Morphological and transcriptomic analysis of attenuated and virulent strains of Phytophthora infestans

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Abstract
Phytophthora infestans is a hemibiotroph Oomycete that primarily infects tomato. In this study, the growth status and pathogenicity of attenuated and virulent strains of Phytophthora infestans were determined. Furthermore, RNA-seq technology was used to explore the differences in gene transcription levels between attenuated and virulent strains. Gene Ontology (GO) classification and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed on the differentially expressed genes (DEGs) obtained by sequencing, and the significant DEGs related to the growth and pathogenicity of the strains were screened from the significantly enriched pathways. The results revealed that compared with the virulent strain, the growth of the attenuated strain was inhibited, the structure of hyphae was destroyed, and the disease index was decreased. The differences in the growth status and disease index of the attenuated strain were related to changes in several metabolic pathways, and the DEGs in the metabolic pathways indicated alterations in the attenuated strain growth and pathogenicity. There were 2,651 DEGs in the attenuated strain, of which 1,086 were upregulated and 1,565 were downregulated. The inhibited growth of the attenuated strain was associated with accumulation of excessive glucose, decomposition of serine/glycine, and reduction of tryptophan synthesis. The reduced pathogenicity of the strain was associated with degradation of the cell wall and reduced formation of melanin and α-keto butyric acid. These results could offer insights into the mechanisms of attenuation of Phytophthora infestans.

Keywords Phytophthora infestans · Attenuated strain · Growth and pathogenicity · RNA-seq · Bioinformatics analysis · DEGs

Introduction
Tomato late blight is an important plant disease that seriously threatens the cultivation and production of tomatoes. In recent years, with the changes in planting methods and the popularization and application of large-scale greenhouses, outbreaks of late blight have become more serious and more frequent (Zhao et al. 2020). Tomato late blight is caused by the infestation of Phytophthora infestans (Mont.) De Bary (Mo et al. 2016; Hu et al. 2018; Liu et al. 2019; Zhi et al. 2020). Tomato leaves, stems, flowers, and fruits can be infected, endangering the entire growth period of the tomato. In severe cases, the stems may rot, the plants can wilt, and the fruits may turn brown (Liu et al. 2020). Phytophthora is presently assigned to class Oomycetes. The hyphae are colorless and thin, without a septum (Yin et al. 2016). The pathogen grows in the interstitial spaces of the host cells, and several filamentous structures are used to penetrate the host cells to absorb nutrients. The white mold on the diseased spots is composed of the septal stalk and sporangium of the pathogen. The host range is relatively narrow, and among cultivated plants, only tomatoes and potatoes are infected (Wa 2019). Although Phytophthora infestans can overwinter by means of oospores, it mainly overwinters as hyphae or sporangia. The spores are spread by air currents, and they infect host plants when the temperature and humidity are suitable (Li 2020). The conditions for infecting tomatoes are
low temperature and high humidity, and the symptoms of late blight may also appear in tropical areas under suitable weather conditions in winter (Tewodros et al. 2019).

In 1965, the French phytologist Grente investigated a susceptible chestnut forest in Italy and found that the pathogens isolated from typical and atypical ulcers were different. Grente called this white strain an attenuated strain of chestnut blight, because the attenuated strain had a cross-protective effect on the virulent strain. This finding opened new directions for the control of chestnut blight (Du and Zhang 2000). Since then, related ideas such as the use of attenuated, low-virulence, or non-pathogenic strains have been examined (Qian et al. 2013). Qian (2013) screened out an attenuated strain of Fusarium oxysporum f. sp. cucumerinum by using fresh root extract of celery and constructed an induction system demonstrating that the attenuated strain could effectively control the blight. Wang et al. (2014) isolated a strain of Botryosphaeria dothidea with abnormal colony morphology, strain LW-1, that showed weak pathogenicity and low virulence when infecting hosts. Ding et al. (2017) isolated the Bd CV1-G1 strain from Botryosphaeria dothidea, a strain that affected the growth of the host but demonstrated weakened pathogenicity. Zhao (2018) screened an attenuated Verticillium dahliae strain Vn-1 that met the evaluation criteria of attenuated strains, and its disease index was 9.43.

Transcriptome refers to the collection of all transcription products in cells, tissues, or organisms under specific physiological conditions. Transcriptome provides a link between genomic genetic information and proteome with biological functions (Yao et al. 2017). Transcriptome sequencing technology has been widely used to study the pathogenic mechanism of fungi, such as Verticillium dahliae, Puccinia triticina, Curvularia lunata, and Magnaporthe oryzae (Duressa et al. 2013; Zhang 2014; Gao et al. 2014; Choi et al. 2015).

In this study, we compared the virulent and attenuated strains of Phytophthora infestans, with the goals of obtaining comprehensive information on the morphology and transcriptome of the attenuated strain and using this information to analyze the relationships between the growth, pathogenicity, and metabolic pathways. This knowledge will help to clarify the mechanism of the attenuated strain. To the best of our knowledge, this is the first experiment that studies using attenuated strains of pathogen Oomycetes for transcriptomic observations.

Materials and methods

Strains and culture medium

The virulent strain of Phytophthora infestans in this experiment was provided by the Institute of Plant Protection, Chinese Academy of Agricultural Sciences; the attenuated strain of Phytophthora infestans was screened and obtained by our research group. Potato dextrose agar (PDA, Becton, Dickinson and Co., MD, USA) medium was used to cultivate the strains.

Determination of strain growth status and pathogenicity

Determination of strain growth status

Under aseptic conditions, we used a hole punch with a diameter of 0.6 cm to sample attenuated and virulent strains of the fungus. An inoculation needle was used to transfer the fungus cake into 30 mL potato dextrose broth (PDB, Becton Dickinson) medium, and the sample was cultured at 25 °C in the dark with shaking at 130 rpm. Samples were collected at 144 h after inoculation. Mycelia were first collected by filtration through a stainless steel sieve (40 mesh), washed with distilled water, and then lyophilized. The weight of dried mycelia was recorded as the biomass (g) (Hu et al. 2017). The fungus cakes were transferred to 20 mL PDA medium with the hyphae face down; one piece was taken from each petri dish and placed in the center of the PDA medium, cultured in a constant temperature incubator at 25 °C for 144 h. The changes of the attenuated strain were examined after 144 h. The changes of the virulent strain were examined through a stainless steel sieve (40 mesh), washed with distilled water, and then lyophilized. The weight of dried mycelia was recorded as the biomass (g) (Hu et al. 2017).

Determination of strain pathogenicity

After 144 h of culture, the fungus cakes of attenuated and virulent strains were washed with sterile water to prepare suspensions. Under the microscope, the spore concentration was adjusted to 5.0×10^4 sporangia/mL, and the prepared suspension was placed in a normal temperature box for 2–3 h. The strains were tested at the 6–8 leaf stages of tomato seedlings. The suspension was sprayed evenly on the front and back sides of the leaves using a sprayer. The front and back sides of leaves were covered with uniform fungal suspension as the standard. The inoculation treatment was carried out at 6–7 p.m., and leaves were kept warm and moist in a small shed after spraying. From the first appearance of symptoms, the diseased plant rate and diseased leaf rate of tomato plants in each group were recorded according to the disease grade standards (Chai et al. 2005). The disease index was calculated once every 3 days, with 24 plants...
in each experimental group and 3 biological replicates per treatment.

The grading standards were as follows: grade 0: asymptomatic; grade 1: ≤5% of the leaf area was infected to form small necrotic spots; grade 2: 6–15% of the leaf area was infected to form restrictive necrotic spots; grade 3: 16–30% of the leaf area was infected, and no necrotic spot was formed on the stem; grade 4: 31–60% of the leaf area was infected, or small necrotic spots were formed on the stem; grade 5: 61–90% of the leaf area was infected, or the stem formed expanded spots; grade 6: 91–100% of the leaf area was infected, or the stem was damaged, or the plants died.

The disease index (%) = \( \frac{\sum \text{(number of diseased leaves at each level} \times \text{corresponding grade value})}{(\text{total number of investigated leaves} \times \text{highest grade value})} \times 100\% \) (Zhu et al. 2020).

**RNA sample preparation**

After 144 h of inoculation, sterile tweezers were used to carefully scrape about 300 mg of the cultured mycelium from each fungus cake, and the samples were placed in premarked cryopreservation tubes (the cryopreservation tube number T represents the attenuated strain; number C represents the virulent strain), frozen in liquid nitrogen, and stored in an ultra-low temperature refrigerator at −80 °C for later use. Each experimental group contained three biological replicates.

**RNA library construction**

Total RNA was extracted from mycelium samples by a mirVana miRNA Isolation Kit (Ambion, Texas, USA) following the manufacturer’s instructions. RNA purity was checked by using a NanoDrop 2000, and the concentration was measured using a Qubit RNA Assay Kit (Life Technologies, CA, USA). RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, Santa Clara, CA, USA). A total of 4 μg RNA per sample was used as input material for the RNA sample preparation. The libraries were constructed using a TruSeq Stranded mRNA LTSample Prep Kit (Illumina, San Diego, CA, USA) according to the manufacturer’s instructions. The libraries were sequenced on an Illumina sequencing platform (HiSeq™ 2500) by OE biotech Co., Ltd. (Shanghai, China).

**Bioinformatics analysis**

Raw reads were processed using Trimmomatic (Bolger et al. 2014). The reads containing adapters, ploy-N, and low-quality sequences were removed to obtain the clean reads. Clean read data were examined for Q30 and GC content to ensure high quality. After removing low-quality sequences, the clean reads were assembled into expressed sequence tag clusters (contigs) and de novo assembled into transcripts by using Trinity version 2.4 (Grabherr et al., 2013) in paired-end mode. The longest transcript was chosen as a unigene based the similarity and length of the sequence for subsequent analysis. The functions of the unigenes were annotated by alignment with the NCBI nonredundant (NR), SwissProt, and clusters of orthologous groups for eukaryotic complete genomes (KOG) databases using blastx (Altschul et al. 1990) with a threshold E-value of 10⁻⁵, and the FPKM value (Trapnell et al. 2010) of each unigene was calculated using eXpress (Roberts and Pachter, 2013). DEGs were identified using the DESeq (Simon and Wolfgang 2013) functions estimateSizeFactors and nbimomTest. P value < 0.05 and ln2 fold change> 1 were set as the threshold criteria for differential expression. GO and KEGG (Kanehisa et al. 2008) enrichment analysis of DEGs was performed to determine the biological functions or pathways involving these genes.

**Significant DEGs analysis of related KEGG pathways**

According to the reported genes, proteins, and enzymes related to the growth and pathogenicity of strains, the related significant DEGs were screened from the significantly enriched pathways between the attenuated and virulent strains. Q value (FDR-adjusted P value) < 0.05 and ln2 fold change> 1 were set as the threshold criteria for significantly differential expression.

**Results**

**Growth status and pathogenicity of strains**

**Growth status of strains**

After 144 h of constant temperature culture at 25 °C, the strains were photographed, observed under microscope, and growth indexes were measured (Fig. 1). Microscopic observation showed that the fungal mycelia presented differential changes. The mean colony diameters of the attenuated and virulent strains were 3.418 cm and 7.508 cm, and the mean mycelial biomasses were 0.224 g and 0.347 g, respectively. The morphological characteristics of attenuated and virulent strains were also quite different (Table 1). By observing the growth status of mycelia, the viability and integrity of the samples were ensured.

**Determination of strains pathogenicity**

Tomato seedlings were inoculated with the suspensions of attenuated and virulent strains at the 6–8 leaf stages. From the first appearance of symptoms, the disease indexes of
different strains were calculated (Table 2) to observe the changes in pathogenicity.

Quality analysis of total RNA

Total RNA quality results were obtained after RNA-seq sequencing of six samples (Table 3). Trinity (Grabherr et al., 2013) software was used to splice and assemble the clean reads data obtained by sequencing, and the transcripts obtained were further spliced to obtain unigene results (Table 4). Subsequent data analysis was performed according to the quality statistics of the sequencing data.

Screening of DEGs

To analyze the differential expression of the attenuated strain (Treat) and the virulent strain (Control), RNA reads were mapped to the genome. Principal component analysis (PCA) indicated that Treat and Control formed separated clusters, indicating distinct transcriptional profiles (Fig. 2a). The attenuated strain was more variable than the virulent strain.

Fig. 1 Comparison of growth status of strains. Note: After the strains were cultured for 144 h, photographs were taken, observed under microscope, and growth indexes were measured. The results were as follows: a Comparison of growth state of strains; b Comparison of microscopic changes of strains. (1) Normal mycelia of the virulent strain (CK); (2–6) mycelia of the attenuated strain. Hyphae wrapped around each other (2) and twisted (3); hyphae became bent, transparent, and local expansion (4); uneven distribution of hyphae thickness (5); protoplast leaked out (6). c Comparison of colony diameter. d Comparison of mycelial biomass. The X-axis lists the sample names, and the Y-axis is the growth index. The results in the figure are expressed as mean ± SD (n = 5). *P < 0.05

Table 1 Morphological characteristics of different strains

| Sample name     | Colony color                  | Colony morphology                        | Aerial hypha                  | Growth rate |
|-----------------|-------------------------------|------------------------------------------|------------------------------|-------------|
| Attenuated strain | The front is white, the back is light pink | Nearly round, flat, and edge regular     | Short, compact, fluffy, and white | Slow        |
| Virulent strain  | The front is light pink, the back is dark pink | Round, obvious raised in the middle, and edge strigose | Long, loose, flocculent, grow upward, and white | Quick       |

Table 2 Disease indexes of tomato seedlings inoculated with different strains (%)

| Days after inoculation | Attenuated strain | Virulent strain |
|------------------------|-------------------|-----------------|
|                        | 3d | 6d | 9d | 12d | 15d | 18d | 21d      |
|------------------------|----|----|----|-----|-----|-----|----------|
| Attenuated strain      | 0.17 | 0.26 | 0.61 | 1.31 | 3.32 | 3.84 | 4.45     |
| Virulent strain        | 1.41 | 5.2 | 7.14 | 8.91 | 12.79 | 16.05 | 19.66  |

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A volcano plot was drawn for the differential transcriptional profile results of Treat and Control (Fig. 2b). The attenuated strain had 2,651 DEGs ($P < 0.05$, $\log_2$ fold change $> 1$) compared to the virulent strain, of which 1,086 DEGs were upregulated and 1,565 DEGs were downregulated. Upregulated genes may positively regulate the pathogenic process, while downregulated genes have an impact on the pathogenicity of fungal pathogens from the aspect of negative regulation.

**GO enrichment analysis**

A GO classification enrichment analysis was performed on the DEGs between the attenuated and virulent strains (Fig. 3). GO terms having a corrected value of $P < 0.05$ were considered to be statistically significant. In the GO database, DEGs are classified into three categories according to their function: biological process, cellular component, and molecular function. There are 23 sub-functional categories.

**Table 3** Quality results of total RNA

| Sample | Raw reads | Clean reads | Total mapped reads | Uniquely mapped | Q30% | GC% |
|--------|-----------|-------------|--------------------|-----------------|------|-----|
| T1     | 48.01 M   | 47.50 M     | 44.34 M            | 43.73 M         | 95.56| 53.01|
|        |           |             | (93.34%)           | (92.06%)        |      |     |
| T2     | 45.73 M   | 45.27 M     | 42.18 M            | 41.62 M         | 95.60| 52.98|
|        |           |             | (93.18%)           | (91.93%)        |      |     |
| T3     | 45.60 M   | 45.35 M     | 42.25 M            | 41.71 M         | 97.16| 52.94|
|        |           |             | (93.17%)           | (91.95%)        |      |     |
| C1     | 47.14 M   | 46.56 M     | 42.95 M            | 42.34 M         | 96.40| 52.68|
|        |           |             | (92.25%)           | (90.93%)        |      |     |
| C2     | 46.30 M   | 45.73 M     | 42.52 M            | 41.91 M         | 96.56| 52.47|
|        |           |             | (92.99%)           | (91.65%)        |      |     |
| C3     | 47.51 M   | 47.20 M     | 43.81 M            | 43.17 M         | 97.04| 52.65|
|        |           |             | (92.82%)           | (91.47%)        |      |     |

**Table 4** Statistics of splicing results

| Term     | All | $\geq 500 \text{ bp}$ | $\geq 1000 \text{ bp}$ | N50 | Total length | Max length | Min length | Average length |
|----------|-----|-----------------------|------------------------|-----|--------------|------------|-------------|----------------|
| Unigene  | 19,843 | 15,324                 | 10,744                 | 2,079 | 28,794,426   | 23,524     | 302         | 1,451.11       |

**Fig. 2** Two-dimensional principal component analysis (PCA) plot (a) and volcano plot (b). Note: a Principal component analysis (PCA) score plot. The virulent strains are indicated by blue dots labeled “Control number,” while the attenuated strains are indicated by red dots labeled “Treat number.” b The volcano plot of DEGs between Treat and Control strains. Red dots denote upregulated significantly different unigenes ($P < 0.05$, $\log_2$ fold change $\geq 1$); green dots denote downregulated significantly different unigenes ($P < 0.05$, $\log_2$ fold change $\leq -1$), and gray dots denote unigenes not significantly different. The X-axis shows the $\log_2$ fold change, and the Y-axis shows the $-\log_{10}$ $P$ value.
within biological processes, among which biological regulation, cellular process, establishment of localization, localization, metabolic process, and response to stimulus were the six categories with the most DEGs; cellular component has 20 sub-functional categories, among which cell, cell part, membrane, membrane part, and organelle were the five categories with the most DEGs. Molecular function has 21 sub-functional categories, among which binding, catalytic activity, and transporter activity were the three categories with the most DEGs. It was worth noting that the DEGs in protein tag were all downregulated, while the DEGs in rhythmic process, electron carrier activity, and protein binding transcription factor activity were all upregulated in the 64 sub-functional categories. These genes may be involved in the growth and pathogenicity of the fungi.

**KEGG enrichment analysis**

A KEGG pathway enrichment analysis was performed on the DEGs between the attenuated and virulent strains, and the top 20 metabolic pathways with the most significant enrichment were selected and used to construct a KEGG enrichment scatter plot (Fig. 4). The results showed that the DEGs were mainly concentrated in starch and sucrose metabolism (ko00500), tyrosine metabolism (ko00350), glycine/serine and threonine metabolism (ko00260), isoquinoline alkaloid biosynthesis (ko00950), and fatty acid degradation (ko00711) pathways. Among the significantly enriched pathways, metabolic and biosynthetic pathways were the most numerous, with 20 pathways accounting for 87% of the total. The results suggest that the key DEGs of these pathways were closely related to the growth and pathogenicity of the fungi.

**Genes involved in growth of strains**

In this study, twenty-seven significant DEGs related to growth were screened (Table 5). Twenty genes were upregulated, and seven genes were downregulated. Among these genes, \( \text{EG} \) (endoglucanase), \( \text{CBHC} \) (cellulose 1,4-beta-cellobiosidase), \( \text{CelB} \) (cellulase), \( \text{glgC} \) (glucose-1-phosphate adenyl transferase), \( \text{bgIX} \) (beta-glucosidase), \( \text{malZ} \) (alpha-glucosidase), \( \text{SGA} \) (glucoamylase), \( \text{amyA} \) (alpha-amylase), and \( \text{IMA} \) (oligo-1,6-glucosidase) encode enzymes involved in glucose production; \( \text{glyA} \) (serine hydroxymethyltransferase), \( \text{GLDC} \) (glycine dehydrogenase), \( \text{AMT} \) (amino methyl transferase), and \( \text{PIPOX} \) (sarcosine oxidase) encode enzymes involved in the decomposition of serine and glycine; \( \text{TrpB} \) (tryptophan synthase beta chain) encodes an enzyme involved in the synthesis of tryptophan. \( \text{EG} \), \( \text{CBHC} \), and \( \text{CelB} \) had the most significantly upregulated expression levels at 44.782, 38.196, and 31.847 times the control group (virulent strain), respectively.

![Fig. 3 GO classification of DEGs (P<0.05). Note: Red indicates the GO sub-functional categories of upregulated DEGs enrichment; green indicates the GO sub-functional categories of downregulated DEGs enrichment. The X-axis lists the names of the GO sub-functional categories, and the Y-axis is the number of genes in the corresponding sub-functional categories and the percentages](image-url)
Genes involved in pathogenicity of strains

In this study, nine significant DEGs related to pathogenicity were screened (Table 6). Four genes were upregulated, and five genes were downregulated, including bglX (beta-gluco- cosidase) encode enzymes involved in cell wall degradation, TYR (tyrosinase) encode enzymes involved in melanin formation, and ilvA (threonine dehydratase) encodes an enzyme involved in α-ketobutyric acid formation. TYR had the most significantly downregulated expression level, 0.082 times that of the control group (virulent strain).

Discussion

Yan et al. (2016) showed that YePSA medium supplemented with a high concentration of glucose could inhibit the sexual reproduction and mycelial growth of Sporisorium scitamineum. Liang et al. (2014) showed that glucose could inhibit the mating ability of Candida albicans. By comparing the significant DEGs between attenuated and virulent strains, we found that the expression levels of twelve coding genes involved in glucose production were all upregulated. This suggests that the attenuated strain might feature inhibition of mycelial growth and sexual reproduction through excessive glucose accumulation.

As a non-essential amino acid, L-serine participates in many biological metabolic processes, especially in cell proliferation and growth (Yang 2016). Glycine is an important raw material for nucleic acid synthesis. Lack of glycine can lead to the obstruction of DNA synthesis and the impairment of cell growth and proliferation (Wei and Yu 2018). The addition of folic acid showed that a high concentration of folic acid was detrimental to the growth of Corynebacterium glutamicum (Ren 2009). Comparison of the significant DEGs between attenuated and virulent strains showed that the expression levels of four coding genes involved in serine and glycine decomposition were upregulated. This suggests that the attenuated strain might show inhibition of the mycelial growth rate through decomposition of serine/glycine and production of folic acid precursors.

Studies have shown that low-activity tyrosinase (TYR) can reduce the browning degree of the SCAU1 strain (Yu 2018). Zhou (2019) found that the mutant strain of Candida albicans could not grow without the ilv2 gene. Studies have shown that tryptophan auxotrophic strains of Mycobacterium tuberculosis cannot survive and reproduce in macrophages (Bange et al. 1996; Gordhan et al. 2002). By comparing the significant DEGs between attenuated and virulent strains, seven downregulated genes related to the growth of the strain were identified. This suggests that the colony color, colony diameter, and mycelial biomass of the attenuated strain might be affected through downregulation of the expression of several related genes.
Beta-glucosidase (bgIX) participates in the metabolism of fungal cell walls; *Phanerochaete chrysosporium* can produce multiple β-1,3-glucosidases to jointly degrade cell walls (Chang 2017). The cell walls of fungi are involved in the formation of biofilms. For pathogenic fungi, the cell wall is important in virulence and pathogenicity, because fungal cell walls protect the strain from the host defense mechanisms (Li 2015).

Tyrosinases in tyrosine metabolism are key enzymes involved in melanin synthesis (Yang 2019). Melanin is closely related to pathogenicity, and mutant strains that lack melanin production lose the pathogenicity due to lack of sufficient turgor (Zhang 2014). Studies have found that virulence of strains lacking the *ilvC* gene is nearly absent; after the deletion of *ilv2* and *ilv6* in *Magnaporthe oryzae*, the infectivity of mutants *Moilv2* and *Moilv6* is significantly reduced.

### Table 5 Significant DEGs related to the growth of strains

| Pathway | Gene name | Gene ID | Fold change | Type | Q value | Enzyme family               |
|---------|-----------|---------|-------------|------|---------|-----------------------------|
| ko00950 | tynA      | TRINITY_DN2405_c0_g2_i1 | 26.020     | Up   | 1.59E-05 | Primary-amine oxidase       |
| ko00350 | TRINITY_DN6172_c0_g1_i1 | 2.492     | Up   | 8.25E-05 | Endoglucanase               |
| ko00260 | TRINITY_DN9085_c0_g1_i1 | 2.435     | Up   | 0.00085 | Cellulose 1,4-beta-celllobiosidase |
| ko00500 | CBHC      | TRINITY_DN483_c0_g1_i1 | 38.196     | Up   | 0.028 | Cellulase                   |
| ko00260 | TRINITY_DN12353_c0_g1_i1 | 31.847    | Up   | 9.05E-25 | Cellulase                   |
| ko00260 | TRINITY_DN910_c0_g1_i1 | 15.699    | Up   | 0.0040 | Glucose-1-phosphate adenyl transferase |
| ko00260 | TRINITY_DN16729_c0_g1_i1 | 5.725     | Up   | 7.80E-08 | Beta-glucosidase            |
| ko00260 | TRINITY_DN4010_c0_g1_i1 | 5.373     | Up   | 1.80E-05 | Beta-glucosidase            |
| ko00260 | TRINITY_DN3408_c0_g2_i1 | 3.701     | Up   | 7.03E-09 | Beta-glucosidase            |
| ko00260 | TRINITY_DN9742_c0_g1_i1 | 2.289     | Up   | 0.021  | Beta-glucosidase            |
| ko00271 | TRINITY_DN9963_c0_g1_i1 | 4.462     | Up   | 0.00043 | Alpha-glucosidase           |
| ko00271 | TRINITY_DN3369_c0_g1_i1 | 4.136     | Up   | 2.44E-12 | Glucoamylase                |
| ko00271 | TRINITY_DN15061_c0_g1_i1 | 3.404     | Up   | 0.0013 | Alpha-amylose               |
| ko00271 | TRINITY_DN707_c0_g1_i1 | 2.278     | Up   | 0.00052 | Oligo-1,6-glucosidase       |
| ko00271 | TRINITY_DN14981_c0_g1_i1 | 2.779     | Up   | 2.62E-07 | Serine hydroxymethyl transferase |
| ko00271 | TRINITY_DN16738_c0_g1_i1 | 2.535     | Up   | 7.80E-07 | Glycine dehydrogenase       |
| ko00271 | TRINITY_DN1768_c0_g1_i1 | 2.278     | Up   | 2.17E-05 | Amino methyl transferase    |
| ko00271 | TRINITY_DN12573_c0_g1_i1 | 2.079     | Up   | 0.017  | Sarcosine oxidase           |
| ko00271 | TRINITY_DN1530_c0_g2_i1 | 12.296    | Up   | 0.024  | Acetyl-CoA C-acetyltransferase |
| ko00271 | TRINITY_DN16117_c0_g1_i1 | 0.457     | Down | 5.49E-05 | Tyrosinase                  |
| ko00271 | TRINITY_DN12772_c0_g1_i1 | 0.342     | Down | 0.0073 | Tyrosinase                  |
| ko00260 | TRINITY_DN17109_c0_g1_i1 | 0.244     | Down | 2.68E-07 | Tyrosinase                  |
| ko00260 | TRINITY_DN2405_c0_g1_i1 | 0.082     | Down | 4.07E-07 | Tyrosinase                  |
| ko00260 | TRINITY_DN8611_c0_g1_i1 | 0.491     | Down | 0.0019 | Succinate-semialdehyde dehydrogenase |
| ko00260 | TRINITY_DN8283_c0_g1_i1 | 0.461     | Down | 0.0028 | Threonine dehydratase       |
| ko00260 | TRINITY_DN13540_c0_g1_i1 | 0.190     | Down | 0.0017 | Tryptophan synthase beta chain |

### Table 6 Significant DEGs related to the pathogenicity of strains

| Pathway | Gene name | Gene ID | Fold change | Type | Q value | Enzyme family               |
|---------|-----------|---------|-------------|------|---------|-----------------------------|
| ko00500 | bgIX      | TRINITY_DN16729_c0_g1_i1 | 5.725     | Up   | 7.80E-08 | Beta-glucosidase            |
| ko00950 | TRINITY_DN4010_c0_g1_i1 | 5.373     | Up   | 1.80E-05 | Beta-glucosidase            |
| ko00260 | TRINITY_DN3408_c0_g2_i1 | 3.701     | Up   | 7.03E-09 | Beta-glucosidase            |
| ko00260 | TRINITY_DN9742_c0_g1_i1 | 2.289     | Up   | 0.021  | Beta-glucosidase            |
| ko00950 | TYR       | TRINITY_DN16117_c0_g1_i1 | 0.457     | Down | 5.49E-05 | Tyrosinase                  |
| ko00350 | TRINITY_DN12772_c0_g1_i1 | 0.342     | Down | 0.0073 | Tyrosinase                  |
| ko00350 | TRINITY_DN17109_c0_g1_i1 | 0.244     | Down | 2.68E-07 | Tyrosinase                  |
| ko00350 | TRINITY_DN2405_c0_g1_i1 | 0.082     | Down | 4.07E-07 | Tyrosinase                  |
| ko00260 | ilvA      | TRINITY_DN8283_c0_g1_i1 | 0.461     | Down | 0.0028 | Threonine dehydratase       |
| ko00260 | trpB      | TRINITY_DN13540_c0_g1_i1 | 0.190     | Down | 0.0017 | Tryptophan synthase beta chain |

Beta-glucosidase (bgIX) participates in the metabolism of fungal cell walls; *Phanerochaete chrysosporium* can produce multiple β-1,3-glucosidases to jointly degrade cell walls (Chang 2017). The cell walls of fungi are involved in the formation of biofilms. For pathogenic fungi, the cell wall is important in virulence and pathogenicity, because fungal cell walls protect the strain from the host defense mechanisms (Li 2015). Tyrosinases in tyrosine metabolism are key enzymes involved in melanin synthesis (Yang 2019). Melanin is closely related to pathogenicity, and mutant strains that lack melanin production lose the pathogenicity due to lack of sufficient turgor (Zhang 2014). Studies have found that virulence of strains lacking the *ilvC* gene is nearly absent; after the deletion of *ilv2* and *ilv6* in *Magnaporthe oryzae*, the infectivity of mutants *Moilv2* and *Moilv6* is significantly reduced.
MoIv6 decreased significantly (Zhou 2019). In comparisons of the significant DEGs between attenuated and virulent strains, the expression levels of four coding genes involved in cell wall degradation were all upregulated; five coding genes involved in melanin and α-ketobutyric acid formation were all downregulated. This suggests that the attenuated strain might be affected by the expression of genes related to the cell wall and the virulence factors, thereby affecting the pathogenicity of the strain.

Fungi (e.g., Oomycetes) are heterothrophic eukaryotes (Hage et al. 2021), and the substances and energy required for their growth must be absorbed from the outside. Carbon and nitrogen are essential elements for living organisms (Chang 2017; Ueda et al. 2017). The observation showed that the weakened growth viability of the attenuated strain might result in the reduction of nutrients absorbed by the attenuated strain from the outside, which put it in a “starvation” state, accelerated the carbon and nitrogen metabolism inside the strain, and promoted the upregulated expression of coding genes involved in the decomposition of starch, sucrose, and amino acids.

**Conclusion**

In this study, compared with the virulent strain, the morphology and growth of the attenuated strain was significantly inhibited, and the field disease index was decreased. RNA-seq results showed that the growth of the attenuated strain was inhibited by accumulation of excessive glucose, decomposition of serine/glycine, and reduction of the synthesis of tryptophan. The pathogenicity of the strain was then reduced by degrading the cell wall and decreasing the formation of melanin and α-ketobutyric acid. Through the morphological and transcriptomic comparative analysis between attenuated and virulent strains of *Phytophthora infestans*, the reasons for the difference in growth and pathogenicity between the strains were further revealed, and the results thus laid a foundation for the elucidation of the mechanisms of attenuated strains. To the best of our knowledge, this study provides the first dataset on the transcriptome of attenuated strains of pathogen Oomycetes.

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**Author contribution** Xingfu Yun proposed research ideas and experimental design; Jiafang Liu conducted experimental research and data analysis, and was responsible for writing the first draft of the manuscript; Lei Li and Yong Wang participated in the revision and correction of the manuscript.

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**Data availability** Please contact author for data requests.

**Declarations**

**Ethics approval and consent to participate** Compliance with ethical standards.

**Consent for publication** Not applicable.

**Competing interests** The authors declare no competing interests.

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