Identification of saponins detoxification genes in I. mors-panacis G3B inducing root rot of Panax notoginseng by RNA-Seq

Guohong Zeng (✉ zgh20180105@zstu.edu.cn)  
Zhejiang Sci-Tech University  https://orcid.org/0000-0002-1355-4569

Jin Li  
Zhejiang Sci-Tech University

Yuxiu Ma  
Zhejiang Sci-Tech University

Qian Pu  
Zhejiang Sci-Tech University

Tian Xiao  
Zhejiang Sci-Tech University

Ruihuan Yang  
Zhejiang Sci-Tech University

Xiufang Hu  
Zhejiang Sci-Tech University

Research Article

Keywords: I. mors-panacis G3B, Panax notoginseng, RNA-Seq, Root rot, Saponins detoxification genes

DOI: https://doi.org/10.21203/rs.3.rs-212506/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Saponins are kinds of antifungal compounds produced by *P. notoginseng* to resist invasion by pathogens. *I. mors-panacis* G3B was the dominant pathogen inducing root rot of *P. notoginseng*, and the abilities to detoxify saponins were the key to infect *P. notoginseng* successfully. To research the molecular mechanisms of detoxifying saponins in *I. mors-panacis* G3B, we used high-throughput RNA-Seq to identify 557 and 1519 differential expression genes (DEGs) in *I. mors-panacis* G3B with saponins treatments for 4 H and 12 H compared with no saponins treatments, respectively. Among these DEGs, we found 93 genes which were simultaneously highly expressed in *I. mors-panacis* G3B with saponins treatments for 4 H and 12 H, they mainly belong to genes encoding transporters, glycoside hydrolases, oxidation-reduction enzymes, transcription factors and so on. In addition, there were 21 putative PHI (Pathogen-Host Interaction) genes out of those 93 up-regulated genes. In this report, we identified virulence associated genes in *I. mors-panacis* G3B which may be related to detoxifying saponins to infect *P. notoginseng* successfully. They provided an excellent starting point for in-depth study on pathogenicity of *I. mors-panacis* G3B and developed appropriate root rot disease management strategies in the future.

Introduction

*Panax notoginseng* (Burkill) F.H. Chen (*P. notoginseng*) is a kind of traditional medicine mainly used for treatment of a variety of diseases such as ischemic cardiovascular diseases and so on. *P. notoginseng* has been cultivated for about 400 years in China since the time of Shizhen Li, which belongs to the *Panax* genus and Araliaceae. Currently, it was mainly cultivated artificially in the mountain area with altitude between 1,200 and 2,000 m of Wenshan prefecture, Yunnan province, China (Guo et al., 2010). Although there had been reports on wild species of *P. notoginseng*, no successful case had been described and only some closely related wild species or variety had been found (Yan et al., 2006). *P. notoginseng* is a perennial medicinal plant that grows in the shade and needs 4~6 years to produce mature roots and accumulate of bioactive compounds such as saponins. The humid production environment and prolonged period of growth exposes the roots to potential infection by different pathogens, which severely restrict the production of *P. notoginseng*, especially in land-limited mountain area.

*P. notoginseng* is vulnerable to be attacked by soil microbes including fungi, bacteria and nematodes because of its long-term cultivation and shady environment. Fungi dominate with increasing years of planting, more than 70 genera were found in the rhizosphere soil of *P. notoginseng* cultivated in wenshan prefecture, and 20 species have been identified (Wang et al., 2003). Pathogenic fungi cause serious diseases such as black spot, circular spot, gray mould, root rot and so on. Among them, root rot is the most common and severe disease, resulting in dramatic crop losses up to 10%-20%, or more than 70% (Wu et al., 2015; Sun et al., 2004). Root rot typically appears at the tip of the taproot destroying the fibrous roots and attacks toward the crown, the core of the root eventually disintegrates and remains hollow, which is also known as disappearing root rot (Rahman and Punja, 2005). Thus, identification of the dominant pathogens of the root rot and clarification their pathogenesis are prerequisites for effective control to maintain the sustainable cultivation of *P. notoginseng*. 

Page 2/14
Root rot is an ubiquitous disease worldwide, occurring in multiple plants with varied pathogens. And *Cylindrocarpon destructans* is a kind of soilborne pathogenic fungi which can cause severe root rot in many hosts including *P. ginseng*. They can be divided into weak and aggressive isolates. However, aggressive isolates cause severe root rot disease especially to *P. gingseng* with limited pathogenicity in other hosts, and were therefore named *C. destructans* f. sp. *Panacis* (Seifert et al., 2003). Then *C. destructans* was reclassified as *Ilyonectria radicicola* (Chaverri et al., 2011). According to multigene molecular analysis especially histone H3 (HIS H3), *I. radicicola* isolates appeared a polyphyletic relationship and each group of isolates is considered to be a different species (Lombard et al., 2013; Cabral et al., 2012). And *C. destructans* f. sp. *panacis* was genetically distinct from the other isolates and clustered in a distinct group named as *Ilyonectria mors-panacis* (Seifert et al., 2003). According to multigene molecular analysis especially histone H3 (HIS H3), *I. radicicola* isolates appeared a polyphyletic relationship and each group of isolates is considered to be a different species (Lombard et al., 2013; Cabral et al., 2012). And *C. destructans* f. sp. *panacis* was genetically distinct from the other isolates and clustered in a distinct group named as *Ilyonectria mors-panacis* (Seifert et al., 2003). On these bases, Mi used culture-dependent and molecular methods to investigate the fungal communities and identify the dominant pathogen of G3B inducing root rot of *P. notoginseng*. G3B is phylogenetically and phenotypically similar to *I. mors-panacis*, so named for *I. mors-panacis* G3B (Mi et al., 2017). Therefore, understanding the genetic properties and pathogenicity of *I. mors-panacis* will provide theoretical supports for inhibiting root rot to maintain sustainable cultivation of *P. notoginseng*.

*I. mors-panacis* experiences complicated pathogenicity processes, which were related to interactions with ginseng root. They mainly include the following steps: when spore or mycelium adheres to the root surface, *I. mors-panacis* rapidly produces high quantities of hydrolytic enzymes such as cellulase and pectinase, allowing rapid invasion of the epidermal layer and fast extension of the inoculum to the cortical and inner tissues; And the ginseng plant starts to secret ginsenosides or phenolic compounds to resist the invasion by pathogens; In response to them, *I. mors-panacis* further produces enzymes that can degrade ginsenosides or phenolic compounds such as glycosidases and polyphenoloxidases; At the same time, *I. mors-panacis* sequesters iron from the ginseng plant to support its growth using siderophores; Once the cell wall components of ginseng plant break down, its defense response declines and *I. mors-panacis* propagates quickly and then root rotting symptoms are established (Farh et al., 2018). During the pathogenicity processes, ginsenosides secreted by ginseng plant have been shown to possess chemical defenses against fungi and therefore act as phytoanticipins (Nicol et al., 2002). However, the antifungal action of ginsenosides is not effective against all potential ginseng pathogens. Oppositely, they have been shown to stimulate the in vitro growth of some pathogens inducing root rot of ginseng plants and this may result from the ability of these pathogens to metabolize ginsenosides via extracellular glycosidases (Andreea Neculai et al., 2009; LF and MA, 2006). In addition, relevant study has shown that the highly aggressive species of pathogens inducing root rot disease produces much more hydrolytic enzyme, the oxidative enzyme and polyphenol oxidase than weakly aggressive, which destroy the plant defensive barriers (Rahman and Punja, 2005). However, the detail involvements of hydrolytic enzymes in detoxifying ginsenosides and more related pathogenesis have yet been studied.

Therefore, the goal of the present study is to identify the genes encoding the ginsenosides degrading enzymes from *I. mors-panacis* G3B. To achieve this objective, we built the transcriptome sequencing platform for high-throughput prediction of all associated genes. This study not only revealed candidate
genes for further functional research on pathogenicity of *I. mors-panacis* but also provided theoretical supports for inhibiting root rot and alleviating replant failure of ginseng plants.

**Materials And Methods**

**Fungal strains and growth conditions**

*I. mors-panacis* G3B used in the study was first isolated from the rhizosphere soil of diseased *P. notoginseng* cultivated in Wenshan, Yunnan Province, China (Mi et al., 2017). The strain was cultured onto a PDA (Potato Dextrose-Agar) plate at 22°C for 18 days.

**Assessment of the tolerance of I. mors-panacis G3B to saponins**

The susceptibility of *I. mors-panacis* G3B to saponins was evaluated by estimating the growth diameters of colonies which were inoculated in PDA agars and those supplemented with Sanqi total Saponins (SAPs) (Solarbio). 5 µL conidial suspension (1×10^7 conidia mL^−1) were respectively applied to the center of PDA agar plates (90 mm in diameter) and those containing SAPs at the concentration of 500 ppm. Inoculated plates were incubated at 22°C and colonies diameters were measured daily from 3 days post-inoculation until the colonies ceased growing. Three samples per treatment were used as replicates and the experiment was conducted three times.

**Assessment of the capacities of I. mors-panacis G3B to degrade saponins**

Conidial suspension of *I. mors-panacis* G3B were inoculated in the PDA liquid medium supplemented with proper amount of saponins. And none *I. mors-panacis* G3B inoculation in the PDA liquid medium containing equivalent saponins were negative controls. According to the standard curves (concentration-peak area) of Rg1 and Rb1 analysed by HPLC, we assayed the concentrations of Rg1 and Rb1 after 0 day, 3 days and 6–12 days inoculation in the PDA culture solution, respectively.

**I. mors-panacis G3B incubation with saponins and RNA extraction**

The conidia of *I. mors-panacis* G3B were inoculated in sabouraud dextrose broth at a final concentration of 1×10^6 conidia mL^−1 and incubated for 36 h at 22°C with 220 rpm. The mycelium was collected by filtration, and washed three times with sterilized water. Subsequently, 0.5 g mycelium were inoculated into 50 mL basal salt solution (M100 medium with glucose excluded) and equivalent basal salt solution added with saponins as sole carbon and nitrogen sources respectively, then incubated at 22°C with 220 rpm for 4 H and 12 H. The mycelium of *I. mors-panacis* G3B at each time point were collected and rinsed with distilled water and then immediately frozen in liquid nitrogen until RNA extraction. The mycelium harvested from *I. mors-panacis* G3B incubated with saponins-free basal salt solution served as controls (4 H and 12 H). The total RNA was extracted using TRIzol Reagent in accordance with manufacturer's protocols and then treated with RNase-free DNase to eliminate genomic DNA contamination. The total
RNA was quantified on the Thermo Scientific NanoDrop 2000 spectrophotometer and the Agilent 2100 Bioanalyzer.

**Library construction and sequencing**

The mRNA was purified and isolated by treating total RNA with Magnetic Oligo (dT) beads. Then, the purified mRNA was sheared to approximately 200 bp fragments prior to cDNA synthesis. Short fragments were purified and ligated to sequencing adapters. Fragments with suitable sizes on the basis of agarose gel electrophoresis were selected as templates for PCR amplification in order to isolate and purify the cDNA fragments for sequencing. Construction of libraries (Illumina Truseq™ RNA sample prep Kit) and sequencing with the Illumina HiSeq 2000 platform were performed by Frasergen (Shanghai, China). The quality of raw RNA-Seq reads was filtered using the following critera: (1) reads including adapter sequencing or empty adapter were filtered; (2) reads for which Ns comprised more than 10% of the total length was discarded; (3) reads with low-quality bases (<Q20) were filtered.

**RNA-Seq reads mapping and annotation**

Hisat2 v 2.1.0 was used to map the RNA-Seq reads with the reference genome for subsequent analysis. The mapped-reads were subjected to de novo transcriptome assembly by using trinity assembly software to obtain high-quality transcript sequence. Then, the assembled sequences were used for a homology search against the NR, String, Swissport and KEGG database by NCBI-Blastx Version 2.2.25 with an E-value of $10^{-5}$.

**Gene expression analysis and DEGs validation**

Reads that aligned uniquely to the reference sequence were used for gene expression quantification that were measured and normalized as the fragments per kilobase of exon per million fragments mapped (FPKM), which is similar to reads per kilobase of exon per million mapped reads (Marioni et al., 2008). Differential expression analysis was performed with edgeR v 3.24 software using the test of fold change ($|\log_2\text{FPKM}| > 1$) and false discovery rate (fdr < 0.05) to estimate the level of differential gene expression by each sample under different induction conditions (Benjamini et al., 2001).

**Identification of virulence associated genes during saponins metabolism**

To identify potential pathogenicity and virulence genes, whole genome blast searches were conducted against protein sequences in the Pathogen-Host Interaction database (PHI database) (version 3.2, http://www.phi-base.org/) ($E < 1 \times 10^{-5}$). Further, we found the partial virulence associated genes identified from the PHI database among differential expression genes at 4 H and 12 H during saponins metabolism.

**Results**
The tolerance of I. mors-panacis G3B to saponins

P. notoginseng can secret secondary metabolites such as saponins that used for anti-microbes. Therefore, we assayed the sensitivity to saponins of I. mors-panacis G3B as dominant pathogen inducing root rot of P. notoginseng. Under saponins stress [PDA supplemented with 500 ppm Sanqi total Saponins (SAPs)], I. mors-panacis G3B growed significantly faster and produced bigger isolated colonies than control (Inoculation on PDA without SAPs) ($P < 0.05$) (Fig. 1). They suggested that I. mors-panacis G3B could degrade saponins and use them as carbon sources, and this might also be the main factor for I. mors-panacis G3B to infect the P. notoginseng successfully.

The capacity of I. mors-panacis G3B to detoxify saponins

To verify the degradation effect on saponins of I. mors-panacis G3B directly, we assayed its abilities to degrade the Rg1 and Rb1. Compared with CK, the concentration of Rg1 and Rb1 in the culture solution inoculation with I. mors-panacis G3B was significantly decreased at 3 days and 6–12 days post inoculation, respectively (Fig. 2). They indicated that I. mors-panacis G3B could degrade different kinds of saponins such as Rg1 and Rb1, and exhibiting different degradation abilities.

Overview of RNA-Seq analysis of I. mors-panacis G3B

In general, saponins exhibit antifungal activities against soil-borne fungi. However, the pathogenic fungi can produce saponins detoxifying enzymes to decrease the fungitoxicitiy. To find the genes which may be related to detoxifying saponins, the transcriptomes of I. mors-panacis G3B which were treated with saponins for 4 H and 12 H were profiled by Illumina HiSeq 2000 RNA-Seq (free-saponins treatment as negative control), two biological replicates and 8 datasets were established. Approximately 30 million 200 bp paired-end reads were generated, the majority of the reads ($\sim 94\%$) were mapped to the I. mors-panacis G3B draft reference genome sequence which had been available from the GenBank under the accession number PPHJ0000000.1 (BioProject: PRJNA431033) and more than 70% unique mapped reads appeared, indicating the high abundance and excellent quality of the sequencing data (Table 1). In this study, one gene was considered to be expressed when its fragments per kilobase per million fragments (FPKM) was greater than or equal to 1 and genes with FPKM between 8 and 32 represented a majority (Fig. 3).
| Samples   | Reads       | Length | Q20 (%) | Q30 (%) | GC content (%) | Mapping reads (ratio %) | Unique reads (ratio %) |
|-----------|-------------|--------|---------|---------|----------------|------------------------|-----------------------|
| Control-4H| 29,058,748  | 200    | 98.4    | 95.4    | 55             | 27,463,859 (94.5)       | 22,698,931 (78.1)     |
| Control-12H| 30,631,115 | 200    | 98.3    | 95.3    | 55.5           | 28,959,571 (94.5)       | 26,064,239 (85.1)     |
| SAP-4H    | 31,643,822  | 200    | 98.4    | 95.5    | 54.2           | 29,920,854 (94.6)       | 23,132,888 (73.1)     |
| SAP-12H   | 30,085,411  | 200    | 98.4    | 95.6    | 54.8           | 28,530,258 (94.8)       | 23,817,812 (79.2)     |

DEGs identification

In order to identify the putative signal transduction and metabolic pathways involved in detoxifying saponins in *I. mors-panacis* G3B, we used RNA-Seq to compare the set of differentially expressed genes (DEGs) between saponins treatments and free-saponins treatments for 4 H and 12 H, respectively. In this section, ‘up-regulated genes’ are genes with higher expression levels when saponins treatment, and ‘down-regulated genes’ are those with lower expression levels under the same conditions. When mycelium of *I. mors-panacis* G3B grown for 4 H and 12 H, the number of DEGs was 557 (247 up-regulated and 310 down-regulated) and 1519 (683 up-regulated and 836 down-regulated) respectively. In addition, 343 genes including 93 up-regulated genes and 249 down-regulated genes presented the same expression pattern in the mycelium of *I. mors-panacis* G3B with saponins treatments compared with free-saponins treatments for 4 H and 12 H.

Core genes encoding saponins detoxifying enzymes

Saponins are kinds of glycosides whose aglycones are triterpenes or spiral steranes, they are composed of saponin units and sugars such as glucose, galactose, rhamnose, arabinose, glucuronic acid, galacturonic acid and so on. And the genes involved in their metabolism are unknown. According to the above RNA-Seq analysis, if a DEG in the *I. mors-panacis* G3B with saponins treatments had a similar expression pattern between 4 H and 12 H post inoculation, it may be involved in signal transduction and metabolic pathway of saponins. Therefore, we mainly focused on the above 93 up-regulated genes simultaneously present in the *I. mors-panacis* G3B treated with saponins for 4 H and 12 H, they mainly included several kinds of transporters, glycoside hydrolases, oxidation-reduction enzymes, transcription factors and so on (Fig. 5). We speculated that they are responsible for transporting and metabolizing saponins, making *I. mors-panacis* G3B resist the antimicrobial activity of saponins and infect *P. notoginseng* successfully.

Virulence associated genes
To find potential virulence-associated genes, the whole genome blast analysis was conducted against the Pathogen-Host Interaction (PHI) gene database, a collection of experimentally verified pathogenicity, virulence and effector genes from fungi, oomycetes and bacteria (Winnenburg et al., 2007). And we identified 2298 putative PHI genes in *I. mors-panacis* G3B (12.5% of its genes). We processed on the assumption that the proof of pathogenicity or virulence of a gene in one fungus may also suggest a pathogenicity or virulence in other fungi (Baldwin et al., 2006). Therefore, the 2298 putative PHI genes may be involved in pathogenicity in *I. mors-panacis* G3B, and we found 21 genes which were simultaneously highly expressed in *I. mors-panacis* G3B treated with saponins for 4 H and 12H among them (Table 2). They may be pathogenicity determinants and involved in detoxifying saponins as antimicrobial compounds.
Table 2
Statistic of virulence-associated genes

| Gene   | Log₂ (Fold Change) (4H/12H) | Description                      | PHI accession number                        |
|--------|-----------------------------|----------------------------------|---------------------------------------------|
| g03093 | 2.72/2.58                  | facilitated glucose transporter   | PHI:538|FRT1|AAU87358|TX:40559|Botrytis |
| g05295 | 3.51/2.96                  | hexose transporter protein        | PHI:538|FRT1|AAU87358|TX:40559|Botrytis |
| g08442 | 2.55/1.71                  | oligopeptide transporter         | PHI:1085|Ptr2|AAO31597|TX:13684|Stagonospora |
| g10172 | 2.81/6.87                  | cycloheximide resistance protein | PHI:26|CaMDR1|CAA37820|TX:5476|Candida |
| g16123 | 6.67/6.61                  | abc-2 type transporter           | PHI:258|GPABC1|CAC40023|TX:5128|Gibberella |
| g17147 | 3.57/3.89                  | sugar transporter                | PHI:538|FRT1|AAU87358|TX:40559|Botrytis |
| g17247 | 5.14/2.13                  | polyamine transporter 4          | PHI:26|CaMDR1|CAA37820|TX:5476|Candida |
| g06404 | 4.12/1.71                  | glycosyl hydrolases family 18 protein | PHI:144|CHT42|AAC05829|TX:29875|Trichoderma |
| g06471 | 6.86/4.78                  | cutinase 3                       | PHI:407|PBC1|CAB40372|TX:76659|Pyrenopeziza |
| g17146 | 2.84/4.57                  | triacylglycerol lipase           | PHI:541|LIP1|AAU87359|TX:332648|Botrytis |
| g02004 | 3.97/1.37                  | n-alkane-inducible cytochrome p450 | PHI:438|BcBOT1 |
| g02512 | 7.71/4.97                  | alcohol dehydrogenase            | PHI:881|MGG_04556|EDJ96020|TX:318829|Magnaporthe |
| g10458 | 4.04/4.13                  | cytochrome p450                  | PHI:438|BcBOT1 |
| g11226 | 5.08/2.71                  | aldehyde reductase               | PHI:1047|CTB6|ABK64183|TX:29003|Cercospora |
| g14274 | 2.20/1.96                  | restculine oxidase               | PHI:716|ZEB1|ABB90284|TX:5518|Fusarium |
| Gene | Log$_2$ (Fold Change) (4H/12H) | Description | PHI accession number |
|------|--------------------------------|-------------|---------------------|
| g00310 | 2.33/1.76 | leptomycin b resistance protein pmd1 | PHI:1018|ABC3|AAZ81480|TX:318829|Magnaporthe |
| g10356 | 3.48/6.24 | atpase | PHI:132|ABC1|AAB86640|TX:318829|Magnaporthe |
| g02523 | 6.05/3.84 | cutinase transcription factor 1 beta | PHI:1021|CTF1|ABR12478|TX:5507|Fusarium |
| g03819 | 2.90/2.14 | cutinase transcription factor 1 beta | PHI:1021|CTF1|ABR12478|TX:5507|Fusarium |
| g06402 | 3.00/1.77 | fungal specific transcription | PHI:1021|CTF1|ABR12478|TX:5507|Fusarium |
| g11614 | 2.57/1.65 | fungal specific transcription factor domain-containing protein | PHI:1021|CTF1|ABR12478|TX:5507|Fusarium |

**Discussion**

Ginsenosides are antifungal compounds that are thought to be secreted by ginseng plants to defend infection by soilborne fungi (Augustin et al., 2011), their concentrations dramatically increase in adventitious hairy roots of *Panax ginseng* when attacked by microbes (Liu et al., 2004). However, ginsenosides showed a rapid reduction in roots infected with the aggressive *I. mors-panacis* isolates (Farh et al., 2017). Relevant study also showed that when the tomato was artificially inoculated with *Cladosporium fulvum* inducing blight spot, α-tomatine secreted by tomato plants reduced because of β-glucosidase produced by pathogen hydrolyzing it, resulting in accumulation of a less fungitoxic compound (Okmen et al., 2013). In the current study, we found the growth of *I. mors-panacis* G3B was significantly increased in media supplemented with saponins, indicating that *I. mors-panacis* G3B could produce associated saponin-hydrolyzing enzymes to metabolize saponins. Therefore, *I. mors-panacis* may use a similar mechanism to detoxify ginsenosides such as Rg1 and Rb1 and so on and resist antifungal activity to infect the ginseng plants successfully.

Recent improvements in next generation sequencing technology and bioinformatics now allows the de novo assembly of highly quality eukaryotic genome (Nowrousian et al., 2010; Li et al., 2010). Previously, we used such an approach to provide the first draft sequences of *I. mors-panacis* G3B inducing root rot of *P. notoginseng*, and thus serve as an excellent starting point for gaining a broad perspective of issues in *P. notoginseng* pathology (Zhu et al., 2019). In this study, we used high-throughput RNA-Seq to
characterise the transcriptome profile of *I. mors-panacis* G3B with saponins treatments for different time periods. The induced responses of *I. mors-panacis* G3B to saponins treatments were characterised to reveal genes involved in the saponins detoxification. Saponins, based on a dammarane carbon skeleton with four trans-oriented rings and side-chains that consist various sugar moieties (mono- and disaccharides of glucose, rhamnose, xylose and arabinose) attached through the C-20 and either the C-3 or C-6 positions (Attele et al., 1999). By comprehensive analysis, a total of 93 unigenes were all up-regulated in mycelium harvested from *I. mors-panacis* G3B treated with saponins for 4 H and 12 H. The above related gene encoding proteins mainly belong to transporters, glycoside hydrolases, oxidation-reduction enzymes, transcription factors and therefore they may be involved in putative signal transduction and metabolic pathways to detoxify saponins.

By using the experimentally verified Pathogen-Host Interaction (PHI) gene reference database (Winnenburg et al., 2007), we found that 12.5% of the genes (2298) in the *I. mors-panacis* G3B genome have significant similarities with genes involved in pathogenicity in other fungi, such as plant pathogens *F. graminearum* and *M. oryzae* and even animal pathogens *C. albicans*, they may be candidate genes controlling pathogenicity. Further, we identified 21 genes which were simultaneously highly expressed in *I. mors-panacis* G3B treated with saponins for 4 H and 12H among the 2298 genes, they may be involved in saponins metabolism for resisting antifungal activity to control pathogenicity.

In conclusion, our study explored the tolerance of *I. mors-panancis* G3B to saponins, which may intervene in the plant defense mechanism against pathogens infecting ginseng plants. The pathogenicity of *I. mors-panacis* G3B may mainly depend on detoxification saponins and we identified associated virulence genes which may be involved in saponins metabolism. They provide an excellent starting point for in-depth study of biological function of related-genes in the further work and gain advanced insights into the pathogenicity of *I. mors-panacis* causing root rot in ginseng plants.

Declarations

Conflict of interest

We declare that we have no conflicts of interest in the authorship or publication of this contribution.

Acknowledgements

This research is supported by the general scientific research project from Education Department of Zhejiang Province (Y201840250), general scientific research project from Zhejiang Sci-Tech University (18042289-Y), Zhejiang Natural Science Foundation of China (LQ20C010006). We are grateful to Dr. Hu for their initial experiments on this project.

References
1. Andreea Neculai M, Ivanov D, Bernards MA (2009) Partial purification and characterization of three ginsenoside-metabolizing β-glucosidases from *Pythium irregulare*. Phytochemistry 70:1948–1957. https://doi.org/10.1016/j.phytochem.2009.09.007

2. Attele AS, Wu JA, Yuan CS (1999) Ginseng pharmacology: multiple constituents and multiple actions. Biochem Pharmacol 58:1685–1693. https://doi.org/10.1016/S0006-2952(99)00212-9

3. Augustin JM, Kuzina V, Andersen SB, Bak S (2011) Molecular activities, biosynthesis and evolution of triterpenoid saponins. Phytochemistry 72:435–457. https://doi.org/10.1016/j.phytochem.2011.01.015

4. Baldwin TK, Winnenburg R, Urban M, Rawlings C, Koehler J, Hammond-Kosack KE (2006) The pathogen-host interactions database (PHI-base) provides insights into generic and novel themes of pathogenicity. Molecular plant-microbe interactions 19:1451–1462. https://doi.org/10.1094/MPMI-19-1451

5. Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I (2001) Controlling the false discovery rate in behavior genetics research. Behav Brain Res 125:279–284. https://doi.org/10.1016/S0166-4328(01)00297-2

6. Cabral A, Groenewald JZ, Rego C, Oliveira H, Crous PW (2012) Cylindrocarpon root rot: multi-gene analysis reveals novel species within the *Ilyonectria radicicola* species complex. Mycological Progress 11:655–688. https://doi.org/10.1007/s11557-011-0777-7

7. Chaverri P, Salgado C, Hirooka Y, Rossman AY, Samuels GJ (2011) Delimitation of *Neonectria* and *Cylindrocarpon* (*Nectriaceae, Hypocreales, Ascomycota*) and related genera with *Cylindrocarpon*-like anamorphs. Stud Mycol 68:57–78. https://doi.org/10.3114/sim.2011.68.03

8. Farh ME, Kim Y, Singh P, Yang D (2017) Cross Interaction Between *Ilyonectria mors-panacis* Isolates Infecting Korean Ginseng and Ginseng Saponins in Correlation with Their Pathogenicity. Phytopathology 107:561–569. https://doi.org/10.1094/PHYTO-05-16-0210-R

9. Farh ME, Kim Y, Kim Y, Yang D (2018) *Cylindrocarpon destructans*/*Ilyonectria radicicola*-species complex: Causative agent of ginseng root-rot disease and rusty symptoms. Journal of Ginseng Research 42:9–15. https://doi.org/10.1016/j.jgr.2017.01.004

10. Guo HB, Cui XM, An N, Cai GP (2010) Sanchi ginseng (*Panax notoginseng* (Burkill) F. H. Chen) in China: distribution, cultivation and variations. Genetic Resources and Crop Evolution 57: 453–460. https://doi.org/10.1007/s10722-010-9531-2

11. LF Y, and B. MA (2006) In vitro metabolism of ginsenosides by the ginseng root pathogen *Pythium irregulare*. Phytochemistry 67:1740–1749. https://doi.org/10.1016/j.phytochem.2005.06.030

12. Li R, Fan W, Tian G, Zhu H, He L, Cai J, Huang Q, Cai Q, Li B, Bai Y, Zhang Z, Zhang Y, Wang W, Li J, Wei F, Li H, Jian M, Li J, Zhang Z, Nielsen R, Li D, Gu W, Yang Z, Xuan Z, Ryder OA, Leung FC, Zhou Y, Cao J, Sun X, Fu Y, Fang X, Guo X, Wang B, Hou R, Shen F, Mu B, Ni P, Lin R, Qian W, Wang G, Yu C, Nie W, Wang J, Wu Z, Liang H, Min J, Wu Q, Cheng S, Ruan J, Wang M, Shi Z, Wen M, Liu B, Ren X, Zheng H, Dong D, Cook K, Shan G, Zhang H, Kosiol C, Xie X, Lu Z, Zheng H, Li Y, Steiner CC, Lam TT, Lin S, Zhang Q, Li G, Tian J, Gong T, Liu H, Zhang D, Fang L, Ye C, Zhang J, Hu W, Xu A, Ren Y, Zhang G, Bruford MW, Li Q, Ma L, Guo Y, An N, Hu Y, Zheng Y, Shi Y, Li Z, Liu Q, Chen Y, Zhao J, Qu N, Zhao S,
13. Lombard L, Bezuidenhout CM, Crous PW (2013) Ilyonectria black foot rot associated with Proteaceae. Australas Plant Pathol 42:337–349. https://doi.org/10.1007/s13313-012-0188-5

14. Marioni JC, Mason CE, Mane SM, Stephens M, Gilad Y (2008) RNA-seq: An assessment of technical reproducibility and comparison with gene expression arrays. Genome Res 18: 1509–1517. http://www.genome.org/cgi/doi/10.1101/gr.079558.108

15. Mi C, Yang R, Rao J, Yang S, Wei F, Li O, Hu X (2017) Unveiling of dominant fungal pathogens associated with rusty root rot of Panax notoginseng based on multiple methods. Plant Dis 101:2046–2052. https://doi.org/10.1094/PDIS-01-17-0135-RE

16. Nicol RW, Traquair JA, Bernards MA (2002) Ginsenosides as host resistance factors in American ginseng (Panax quinquefolius). Can J Bot 80:557–562. https://doi.org/10.1139/B02-034

17. Nowrousian M, Stajich JE, Chu M, Engh I, Espagne E, Halliday K, Kamerewerd J, Kempken F, Knab B, Kuo HC, Osiewacz HD, Poggeler S, Read ND, Seiler S, Smith KM, Zickler D, Kuck U, Freitag M (2010) De novo assembly of a 40 Mb eukaryotic genome from short sequence reads: Sordaria macrospora, a model organism for fungal morphogenesis. PLoS Genet 6:e1000891. https://doi.org/10.1371/journal.pgen.1000891

18. Okmen B, Etalo DW, Joosten MH, Bouwmeester HJ, de Vos RC, Collemare J, and P. J. de Wit. (2013) Detoxication of α-tomatine by Cladosporium fulvum is required for full virulence on tomato. New Phytologist 198: 1203–1214. https://doi.org/10.1111/nph.12208

19. Rahman M, Punja ZK (2005) Factors Influencing Development of Root Rot on Ginseng Caused by Cylindrocarpon destructans. Phytopathology 95:1381–1390. https://doi.org/10.1094/PHYTO-95-1381

20. Seifert KA, McMullen CR, Yee D, Reeleder RD, Dobinson KF (2003) Molecular Differentiation and Detection of Ginseng-Adapted Isolates of the Root Rot Fungus Cylindrocarpon destructans. Phytopathology 93:1533–1542. https://doi.org/10.1094/PHYTO.2003.93.12.1533

21. Wang YH, Qiu C, Wang DW, Hu ZF, Yu BY, Zhu DN (2011) Identification of multiple constituents in the traditional Chinese medicine formula Sheng-Mai San and rat plasma after oral administration by HPLC-DAD-MS/MS. J Pharm Biomed Anal 54:1110–1127. https://doi.org/10.1016/j.jpba.2010.11.034

22. Winnenburg R, Urban M, Beacham A, Baldwin TK, Holland S, Lindeberg M, Hansen H, Rawlings C, Hammond-Kosack KE, Kohler J (2007) PHI-base update: additions to the pathogen host interaction database. Nucleic Acids Res 36:D572–D576. https://doi.org/10.1093/nar/gkm858

23. Wu Z, Hao Z, Zeng Y, Guo L, Huang L, Chen B (2015) Molecular characterization of microbial communities in the rhizosphere soils and roots of diseased and healthy Panax notoginseng. Antonie Van Leeuwenhoek 108:1059–1074. https://doi.org/10.1007/s10482-015-0560-x

24. Zhu B, Wang S, Mi C, Yang R, Zen G, Hu X (2019) Genome Sequence Resource for Ilyonectria mors-panacis, Causing Rusty Root Rot of Panax notoginseng. Mol Plant Microbe Interact 32:1468–1471.
25. Liu J, Ding JY, Zhou QY, He L, Wang ZT (2004) Studies on influence of fungal elicitor on hairy root of *Panax ginseng* biosynthesis ginseng saponin and biomass. China Journal of Chinese Materia Medica 29:302–305

26. Sun Y, Ke J, Ma N, Chen Z, Wang C, Cui X (2004) Effects of root rot on saponin content in *Panax notoginseng*. Journal of Chinese Medicinal Mater 27:79–80

27. Yan XL, Xiao FH, Wen GS, Li X, Duan CL (2006) Investigation and collection of wild *Panax* species in Yunnan province. Journal of Yunnan Agricultural University 21:419–423