersed in 1 mM GABA (γ-aminobutyric acid, Sigma-Aldrich, China) solution for 24 h. After the potato tubers soaking treatment, the potato tubers were placed in a ventilated space for 1 day to allow them to fully dry and then placed in a box (5 L) in a dark room with the room temperature set to 23 ± 2 °C. The conditions of the control group were the same as for the treated groups except that H_2O replaced GABA for immersion of the tubers. The bud eye regions (height 5 mm × radius 7 mm)

were collected during placement on day 30 with an iron tube, placed in 2 mL centrifuge tubes, and immediately frozen in liquid nitrogen for long-term storage at −80 °C.

2.3. Measurement of POD Activity and Soluble Sugar

The quantitative determination of POD activity was carried out as Yang et al. described [32]. One POD activity unit (U) was defined as a change of 0.01 in absorbance of 0.01 at OD_{470} per minute. The specific activity of the enzyme was expressed as a U/g protein. The soluble sugar content was determined by the soluble sugar kit (Nanjing Jiancheng Biotech Co., Nanjing, China). Three biological replicates were performed in this study.

2.4. Proteomic Analysis

On the 30th day, tuber bud eye region samples were ground to powder in liquid nitrogen in different groups, CK and 10 mL GSO treatment. Weighing 100 mg of powder was thoroughly mixed with 500 µL of urea lysis buffer (8 M urea, 100 mM $NaHPO_4$, 10 mM dithiothreitol, 1% Triton-100, and 1% protease inhibitor cocktail pH8.5) [33]. First, the protein homogenates from the bud eye regions were diluted with 30 mM NH_4HCO_3 at a 5-fold dilution and then treated with 5 mM dithiothreitol at 55 °C for 45 min, followed by 11 mM iodoacetamide at 25 °C for 30 min in the dark. Then, the protein was digested with lysyl endopeptidase (Promega, Madison, WI, USA) at a 1:100 (w/w) ratio at 37 °C for 6 h. Subsequently, a second digestion was performed using trypsin (Promega, Madison, WI, USA) at a 1:50 (w/w) ratio for another 6 h. After trypsin digestion and drying, the peptides were dissolved in 0.5 M triethylammonium bicarbonate. The Tandem Mass Tag (TMT) labeling procedure was performed using a 16-plex TMT system (Thermo, Waltham, MA, USA) [11]. After that, the peptides were desalted with a Strata X C18 SPE column (Phenomenex, Torrance, CA, USA) and vacuum dried. A solvent containing 0.1% formic acid in water was used. On an EASY-nLC 1000 UPLC system, tryptic peptides were treated with 25% solvent B containing 0.1% formic. The peptides were subjected to tandem mass spectrometry (MS/MS) using a Q ExactiveTM Plus system (Thermo, Waltham, MA, USA) coupled to an online UPLC.

2.5. Bioinformatics Analysis of DAPs

The MaxQuant search engine (v.1.5.2.8, Matrix Science Inc., Boston, MA, USA) was used for the processing of the resulting MS/MS data. Proteins that exhibited changes of more than 1.2-fold or less than 0.83-fold were considered as DAPs (Differentially Abundant Proteins). First search, the mass tolerance was set at 20 ppm, for the main search at 5 ppm, and the fragmented ions at 0.02 Da. Peptides were required to score at least >40. The Blast2GO program was used to obtain DAP functional annotations. For the proteomic annotation, the Gene Ontology (GO) enrichment analysis was performed using the UniProt-GOA database, and the protein metabolic pathways were determined using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Using TargetP1.1, subcellular localization of DAP prediction was performed.

2.6. qRT-PCR Analysis

Total RNA was extracted from the bud eye region samples using TRIzol reagent (TaKaRa, Tokyo, Japan) and was used to generate cDNA with a reverse transcriptase kit (Thermo, Waltham, MA, USA). Gene expression levels were calculated using the formula $2^{-\triangle\triangle Ct}$. A CFX96 Touch Real-Time PCR System (Bio-Rad, Richmond, CA, USA) was used for qRT-PCR. The primer sequences are listed in Table S1.

2.7. Statistical Analysis

Data are expressed as the mean value ± SE (n = 3). Statistical analysis was performed using unpaired SPSS 14.0 software with Student's *t*-test followed by Bonferroni post-hoc test, and $p \leq 0.01$ and $p \leq 0.05$ were considered statistically significant.

3. Results

3.1. Effects of GSO Treatment on Potato Tuber Physiology

By treating three varieties of potato tubers with different concentrations of GSO, it was found that GSO treatment reduced sprout growth. We found that fumigating each variety of potato tubers with 10 mL of GSO significantly reduces potato sprout growth (Figure S1). Later, Favorita potato tubers were treated with 10 mL of GSO. Photographs of the sprouting potatoes were obtained after 60 d of storage, and the results suggested that tuber sprouts grew slower after 10 mL GSO treatment than after the control treatment (Figure 1A). Sprout length was measured, and on the 60th day, the average sprout length was 55 mm in the control group and 32 mm in the treatment group; thus, the treatment decreased sprout length by 1.71-fold. Therefore, GSO significantly inhibited tuber sprouting (Figure 1B). To measure the physiological responses of the bud eye regions to GSO treatment, soluble sugar content, and POD activity were measured; the soluble sugar content was decreased by 1.23-fold (Figure 1C), and the POD activity was increased by 1.22-fold (Figure 1D).

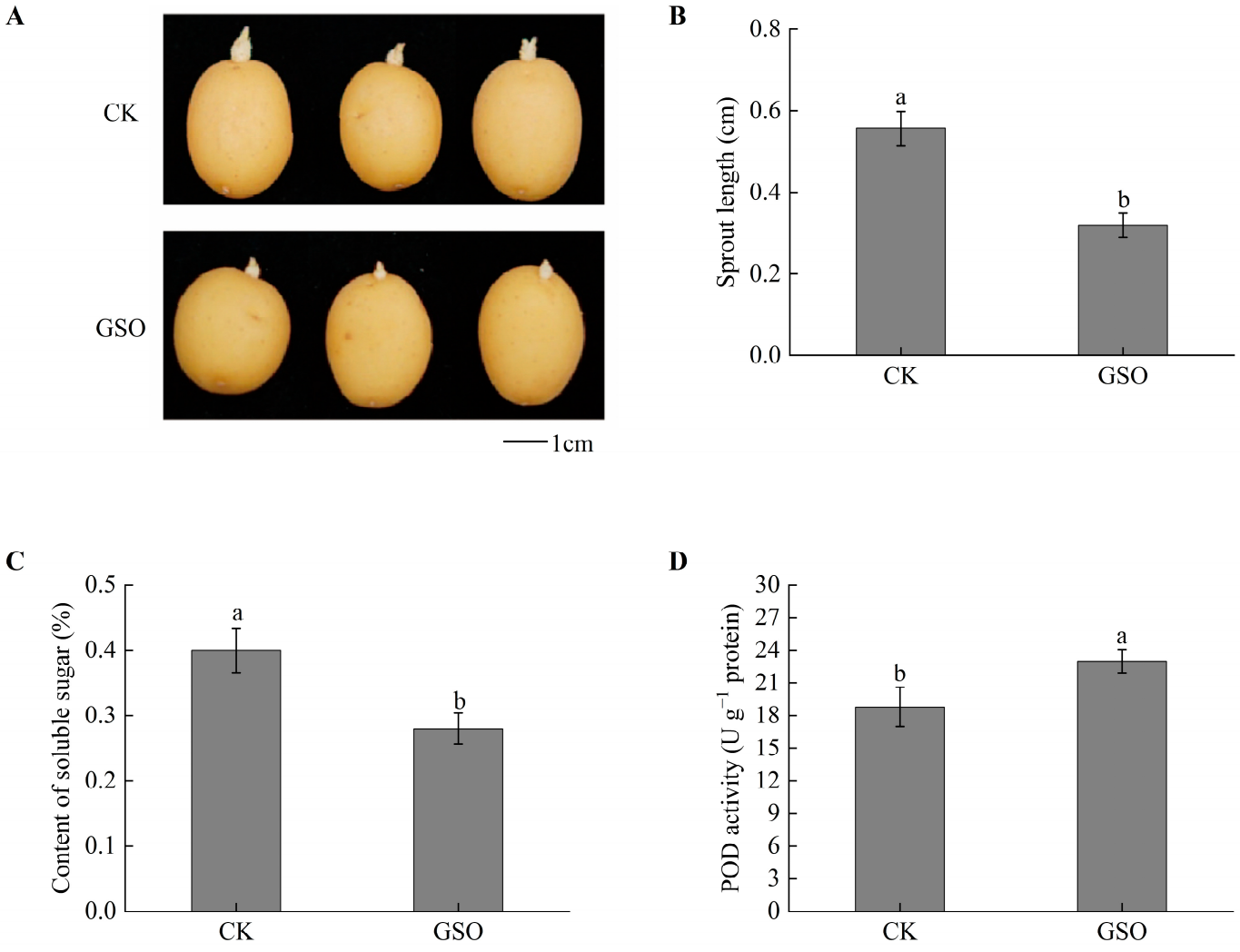

Figure 1. Grape seed oil (GSO) treatment delayed potato sprouting. (**A**) Sprout length phenotype. (**B**) Statistical results of sprout length. (**C**) Soluble sugar content. (**D**) peroxidase (POD) activity. Values are the mean ± SD (n = 5), and different letters indicate significant differences ($p \leq 0.05$) between treatments. Five independent experiments were performed. CK indicates control, and GSO indicates GSO treatment.

3.2. Subcellular Location and Protein Domain Analysis of DAPs

The proteomic data showed that 10 mL GSO treatment dramatically changed the protein abundance in Favorita potato bud eye regions at storage 30 d. Among the quantifiable proteins, a total of 335 proteins were regarded as DAPs. The relevant information for all DAPs is shown in Table S2. Among these proteins, 206 were upregulated (Figure 2A) and 129 were downregulated (Figure 2B). The subcellular localization of proteins was predicted based on the analysis of amino acid sequences, and the subcellular locations of 335 DAPs were predicted using Target P1.1 software. The subcellular locations of 335 DAPs were predicted by Target P1.1 software. The results demonstrated that 120 proteins were localized in the cytoplasm, of which 74 were increased; 83 proteins were localized in chloroplasts, of which 45 were increased; and 48 proteins were localized in the nucleus, of which 30 were increased. Consequently, these were the top three subcellular locations of the proteins. The extracellular space, mitochondrion, plasma membrane, and vacuolar membrane had the lowest numbers of proteins, with 33, 18, 11, and 8, respectively (Figure 2A,B). According to the protein domain analysis, the three most upregulated protein groups were trypsin and protease inhibitors, the pathogenesis-related protein Bet v I family, and the glycosyl hydrolase family 1, and the LIM domain, galactose binding lectin domain and tubulin C-terminal domain were all downregulated (Figure 2C).

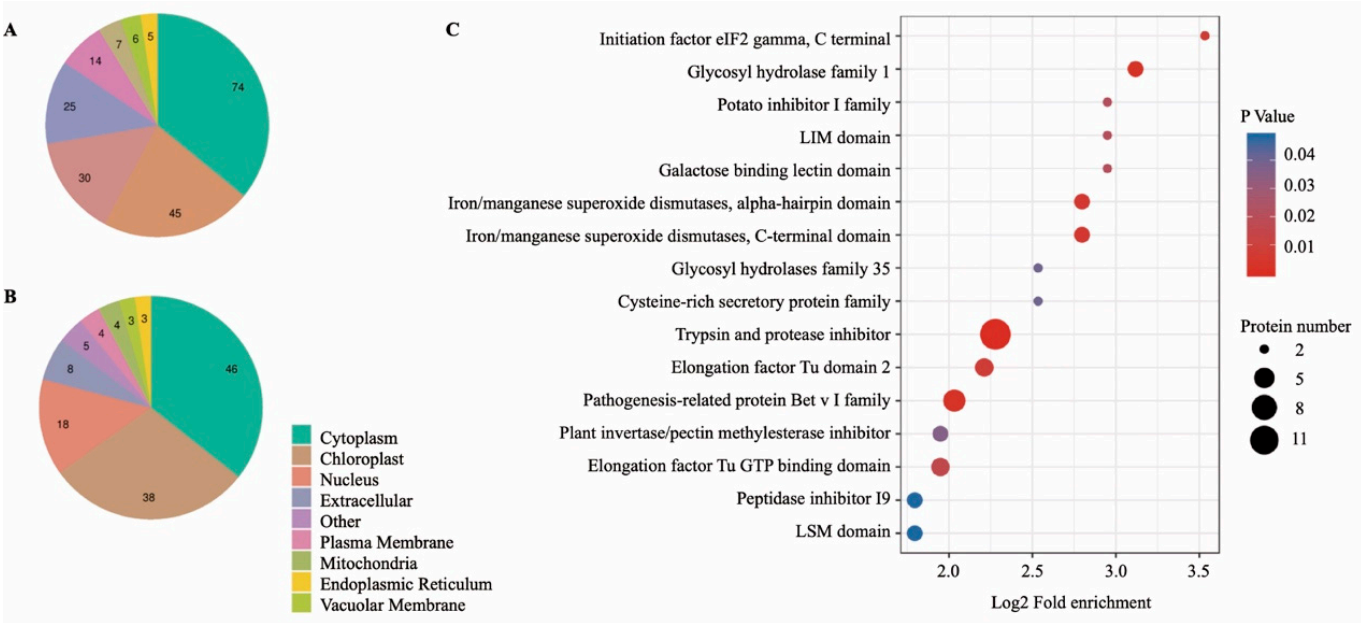

Figure 2. Analysis results of subcellular localization and protein domains. (**A**) upregulated subcellular localization analysis; (**B**) downregulated subcellular localization analysis; (**C**) protein domain analysis. Numbers represent the amount of protein.

3.3. COG/KOG Functional Annotation

The COG/KOG database contained annotations for 238 proteins (Figure 3). The COG pathway analysis of all DAPs is shown in Supplementary Table S3. For all functional ontologies, posttranslational modification, protein turnover, and chaperones had the most proteins. Biogenesis and carbohydrate transport, translation, ribosomal structure, and metabolism groups also contained a relatively high number of proteins. Nuclear structure, cell wall/membrane/envelope biogenesis and defense mechanisms had fewer proteins. In addition, 20 proteins were categorized as functionally unknown proteins.

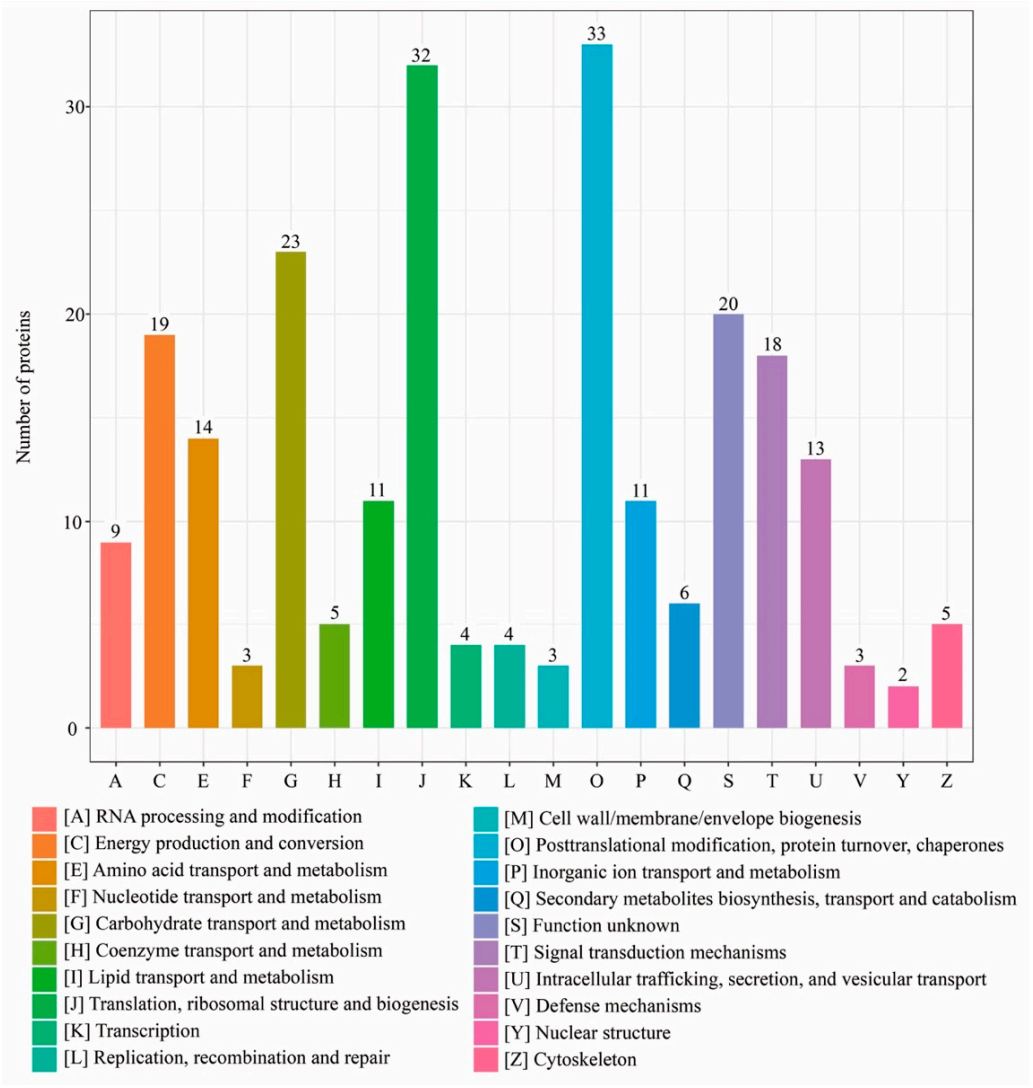

Figure 3. Distribution of COG/KOG functional classes. The number represents the amount of protein.

3.4. GO and KEGG Analysis of DAPs

All DAPs were annotated and classified according to biological process (BP), cellular component (CC), and molecular function (MF) terms according to the GO database. The primary BP terms were cellular metabolic process, organic substance metabolic process, and primary metabolic process; the primary CC terms were intracellular, membrane-bounded organelle, and intracellular organelle; and the primary MF terms were organic cyclic compound binding, heterocyclic compound binding, and hydrolase activity (Figure 4). Next, the biological metabolic pathways were examined by KEGG analysis, which indicated that the pathways involving "oxidative phosphorylation" followed by "biosynthesis of secondary metabolites—other", "starch and sucrose metabolism" and "glutathione metabolism" were the most upregulated clusters. The pathways involving "glyoxylate and dicarboxylate metabolism", "butanoate metabolism" and "circadian rhythm—plant" were the three most downregulated clusters (Figure 5).

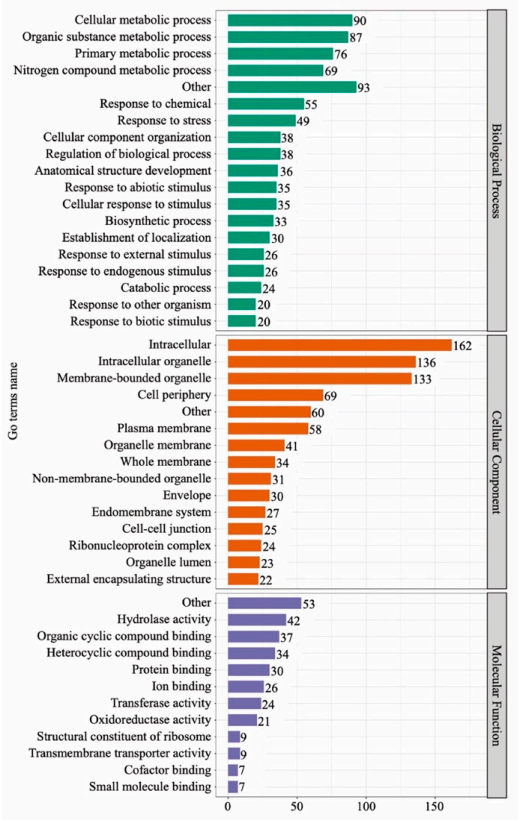

Figure 4. GO classifications of DAPs. Note that green represents biological processes, orange represents cellular components, and purple represents molecular functions. Numbers represent the amount of protein.

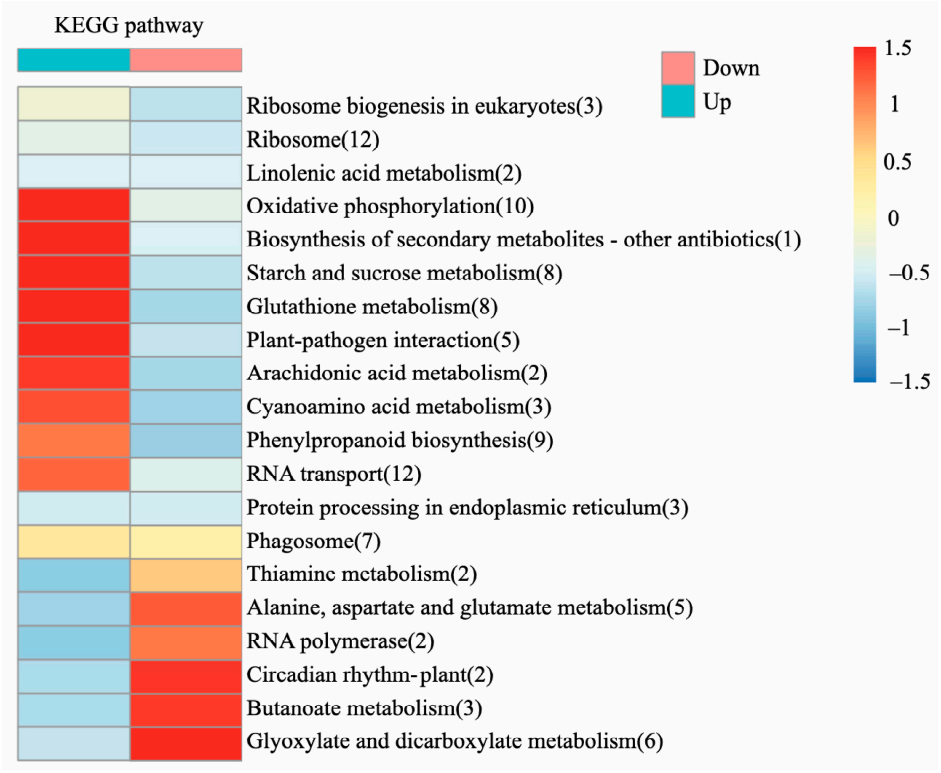

Figure 5. KEGG pathway analysis of DAPs. Note: numbers indicate the amount of protein. Red indicates increased protein abundance; blue indicates decreased protein abundance.

3.5. Protein-Protein Interaction Network Analysis

A total of 70 DAP interaction networks were constructed, and among the DAPs, 46 were upregulated and 24 were downregulated (Figure 6). Forty-eight interacting proteins belonged to "RNA transport and splicing", such as glutathione peroxidase, ubiquitin-conjugating enzyme E2 36-like, DNA-directed RNA polymerase subunit, and cytochrome b-c1 complex subunit 6 (Figure 6A). Nine interacting proteins belonged to "alanine, aspartate, and glutamate metabolism", such as glycine cleavage system P protein, malate dehydrogenase, and arginine biosynthesis bifunctional protein ArgJ (Figure 6B). Thirteen interacting proteins belonged to "glutathione metabolism", such as glutathione peroxidase, superoxide dismutase, and probable glutathione S-transferase (Figure 6C). Information about the protein interactions is shown in Table S4.

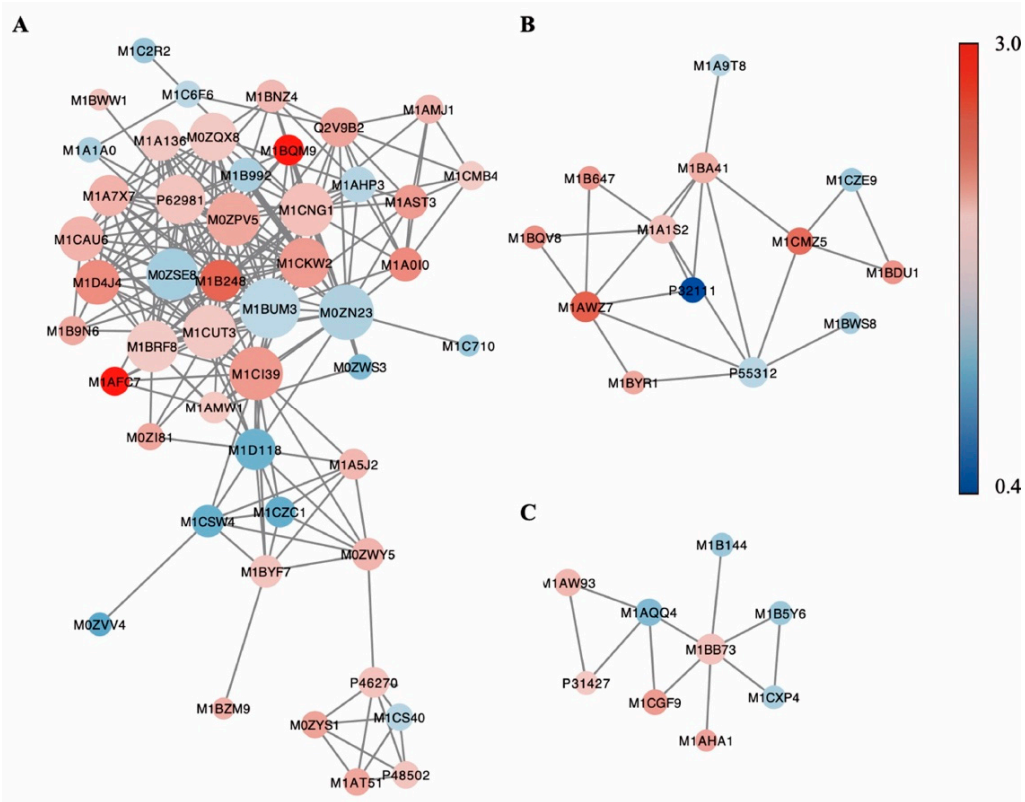

Figure 6. Protein–protein interaction networks. (**A**). RNA transport and splicing. (**B**). Alanine, aspartate and glutamate metabolism. (**C**). Glutathione metabolism. Red indicates increased protein abundance; blue indicates decreased protein abundance.

3.6. Complementation of the Proteomic Results via qRT-PCR

In our study, a total of eight proteins with proteomic data were randomly selected, and their accuracy was verified using quantitative real-time PCR (qRT-PCR) (Figure 7). We selected that 5 proteins were upregulated, including thioredoxins (TRX), glutathione S-transferase (GST), furcatin hydrolase (FH), universal stress protein A-like (UspA) and glucose-6-phosphate (G6P), and three proteins were downregulated, including GAD, pectinmethylesterase inhibitor (PMEI) and phytochrome (PHY). The gene expression data, except for the PHY gene, showed the same tendencies as the proteomic data (Figure 7). The primer sequences for eight genes are listed (Table S1).

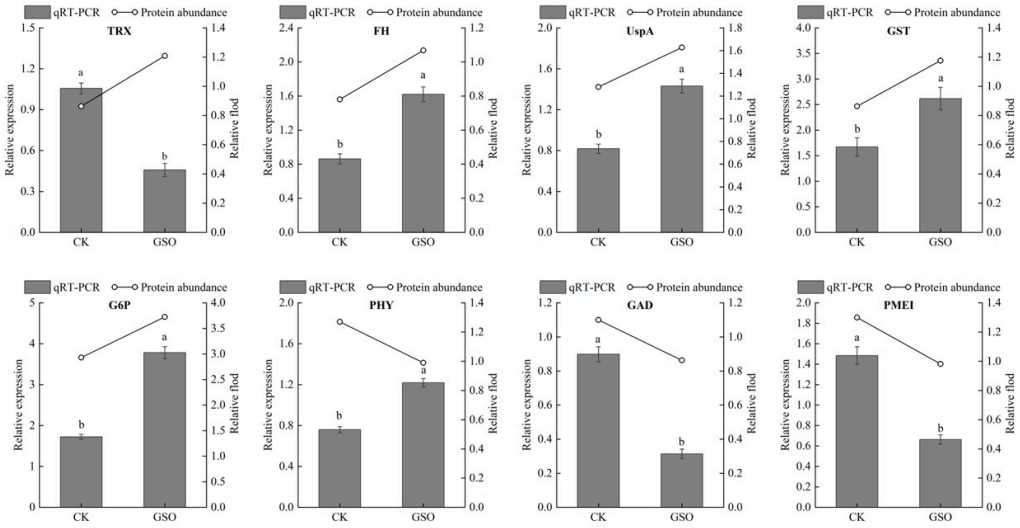

Figure 7. Complementation of the proteomic results by qRT-PCR. Note that the values are means ± SD (n = 3), and different letters indicate significant differences ($p < 0.05$) between the treatments. Three independent experiments were performed. The tubers fumigated with control and GSO were detected at 30 d. TRX, thioredoxins; FH, furcatin hydrolase; UspA, universal stress protein A-like; GST, glutathione S-transferase; G6P, glucose-6-phosphate; PHY, phytochrome; GAD, glutamate decarboxylase; PMEI, pectinmethylesterase inhibitor.

3.7. Analysis of Important DAPs in Tuber after GSO Treatment

After analysis, we found that 29 DAPs were involved in ROS and energy metabolism after GSO treatment; among them, 15 were involved in the ROS metabolism pathway, and 14 were involved in the energy metabolism pathway (Table 1). Among the DAPs, gamma aminobutyrate transaminase 1 and GAD are key proteins participating in GABA synthesis. After the 30 d storage experiment, we discovered that the tubers subjected to the 1 µm/L GABA treatment for 24 h had longer sprouts than the CK tubers. GABA could promote tuber sprouting. To study the role of the GABA sprouting process in tubers, we identified 8 genes in the GABA synthesis pathway, and qRT-PCR experiments were performed at 30 d. The results showed that the expression levels of six genes, including 4-aminobutyraldehyde dehydrogenase (*ABALDH*), arginine decarboxylase (*ADC*), diamine oxidase (*DAO*), *GAD*, ornithine decarboxylase (*ODC*), and polyamine oxidase (*PAO*), were upregulated after GABA treatment. The expression of pyrroline dehydrogenase (*PDH*) was downregulated, and the expression of carbamoylputrescine amidohydrolase (*CPAH*) was not changed after GABA treatment (Figure 8). According to the above results, GABA is correlated with the sprouting process in potato tubers. The primer sequences for eight genes are listed (Table S1).

Table 1. Proteins involved in ROS and energy metabolism.

Protein Accession	Protein Annotation	Fold	*p*-Value
M0ZIL5	Peroxidase	1.398	0.037185
M1A1S2	Glutathione peroxidase	1.257	0.0127168
M1A251	Peroxidase	1.236	0.00067654
M1A2Y4	Peroxidase	1.300	0.0020564
M1A7Q6	Perakine reductase	1.765	0.029841
M0ZIL5	Peroxidase	1.398	0.037185
M1AWZ7	Glutathione peroxidase	1.963	0.027001
M1BDU1	Superoxide dismutase	1.533	4.6877×10^{-6}
M1BQV8	Microsomal glutathione S-transferase 3	1.566	0.00078101
M1BWS8	Peroxisomal (S)-2-hydroxy-acid oxidase GLO1	0.771	0.00079654

Table 1. *Cont.*

Protein Accession	Protein Annotation	Fold	*p*-Value
M0ZIL5	Peroxidase	1.398	0.037185
M1CMY9	Superoxide dismutase	1.89	0.000004788
M1CMY9	Superoxide dismutase	1.89	0.000004788
M1D5G4	Peroxidase	0.797	0.0186962
P55312	Catalase isozyme 2	0.827	0.00132391
K9MBD0	Beta-1,3-glucanase 2	0.781	0.0183778
K9MBL3	Beta-1,3-glucanase 11	0.831	0.0153825
M0ZKH6	Glucose-6-phosphate/phosphate translocator 1	1.93	0.0095205
M1AAD9	Epidermis-specific secreted glycoprotein EP1-	1.22	0.025897
M1AUM5	Gamma aminobutyrate transaminase 1	0.784	0.00056108
M1B144	Glutamate decarboxylase	0.748	0.000080024
P07745	Patatin	1.211	0.0187844
P15478	Patatin-T5	1.32	0.00065612
P25083	ADP, ATP carrier protein	0.609	0.029918
P80595	Apyrase	1.454	0.0082
Q00081	Glucose-1-phosphate adenylyl transferase	1.204	0.0075754
Q00775	Granule-bound starch synthase 1	1.427	1.7086×10^{-6}
Q2MY36	Patatin-15	1.213	0.0078564
Q2MY44	Patatin-07	1.319	0.0076968

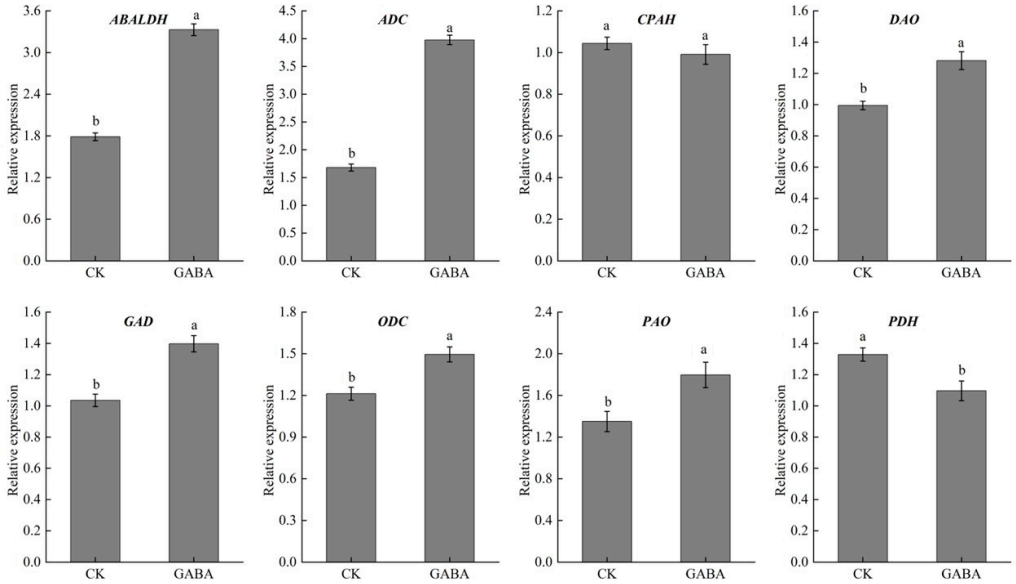

Figure 8. Effect of exogenous GABA treatment on the *ABALDH*, *ADC*, *CPAH*, *DAO*, *GAD*, *ODC*, *PAO and PDH* in the potato tubers. Note that the values are means ± SD (n = 3), and different letters indicate significant differences ($p < 0.05$) between the treatments. Three independent experiments were performed. The tubers treated with CK and GABA were detected at 30 d. Abbreviations: *ABALDH*, 4-aminobutyraldehyde dehydrogenase; *ADC*, arginine decarboxylase; *CPAH*, carbamoylputrescine amidohydrolase; *DAO*, diamine oxidase; *GAD*, glutamate decarboxylase; *ODC*, ornithine decarboxylase; *PAO*, polyamine oxidase; *PDH*, pyrroline dehydrogenase.

4. Discussion

In this study, we revealed that GSO treatment inhibited the growth of potato buds (Figure 1A), and POD enzyme activity increased after treatment (Figure 1D), which indicates that high POD enzyme activity is necessary for sprouting [34]. Our recent research indicates that snakin-2 physically interacts with POD to play a role in the tuber sprouting process [35,36]. The soluble sugar content decreased after GSO treatment (Figure 1C).

Previous studies reported that the accumulation of soluble sugar was related to starch degradation, which was increased only at the time of bud sprouting [34]. The reduction in soluble sugar content slows the growth of potato sprouts and the supply of nutrients and inhibits potato sprouting. Therefore, we speculate that GSO treatment affects the activities of POD and soluble sugar content to inhibit the growth of potato buds.

In our study, KEGG pathway analysis indicated "RNA transport", "oxidative phosphorylation" and "phenylpropanoid biosynthesis" pathways (Figure 5). Previous studies have shown that "RNA transport" related genes were highly expressed in dormancy tuber, and strongly down-regulated in sprouting tubers [11]. "Oxidative phosphorylation" related genes are activated when tubers sprout, anaerobic respiration during tuber dormancy, and genes related to "oxidative phosphorylation" after dormancy is broken are activated tubers for aerobic respiration, satisfying a large amount of energy for bud growth [37]. "Oxidative phosphorylation" plays an important role in dormancy release in potato tubers. Similarly, in previous studies, "oxidative phosphorylation" was affected by treatment with camphor and CIPC (a bud inhibitor) during tuber sprouting [33,38]. Li et al. reported that the molecular mechanism of camphor inhibition of potato tuber sprouting also disrupts the physiological process of "phenylpropanoid biosynthesis" to prolong tuber sprouting, which is similar to the molecular mechanism of inhibition of tuber sprouting after GSO treatment [33]. Therefore, based on our speculation, the CK group entered the germination stage after 30 days, while GSO would extend the dormancy period of potatoes by impacting processes such as "RNA transport", "oxidative phosphorylation", and "phenylpropanoid biosynthesis".

Reactive oxygen species (ROS) are produced by all living organisms, and the excessive accumulation of ROS results in oxidative stress, particularly in the form of oxidization of some functionally important proteins [39,40], thereby inhibiting tuber sprouting [11,15,35,41]. One indirect function of GSTs is to remove ROS. The rise in ROS content is an early step in the process of potato dormancy break [15]. GST expression was increased at both the RNA and protein level after GSO treatment (Figure 5). In previous reports, inhibitors of ROS accumulation can reduce the germination of grass and ginger [42,43]. Among them, peroxiredoxin thioredoxins (TRX) are involved in metabolism and seed germination processes [2,44]. Two close and reactive cysteine residues in a conserved motif perform this function: WCG/PPC [2,45]. They can also protect cells from oxidative damage by producing peroxiredoxins [46]. In order to maintain proteins in the correct redox state, protein disulfide isomerase may also be involved. TRX expression was increased at the RNA level and reduced at the protein level after GSO treatment (Figure 5). Therefore, we can speculate that in potatoes, GSO treatment can maintain redox homeostasis and inhibit sprouting.

Abscisic acid (ABA) and gibberellin (GA) are two major endogenous phytohormones that play vital roles in dormancy and germination processes [47,48]. An increasing number of reports have shown that phytochromes are related to light-mediated seed germination [49,50]. *Phytochrome* (*PHY*) expression was increased at the RNA level and reduced at the protein level after GSO treatment (Figure 5). The *AtUSP* gene, which is indirectly related to GA signaling, is clearly expressed in specific organs and is regulated by ABA. Reduced expression of the *AtUSP* gene leads to slower germination supports the involvement of the studied USP protein in the regulation of *Arabidopsis* seed germination [51,52]. USP expression was increased at both the RNA level and the protein level after GSO treatment (Figure 5). The tubers maintain homeostasis after GSO treatment, delay the perception of the external environment, and inhibit sprouting.

Glucose-6-phosphate/phosphate (G6P) expression was reduced at both the RNA and protein levels after GSO treatment in our study (Figure 5). Glucose-6-phosphate/phosphate translocators mediate the import of G6P into plastids for starch synthesis [53,54]. G6P is the precursor of synthetic starch [55]. Overexpression of G6P in *Arabidopsis* is upregulated during early seed development and then downregulated. It can be inferred that sugar and starch metabolism is altered to inhibit sprouting after GSO treatment. Furcatin hydrolase (FH) is a unique disaccharide-specific carbamate that hydrolyses furoic acid to carbamate

and p-allylphenol [56]. In our study, FH was increased at both the RNA and protein levels (Figure 7); therefore, we hypothesized that FH plays an important role in maintaining the dormant state of potato tubers.

Gao et al. reported that GABA inhibited the browning of fresh-cut potatoes by reducing polyphenol oxidase activity and ROS content [29]. Gamma aminobutyrate transaminase (GAD) is the rate-limiting enzyme for GABA synthesis, and it can catalyze the irreversible decarboxylation of L-glutamic acid to produce GABA and CO_2 [57]. In our study, the GAD expression level was decreased at both the RNA and protein levels after GSO treatment (Table 1 and Figure 7). Then, we detected the expression levels of eight genes in the GABA synthesis pathway. The results suggested that *ABALDH*, *ADC*, *DAO*, *GAD*, *ODC*, and *PAO* were upregulated after GABA treatment, the expression level of *CPAH* was not changed, and *PDH* was downregulated after treatment (Figure 8). For GABA synthesis, *PAO* can maintain polyamine homeostasis in cells [58]. Polyamine (PA) catabolism can afford materials, and *ODC* and *ADC* are vital enzymes involved in this GABA synthesis pathway [59]. Endogenous GABA concentrations increase during the seed germination processes of barnyard millet and wheat [60,61], and Sheng et al. also reported that exogenous GABA might promote barley seed germination by improving α-amylase activity to produce more soluble sugars [27]. Li et al. reported that exogenous GABA promotes potato tuber sprouting by altering ROS signaling pathways [41]. Hence, we proposed that exogenous GABA could enhance endogenous GABA content by increasing the expression level of synthesis-related genes to accelerate tuber sprouting. We hypothesize that GSO inhibits potato sprouting by inhibiting the synthesis of GABA in potato tubers. Subsequently, multi-omics joint analysis is used for further exploration [62].

5. Conclusions

In our research, we found for the first time that GSO inhibited the growth of tuber buds after 60 days of treatment. Then, comparative proteomic analysis showed that 335 proteins were enriched after 30 days of GSO treatment. After analysis, we found that 15 ROS-related proteins and 14 proteins involved in energy metabolism were DAPs. Among them, gamma aminobutyrate transaminase 1 and aminobutyrate transaminase decreased in abundance after GSO treatment. Meanwhile, the transcription level of genes related to GABA synthesis increased significantly according to qRT-PCR analysis. The data presented herein suggested that GSO treatment delayed the sprouting of potato tubers by altering GABA synthesis. Our research provided a theoretical basis for the application of GSO in potato storage and identified many target genes related to potato storage.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9080890/s1, Figure S1: Change in morphology of different varieties of potato tubers after grape seed oil treatment. Table S1: Primer sequences. Table S2: Information on differentially abundant proteins. Table S3: COG analysis of DAPs. Table S4: Protein information of the interaction network.

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