Transcriptomic analysis reveals the mechanism of host growth promotion by endophytic fungus of *Rumex gmelinii* Turcz

Changhong Ding1 · Shouyu Wang1 · Jiabin Li1 · Zhenyue Wang1

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Abstract

*Rumex gmelinii* Turcz. (RGT) is a medicinal plant of the genus *Rumex*, family Polygonaceae. Our research group isolated an endophytic fungus, *Plectosphaerella cucumerina* (Strain J-G) from RGT, which could significantly promote host growth when co-cultured with host seedlings. In this study, we used transcriptome analysis and verification experiments to explore the molecular mechanisms underlying this growth-promoting effect. We found that, during co-culture with Strain J-G, the expression of genes encoding key enzymes in amino acid metabolism and carbohydrate synthesis and metabolism were up-regulated in RGT tissue culture seedlings, providing additional substrate and energy for plant growth. In addition, the expression of genes encoding the responder of RGT seedlings to hormones, including auxin and cytokinin, were significantly enhanced, promoting plant growth and development. Furthermore, RGT seedling defense systems were mobilized by Strain J-G; therefore, more secondary metabolites and substances involved in stress resistance were produced, ensuring normal plant growth and metabolism. The research showed Strain J-G significantly promote the accumulation of biomass and effective components of RGT, which provide basis for its application. This research also provides a reference method for the study of growth-promoting mechanism of endophytic fungi.

Keywords  *Rumex gmelinii* Turcz. (RGT) · Endophytic fungus · Growth promoting · Transcriptome · Mechanism

Introduction

*Rumex gmelinii* Turcz. (RGT) is a medicinal plant of the genus *Rumex*, family Polygonaceae, which is distributed in northeastern and northern China. The root of RGT is used as medicine (Zhang et al. 2008) and is cold-natured, bitter tasting, non-toxic, and beneficial to the lungs and hearts. It can be used to the treatment of constipation, carbuncles, swelling, scabies and other diseases. The main active components of RGT are resveratrol, polydatin, musizin, chrysophanol, emodin, and physcion (Wang et al. 2009; Wang et al. 2005).

Endophytic fungi are frequently present in various plant tissues and organs of plants, including roots, stems, leaves, and flowers, and can be separated from sterilized plant tissues or organs (Guo 2018; Katoch and Pull 2017). Some endophytic fungi can promote plant growth (Jia et al. 2016; Waqas et al. 2012), enhance plant stress resistance, including antifungal infection (Morales-Sánchez et al. 2021), insect resistant (Ratnaweera et al. 2020) and salt tolerant (Mastouri et al. 2012). Some endophytic fungi can increase the accumulation of effective components in plants (Xing 2018). In recent years, increasing attention has been paid to the role of endophytic fungi in promoting the growth of their hosts and strains that can promote host growth have been screened from various medicinal plants, including *Rehmannia glutinosa* (Chen et al. 2011), *Salvia miltiorrhiza* (Zhou et al. 2018), *Anoectochilus roxburghii* (Zhou et al. 2018), *Artemisia annua* (Wu et al. 2018), and *Santalum album* (Liu et al. 2018).

Strain J-G with white colony has been isolated and screened from RGT. Our research found that Strain J-G could promote the growth of the host significantly when co-cultured with the host (Li et al. 2018). Some endophytic fungi can produce plant hormones, such as indoleacetic acid and gibberellin, which can promote plant growth (Asaf et al. 2019). But according to our study, Strain J-G cannot produce indoleacetic acid or gibberellin. Furthermore,
some endophytic fungi can fix nitrogen and dissolve phosphorus and potassium, which can increase the absorption of inorganic elements such as nitrogen, phosphorus and potassium, thereby, promoting plant growth (Yuan et al. 2016). Although many endophytic fungi that can promote plant growth have been detected, there are few studies on the mechanisms underlying this phenomenon, and those are published generally focus on hormones (Asaf et al. 2019), enzyme activity (Wonglom et al. 2020), and signal transduction (Sun et al. 2020). However, organisms are a whole entities, where every biological phenomenon may be associated with a variety of physiological processes, and be the result of various different reactions. Therefore, we applied an omics approach to conduct a comprehensively study of the mechanisms by which Strain J-G promotes the growth of RGT.

A transcriptome is the sum of the transcriptional products of all genes in a particular organism in a certain state (Wang et al. 2015), and represents the link between the genome and the proteome. The transcriptome analysis is now a mature and popular technology in the field of biology that focuses on the expression of functional genes that can describe the molecular mechanisms underlying biological processes. This approach provides both high-throughput information on gene expression at the RNA level and reveals the relationships between gene expression and biological phenomena, allowing characterization of the physiological activities and determination of metabolic characteristics. Transcriptome analysis is highly appropriate for the study of the mechanisms underlying interactions between plants and fungi (Liu et al. 2017; Lamdan et al. 2015).

The objective of this study was to explore the molecular mechanism of host growth promotion by Strain J-G, using transcriptome analysis to provide a basis for the application of endophytic fungi.

Analysis of the growth-promoting effects of Strain J-G

RGT tissue culture seedlings at the same stage of growth state (with three true leaves) were implanted into Murashige and Skoog (MS) solid medium on culture plates, with one seedling per plate. These seedlings were cultured for 5 d in a light incubator, temperature (day/night) 25 °C/18 °C, day/night 14 h/10 h, light intensity 3000 lx for day time. The Strain J-G was inoculated 2 cm away from the RGT tissue culture seedling. Then, co-cultured for 25 d in a light incubator, temperature (day/night) 25 °C /18 °C, day/night 14 h/10 h, light intensity 3000 lx for day time, six replicates were were conducted for each condition. The treatment conditions for the control group were the same, but with no Strain J-G inoculation; six replicates were conducted. Three replicates of the co-culture groups (G1) and control groups (C1) were used to study physiological indicators, and the other three replicates were used for transcriptome analysis.

RNA extraction

Total RNA was extracted from the co-culture and the control groups using Total RNA Extractor (Shanghai Shengong). The integrity of RNA samples was evaluated by electrophoresis on 1% agarose gels, and sample purity determined using a Keao k5500 spectrophotometer. The concentrations of RNA samples were determined using an Agilent 2100 RNA Nano 6000 Assay Kit.

Library construction and sequencing

After the total RNA samples were evaluated and quantified, the mRNAs were enriched using Oligo (dT) magnetic beads, and fragment buffer was added to the obtained mRNAs to generate short fragments. The fragmented mRNAs samples were used as template to synthesize first strand of cDNA, using six nucleotide random primers. Then buffers, dNTPs, RNaseH, and DNA Polymerase I were added to synthesize second strand cDNA. The obtained cDNAs were purified using a QIAQuickPCR kit and eluted in EB buffer. Next, the terminals of double-stranded cDNAs were repaired by addition of added adenine nucleotides and sequencing connectors. Finally, target size fragments were recovered by agarose gel electrophoresis and amplified by PCR to complete library preparation. The constructed library was sequenced on the Illumina platform, using the PE150 sequencing strategy.
Data quality control

Raw data were processed using Perl scripts to ensure the quality of data used in further analyses. Adaptor-polluted reads, low-quality reads, reads with number of N bases accounting for more than 5% were removed (Pang et al. 2018). The clean data obtained after filtering was subjected to statistics analyses of quality and quantity, including Q30 values.

Assembly, coding regions prediction and annotation

Trinity software was used for sequence assembly. TransDecoder was used to identify open reading frames (ORFs) of the assembled transcripts. Trinotate was used to annotate predicted ORFs and transcripts.

Analysis of differential gene expression

HTSeq v0.6.0 was used for each gene in each sample, and RPKM (Reads Per Kilobase Million Mapped Reads) was used to estimate the expression level of certain genes in each sample (Ren et al. 2019). DESeq2v1.4.5 was used for differential gene expression analysis. The p values were assigned to each gene and adjusted using the Benjamini and Hochberg approach for controlling the false discovery rate. Transcripts with q ≤ 0.05 and |log2_ratio| ≥ 1 were identified as differentially expressed genes (DEGs). Heat maps were constructed, based on sample expression levels.

GO and KEGG analysis

Hypergeometric test was used for analysis of GO (Gene Ontology) enrichment of DEGs. In GO analysis, q value was acquired by calculation and adjustment of p value. The GO terms with q < 0.05 were supposed to be remarkably enriched.

Hypergeometric test was used for analysis of KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment of DEGs. In KEGG analysis, q value was acquired by adjustment by multiple comparisons. KEGG terms with q < 0.05 were supposed to be remarkably enriched.

Verification by real-time PCR

Real-time PCR was used to evaluate the expression levels of six genes involved in energy metabolism and plant hormone signal transduction pathways in co-cultured RGT seedlings. GAPDH was used as an internal reference gene. Expressions’ levels of genes of phenylalanine aminolyase (PAL), cinnamoyl coenzyme A reductase (CCR), β-fructofuranosidase (INV), starch synthetase (GlgA), auxin response protein IAA (IAA) and cytokinin receptor (CYT) were evaluated.

A Bio-Rad iQ-5 Fluorescence Quantitative Analyzer was used to generate CT values of target genes and an internal reference gene, following completion of PCR amplification. Gene expressions were calculated using $2^{-\Delta\Delta CT}$. $\Delta\Delta CT = (CT_{Objective} - CT_{Internal Reference})_G1 - (CT_{Objective} - CT_{Internal Reference})_C1$.

Verification by content variation of resveratrol and polydatin

The RGT roots of co-culture and the control groups were taken, and rinsed the culture medium on the root surface with distilled water. Dried in an oven (40 °C) to constant weight, then crushed and sieved by a 60 mesh sieve. 0.2 g powder was refluxed and extracted with 50% ethanol for 4 h, then filtered. The filtrate was evaporated to dryness and diluted with methanol to 2 ml. Three replicates for each group.

A Dimma, Diamonsil C18 column (5 μm, 250 mm × 4.6 mm) and Phenomenex ODS-C18 (4.0 mm × 3.0 mm) protective column were used. The column temperature was set at 40 °C. Mobile phase A was methanol and D was 0.1% phosphoric acid water. Gradient elution conditions: mobile phase from 30% methanol to 100% methanol for 60 min, then 100% methanol for 20 min; flow rate was 1.0 ml/min; tested wavelength was 254 nm. The sample injection volume was 10 μl. The content of resveratrol and polydatin could be detected and quantified by standard curve from our previous study (resveratrol $Y = 9.25 \times 10^6X - 6.07 \times 10^4$; polydatin $Y = 4.40 \times 10^6X - 6.33 \times 10^4$).

Results and discussion

Identification of the endophytic fungus Strain J-G

BLAST comparisons of ITS I sequences showed that Strain J-G was 100% similar to Plectosphaerella cucumerina. The obtained sequence has been submitted to GenBank (Accession No. MT068424). We constructed a neighbor-joining tree (Fig. 1-A), which showed that Strain J-G was on the same branch as Plectosphaerella cucumerina (EU030361.1). Therefore, Strain J-G was identified as species Plectosphaerella cucumerina. Colonies of Strain J-G were white with regular edges and dense mycelia (Fig. 1-B). Strain J-G was deposited in the Traditional Chinese Medicine Resources Laboratory of Heilongjiang University of Traditional Chinese Medicine.
The growth-promoting effects of Strain J-G

Compared with the control group, the dry weight of roots increased by 161% (Fig. 2-A) and the difference was significant. The dry weight of the above-ground parts of co-cultured group was significantly higher than that of the control group (Fig. 2-B). Thus, the growth-promoting effect of Strain J-G on its host was clearly comprehensive and significant.

Analysis of filtered transcriptome data

The transcriptome data after filtering were submitted to SRA (SRR11308217). The total numbers of filtered high-quality sequences for the three replicates (G1a, G1b, G1c) in the co-cultured group were 63,700,940, 63,696,030, and 63,412,070, and the proportions of the filtered high-quality sequences to unfiltered sequences were 95.71%, 95.03% and 94.82%, respectively, indicating that the sequencing quality was acceptable. The total numbers of filtered high-quality sequences for the three replicates (C1a, C1b, C1c) in the control group were 61,116,748, 56,930,540, and 58,908,308. The proportions of the filtered high-quality sequences to the original ones were 97.45%, 97.25%, and 97.82%, respectively, indicating that the sequencing quality was acceptable.

Differential gene expression

According to the expression analysis, there were a total of 41,558 significantly differentially expressed unigenes between co-cultured and control groups, of which 31,481 were up-regulated and 10,077 were down-regulated. Comparisons between the co-cultured and the control groups were used to generate scatter diagrams showing genes with log2foldchange differences in expression (Fig. 3). In Fig. 3, the yellow dots represented genes that were significantly up-regulated and the blue dots represented the genes that were significantly down-regulated, while the gray dots represented other genes that were not significant differentially expressed. There were more significant differences in the expression of up-regulated than down-regulated genes.

GO analysis

34,553 different expressed genes mapped to the GO classification, including 25,756 up-regulated genes and 8797 down-regulated genes. Bubble diagrams of the ten Biological Process (BP), Cellular Component (CC), and Molecular Function (MF) GO items showing the most significant enrichment of unigenes are presented in Fig. 4. Unigenes were enriched for BP items including: metabolic process, heterocycle biosynthetic process, and organic cyclic compound biosynthetic process, among which the most annotated and enriched item was metabolic process. For CC terms, unigenes were enriched and annotated for items including intrinsic component of...
membrane, integral component of membrane, and extracellular region, among which the most annotated item was integral component of membrane, and the most enriched item was extracellular region. The most highly annotated and enriched items for MF were catalytic activity, transition metal ion binding, and DNA binding, among which the most annotated and enriched item was catalytic activity.

Statistical analysis of the results of annotation analysis of differentially expressed unigenes for secondary GO items is presented in Fig. 5. The horizontal ordinate was the secondary GO items with different expressed unigenes’ annotation. The left ordinate indicated the proportion of up-regulated different expressed unigenes (the number of up-regulated differentially expressed unigenes in this entry × 100%/the number of all up-regulated differentially expressed unigenes) and down-regulated different expressed unigenes. The right ordinate indicated the quantities of up-regulated different expressed unigenes and down-regulated different expressed unigenes (unit sizes of up-regulated and down-regulated unigenes were different), there were significant differences in CC, BP, and MF, with differentially expressed unigenes most obvious among CC and BP items.

**KEGG analysis**

6670 different expressed genes mapped to 137 KEGG pathways, of which 4908 were up-regulated and 1762 were down-regulated. Data from all samples were combined and subjected to KEGG enrichment analysis; a diagram of their distribution, according to q values indicating significant enrichment is presented in Fig. 6. Pathways including phenylpropanoid biosynthesis, cyanoamino acid metabolism, tyrosine metabolism, starch and sucrose metabolism, phenylalanine metabolism, steroid biosynthesis, base excision repair, pentose and glucuronate interconversions, plant hormone signal transduction, biosynthesis of unsaturated fatty acids, alpha-linolenic acid glycine, serine and threonine metabolism, and isoquinoline alkaloid biosynthesis were significantly enriched.

**Analysis of growth-promoting mechanism**

Comparisons of the differentially expressed unigenes between the control and co-cultured groups demonstrated that there were significant differences in gene expression in numerous pathways, including biosynthetic pathway, amino acids metabolism pathway, saccharides metabolism and transformation. By comparing the differential expression of these genes, we could speculate the mechanism of host growth promotion by Strain J-G.

**Biosynthetic pathway**

In the pathway of phenylpropanol biosynthesis (Table 1), unigenes homologous to genes encoding phenylalanine ammonia lyase (PAL) were up-regulated, while those for cinnamoyl-CoA reductase (CCR) and shikimic acid o-hydroxycinnamoyl transferase [EC:2.3.1.133] were significantly down-regulated. Under these circumstances, the formation of resveratrol and polydatin, which are main effective components of RGT could be promoted via the enzymatic reaction. PAL is a regulatory enzyme important in secondary metabolism, whose activity is controlled by numerous internal and external factors. Infections with...
viruses, bacteria, and fungi can increase PAL gene expression, thereby promoting secondary metabolite production and enhancing plant resistance, which is a method of plant self-protection. Co-cultured with Strain J-G improved the resistance and living ability of RGT.

In the steroid biosynthetic pathway (Table 1), farnesyl diphosphate farnesyl transferase (FDFT₁) was down-regulated; this infers that steroid biosynthesis may have been inhibited to some extent in co-cultured seedlings.
In unsaturated fatty acid biosynthesis, 26 unigenes were up-regulated and 13 down-regulated, among which genes for acyl-CoA thioesterase 7 [EC:3.1.2.2] and acyl-CoA thioesterase II (TesB) were up-regulated. These enzymes catalyze the synthesis of α-linolenic acid, hexadecanoic acid, stearic acid, and oleic acid, which are important in plant resistance responses. These data infer that, although plants and endophytic fungi can coexist peacefully, the presence of the endophytic fungi does stimulate mobilization of plant defense mechanisms.

Amino acids metabolism pathway

In the cyanamide acid metabolism pathway (Table 1), formamidase [EC: 3.5.1.49] was up-regulated, promotes NH₃ synthesis and nitrogen metabolism. Glycine methyltransferase (GlyA), which promotes the metabolism and transformation of glycine, serine, threonine, and cysteine, was also up-regulated. In glycine, serine and threonine metabolism, 78 unigenes encoding Amino acid invertases were up-regulated, which enhanced the metabolism and conversion of amino acids.

In the tyrosine pathway (Table 1), tyrosine aminotransferase [EC: 2.6.1.5], aromatic amino acid aminotransferase I [EC: 2.6.1.57], histidinol-phosphate aminotransferase (HisC), and aspartate aminotransferase [EC:2.6.1.1] were up-regulated. The mutual transformation of 4-hydroxyphenylpyruvate with tyrosine can be promoted by these enzymes. Furthermore, 4-hydroxyphenylpyruvate dioxygenase (HPD), maleylacetoacetate isomerase (MaiA), acetylase, and other enzymes were also up-regulated. These enzymes catalyze the synthesis of acetoacetate and fumarate, which enters the citric acid cycle to promote substrate metabolism and energy formation. Upregulation of 4-hydroxy-2-oxyheptanediade aldehyde (Hpal) and succinate semialdehyde dehydrogenase/glutarate semialdehyde dehydrogenase (GabD) was also observed. These factors catalyze the conversion of 2, 4-dihydroxyhept-2-enedioate to succinate, which enters the citric acid cycle to enhance the metabolism and energy generation. In addition, monoamine
oxidase, alcohol dehydrogenase, and aldehyde dehydrogenase (NAD(P)\(^+\)) [EC: 1.2.1.5] were up-regulated.

Through the metabolism of amino acids, we speculated the co-culture of Strain J-G and RGT tissue culture seedlings could promote the conversion between amino acids and provide the necessary material basis for the growth of plants. Meanwhile, intermediate products were generated to enter the citric acid cycle to accelerate metabolism, so as to promote the growth of RGT tissue culture seedlings.

**Saccharides’ metabolism and transformation**

In starch and sucrose metabolism (Table 1), β-fructofuranosidase (INV) was up-regulated, promoted the formation of 6-phosphate-glucose. α-glucosidase [EC: 3.2.1.20] was up-regulated, produced more fructose and glucose. Glucan 1,3-β-glucosidase [EC: 3.2.1.58], 1,3-β-glucanase [EC: 2.4.1.34], endoglucanase [EC: 3.2.1.4], cellulase 1,4-β-fibreglucosidase [EC: 3.2.1.91], α-trehalase [EC: 3.2.1.28] were up-regulated, catalyzed the conversion of glucoside, cellulose, and trehalose to glucose. Therefore, respiration substrates required for increased energy provision were up-regulated in co-cultured RGT tissue culture seedlings. Furthermore, starch synthetase (GlgA) and 1,4-α-glucan branching enzyme [EC: 2.4.1.18] were up-regulated. Hence, co-culture of Strain J-G and RGT tissue culture seedlings promoted both glucose generation and starch accumulation.

In the pentose and glucuronide interconversion pathway (Table 1), galacturonidase [EC:3.2.1.67] was up-regulated, which can catalyze diamino-galactose to generate galactose, which participates in various metabolic processes. Upregulation of L-threonine-3-deoxyhexanoic acid aldolase (GAAC), which is beneficial for galactose entry into the glycerol metabolism pathway, providing energy to drive the growth of RGT tissue culture seedlings, was also observed. Alcohol dehydrogenase (NADP\(^+\); AKR1A1) was up-regulated, and can catalyze the formation of D-glucuronate from gluonic acid. Furthermore, UDP-glucose-1-phosphouridyltransferase (UGP2) and UDP-glucose-6-dehydrogenase (UGDH) were up-regulated; these enzymes catalyze UDP-D-glucurionate production from glycolysis-fructose phosphate. Moreover, UDP glycopyrophosphorylase (USP), glucuronosyl transferase (UGT), and β-glucuronidase [EC: 3.2.1.31] were also up-regulated, and may promote glucuronate generation, providing increased substrate for synthesis of aminosaccharide and ribose.

It can be inferred that the variation of saccharides metabolism in co-cultured RGT tissue culture seedlings increased the substrates of respiration and promoted metabolism of plants. Therefore, it could provide more materials and energy for the growth of RGT tissue culture seedlings.

**Plant hormone signal transduction pathway**

In the plant hormone signal transduction pathway (Table 1), auxin influx carrier (AUX1, from the LAX family), auxin-responsive protein IAA (IAA), auxin response factor (k4486), the auxin-responsive GH3 gene family (GH3), and a SAUR family protein (SAUR) were up-regulated, while transport inhibitor responder 1 (TIR1) was down-regulated. These changes are predicted to promote cell responses to growth-promoting hormones, thereby accelerating RGT seedlings growth. Arabidopsis histidine kinase 2/3/4 (a cytokinin receptor; AHK2_3), histidine-containing phosphotransfer protein (AHP), a two-component response regulators of the ARR-B family (ARR-B), and two-component response regulators from the ARR-A family were up-regulated, which could enhance the response of RGT seedlings to cytokinin, thereby promoting cell division. Ethylene-insensitive protein 2 (EIN2), ethylene-insensitive protein binding factor 1/2 (EBF1/2), and ethylene-responsive transcription factor 1 (ERF1) were down-regulated, which could delay plant senescence. Furthermore, BRK1, BR11, BSK, TCH4, and CYCD3, which are important in responses to brassinosteroids, were up-regulated, which could promote cell growth and division in seedlings.
Table 1  Summary of some differently expressed genes of RGT co-cultured with endophytic fungus strain J-G

| Unigene ID          | Putative function                                      | Log2 fold | Pathway                                      |
|---------------------|--------------------------------------------------------|-----------|----------------------------------------------|
| **Phenylpropanol biosynthesis** |                                                        |           |                                              |
| TRINITY_DN33839_c3_g2 | Phenylalanine ammonia lyase (PAL)                      | + 4.8     | Phenylpropanol biosynthesis                   |
| TRINITY_DN37056_c0_g2 | Cinnaoyl-CoA reductase (CCR)                           | − 2.9     | Phenylpropanol biosynthesis                   |
| TRINITY_DN31663_c0_g1 | Shikimate O-hydroxy–cinnamoytransferase                | − 3.2(9.6)| Phenylpropanol biosynthesis                   |
| **Steroid biosynthetic pathway** |                                                        |           |                                              |
| TRINITY_DN36602_c0_g1 | Farnesyl diphosphate farnesyl transferase (FDFT1)      | − 3.38    | Steroid biosynthetic                          |
| **Cyanamide acid metabolism** |                                                        |           |                                              |
| TRINITY_DN29473_c0_g4 | Formamidase                                            | + 13.02   | NH3 synthesis and nitrogen metabolism         |
| TRINITY_DN25667_c0_g1 | Glycine methyl transferase (GlyA)                      | + 13.34   | Metabolism and transformation of amino acids |
| **Tyrosine pathway** |                                                        |           |                                              |
| TRINITY_DN36945_c0_g1 | Tyrosine aminotransferase                             | + 2.99    | Transformation of 4-hydroxy-phenylpyruvate with tyrosine |
| TRINITY_DN12337_c0_g1 | Aromatic amino acid aminotransferase I                 | + 13.13   | Transformation of 4-hydroxy-phenylpyruvate with tyrosine |
| TRINITY_DN14878_c1_g1 | Phosphohistidine aminotransferase (HisC)               | + 14.38   | Transformation of 4-hydroxy-phenylpyruvate with tyrosine |
| TRINITY_DN23316_c0_g1 | Aspartate aminotransferase                            | + 11.53   | Transformation of 4-hydroxy-phenylpyruvate with tyrosine |
| TRINITY_DN10312_c0_g1 | 4-hydroxyphenylpyruvate dioxygenase (HPD)              | + 8.89    | Material metabolism and energy formation     |
| TRINITY_DN14117_c0_g1 | Maleylacetate isomerase (MaiA)                        | + 12.14   | Material metabolism and energy formation     |
| TRINITY_DN44336_c0_g1 | Fumarylacetatoacetase                                 | + 7.71    | Material metabolism and energy formation     |
| TRINITY_DN2328_c0_g1 | 4-hydroxy-2-oxyheptanediol aldehyde (Hpal)            | + 9.19    | Material metabolism and energy formation     |
| TRINITY_DN15731_c0_g1 | Succinate semialdehyde dehydrogenase/glutarate semialdehyde dehydrogenase (GabD) | + 16.98 | Material metabolism and energy formation |
| TRINITY_DN23770_c0_g2 | Monoamine oxidase, alcohol dehydrogenase and aldehyde dehydrogenase (NAD (P)+) | + 9.63 | Metabolism and conversion of amino acids |
| **Starch and sucrose metabolism** |                                                        |           |                                              |
| TRINITY_DN27483_c0_g5 | β-fructofuranosidase (INV)                            | + 1.24(2.4)| 6-phosphate-glucose formation                |
| TRINITY_DN10513_c0_g1 | α-glucosidase                                          | + 8.41    | Conversion between saccharides               |
| TRINITY_DN33633_c0_g3 | Glucan 1,3-β-glucosidase                              | + 3.73    | Conversion between saccharides               |
| TRINITY_DN23967_c0_g2 | 1,3-β-glucanase                                       | + 15.13   | Conversion between saccharides               |
| TRINITY_DN54426_c0_g1 | Endoglucanase                                         | + 10.89   | Conversion between saccharides               |
| TRINITY_DN55106_c0_g1 | Cellulase 1,4-β-fibreglucosidase                      | + 8.47    | Conversion between saccharides               |
| TRINITY_DN15591_c0_g1 | α -trehalase                                          | + 10.36   | Conversion between saccharides               |
| TRINITY_DN27249_c0_g1 | Starch synthetase (GlgA)                              | + 1.97    | Starch synthesis                            |
| TRINITY_DN19103_c0_g2 | 1,4-α-glucan branching enzyme                         | + 12.02   | Starch synthesis                            |
| **Pentose and glucuronide interconversion pathway** |                                                        |           |                                              |
| TRINITY_DN19953_c0_g3 | Galacturonidase                                        | + 8.91    | Diamino-galactose to galactose               |
| TRINITY_DN32703_c1_g6 | L-threonine-3-deoxyhexanoic acid aldolase (GAAC)      | + 13.86   | provide energy                              |
| TRINITY_DN2277_c0_g1 | Alcohol dehydrogenase (NADP+) (AKR1A1)                | + 9.26    | D-Glucuronate formation                      |
| TRINITY_DN27315_c0_g3 | UTP-glucose-1-phosphouridinyltransferase (UGP2)       | + 15.79   | UDP-D-Glucuronate formation                  |
| TRINITY_DN26407_c0_g2 | UDP-glucose-6-dehydrogenase (UDGH)                    | + 0.62    | UDP-D-Glucuronate formation                  |
| TRINITY_DN27361_c0_g2 | UDP glycopyrophosphorylase (USP)                       | + 0.51    | Glucuronate formation                        |
| TRINITY_DN12877_c0_g1 | β-glucuronidase                                       | + 9.52    | Glucuronate formation                        |
| **Plant hormone signal transduction pathway** |                                                        |           |                                              |
| TRINITY_DN26407_c0_g2 | Auxin influx carrier (AUX1 LAX family)                | + 0.62    | Growth-promoting hormones response           |
| TRINITY_DN24511_c0_g1 | Auxin-responsive protein IAA (IAA)                    | + 2.89    | Growth-promoting hormones response           |
Through these phenomena, we speculated the growth and division of RGT tissue culture seedlings were accelerated, while senescence was delayed.

Verification of the expression of key genes in co-cultured RGT tissue culture seedlings

To verify the results of transcriptome analysis and further explore the effect of co-culture on the growth process and secondary metabolites of RGT tissue culture seedlings, the representative genes with significant different expression related to growth promotion and quality improvement were screened from the transcriptome results, then analyzed quantitatively by real-time PCR. Phenylalanine ammonia lyase (PAL), cinnamoyl COA reductase (CCR) are in the pathway of Phenylpropanol biosynthesis, both of them are key enzyme for the synthesis of resveratrol and polydatin, and play an important role in the quality control of medicinal materials. β-fructofuranosidase (INV) and starch synthase (GlgA) are in starch and sucrose metabolism. One catalyzes the formation of 6-phosphate-glucose, the other catalyzes the synthesis of starch. The result of the action of these two key enzymes increases the accumulation of saccharides and provides sufficient energy for the growth of RGT, which is of great significance for growth promoting. Iuxin-responsive protein IAA (IAA) and cytokinin receptor (CYT) were in plant hormone signal transduction pathway. These two functional proteins can improve the sensitivity of RGT to growth hormone and cytokinin, and are significant in plant growth.

As shown in Fig. 7, genes encoding PAL, INV, GlgA, IAA, and CYT were significantly up-regulated in the co-cultured group, while that for CCR was significantly down-regulated compared with the control group. These results were consistent with our findings from transcriptome analysis.

Verification of the accumulation promotion of resveratrol and polydatin

The contents of resveratrol and polydatin in RGT roots of co-culture group were significantly higher than those in the control group (Fig. 8). This was consistent with the trend of up-regulated expression of key enzyme genes in resveratrol and polydatin synthesis pathway in transcriptome research. We speculated that co-cultured with Strain J-G can promote the synthesis of these two components in RGT seedlings. Resveratrol and polydatin are compounds with many beneficial effects, including antitumor, antibiosis, antioxidant, anti-inflammatory, anti-cardiovascular disease, anti-aging, anti-neurodegenerative diseases (Jamshid et al. 2018; Wang et al. 2018). Therefore, endophytic fungus Strain J-G can significantly promote the accumulation of biomass and effective components of RGT.
Conclusion

Through transcriptome analysis and verification experiments, the mechanism of host growth promotion by endophytic fungus Strain J-G of RGT could be speculated as follows. During the co-culture of the endophytic fungus J-G and RGT seedlings, amino acid metabolism, and saccharides synthesis and metabolism were promoted, which likely provided additional substrate and energy for RGT seedling growth. Responses to the hormones, auxin and cytokinin were also enhanced, which would contribute to the growth and development of RGT seedlings. In addition, the presence of Strain J-G also activated RGT seedling defense mechanisms, generating increased levels of secondary metabolites resveratrol and polydatin, and various substances involved in stress resistance responses, to ensure RGT seedling growth and metabolic activity.

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**Author contributions** CD and ZW designed this research. KL, JL did a study on the growth-promoting effect of endophytic fungus, CD, JL and WS performed the related experimental research and analysis of transcriptome. CD and WS drafted the manuscript. All authors read and approved the final manuscript.

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**Data availability** Transcriptome data accession: SRR11308217; ITS gene accession: MT068424.

**Code availability** Not applicable.

**Declarations**

**Competing interest** The authors have no relevant financial or non-financial interests to disclose. The authors have no conflicts of interest to declare that are relevant to the content of this article. All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest to declare that are relevant to the content of this article. All authors have no financial or proprietary interests in any material discussed in this article.

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**References**

Asal LS, Khan AL, Waqas M, Kang SM, Hamayun M, Lee IJ, Hussain A (2019) Growth-promoting bioactivities of Bipolaris sp. CSL-1 isolated from Cannabis sativa suggest a distinctive role in modifying host plant phenotypic plasticity and functions. Acta Physiol Plant 41:65–73. https://doi.org/10.1007/s11738-019-2852-7

Chen BB, Wang M, Hu YL, Lin ZP, Yu RM, Huang LQ (2011) A preliminary study on the growth promoting effect of Rehmannia Glutinosa endophytic fungi. China J Chin Materia Med 36:1137–1140

Guo SX (2018) Research status and development trend of endophytic fungi in medicinal plants. Mycosystema 37:1–13

Jamshid T, Soghra M, Fatemeh SB, Hossein H (2018) Protective effects of Vitis vinifera (grapes) and one of its biologically active constituents, resveratrol, against natural and chemical toxicities: a comprehensive review. Phytother Res 32:2164–2190

Jia M, Chen L, Xin HL, Zheng CJ, Rahman K, Han T, Qin LP (2016) A friendly relationship between endophytic fungi and medicinal plants: a systematic review front. Microbial. https://doi.org/10.3389/fmicb.2016.00906

Katoh M, Pull S (2017) Endophytic fungi associated with Monarda citriodora, an aromatic and medicinal plant and their biocontrol potential. Plant Biol 55:1528–1535. https://doi.org/10.1080/13880209.2017.1309054

Lamdan N, Shalaby S, Ziv T, Kenerley CM, Horwitz BA (2015) Secretome of Trichoderma interacting with maize roots: role in induced systemic resistance. Mol Cell Proteomics 14:1054–1063. https://doi.org/10.1074/mcp.M114.046607

Li JB, Ding CH, Zhang S, Guo H, Cui HH, Wang ZY (2018) Preliminary study on the growth promoting effect of endophytic Fungi from Rumex tomentosa. Int Tradit Chin Med 35:6–9. https://doi.org/10.19656/j.ijtcmm.1002.2406.180002

Liu KH, Ding XW, Narsing RMP, Zhang B, Zhang GZ, Liu FH, Liu XB, Mao M, Li WJ (2017) Morphological and transcriptomic analysis reveals the osmoadaptive response of endophytic fungus Aspergillus monteviendensis ZYD4 to high salt stress. Front Microbiol 8:1789–1799. https://doi.org/10.3389/fmicb.2017.01789

Liu J, Liu YM, Xv ZC, Wang ZY, Huang YL, Deng ZJ (2018) Studies on the diversity of endophytic fungi of Santalum Album and its antibacterial and growth-promoting properties. China J Chin Mater Med 43:3477–3483

Mastouri F, Björkman T, Harman GE (2012) Trichoderma harzianum enhances antioxidant defense of tomato seedlings and resistance to water deficit. Mol Plant Microbe Interact 25:1264–1271. https://doi.org/10.1094/MPMI-09-11-0240

Morales-Sánchez V, Díaz CE, Trujillo E, Muoz E, Gonzalez-Coloma A (2021) Bioactive metabolites from the endophytic fungus Aspergillus sp SPH2. J Fungi 7:109

Pang W, Fu P, Li X, Zhan Z, Yu S, Piao Z (2018) Identification and mapping of the clubroot resistance gene CRd in Chinese cabbage (Brassica rapa ssp. pekinensis). Front Plant Sci 9:653. https://doi.org/10.3389/fpls.2018.00653

Ratnaweera PB, Nisansala JM, Jayasundara M, Suseema HHM, Herath D, Williams DE, Rajapaksha SU, Prabath Nishantha KMDW, Edilip de Silva, Andersen RJ (2020) Antifeedant, contact toxicity and oviposition deterrent effects of phyllistine acetate and phyllistine isolated from the endophytic fungus Diaportha mirtiae against Plutella xylostella larvae. Pest Management Science. 76(4):1541–1548. https://doi.org/10.1002/ps.3673

Ren J, Sun C, Chen L, Hu J, Huang X, Liu X et al (2019) Exploring differentially expressed key genes related to development of folicle by RNA-seq in Peking ducks (Anas Platyrhynchos). PLoS ONE 14:e0209061. https://doi.org/10.1371/journal.pone.0209002

Sun X, Wang N, Li P, Jiang ZY, Liu XY, Wang MC, Su ZZ, Zhang CL, Lin FC, Liang Y (2020) Endophytic fungus Falciphora oryzae promotes lateral root growth by producing indole derivatives after sensing plant signals. Plant Cell Environ 43:358–373. https://doi.org/10.1111/pce.13667

Wang ZY, Zuo YM, Kang YH, Cong Y, Song XL (2005) Study on the chemical composition of Rumex gmelinii Turcz. (II). Chin Tradit Herb Drugs 11:1626–1627. https://doi.org/10.3321/j.isi.2005.11.011

Wang ZY, Chen JM, Wang QB, Kang YH, Cong Y, Liu YX (2009) Study on the chemical composition of Rumex gmelinii Turcz. (IV). Chin Tradit Herb Drugs 40:1352–1355. https://doi.org/10.3321/j.isi.2009.09.003

Wang XL, Huang LQ, Yuan Y, Cha LP (2015) Research progress on transcriptome of medicinal plants. China J Chin Mater Med 40:2055–5012. https://doi.org/10.4268/cjcmmm.20151101

Wang FM, Hu ZA, Liu XY, Feng JQ, Augsburger RA, Gutmann JL, Glickman GN (2018) Resveratrol represses tumor necrosis factor-α-Jun N-terminal kinase signaling via autophagy in human dental pulp stem cells. Arch Oral Biol 97:116–121. https://doi.org/10.1016/j.archoralbio.2018.10.020
Waqas M, Khan AL, Kamran M, Hamayun M, Kang SM, Kim YH (2012) Endophytic fungi produce gibberellins and indoleacetic acid and promotes host-plant growth during stress. Molecules 17:10754–10773. https://doi.org/10.3390/molecules170910754

Wonglom P, Ito SI, Sunpapao A (2020) Volatile organic compounds emitted from endophytic fungus Trichoderma asperellum T1 mediate antifungal activity, defense response and promote plant growth in lettuce (Lactuca sativa). Fungal Ecol 43:100867–100875. https://doi.org/10.1016/j.funeco.2019.100867

Wu XL, Cui GL, Liu F, Li LY (2018) Isolation and molecular identification of endophytic fungi from Artemisia Annua and study on the growth promoting effect of Trichoderma Viride on Artemisia Annua. J Trop Subtrop Bot 26:56–64

Xing XK (2018) Endophytic fungal resources of medicinal plants: a treasure trove to be developed. Mycosystema 37:14–21

Yuan M, Tan SJ, Sun JG (2016) Isolation, identification and biological characteristics of endophytic nitrogen-fixing bacteria in rice and their effects on cadmium uptake by rice seedlings. Scientia Agricultura Sinica 49:3754–3768. https://doi.org/10.3864/j.issn.0578-1752.2016.19.008

Zhang GQ, Zhao HP, Wang ZY, Cheng JR, Tang XM (2008) Research advances in chemical composition and pharmacological activities of Rumex. World Sci Technol-Mod Tradit Chin Med 10:86–93. https://doi.org/10.3969/j.issn.1674-3849.2008.05.020

Zhang SB, Hou L, Pan Q, Wang XM, Cui QH, Tian JZ, Ma LY (2015) Research progress of high-throughput transcriptome of traditional chinese medicine. China J Chin Mater Med 39:1553–1558. https://doi.org/10.4268/cjcmm20140902

Zhou K, Wu WD, Song XQ, Zhou Y (2018) Screening and identification of endophytic fungi from Anoectochilus Roxburghii. J Trop Biol 9:440–444

Zhou LS, Tang S, Guo SX (2018) Endophytic fungus cladosporium sp effect on Salvia Miltiorrhiza growth and salvianolic acid content. Mycosystema 37:95–101

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