Transcriptome analysis reveals underlying immune response mechanism of fungal disease in Gastrodia elata Bl. f. glauca S. Chow

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Research article

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Abstract

Background

Gastrodia elata Bl. f. glauca S. Chow is a rare medicinal plant. G. elata f. glauca is unavoidably infected by pathogens in their growth process, for they are usually grown in a semi-wild state. If it happens, that will seriously affect the yield and quality of their tubers. In previous work, we have successfully isolated and identified the pathogenic fungus from infected tubers in G. elata f. glauca. In this study, healthy and fungal diseased mature tubers of G. elata f. glauca from Changbai Mountain area were used as experimental materials.

Results

A total of 7540 differentially expressed Unigenes (DEGs) were identified (FDR < 0.01, log2FC > 2). Further analysis revealed that transcription factors (TFs) in plant hormone signal transduction pathway and plant pathogen interaction pathway, like WRKY22, GH3, TIFY/JAZ, ERF1, WRKY33, TGA, played vital role in fungal disease response. They mainly involved signal transduction, environmental adaptation, biosynthesis/metabolism of secondary metabolites. In addition, we found jasmonic acid/ethylene is the noteworthy signaling pathway in response to fungal disease in G. elata f. glauca.

Conclusions

The results reveal an underlying immune response mechanism of fungal disease in G. elata f. glauca and provide a novel insight into the breeding of disease resistant varieties of G. elata f. glauca.

Background

Gastrodia elata Bl. f. glauca S. Chow is a form of Gastrodia elata Bl. G. elata Bl., called tianma in Chinese, is a perennial monocotyledon. Its dry tuber is usually used as a precious traditional Chinese medicine Gastrodiae Rhizoma. It is recorded that Gastrodiae Rhizoma has the functions of resting wind and relieving spasmodic, calming liver and inhibiting yang, dispelling wind and relaxing channels and collaterals. Clinically, it is commonly used in children with infantile convulsions, epileptic convulsions, tetanus, headache and vertigo, hand and foot failure, limb numbness, rheumatism arthralgia and other symptoms[1]. According to Compendium of Materia Medica, taking for a long time can help the vital essence run smoothly in body, relax one's body and prolong one's life. Sheng Nong's Herbal Classic lists it as one of the top grades, which means it is tonic and nontoxic. Modern pharmacological research has shown that Gastrodiae Rhizoma has the effects of analgesia[2, 3], anti-inflammation[4], antiasthma[5], antioxidation[6–9], antidepress[10–13], anticonvulsion[14, 15], anti-osteoporosis[16, 17], neuroregulation[18, 19], neuroprotection[20–23], lowering blood pressure[24, 25], reducing blood lipid[25, 26], improving memory[27, 28], inhibiting melanin synthesis and so on. It also has auxiliary therapeutic effect on Alzheimer's disease (AD)[27] and Parkinson's disease (PD)[6, 7, 20, 22] which are the common degenerative diseases nowadays.
G. elata Bl. is an obligate fungal heterotrophic plant with highly degraded leaves and bracts. More than 80% of its life cycle exists underground in the form of tuber, depending almost entirely on fungi to provide nutrient[29]. It is closely related to at least two types of fungi: *Mycena* to promote seed germination and *Armillaria Mellea* to ensure reproductive growth. The growth and development of *G. elata* Bl. usually goes through seed, protocorm, juvenile tuber (also called mima in Chinese), immature tuber (also called baima in Chinese), mature tuber (also called jianma in Chinese), scape, flower, and fruit. During the growth and development of *G. elata*, it is susceptible to infection by non-essential fungi such as *Penicillium*[30], *Ilyonectria robusta*[31] and *Trichoderma hamatum*[32]. Of course, it can cause serious damage to the planting industry[33–38]. Now, it could make this possible to breed varieties with stable heredity by genomics-based techniques.

Wild *G. elata* is found in many countries such as Nepal, Bhutan, India, Japan, North Korea, New Zealand, Australia, Siberia and China. According to the bible *Flora of Yunnan*, there are six varietas of *G. elata*, and they are *G. elata* Bl. f. *pilifera* Tuyama, *G. elata* Bl. f. *viridis* Makino, *G. elata* Bl. f. *glauca* S. Chow, *G. elata* Bl. f. *alba* S. Chow, *G. elata* Bl. f. *elata* and *G. elata* Bl. f. *flavida* S. Chow. In China, we called them maotianma, lvitianma, wutianma, songtianma, hongtianma, huangtianma, respectively. Among them, *G. elata* f. *glauca* is one of the most popular in the market because of its good shape and high dry rate. In China, *G. elata* Bl. f. *glauca* is mainly distributed in northeastern Yunnan, western Guizhou, southern Sichuan and Changbai Mountain area.

So far, the most studied *G. elata* Bl. variety is *G. elata* Bl. f. *glauca* in Zhaotong City, Yunnan Province. However, the genomics research of *G. elata* Bl. f. *glauca* genetics in Changbai Mountain area is almost blank. In fact, *G. elata* Bl. f. *glauca* is not only a traditional Chinese medicinal material in Changbai Mountain, but also one of the most vital special economic crops in Jilin Province. Therefore, whether it is thought from the breeding of high-quality traditional Chinese medicine germplasm resources, or considered on the improvement of local economic development level, the research on disease response mechanism of *G. elata* Bl. f. *glauca* in Changbai Mountain area has important production significance and application value. In this study, we made a detailed comparison between healthy and fungal diseased *G. elata* Bl. f. *glauca* tubers by means of transcriptome sequencing and metabolic pathway analysis. That would provide a new insight for the breeding of disease resistant varieties of *G. elata* Bl. f. *glauca*.

**Results**

**Sequencing overview**

A total of 45.88 GB clean data were generated by sequencing platform, and clean data number of each sample was more than 6.23 GB. GC content ranged from 47.16% to 49.09%, and Q30 of each sample was above 92.92% (Additional file: Table S1). The sequencing results showed that sequencing fragments had high randomness and reliability (Additional file: Figure S1A). After transcript *de novo* assembly, 60324 Unigenes in total were obtained, and the N50 was 2409 kb. Furthermore, 19670 (32.61%) of them were over 1 kb in length (Additional file: Figure S1B). All these indicative data display high assembly integrity.

**Functional annotation and differential expression analysis**

DEGs annotation and function classification
A total of 5140 DEGs were annotated to seven databases. nr (RefSeq non-redundant proteins) had the largest number of annotated DEGs (5066), while KEGG had the least (1745) (Fig.1a). The venn diagram of function annotation in different database as Fig.1b. It was learned that those DEGs between healthy and fungal diseased samples chiefly classified into “signal transduction mechanisms”, “carbohydrate transport and metabolism”, “defense mechanisms”, “energy production and conversion”, “general function prediction only”, “posttranslation modification, protein turnover, chaperones”, “translation, ribosomal structure and biogenesis” (Fig.1c, d). We can believe that G. elata Bl. f. glauca possibly respond to fungal disease by enhancing or weakening these physiological or biological activities.

**GO enrichment and KEGG enrichment analysis**

Using GO database, 2482 DEGs were enriched into 3958 GO terms. GO terms are usually classified into 3 categories: biological process (BP), cellular component (CC), molecular function (MF). Here, 2363 (59.70%) of these GO terms fell into BP, 509 (1.49%) belong to CC, and 1086 (27.44%) were part of MF. Noteworthily, 36 GO terms were about signal transduction, and 24 GO terms were relevant to hormone. By Kolmogorov-Smirnov test, 421 GO terms were significantly enriched (P-value<0.05). Part of them were showed in Additional file: Table S2 and top 30 were displayed as Fig.2a.

Using KEGG database, 122 pathways were enriched and top 50 was showed as Figure 2c. The enrichment degree was based on the P-value and enrichment factor (Fig. 2b). 9 pathways were significantly enriched (p<0.05), and they fell into 3 pathway categories: metabolism, environmental information processing, organismal systems. Specific pathway names displayed in Table 1.

| Pathway category       | Pathway description                     | Specific pathway            | ko ID       | DEG  | All Unigene | P-value |
|------------------------|-----------------------------------------|----------------------------|-------------|------|-------------|---------|
| Metabolism             | Carbohydrate metabolism                 | Starch and sucrose         | ko00500     | 49   | 169         | 0.041   |
|                        | metabolism                             |                            |             |      |             |         |
|                        | Metabolism cofactors and vitamins        | Ubiquinone and other       | ko00130     | 12   | 32          | 0.047   |
|                        | Terpenoids and polyketides              | terpenoid-quinone biosynthesis |          |      |             |         |
|                        | Metabolism terpenoids and polyketides   | Brassinosteroid biosynthesis | ko00905     | 7    | 11          | 0.005   |
|                        | of and flavonoids                       | Diterpenoid biosynthesis   | ko00904     | 7    | 12          | 0.009   |
|                        | Biosynthesis of other secondary         | Flavone and flavonol       | ko00944     | 5    | 5           | 0.001   |
|                        | metabolites                            | biosynthesis               |             |      |             |         |
|                        | Phenylpropanoid biosynthesis            | Phenylpropanoid biosynthesis | ko00940     | 36   | 96          | 0.001   |
|                        | Flavonoid biosynthesis                  | Flavonoid biosynthesis     | ko00941     | 14   | 32          | 0.008   |
| Environmental          | Signal transduction                     | Plant hormone signal       | ko04075     | 44   | 136         | 0.008   |
| Information Processing |                                          | transduction               |             |      |             |         |
| Organismal Systems     | Environmental adaptation                 | Plant-pathogen interaction | ko04626     | 38   | 122         | 0.023   |

Table 1 KEGG pathway enrichment analysis (p<0.05)
Differential expression analysis

A total of 7540 DEGs were identified. 4326 of these DEGs were up-regulated in diseased group, and 3214 were down-regulated (Fig.3a, b). In addition, 40440 Unigenes did not demonstrate significantly differential expression. In other words, DEGs between healthy and diseased samples accounted for 15.71% of all Unigenes.

Transcription factor prediction

By the standard of FDR<0.01 and FC>2, 1295 DEGs were identified as transcription factors with transcription factor prediction tool (Fig.4). Here, transcription factor family covers transcription factor (TF), transcription regulator (TR), protein kinases (PK). It could be clear to see that many DEGs were the members of transcription factor families MYB, ERF, C2H2, NAC, bHLH, C3H, WRKY, bZIP, GRAS, PHD, SNF2, SET. Coincidently, most of those transcription factor families have been proved that their expression or regulation can directly or indirectly affect plant disease resistance[39-58]. Exceptionally, present reports about C3H are mainly related to cold resistance, rather than disease resistance[59, 60].

KEGG pathways analysis

So far, it has been proved that plant disease resistance is relative to plant-pathogen interaction, plant hormone signal transduction, and other pathways about certain secondary metabolite biosynthesis or metabolism[56, 61-64]. Consistently, we got similar results in this study (Fig.5-7, Table 1).

In plant-pathogen interaction (Fig.5), genes except WRKY1/2 were all up-regulated. They were CDPK (calcium-dependent protein kinase), Rboh (respiratory burst oxidase homolog), CNGC (cyclic nucleotide gated channel), calcium-binding protein CML (calmodulin-like protein), LRR (leucine-rich repeat) receptor-like serine/threonine-protein kinase FLS2, MEKK1 (mitogen-activated protein kinase kinase kinase 1), MKK4/5 (mitogen-activated protein kinase kinase 4/5), WRKY transcription factor 33, WRKY transcription factor 22, RIN4 (RPM1-interacting protein 4), serine/threonine-protein kinase PBS 1, molecular chaperone HtpG. Biological processes these up-regulated genes principally involved were hypersensitive response (HR), cell wall reinforcement, defense-related gene induction, phytoalexin accumulation and miRNA production. Some of these genes were involved in PAMP-triggered immunity. Only WRKY transcription factor 2 displayed down-regulated expression, and it was connected with HR, defense-related gene induction and programmed cell death.

Further to analyze the map of plant hormone signal transduction (Fig.6), we learned that GH3 (auxin responsive glycoside hydrolase 3 gene family), AHP (histidine-containing phosphotransfer protein), ARR-B (two-component response regulator ARR-B family), PIF4 (phytochrome-interacting factor 4), ERF1 (ethylene-responsive transcription factor 1), JAZ (jasmonate ZIM domain-containing protein) were up-regulated. In the same pathway, AUX1 (auxin influx carrier), ARF (auxin response factor), CRE1 (cytokinin receptor enzyme), DELLA protein, PP2C (protein phosphatase 2C), EIN2 (ethylene-insensitive protein 2), BZR1/2 (brassinosteroid resistant 1/2), JAR1 (jasmonic acid-amino synthetase), COI1 (coronatine-insensitive protein 1), transcription factor TGA showed down-regulated. As it described, transcription factor TGA is connected with disease resistance. DEGs in this metabolic pathway involved many biological processes, such as cell enlargement, plant growth, cell division, shoot initiation, stem growth, stomatal closure, seed dormancy, fruit ripening, senescence, monoterprenoid biosynthesis, indole alkaloid biosynthesis, cell elongation, of course, disease resistance as well. Interestingly, above biological processes usually accompanied by phosphorylation (+p), dephosphorylation (-p),
ubiquitination (+u). Phosphorylation and ubiquitination are common post-translational modification of proteins. They play an important role in pattern-triggered immunity (PTI), and simultaneously be necessary to receptor complex activation signals and cell homeostasis. Noteworthily, phytohormone played a vital role in this pathway. Specifically, they included jasmonic acid (JA), salicylic acid (SA), ethylene (ET), brassinosteroid (BR), auxin, cytokinin, gibberellin, abscisic acid.

Furthermore, numerous DEGs regulating secondary metabolites biosynthesis, like ubiquinone, brassinosteroid, diterpenoid, phenylpropanoid, flavone and flavonol, revealed active state. In addition, genes related metabolic process of secondary metabolites also revealed significant differential expression. In starch and sucrose metabolism pathway, DEGs related to fructose and glucose synthesis is up-regulated, while DEGs linked to starch and glycogen production showed down-regulated expression.

In particular, brassinosteroid is one of crucial phytohormone closely related to plant growth and stress response. In brassinosteroid biosynthesis map, CYP90D2 (steroid 3-oxidase) showed up-regulated expression; CYP90A1 (cytochrome P450 family 90 subfamily A polypeptide 1) displayed down-regulated expression; CYP734A1/BAS1 (PHYB activation tagged suppressor 1) was mix-regulated, with two genes up-regulated and one gene down-regulated (Fig.7).

In summary, fungal disease response is a complex process involving multiple biological processes. And meanwhile, there is more than one gene participated in this process. Interestingly, one gene was not appeared in single pathway. That is to say, one gene may perform more than one function simultaneously. Anyway, these significantly enriched pathways would well reveal the underlying response mechanism of fungal disease in *G. elata* Bl. f. *glauca*.

### Potential immune response mechanism of fungal disease in *G. elata* Bl. f. *glauca*

Actually, in this study, many genes related to stress response and disease resistance demonstrated high expression and significant difference. Moreover, They were members of certain transcription factor families, like WRKY, GH3, JAZ, CML, ERF, TGA. Furthermore, these genes were closely connected with derivatives of jasmonic acid, salicylic acid, brassinosteroid, ethylene and auxin. By BLAST (blast.ncbi.nlm.nih.gov/Blast.cgi), it is revealed that amino acid sequences of four JAZ genes in *G. elata* family were highly similar to certain sequences in *Dendrobium catenatum*, *Phalaenopsis equestris*, *Apostasia shenzhenica* (Figure 8). Noteworthily, they were all belong to TIFY10 family.

Finally, we found 10 candidate genes responding to fungal disease in *G. elata* Bl. f. *glauca* (Fig.8; Table 2). Seven of them participated in plant hormone signal transduction (ko04075) pathway, and three were part of plant-pathogen interaction (ko04626) pathway.

| Table 2 Information of disease resistance genes. ko04626: plant-pathogen interaction; ko04075: plant hormone signal transduction. K13425: WRKY22; K14487: GH3; K13464: JAZ; K13448: CML; K14516: ERF1; K13424: WRKY33; K14431: TGA. |
| gene ID           | FDR          | log2FC       | regulated | pathway | KEGG entry | nr annotation                                                                 |
|------------------|--------------|--------------|-----------|---------|------------|--------------------------------------------------------------------------------|
| c65017.graph_c0  | 8.65E-170    | 10.60551     | up        | ko04626 | K13425     | probable WRKY transcription factor 27, [Phalaenopsis equestris]                  |
| c32310.graph_c0  | 7.67E-97     | 9.531941     | up        | ko04075 | K14487     | probable indole-3-acetic acid-amido synthetase GH3.1 [Phalaenopsis equestris]   |
| c75818.graph_c1  | 5.48E-19     | 5.547811     | up        | ko04075 | K13464     | protein TIFY 10a-like [Dendrobium catenatum]                                   |
| c76234.graph_c1  | 1.23E-15     | 5.462372     | up        | ko04075 | K13464     | protein TIFY 10c-like [Dendrobium catenatum]                                   |
| c60520.graph_c0  | 1.11E-77     | 4.956140     | up        | ko04075 | K13464     | protein TIFY 10a-like [Dendrobium catenatum]                                   |
| c75190.graph_c0  | 3.26E-110    | 4.430928     | up        | ko04626 | K13448     | probable calcium-binding protein CML18 [Phalaenopsis equestris]                |
| c78203.graph_c0  | 7.49E-63     | 4.276427     | up        | ko04075 | K14516     | ethylene-responsive transcription factor 1B-like [Dendrobium catenatum]        |
| c78388.graph_c1  | 1.21E-46     | 3.499401     | up        | ko04075 | K13464     | protein TIFY 10a-like [Dendrobium catenatum]                                   |
| c71906.graph_c0  | 6.55E-51     | 2.652510     | up        | ko04626 | K13424     | WRKY transcription factor WRKY24-like isoform X1 [Dendrobium catenatum]        |
| c74033.graph_c1  | 8.03E-27     | -2.15672     | down      | ko04075 | K14431     | transcription factor TGA1-like [Dendrobium catenatum]                          |

**Discussion**

According to research in available, plant disease response mechanisms mainly include PAMP-triggered immunity (PTI), effector-triggered immunity (ETI) and systemic acquired resistance (SAR). ETI is usually accompanied by the occurrence of hypersensitivity reaction (HR), giving rise to programmed cell death (PCD). Moreover, ETI can also induce SAR. As is known to all, PTI and SAR are non-specific immunity, while ETI is specific immunity. From what we study, it can be concluded that the disease response mechanism of *G. elata* Bl. f. *glauc*a involves all above three kinds of mechanisms in the whole process of infection. Resistance genes (R genes) were classified into nine types based on intracellular and extracellular pathogen recognition mechanisms[65]. Here, we discovered potential R genes in *G. elata* Bl. f. *glauc*a were probably the members of
transcription factor families like WRKY, GH3, TIFY/JAZ, CML, ERF, TGA. Coincidentally, it has been reported that these above transcription factors did be widely involved in various defense responses[56, 66–78]. Interestingly, GH3 and CML can also regulates fruit development[79, 80]. However, it still needs further study on how these genes perform their functions in respond to fungal disease in *G. elata* Bl. f. *glauca*.

In fact, plant hormones also have a vital role in the process of plant-pathogen interaction. Simultaneously, ETI itself can also induce and generate certain special plant hormone signal[80]. In this research, we found a large number of DEGs annotated to signal transduction mechanisms by means of functional annotation. Furthermore, lots of DEGs were markedly enriched into plant hormone signal transduction pathway in which mainly involved jasmonic acid and ethylene using the KEGG database. Consistently, it has been reported that auxin[81, 82], cytokinins[83, 84], ethylene[82, 85–87], gibberellin[88], abscisic acid[82, 89, 90], brassinosteroids[87], salicylic acid[82, 85, 91], jasmonic acid[82, 85, 91–93], strigolactones[94] can actively participate in disease response. Among them, salicylic acid pathway and jasmonic acid/ethylene pathway are considered as the most common plant hormone signal transduction pathways in response to biological or abiotic stress. It could even be said that the plant resistance to pathogen is initially stimulated by gene expression regulated by transcription factors and ultimately be mediated by plant hormones. Therefore, it is necessary to study phytohormone metabolism of *G. elata* Bl. f. *glauca* in the following work.

In addition, some DEGs in ubiquinone and other terpenoid-quinone biosynthesis pathway, like 4CL, were also significant differential expression between healthy and diseased group. 4CL belongs to the plant phenylpropane derivative, which is related to the synthesis of flavonoids and lignin and is a key enzyme in the biosynthetic pathway. A report indicates that Fm4CL-like 1 is involved in secondary cell wall development and lignin synthesis and it play an important role in osmotic stress by affecting cell wall and stomatal development[95]. This may be a part of fungal disease response mechanism in *G. elata* Bl. f. *glauca*.

**Conclusions**

In conclusion, fungal disease response mechanism in *G. elata* Bl. f. *glauca* is quite complicated. Firstly, JA/ET signal transduction play a positive role in regulation. The up-regulation expression of JAZ and ERF1 indirectly induces ubiquitin mediated proteolysis. Secondly, SA signal transduction reveals negative regulation. The down-regulation expression of TGA indirectly triggered disease resistance. Thirdly, brassinosteroid biosynthesis also makes contributions to fungal disease response. CYP90A1 and CYP90D2 display down-regulation and up-regulation, respectively. Last but not least, auxin signaling pathway involves in fungal disease response actively. However, JA/ET signaling pathway is undoubtedly the most highlighted. As the candidate genes response to fungal disease in *G. elata* Bl. f. *glauca*, their specific functions still need to be further verified. Of course, more insight into the molecular mechanisms of fungal disease response also requires to be revealed.

**Methods**

**Plant material and growth conditions**

Three healthy tubers (HGe) and three fungal diseased tubers (DGe) were used in this experiment. They were identified as mature tubers of *G. elata* Bl. f. *glauca* S. Chow by Professor Yugang Gao in Jilin Agricultural University.
These samples were collected from a planting base (126°44'20"E, 42°24'30"N) in Jingyu County, Baishan City, Jilin Province. Jingyu County is located in the western foot of Changbai Mountain and the upper reaches of Songhua River, with average altitude 775 m, annual average temperature 2.5°C, effective accumulated temperature 2224°C, annual average rainfall 767.3 mm, frost-free period 110 d or so.

**RNA extraction**

Fresh *G. elata* Bl. f. *glauca* tubers used for RNA extraction were washed with sterile water, and after surface disinfection, 100 mg or so healthy tissue was cut near the infected tissue from diseased tubers. Tissues were taken from the same part of healthy tubers to keep uniformity between the two samples, each of which has three biological replications. The total RNA was extracted from each tissue using RNAprep Pure Plant Total RNA Extraction Kit (Polysaccharides & Polyphenolics-rich) (centrifugal column type, catalog No. DP441). RNA was quantified in an Implen NanoPhotometer N50 ultra-micro ultraviolet spectrophotometer (Thermo Scientific; [www.thermofisher.com](http://www.thermofisher.com)). The purity and integrity of RNA was determined in an Agilent 2100 Bioanalyzer (Agilent; [www.agilent.com](http://www.agilent.com)). Finally, qualified total RNA was obtained, and the quality indicators were shown in Additional file: Table S2.

**cDNA library construction and sequencing**

cDNA library was constructed and sequenced by Biomarker Technologies ([www.biomarker.com.cn](http://www.biomarker.com.cn)). Follow steps are required to build the library: purification and fragmentation of mRNA, synthesis and purification of double-stranded cDNA, the end repair or dA tail addition, junction ligation and USER (uracil-specific excision reagent) enzyme digestion, ligated products purification and fragments size classification, library amplification, magnetic bead purification or sorting of amplification products, library quality control. cDNA library was checked for quality and quantity using Agilent Bioanalyzer (Agilent; [www.agilent.com](http://www.agilent.com)). All RNA sequences of 150 bp between 5'-terminal and 3'-terminal was sequenced through Illumina Noveseq high-flux sequencing platform. Paired-end sequencing data was generated for each sample with 2×150 bps read lengths.

**Reads mapping and transcript de novo assembly**

The resulting reads called raw data are stored in fastq format. The raw data of each sequencing sample includes two fastq files containing reads determined at both ends of all cDNA fragments. The quality of raw reads was assessed using the fastqc program ([http://www.bioinformatics.babraham.ac.uk/projects/fastqc/](http://www.bioinformatics.babraham.ac.uk/projects/fastqc/)). Data filtering on raw data to remove low quality reads and reads containing connector or poly-N, we obtained high quality clean data.

Using Trinity software with default parameters, the sequence assembly of clean data is carried out in combined assembly. In this way, the sequencing depth can be increased indirectly, and transcripts with low expression abundance in *G. elata* Bl. f. *glauca* RNA samples can be assembled more completely. Clean data of each sample was aligned with assembled transcript or Unigene library to obtain mapped reads that matched transcript or Unigene library.

**Gene expression and annotation**
A Unigene supported by a minimum of three mapped high-quality reads was considered as expressed. DEGs were evaluated with the DESeq2 package. Benjamini-Hochberg method was used to correct the significant P-value obtained from the original hypothesis test. Finally, FDR (false discovery rate, corrected P-value) was used as one of the key indexes of DEGs identification. This is done for the sake of reducing the false positive caused by independent statistical hypothesis test to a large number of gene expression values. FC (fold change) means the ratio of gene expression levels between healthy tubers and diseased tubers. Negative log2FC values indicate higher expression in healthy samples, while positive log2FC values showed higher expression in disease ones. In addition, the gene expression abundance, described by FPKM (reads per kilobase of exon model per million mapped reads) value, is also a factor to be considered for DEGs identification. When gene expression abundance is small, that is to say, be with low signal values, it may not be detected in subsequent validation.

In organisms, different gene products coordinate with each other to perform biological functions, and enrichment analysis helps to further interpret the gene functions. All Unigenes were annotated into databases such as Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), COG (clusters of orthologous groups), KOG (clusters of euKaryotic Orthologous Groups), eggNOG (Evolutionary Genealogy of Genes: Non-supervised Orthologous Groups). The differential expression and enrichment analysis were conducted using Fisher's exact test to obtain an adjusted P-value (less than 0.05) with an FDR correction.

**Supplementary Information**

**Additional file 1:** Table S1. Sequencing and reads mapping.

**Additional file 3:** Figure S1. (a) Raw data quality. (b) de novo assembly.

**Additional file 2:** Table S2. GO terms enrichment of DEGs. KS: Kolmogorov-Smirnov test (p<0.01).

**Additional file 3:** Table S3. Concentration, purity and integrity of total RNA.

**Abbreviations**

DEG: differentially expressed Unigene; TF: transcription factor; AD: Alzheimer's disease; PD: Parkinson's disease; BP: biological process; CC: cellular component; MF: molecular function; KS: Kolmogorov-Smirnov test; nr: RefSeq non-redundant proteins; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; COG: clusters of orthologous groups, KOG: clusters of euKaryotic Orthologous Groups; eggNOG: Evolutionary Genealogy of Genes: Non-supervised Orthologous Groups; TR: transcription regulator, PK: protein kinases; CDPK: calcium-dependent protein kinase; Rboh: respiratory burst oxidase homolog; CNGC: cyclic nucleotide gated channel; CML: calmodulin-like protein; LRR: leucine-rich repeat; MEKK1: mitogen-activated protein kinase kinase kinase 1; MKK4/5: mitogen-activated protein kinase kinase 4/5; RIN4: RPM1-interacting protein 4; HR: hypersensitive response; GH3: glycoside hydrolase 3; AHP: histidine-containing phosphotransfer protein; ARR-B: two-component response regulator ARR-B family; PIF4: phytochrome-interacting factor 4; ERF1: ethylene-responsive transcription factor 1; JAZ: jasmonate ZIM domain-containing protein; AUX1: auxin influx carrier; ARF: auxin response factor; CRE: cytokinin receptor enzyme; PP2C: protein phosphatase 2C; EIN2: ethylene-insensitive protein 2; BZR1/2: brassinosteroid resistant 1/2; JAR1: jasmonic acid-amino synthetase; COI1:
coronatine-insensitive protein 1; +p: phosphorylation; -p: dephosphorylation; +u: ubiquitination; PTI: pattern-triggered immunity; JA: jasmonic acid; SA: salicylic acid; ET: ethylene; BR: brassinosteroid; ETI: effector-triggered immunity; SAR: systemic acquired resistance; R genes: Resistance genes; HGe: healthy tubers; DGe: diseased tubers; FDR: false discovery rate; FC: fold change.

Declarations

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Author's contributions

YG contributed in research conceiving, material collection, and paper revision. PZ provided technical assistance. YX carried out the experiment. YW participated in study design, experiment progress, and manuscript writing. All authors read and approved the final manuscript.

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Availability of data and materials

The sequence data generated during the current study are available in the NCBI SRA repository via accession numbers SAMN14380862 and SAMN14380861 (www.ncbi.nlm.nih.gov/bioproject/PRJNA612737). All data analyzed during this study are included in this published article and its additional files.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declared that they have no competing interests.

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Figures
Figure 1

Function annotation and classification of DEGs. (a) DEGs functional annotation in different databases. (b) Venn diagram of function annotation in different database. (c) eggNOG function classification. (d) KOG function classification.
Figure 2

(a) GO enrichment. (b) Statistics of pathway enrichment. (c) KEGG pathway enrichment. Each circle in figure B represents a KEGG pathway.
Figure 3

(a) Volcano map of DEGs. (b) MA plot of DEGs.
Figure 4

Transcription factor prediction. The x-axis shows transcription factor family name.
Figure 5

Plant-pathogen interaction pathway map. Red marks indicate up-regulated expression; blue marks indicate down-regulated expression.
Figure 6

Plant hormone signal transduction pathway. Red marks indicate up-regulated expression; blue marks indicate down-regulated expression.

Figure 7

Brassinosteroid biosynthesis. Red marks indicate up-regulated expression; blue marks indicate down-regulated expression.
Figure 8

Phylogenetic tree of TIFY10 in Orchidaceae plant.
Figure 9

Cluster heatmap of disease resistance related genes. The change in color from red to green indicates the change in log2FPKM value from large to small.

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