Transcriptomic Analysis Reveals the Mechanism of Host Growth Promotion by Endophytic Fungus of *Rumex Gmelinii* Turcz.

Changhong Ding (598808726@qq.com)
Heilongjiang University of Traditional Chinese Medicine: Heilongjiang University of Chinese Medicine
https://orcid.org/0000-0002-1502-9434

Shouyu Wang
Heilongjiang University of Traditional Chinese Medicine: Heilongjiang University of Chinese Medicine

Jiabin Li
Heilongjiang University of Traditional Chinese Medicine: Heilongjiang University of Chinese Medicine

Zhenyue Wang
Heilongjiang University of Traditional Chinese Medicine: Heilongjiang University of Chinese Medicine

Research Article

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Abstract

Rumex gmelinii Turcz. (RGT) is a medicinal plant of Rumex in Polygonaceae. Our research group isolated an endophytic fungus plectosaphaerella cucumerina (Strain J-G) from RGT, which could promote the growth of the host significantly when was co-cultured with the host. In this study, we mainly used transcriptomic analysis to explore the molecular mechanism of the growth promoting effect. It was found that during the process of co-culture with Strain J-G, metabolism of amino acids, the synthesis and metabolism of carbohydrates in RGT tissue culture seedlings were all promoted, which provided sufficient material and energy basis for the growth of plants. In addition, response to hormones such as auxin and cytokinin in RGT tissue culture seedlings was improved significantly, which promoted the growth and development of plants. The repairing ability of DNA in RGT tissue culture seedlings was also enhanced to provide a good guarantee for the growth of plants. Meanwhile, defense system of RGT tissue culture seedlings was also mobilized by the existence of Strain J-G, therefore more secondary metabolites and stress resistant substances were produced to ensure the normal growth and metabolism of plants.

Introduction

Rumex gmelinii Turcz. (RGT) is a medicinal plant of *Rumex* in Polygonaceae, which is distributed in Northeast and North of China. The root of RGT is used as medicine (Zhang GQ et al. 2008) which is cold-natured, bitter in taste, non-toxic, and beneficial to lung and heart. It can be used for the treatment of constipation, carbuncle, swelling, scabies and other diseases. It mainly contains resveratrol, polydatin, musizin, chrysophanol, emodin, physcion and other components (Wang ZY et al. 2009; Wang ZY et al. 2005).

Endophytic fungi widely exist in various tissues and organs of plants, such as roots, stems, leaves, and flowers, and can be separated from sterilized tissues or organs of plants (Guo SX 2008; Katoch M and Pull S 2017). Some endophytic fungi can promote plant growth (Jia M et al. 2016), enhance the ability of plants to resist adversity (Waqas et al. 2012) and increase the accumulation of effective components in plants (Xing XK 2018). In recent years, more and more attention has been paid to the role of endophytic fungi in promoting the growth of host. Some endophytic fungi that can promote the growth of the host have been screened from various medicinal plants, such as *Rehmannia glutinosa* (Chen BB et al. 2011), *Salvia Miltiorrhiza* (Zhou LS et al. 2018), *Anoectochilus roxburghii* (Zhou K et al. 2018), *Artemisia annua* (Wu XL et al. 2018) *Santalum album*(Liu J et al. 2018).

Strain J-G has been isolated and screened from RGT which can promote the growth of the host significantly when is co-cultured with the host. There are many reasons for the promotion of growth. Maybe endophytic fungi in RGT form plant hormones of growth promoting, or promote the synthesis of certain plant hormones, it may also be associated with various metabolisms. It is found that some endophytic fungi can produce plant hormones such as indoleacetic acid (IAA) and gibberellin (GAs), which can promote the growth of plants (Asaf LS et al. 2019). There are also some endophytic fungi have the abilities to fix nitrogen, dissolve phosphorus and dissolve potassium, which can increase the

absorption of inorganic elements such as nitrogen, phosphorus and potassium, so as to promote the growth of plants (Yuan M et al. 2016). Although many endophytic fungi that can promote the growth of plants have been found, there are few studies on the mechanism of growth-promoting, which usually focuse on hormone (Asaf LS et al. 2019), enzyme activity (Prisana Wonglom et al. 2020), and signal transduction (Sun X et al. 2020). But the organism is a whole, every biological phenomenon may be associated with a variety of physiological processes, and is the result of a variety of reactions. Therefore, we intended to use the method of omics to comprehensively study the growth-promoting mechanism of RGT by Strain J-G.

The transcriptome is the sum of the transcriptional products of all genes in a particular organism in a certain state (Wang XL et al. 2015; Zhag SB et al. 2014), which is the link between the genome and the proteome. The transcriptome focuses on the expression of functional genes that can describe the molecular mechanism in biological processes. Now transcriptome analysis has become a more mature and popular technology in the field of Biology. Through transcriptome analysis, not only the high-throughput information of gene expression at RNA level can be obtained, but also the internal relationship between gene expression and life phenomena can be revealed, so as to characterize the physiological activity of life and determine its metabolic characteristics. Many researches have shown that transcriptome analysis is highly feasible in studying the interaction mechanism between plants and fungi (Liu KH et al. 2017; Lamdan NL et al.2015).

The objective of this study was to explain the molecular mechanism of host growth promotion by Strain J-G of RGT based on transcriptome analysis. In order to provide basis for the application of endophytic fungi.

Methods

Isolation and identification of endophytic fungus strain J-G

Strain J-G is an endophytic fungus isolated from RGT. The genomic DNA of Strain J-G was extracted and amplified the ITS □ sequence by PCR. Then the sequencing result was entered into GenBank for comparison. After that sequences with high homology to ITS □ of Strain J-G were selected, downloaded, and input into the software MEGA5 to test. The method of neighbor joining was used to establish phylogenetic tree.

Study on the growth-promoting effect of strain J-G

RGT tissue culture seedlings with the same growth state were selected and implanted into MS solid medium, one seedling per medium. These seedlings were placed in a light incubator, temperature (day / night) 25°C / 18°C, photoperiod 14/10 h, light intensity 3000 lx, cultured for 5 d. The Strain J-G was inoculated on the medium 2cm away from the RGT tissue culture seedling with an inoculating needle. Then these seedlings were placed in a light incubator, emperature (day / night) 25°C / 18°C, photoperiod 14/10 h, light intensity 3000 lx, co-cultured for 25 d, six repetitions were set under the same conditions.

The other treatment conditions of the control group were the same except no inoculation of Strain J-G, six repetitions were set under the same conditions. Three replicates of co-culture group and control group were used in the study of physiological indicators, and the other three replicates were used in the analysis of transcriptome.

RNA extraction

Total RNA of both co-culture group and the control group was extracted by Total RNA Extractor (Shanghai Shengong). Integrity of RNA samples was detected by 1% agarose electrophoresis, and Keao k5500 spectro-photometer was used to detected the purity of samples. Agilent 2100 RNA Nano 6000 Assay Kit was used to detected the concentration of RNA samples.

Library construction and sequencing

After the total RNA samples were detected qualified, the mRNAs were enriched by magnetic beads with Oligo (dT), and fragment buffer was added to the obtained mRNAs to make the fragments into short fragments. The fragmented mRNAs were used as templates to synthesize the first strands of cDNA with six base random primers. Then buffers, dNTPs, RNaseH, and DNA Polymerase I were added to synthesize the second strand of cDNA continually. The obtained cDNAs were purified by QIAQuickPCR kit and were eluted with EB buffer. After that, the double stranded cDNAs were treated with terminal repair, added base A and sequencing connector. Finally, the target size fragments were recovered by agarose gel electrophoresis and were amplified by PCR to complete the entire library preparation. The constructed library was sequenced using the Illumina platform, and the sequencing strategy was PE150.

The quality control of the data

Raw data were processed with Perl scripts to ensure the quality of data used in further-analysis. Adaptor-polluted reads, low-quality reads, reads with number of N bases accounting for more than 5 % were removed (Pang W 2018).The obtained clean data after filtering would be carried out on statistics analyses on its quality, data quantity and quality, including Q30.

Assembly, coding regions prediction and annotation

The software Trinity was used for assembly. TransDecoder was used to identify the open reading frame (ORF) of the assembled transcripts. Trinotate was used to annotate the predicted ORF and transcripts.

Analysis of gene different expression

HTSeq v0.6.0 was used to count the Reads for each gene in each sample, and RPKM (Reads Per Kilobase Millon Mapped Reads) was used to estimate the expression level of certain genes in each sample Ren J 2019 .DESeq2v1.4.5was used for differential gene expression analysis. The P-value were assigned to each gene and adjusted by the Benjamini and Hochberg approach for controlling the false discovery rate.

Genes with q≤0.05 and |log2_ratio|≥1were identified as differentially expressed genes (DEGs). Then heat maps were made based on the expression of samples.

GO and KEGG analysis

Hypergeometric test was used foranalysis 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 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 to be remarkably enriched.

Verification by real-time PCR

Real time PCR was used to detect the expression level of six genes in energy metabolism and plant hormone signal transduction pathway of co-cultured RGT tissue culture seedlings. GAPDH was taken as internal reference gene. The gene expressions 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 detected.

Bio-Rad iQ-5 Fluorescence Quantitative Analyzer provided CT values of target genes and internal reference gene after amplifications were completed. Gene expressions were calculated by $2^{-\triangle\triangle CT}$. $\triangle\triangle$CT=(CT Objective - CT Internal Reference)Co-culture Group-(CT Objective - CT Internal Reference) Control Group

Results

Identification of endophytic fungus strain J-G

The blast comparison of ITS sequence showed that the Strain J-G was 100% similar to plectosaphaerella cucumerina. The sequence obtained has been submitted to GenBank, No. MT068424. In the NJ tree (Fig. 1-A), Strain J-G was in the same branch with Plectosphaerella cucumerina (EU030361.1). Therefore, Strain J-G was identified as Plectosphaerella cucumerina. The colony of its Strain was 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.

Compared with the control group, the fresh weight of the roots of co-cultured RGT tissue culture seedlings increased by 176% (Fig. 2-A), and the dry weight of roots increased by 161% (Fig. 2-B). These two differences were significant. Both the fresh weight and dry weight of the above-ground parts of co-cultured group were significantly higher than that of the control group (Fig. 2-C, D). Thus, it could be seen that the growth promoting effect of Strain J-G on host was comprehensive and significant.

RNA extraction and detection

The results of RNA extraction and detection were shown in the Table 1. The Detection from all aspects showed that the quality of total RNA of both the co-cultured group (G1a, G1b, G1c) and the control group (C1a, C1b, C1c) was qualified, and downstream experiments could be carried out.

Table 1 Total RNA test results of samples

Sample	Concentration (ng/μL)	Volume (μL)	Total (μg)	OD260/280	OD260/230	25S/18S*	RIN*
C1a	1245	25	31.1	2.1	1.9	1.2	8.4
C1b	1002	25	25.1	2.1	1.9	1.3	8.1
C1c	594	25	14.9	2.1	1.9	1.6	8.9
G1a	480	25	12.0	2.1	0.8	1.3	8.9
G1b	387	25	9.7	2.1	0.9	1.0	7.1
G1c	423	25	10.6	2.1	1.5	1.4	8.4

RIN* : RNA Intergrity Number, RNA integrity index

Test results of transcriptome data after filtering

The transcriptome data after filtering were submitted to SRA (SRR11308217), the test results of it were shown in Fig.3. The total numbers of filtered high-quality sequences of the three replicates (G1a, G1b, G1c) in the co-cultured group were 63,700,940, 63,696,030, and 63,412,070. The proportions of the filtered high-quality sequences to the original ones were 95.71%, 95.03% and 94.82%, which indicated that the sequencing qualities were acceptable. The total numbers of filtered high-quality sequences of 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%, which indicated that the sequencing qualities were acceptable.

The basic situation of gene different expression

According to the expression analysis, there were a total of 34,553 significantly different expressed unigenes between co-cultured group and the control, of which 25,756 were up-regulated and 8,797 were down-regulated.

According to the comparison between co-cultured group and the control, the scatter diagrams of log2foldchange of multiples of gene expression differences were obtained (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 the other genes that were not significant differentially expressed. It was showed that the up-regulated genes had more significant expression differences.

The results of GO analysis

The bubble diagram of the ten GO items with the highest significance of unigenes enrichment in Biological Process (BP) were shown in Fig. 4. The larger the bubble was, the higher the Rich_Ratio was, the redder the color was, the higher degree of enrichment was. It could be seen from Fig. 4 that the unigenes in the ten items, such as metabolic process, heterocycle biosynthetic process and organic cyclic compound biosynthetic process were more annotated, among which the most annotated and enriched item was the metabolic process.

The bubble diagram of the ten GO items with the highest significance of unigene enrichment in Cellular Component(CC) were shown in Fig.4. It showed that the unigenes in the ten items, such as intrinsic component of membrane, integral component of membrane and extracellular region were more annotated, among which the most annotated item was integral component of membrane, and the most enriched item was extracellular region.

The bubble diagram of the ten GO items with the highest significance of unigenes enrichment in Molecular Function(MF) were shown in Fig.4. It showed that the unigenes in the ten items, such as catalytic activity, transition metal ion binding and DNA binding were more annotated, among which the most annotated and enriched item was the catalytic activity.The statistics of annotation results of different expressed unigenes in secondary Go items were shown 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 /down-regulated different expressed unigenes. The right ordinate indicated the quantitis of up-regulated different expressed unigenes /down-regulated different expressed unigenes, among which there were significant differences in gene expression of cellular component, biological process, and molecular function. In terms of the distribution, the different expressed unigenes in cellular component and biological process were more obvious.

The results of KEGG analysis

All the samples enriched KO were combined and the distribution diagram was made according to the enrichment significance q value of the sample in the KO (Fig.6). It was shown that pathways of 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, Isoquinoline alkaloid biosynthesis were significantly enriched.

Gene expression differences of co-cultured RGT tissue culture seedlings

By comparing the differentially expressed unigenes between the control group and the co-cultured group, it was found that there were significant differences in gene expression in many pathways, such as Phenylalanine biosynthesis pathway, Phenylalanine metabolism, Starch and sucrose metabolism, Plant hormone signal transduction and so on.

In the pathway of Phenylpropanol biosynthesis (Table 2), unigenes homologous to genes of phenylalanine ammonia lyase (PAL) were up-regulated, while unigenes homologous to 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 could be promoted through the enzymatic reaction.

In Cyanamide acid metabolism pathway (Table 2), unigenes homologous to genes of Formamidase [EC: 3.5.1.49] were up-regulated, which promoted NH3 synthesis and nitrogen metabolism. Unigenes homologous to glycine methyl transferase (GlyA) were up-regulated, which promoted the metabolism and transformation of glycine, serine, threonine and cysteine.

In the Tyrosine pathway (Table 2), it was observed that unigenes homologous to genes of tyrosine aminotransferase [EC: 2.6.1.5], aromatic amino acid aminotransferase I [EC: 2.6.1.57], phosphohistidine aminotransferase (HisC) and aspartate aminotransferase[EC:2.6.1.1] were up-regulated. The mutual transformation of 4-hydroxy-phenylpyruvate with tyrosine could be promoted by these enzymes. Unigenes homologous to genes of 4-hydroxyphenylpyruvate dioxygenase (HPD), maleylacetate isomerase (MaiA), fumarylacetoacetase [EC:3.7.1.2] and other enzymes were up-regulated, which could catalyze the synthesis of acetoacetate and fumarate, and eventually fumarate entered the citric acid cycle to promote the material metabolism and energy formation. Meanwhile, unigenes homologous to genes of 4- hydroxy-2 - oxyheptanediate aldehydase (Hpal) and succinate semialdehyde dehydrogenase/glutarate semialdehyde dehydrogenase (GabD) were up-regulated, which could catalyze the conversion of 2, 4-dihydroxyhept-2-enedioate to succinate, then succinate entered the citric acid cycle to enhance the material metabolism and energy formation. In addition, unigenes homologous to genes of monoamine oxidase, alcohol dehydrogenase and aldehyde dehydrogenase (NAD (P) +) [EC: 1.2.1.5] were up-regulated, which could promote the metabolism and conversion of amino acids, in order to provide more materials for the growth of RGT tissue culture seedlings.

In Glycine, serine and threonine Metabolism, 78 unigenes homologous to genes of Amino acid invertases were up-regulated, which enhanced the metabolism and conversion of amino acids.

In Starch and sucrose metabolism (Table 2), unigenes homologous to genes of β-fructofuranosidase (INV) were up-regulated, which could promote the formation of 6-phosphate-glucose. Unigenes homologous to genes of α-glucosidase [EC: 3.2.1.20] were up-regulated, which could produce more

fructose and glucose. Unigenes homologous to genes of 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], cellulose 1,4-β-fibreglucosidase [EC: 3.2.1.91], α -trehalase [EC: 3.2.1.28] were up-regulated, which could catalyze the conversion of glucoside, cellulose, and trehalose to glucose. Therefore, substrates of respiration increased for energy provision of RGT tissue culture seedlings. Meanwhile, unigenes homologous to genes of starch synthetase (GlgA), 1,4-α-glucan branching enzyme [EC: 2.4.1.18] were up-regulated, which could catalyze the synthesis of starch. Therefore, it could be seen that the co-culture of the Strain J-G and RGT tissue culture seedlings not only promoted the formation of glucose, but also enhanced the accumulation of starch.

Table 2 Summary of some differently expressed genes of rgtco-cultured with endophytic fungus strain J-G

Unigene ID	Putative funtion	Log2 fold	Pathway
Phenylpropanol biosynthesis			
TRINITY_DN33839_c3_g2	Phenylalanine ammonia-lyase (PAL)	+4.8	Phenylpropanol biosynthesis
TRINITY_DN37056_c0_g2	cinnamoyl-CoA reductase (CCR)	-2.9	Phenylpropanol biosynthesis
TRINITY_DN31663_c0_g1	Shikimate O-hydroxy--cinnamoytransferase	-3.2(9.6)	Phenylpropanol biosynthesis
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	Fumarylacetoacetase	+7.71	Material metabolism and energy formation
TRINITY_DN2328_c0_g1	4- hydroxy-2 - oxyheptanediate aldehydase (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	Cellulose 1,4-β-fibreglucosidase	+8.47	Conversion between saccharides
TRINITY_DN15591_c0_g1	α - trehalase	+10.36	Conversion between saccharides
TRINITY_DN27249_c0_g2	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	diaminogalactose 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 (UGDH)	+0.62	UDP-D-Glucuronate formation
TRINITY_DN27361_c0_g2	UDP glycopyrophosphorylase (USP)	+0.51	Glucoronate formation
TRINITY_DN12877_c0_g1	β-glucuronidase	+9.52	Glucoronate formation
Steroid biosynthetic pathway			
TRINITY_DN36602_c0_g1	Farnesyl diphosphate farnesyl transferase (FDFT1)	-3.38	Steroid biosynthetic
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
TRINITY_DN37131_c0_g1	Auxin response factor (k4486)	+4.46	Growth-promoting hormones response
TRINITY_DN27355_c2_g1	Auxin responsive GH3 gene family(GH3)	+0.64	Growth-promoting hormones response
TRINITY_DN24352_c0_g1	SAUR family protein (SAUR)	+5.98	Growth-promoting hormones response
TRINITY_DN26677_c2_g1	Transport inhibitor responder 1 (TIR1)	-2.61	Growth-promoting hormones response
TRINITY_DN28612_c0_g1	Arabidopsis histidine kinase 2/3/4 (cytokinin receptor) (AHK2_3)	+5.44(43.64)	Cytokinin response
TRINITY_DN23829_c0_g1	Histidine-containing phosphotransfer peotein (AHP)	+8.21	Cytokinin response

TRINITY_DN31927_c0_g3	Two-component response regulator ARR-B family (ARR-B)	+3.47	Cytokinin response
TRINITY_DN25935_c0_g2	Two-component response regulator ARR-A family	+8.95	Cytokinin response
TRINITY_DN36970_c0_g1	Ethylene-insensitive protein 2 (EIN2)	-0.16	delay plant senescence
TRINITY_DN24552_c0_g1	Ethylene-insensitive protein 2 (EBF1 2)	-2.85	delay plant senescence
TRINITY_DN26260_c0_g1	Ethylene-responsive transcription factor 1 (ERF1)	-2.27	delay plant senescence
TRINITY_DN30065_c0_g2	BAK1	+2.64	Brassinosteroids response
TRINITY_DN32023_c0_g1	BRI1	+3.71	Brassinosteroids response
TRINITY_DN34069_c0_g2	BSK	+2.76	Brassinosteroids response
TRINITY_DN32302_c2_g1	TCH4	+3.66	Brassinosteroids response
TRINITY_DN24841_c0_g2	CYCD3	+5.63	Brassinosteroids response
TRINITY_DN33321_c0_g1	Jasmonic acid-amino synthetase (JAR1)	+2.87	Jasmonic acid synthesis
TRINITY_DN25813_c0_g1	Coronatine-insensitive protein 1 (COI-1)	+2.91	Plant hormone signal transduction
TRINITY_DN36389_c1_g2	Jasmonate ZIM domain-containing protein (JAZ)	+2.61	Plant hormone signal transduction
TRINITY_DN39357_c0_g1	Transcription factor MYC2 (MYC2)	+2.63	Plant hormone signal transduction
TRINITY_DN43631_c3_g2	Regulatory protein NPR1 (NPR1)	+3.44	improvement of plant disease resistance
TRINITY_DN30786_c1_g1	Transcription factor TGA	+4.08	improvement of plant disease resistance

In the Pentose and glucuronide interconversion pathway (Table 2), unigenes homologous to genes of galacturonidase [EC:3.2.1.67] were up-regulated, which could catalyze diaminogalactose to form

galactose, and galactose could participate in various metabolisms. Unigenes homologous to genes of L-threonine-3-deoxyhexanoic acid aldolase (GAAC) were up-regulated, which was beneficial for galactose to enter into the glycerol metabolism pathway and provide more energy for the growth of RGT tissue culture seedlings. Unigenes homologous to genes of alcohol dehydrogenase (NADP+) (AKR1A1) were up-regulated, which could catalyze gulonic acid to form more D-Glucuronate. Meanwhile, unigenes homologous to genes of UTP-glucose-1-phosphouridinyltransferase (UGP2) and UDP-glucose-6-dehydrogenase (UGDH) were up-regulated, which could catalyze the production of glycolysis– fructose phosphate to form more UDP-D-Glucuronate. And unigenes homologous to genes of UDP glycopyrophosphorylase (USP), glucuronosyl transferase (UGT), β-glucuronidase [EC: 3.2.1.31] were also up-regulated, which could promote the formation of glucoronate. As a result, a larger amount of glucoronate was produced to provide more materials for synthesis of aminosaccharide and ribose.

In the Steroid biosynthetic pathway (Table 2), unigenes homologous to genes of farnesyl diphosphate farnesyl transferase (FDFT1) were down-regulated. It could be inferred that biosynthesis of steroid would be inhibited to some extent.

In Basal resection and repair processes, 38 unigenes homologous to genes of most enzymes used in eukaryotic basal resection and repair were significantly up-regulated, only 13 unigenes homologous to genes of DNA-3-methyladenine glycosylase (MPG) and poly (ADP ribose) polymerase (PARP) were down-regulated. It could be inferred that the repair function of DNA was improved.

In the Plant hormone signal transduction pathway (Table 2), unigenes homologous to genes of auxin influx carrier (AUX1 LAX family), auxin-responsive protein IAA (IAA), auxin response factor (k4486), auxin responsive GH3 gene family(GH3) and SAUR family protein (SAUR) were up-regulated, while unigenes homologous to genes of transport inhibitor responder 1 (TIR1) were down-regulated, which promoted the response of cells to growth-promoting hormones, so as to accelerate the growth of RGT tissue culture seedlings. Unigenes homologous to genes of arabidopsis histidine kinase 2/3/4 (cytokinin receptor) (AHK2_3), histidine-containing phosphotransfer peotein (AHP), two-component response regulator ARR-B family (ARR-B) and two-component response regulator ARR-A family were up-regulated, which could enhance the response of RGT tissue culture seedlings to cytokinin, thereby cell division of seedlings was promoted. Unigenes homologous to genes of ethylene-insensitive protein 2 (EIN2), ethylene-insensitive protein 2 (EBF1 2), ethylene-responsive transcription factor 1 (ERF1) were down-regulated, which could delay plant senescence. Unigenes homologous to genes of BAK1, BRI1, BSK, TCH4, and CYCD3 that played an important role in the response of brassinosteroids were up-regulated, which could promote cell growth and cell division of RGT tissue culture seedlings. Unigenes homologous to genes of jasmonic acid-amino synthetase (JAR1) were up-regulated, which could promote synthesis of jasmonic acid. Unigenes homologous to genes of coronatine-insensitive protein 1 (COI-1), jasmonate ZIM domain-containing protein (JAZ) and transcription factor MYC2 (MYC2) were up-regulated. Because jasmonic acid can induce the expression of resistance genes, these differential expression of genes might be related to the infection of Strain J-G. Meanwhile, unigenes homologous to genes of regulatory protein NPR1 (NPR1) and the transcription factor TGA which were also related to the improvement of plant

disease resistance were up-regulated, it could be related to the infection of endophytic fungus and the resistance of the host.

In the Biosynthesis of unsaturated fatty acids, 26 unigenes were up-regulated and 13 unigenes were down-regulated, among which unigenes homologous to genes of acyl-CoA thioesterase 7[EC:3.1.2.2] and acyl-CoA thioesterase II (TesB) were up-regulated. These enzymes could catalyze the synthesis of α-linolenic acid, hexadecanoic acid, stearic acid and oleic acid, which played an important role in the resistance of plants.

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

In order 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, six genes of key enzymes, such as genes of phenylalanine ammonia lyase (PAL), cinnamoyl COA reductase (CCR), β-fructofuranosidase (INV), starch synthase (GlgA), auxin response protein IAA (IAA), cytokinin receptor (CYT) of RGT tissue culture seedlings were selected for quantitatively analysis of expression by real-time PCR.

As can be seen from the results (Fig. 7), genes of PAL, INV, GlgA, IAA, CYT were significantly up-regulated in co-cultured group and gene of CCR was significantly down-regulated compared with the control group. The results of these gene expression tests were consistent with those of the transcriptome analysis.

Discussion

PAL is an important regulatory enzyme in secondary metabolism, whose activity is controlled by many internal and external factors. The infection of viruses, bacteria and fungi can increase the expression of PAL gene, so as to promote the production of secondary metabolites and enhance the resistance of plants, which is a mean of plant self-protection. It could be seen from the regulation of phenylalanine metabolism that the up-regulated expression of synthesis related enzymes could produce more phytoalexin--resveratrol and polydatin. Jasmonate can induce the expression of resistance gene, unigenes homologous to genes of JAR1 were up-regulated, which promote the synthesis of jasmonate. Unigenes homologous to COI-1□MYC2 were also up-regulated, which activate the expression of jasmonate responsive genes. Unigenes homologous to regulatory protein NPR1 (NPR1) and transcription factor TGA were up-regulated, which were related to the improvement of plant disease resistance. The up-regulated expression of 26 unigenes in the Biosynthetic pathway of unsaturated fatty acids promoted the synthesis of unsaturated fatty acids for plant stress resistance. Hence it could be inferred that although plants and endophytic fungi could coexist peacefully, the presence of endophytic fungi still stimulated plants to mobilize the defense mechanism into defensive status.

Although RGT tissue culture seedlings were in the state of defensive, the growth of these seedlings were not inhibited. The results of the experiment conformed that co-culture with Strain J-G could significantly

promote the growth of RGT tissue culture seedlings. This mainly because Strain J-G not only enhanced defense responses of RGT tissue culture seedlings, but also promote the metabolism of them.

The variation of gene expression in the pathway of Cyanoamino acid metabolism not only promoted the metabolism of nitrogen, but also enhanced metabolism and transformation of glycine, serine, threonine and cysteine. The up-regulated expression of genes in the pathway of Glycine, serine and shreonine metabolism promoted the transformation between amino acids as well. In Tyrosine metabolism pathway, the up-regulated expression of tyrosine transaminase, acetylase and other enzymes could catalyze the synthesis of acetylacetone and fumarate, and the up-regulated expression of genes of Hpal and succinate semialdehyde dehydrogenase / glutaric acid semialdehyde dehydrogenase promoted the synthesis of succinate. Substances such as acetylacetone, fumarate and succinate could all enter the citric acid cycle to promote material metabolism and energy formation. Through the regulation of these pathways of amino acid metabolism, it could be inferred that 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.

The expression of 97 unigenes in Starch and sucrose metabolism were up-regulated. These unigenes homologous to enzymes which promoted the formation of fructose and glucose and the accumulation of starch components. The expression of 45 unigenes in pathway of Pentose and glucuronide interconversion were up-regulated which accelerated glyceride metabolism, thus provided more energy for the growth of RGT tissue culture seedlings. Meanwhile, the formation of glucuronic acid were also enhanced, which could provide more materials for synthesis of aminosaccharide and ribose. It could be inferred from the variation of carbohydrate metabolism that co-culture of Strain J-G and 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.

In the Plant hormone signal transduction pathway, the expression of 108 unigenes were up-regulated and 48 unigenes were down-regulated. The up-regulated expressions of unigenes homologous to responsive protein, responsive factors and receptors to growth hormones, cytokinins and brassinosteroids enhanced the response to these hormones. Meanwhile, the down-regulated expression of important genes inhibited the process of senescence. Though these phenomena it could inferred that the growth and division of RGT tissue culture seedlings were accelerated, while senescence was delayed.

In addition, the function of gene repair was improved. Base-excision repair (BER) is the main approach to DNA damage repair, which can treat small base damage caused by oxidative and alkylation damage. BER is usually defined as DNA repair initiated by damage specific DNA glycosylase, and is performed by either of the two sub-pathways: short patch BER and long patch BER. Each sub-pathway of BER depends on the formation of protein complexes, which gather at DNA damage sites and promote restoration in a coordinated manner. This complex process seems to increase the specificity and efficiency of the BER pathway, thus, the maintenance of genomic integrity is promoted by preventing the accumulation of

highly toxic repair intermediates. Most unigenes homologous to genes in the BER pathway were up-regulated, therefore the repair function of DNA was improved, which provided a good guarantee for the growth of RGT tissue culture seedlings.

Conclusion

During the co-culture of the endophytic fungus J-G and RGT tissue culture seedlings, metabolism of amino acids, synthesis and metabolism of carbohydrates were promoted, which provided sufficient materials and energies for the growth of RGT tissue culture seedlings. The responses to hormones such as auxin and cytokinin were improved, which enhanced the growth and development of RGT tissue culture seedlings. The DNA repair capacities were improved, which provided a good guarantee for the growth of RGT tissue culture seedlings. In addition, the existence of Strain J-G also activated the defense mechanism of RGT tissue culture seedlings, therefore more secondary metabolites and various stress-resistant substances were produced to ensure the growth and metabolism of RGT tissue culture seedlings.

Declarations

Author contributions CD and ZW designed this research. KL, JL did a study on the growth promoting effect of endophyticfungus, CD, JL and WS performed the related experimental research and analysis of transcriptome. CD and WS drafted the manuscript.

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Data availability and material Not applicable.

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Ethical approval Not applicable

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Conflicts of Interest

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Figures

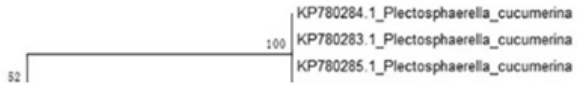

Figure 1

Endophytic fungus Strain J-G

Figure 2

Effects of Strain J-G on Various Physiological Indexes of Rumex gmelinii Turcz. Cultured Seedlings (n=3 *P < 0.05)

* represents a significant difference from the blank control (CK)

Figure 3

log2foldchange and Volcanic map of G1-C1 comparison

A Scatter diagram of log2foldchange value distribution B Volcanic map

Figure 4

Bubble diagram of the 10 GO items with the highest enrichment significance

Figure 5

Statistics of annotation results of different expressed genes in secondary GO items.

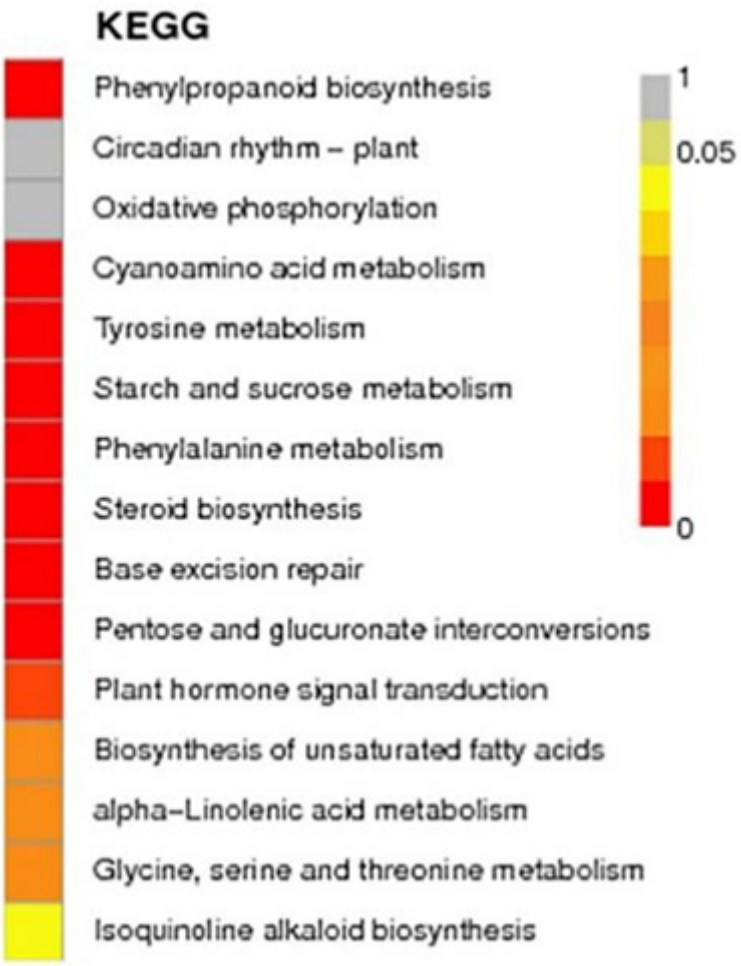

Figure 6

Significance of KEGG enrichment

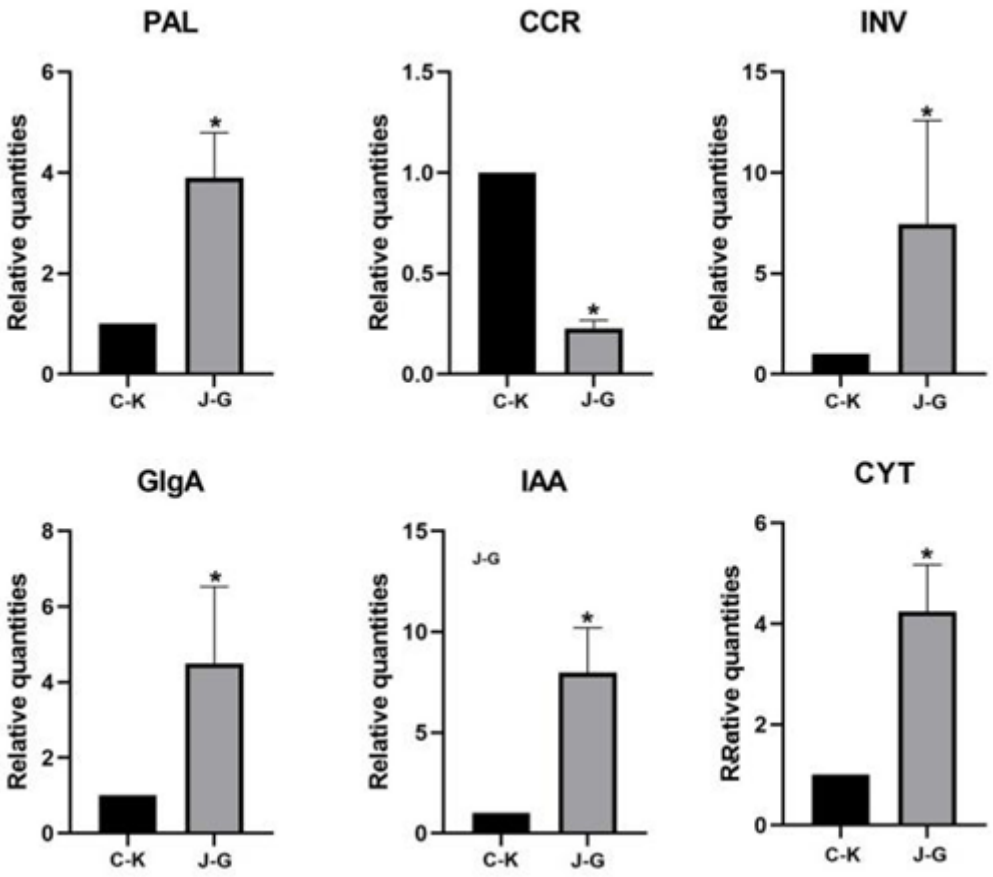

Figure 7

Different expression of each gene ($p < 0.05$, $n=3$)