

Transcriptome Analysis of *Dryadomyces quercus-mongolicae*, a Fungus Associated with Korean Oak Wilt Disease that Causes Oak Mortality in South Korea

Hyowon Park ¹, Jorge R. Ibarra Caballero ², Jane E. Stewart ², Ned B. Klopfenstein ³, Uk Lee ^{4*}, and Mee-Sook Kim ^{5*}

¹Department of Plant Science and Technology, Chung-Ang University, Anseong 17546, Korea

²Department of Agricultural Biology, Colorado State University, Fort Collins, CO 80523, USA

³USDA Forest Service-Rocky Mountain Research Station, Moscow, ID 83843, USA

⁴Special Forest Resources Division, National Institute of Forest Science, Suwon 16631, Korea

⁵USDA Forest Service-Pacific Northwest Research Station, Corvallis, OR 97331, USA

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Korean oak wilt disease associated with *Dryadomyces quercus-mongolicae* recently emerged as a major tree disease in South Korea. A comprehensive transcriptome analysis is presented for *D. quercus-mongolicae* grown *in vitro* on three different culture media, identifying nearly 7,000 expressed transcripts. Most transcripts are associated with proteins essential for fungal survival

and growth. The 40S ribosomal protein S25, ceramide very long chain fatty acid hydroxylase, Epl1 protein, and ADP/ATP translocase are particularly important due to their critical roles in the metabolism and environmental adaptation of fungi. Gene ontology analyses revealed that 39.4%, 61.2%, and 43.3% of transcripts were successfully annotated to biological process, molecular functions, and cellular component aspects, respectively. Furthermore, key metabolic pathways were elucidated, including sphingolipid metabolism, L-tryptophan biosynthesis, and glycolysis, which provide important information on physiological functioning of *D. quercus-mongolicae*. Overall, these findings provide key information on fundamental biological mechanisms of *D. quercus-mongolicae*.

Keywords : oak wilt, *Platypus koryoensis*, *Quercus mongolica*, *Raffaelea quercus-mongolicae*, transcriptome

*Co-corresponding authors.

U. Lee

Phone) +82-31-290-1161, FAX) +82-31-290-1050

E-mail) rich26@korea.kr

M.-S. Kim

Phone) +1-541-750-7150, FAX) +1-541-750-7760

E-mail) meesook.kim@usda.gov

ORCID

Hyowon Park

https://orcid.org/0000-0002-6375-389X

Jorge R. Ibarra Caballero

https://orcid.org/0000-0001-6007-5168

Jane E. Stewart

https://orcid.org/0000-0001-9496-6540

Ned B. Klopfenstein

https://orcid.org/0000-0002-9776-3973

Uk Lee

https://orcid.org/0000-0003-1934-4455

Mee-Sook Kim

https://orcid.org/0000-0001-7073-6708

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Outbreaks of oak mortality (mainly *Quercus mongolica*) were observed in Seongnam, South Korea in 2004, and this mortality continued to spread throughout the entire country (Kim et al., 2009). In subsequent years, the mortality rate for oak species has continued to rise due to the disease known as Korean oak wilt (Kim et al., 2016). The fungus *Dryadomyces quercus-mongolicae* (formerly *Raffaelea quercus-mongolicae*) (de Beer et al., 2022) was isolated from a dead *Q. mongolica* (Mongolian oak) and subsequently, this species was found to be associated with the mass mortality of oaks in South Korea. *Dryadomyces quercus-mongolicae* is known to be vectored in the

mycelium of the ambrosia beetle, *Platypus koryoensis* (Kim et al., 2009). When these ambrosia beetles attack and penetrate the host trees, *D. quercus-mongolicae* is transmitted, where it appears to subsequently cause disease in the host trees. In response to the fungal infection, oak produce parenchyma cell-derived tyloses within the xylem vessels, which function as a defense mechanism. Although tyloses may act to limit fungal spread within the vascular system, these reactions with accompanying fungal growth can also block water flow in the xylem, which is believed to be a primary cause of Korean oak wilt disease (Kim et al., 2009). However, the precise pathogenic mechanisms of *D. quercus-mongolicae* associated Korean oak wilt have not been clearly established in previous studies (Torii et al., 2014; Yi et al., 2017).

Transcriptomic analyses of plant pathogens can contribute to our understanding of genetic mechanisms related to pathogenicity, disease, and biological functions (DiGustini et al., 2011; Keeling et al., 2012; Toledo-Arana et al., 2009) by examining gene expression at different stages of infection and disease development. Expressed genes vary depending on cell/tissue type, environmental factors, developmental stage, and many other interacting factors and signals. For these reasons, transcriptomic analyses are essential for characterizing the genetic bases of an organism's functions under variable environments. Transcriptomic analyses of fungal pathogens that cause tree diseases can also provide baseline information for examining host-pathogen and host-vector interactions, pathogenesis, general metabolism, development, environmental adaptation, and ecological functions. Information on basic physiological mechanisms of *D. quercus-mongolicae* is currently quite limited. Consequently, the objective of this study was to analyze the functional annotations of expressed transcripts from *D. quercus-mongolicae* grown *in vitro* to provide baseline information toward understanding biological functions of this fungus.

The *D. quercus-mongolicae* isolate (KACC44405) used in this study was obtained from the Korean Agricultural Culture Collection (National Agrobiodiversity Center, Jeonju, South Korea). This isolate was selected for our transcriptomic study because of the available genomic sequence information (Jeon et al., 2017), and it also serves as the type specimen for describing *D. quercus-mongolicae* (as *R. quercus-mongolicae*) (Kim et al., 2009). The fungus was grown under *in vitro* conditions on three culture media that represented a high-carbon source condition (potato dextrose agar; PDA), very low carbon source condition (water agar; WA), and partially resembling host condition (sawdust-amended water agar; SWA). The SWA medium

contained sawdust collected from *Q. mongolica* using a wood-chip grinding machine (FJ Tech, Gimpo, South Korea) that was added to the WA medium before autoclaving. Pieces containing (3 × 3 × 3 mm) fungal inoculum were excised from an actively growing culture and placed onto a sterile, nylon-membrane filter (Millipore Sigma, Burlington, MA, USA) that was overlaid on each of the three culture media, PDA (39 g/L, BD-Difco, Sparks, MD, USA), SWA (100 g/L sawdust with 1.5% WA, BD-Difco), and WA (1.5% agar, BD-Difco), before incubating at 25 ± 1°C. After 7 days, each mycelial sample (100 mg wet weight) was gently scraped off from the nylon membranes overlaying each of the three culture media and immediately placed into pre-chilled, 2.0-mL microtubes containing two stainless steel beads (5-mm diameter). The tubes containing mycelia from each respective medium were stored at -80°C for 5 min, and then mycelial samples were ground three times for 3 min at 40 Hz using TissueLyser (TissueLyser LT, Qiagen, Hilden, Germany). After grinding the tissue, tubes were stored again at -80°C for 3 min before adding 800 µL RNA lysis buffer and again disrupting the mycelia for 1 min at 50 Hz. Total RNA was extracted using the *Quick*-RNA Fungal/Bacterial Mini-Prep Kit (R2014, Zymo Research, Tustin, CA, USA) following the manufacturer's protocol. The resulting RNA concentration was measured using a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA), and RNA integrity number was checked using TapeStation (Agilent Technologies, Santa Clara, CA, USA).

The mRNA was isolated from total RNA using an mRNA purification kit (Zymo Research), and an Illumina cDNA library was generated from isolated mRNA using TruSeq RNA Sample Prep Kit (Illumina Inc., San Diego, CA, USA). The cDNA library was sequenced at Macrogen Korea (Seoul, South Korea) using Illumina HiSeq 2000 (Illumina Inc.), producing a read length of 101 bp. The resulting sequence reads were checked for quality using FastQC v0.12.1 (<https://www.bioinformatics.babraham.ac.uk>). The software Trimmomatic v0.38 (Bolger et al., 2014) was used to remove adapters and remove low-quality sequences, if any. Resulting reads were aligned to the *D. quercus-mongolicae* genome (accession no. NIPS000000000) (Jeon et al., 2017) using HISAT2 version 2.2.1 (Kim et al., 2019), setting the maximum intron length to 6,000 bp. The aligned reads were assembled, and their FPKM values (fragments per kilobase of transcript per million fragments mapped) were estimated with using Cufflinks (version 2.2.1) (Trapnell et al., 2010). This Transcriptome Shotgun Assembly project has been deposited to the Sequence Read Archive (BioProject: PRJNA513385) in National Center for Bio-

technology Information (accession no. SRR32758122 for PDA; SRR32757902 for SWA; and SRR32757901 for WA). All transcripts were annotated using the UniProtKB database using MMseqs2 (Release 14-7e284) (Steinegger and Söding, 2017) to determine the predicted functions of proteins encoded by each transcript. The longest open reading frames were predicted using Orfipy (Singh and Wurtele, 2021). These protein-encoding sequences were analyzed with DeepLoc version 2.0 (Thummuluri et al., 2022) to predict the subcellular localization of putative proteins. Any putative proteins that were localized in the ‘Extracellular’ region were subjected to analysis by EffectorP version 3.0 (Sperschneider and Dodds, 2021) as secreted proteins. Carbohydrate-active enzymes (CAZymes) were predicted using dbCAN3 version 4.0 (Zheng et al., 2023).

The transcriptome libraries from all mycelial samples of *D. quercus-mongolicae* generated a total of 94,951,536 reads. From the PDA sample, a total of 31,183,399 reads were generated (GC content: 57%), and the ratio of aligned reads was 97.40%, while from the SWA sample, a total of 34,052,359 reads were generated (GC content: 57%), and the ratio of aligned reads was 97.49%. Lastly, the reads from the WA sample generated 29,715,778 reads (GC

Table 1. Summary of *Dryadomyces quercus-mongolicae* transcriptome reads mapped to the reference genomes

Samples ^a	Total reads	Read mapping ratio (%)	GC (%)
PDA	31,183,399	97.40	57
SWA	34,052,359	97.49	57
WA	29,715,778	97.13	56

^a*Dryadomyces quercus-mongolicae* was cultured on potato dextrose agar (PDA), sawdust-amended water agar (SWA), and water agar (WA).

content: 56%), with an alignment ratio of 97.13% (Table 1). The number of transcripts generated from PDA, SWA, and WA samples were 6,739, 6,900, and 6,855 transcripts, respectively. A total of 6,679 transcripts were commonly expressed across all three media types (Supplementary Fig. 1). Among all assembled transcripts, 4,368 transcripts were successfully annotated with putative protein and gene names, Gene Ontology (GO) terms, organism, Kyoto Encyclopedia of Genes and Genomes, and UniPathway by searching the Uniprot database using the MMseq2 program.

Table 2. Summary of the 20 most abundant expressed genes analyzed via transcriptome of *Dryadomyces quercus-mongolicae*

Locus	FPKM	Gene name	Protein name
NIPS01000040.1:935728–936762	2,935.787	CMQ_6984	40S ribosomal protein S25
NIPS01000010.1:777871–780111	2,508.836	CDV36_008273	Ceramide very long chain fatty acid hydroxylase
NIPS01000038.1:459664–460614	2,475.233	CMQ_7839	Epl1 protein
NIPS01000040.1:932230–934782	1,909.18	CMQ_7025	ADP/ATP translocase (ADP, ATP carrier protein)
NIPS01000006.1:1512860–1525145	1,679.32	CMQ_6288	Dihydrofolate synthetase fol3
NIPS01000038.1:1644321–1649326	1,644.106	PMAA_003180	Cytochrome c oxidase subunit VIa, putative
NIPS01000006.1:720693–725015	1,477.72	S40288_09971	-
NIPS01000038.1:179668–181740	1,398.754	CMQ_5029	Woronin body major protein
NIPS01000006.1:1591074–1593078	1,330.054	CMQ_5954	An1-type zinc finger protein
NIPS01000042.1:3689934–3692879	1,228.092	HMPREF1624_01111	ABM domain-containing protein
NIPS01000001.1:697370–698372	1,219.778	CMQ_4480	40S ribosomal protein S12
NIPS01000043.1:3908112–3918765	1,193.607	CMQ_3140	Elongation factor 1-alpha
NIPS01000037.1:1241745–1247077	1,177.308	CMQ_8082	Heat shock protein 30
NIPS01000004.1:791158–797155	1,121.673	CMQ_2107	Aar2 domain containing protein
NIPS01000043.1:1262011–1265518	1,104.991	SPSK_09373	dCMP deaminase
NIPS01000042.1:207329–209619	1,097.239	CMQ_2990	Major facilitator superfamily transporter monosaccharide
NIPS01000037.1:856237–859011	1,094.254	CMQ_6093	Major facilitator superfamily transporter
NIPS01000043.1:164114–166546	1,020.814	SPSK_09419	4-Hydroxybenzoate polyprenyltransferase
NIPS01000038.1:31328–32303	909.9773	CH63R_12829	Histone H3
NIPS01000039.1:357309–359088	898.717	BO78DRAFT_370918	Extracellular membrane protein CFEM domain-containing protein

FPKM, fragments per kilobase of transcript per million fragments mapped.

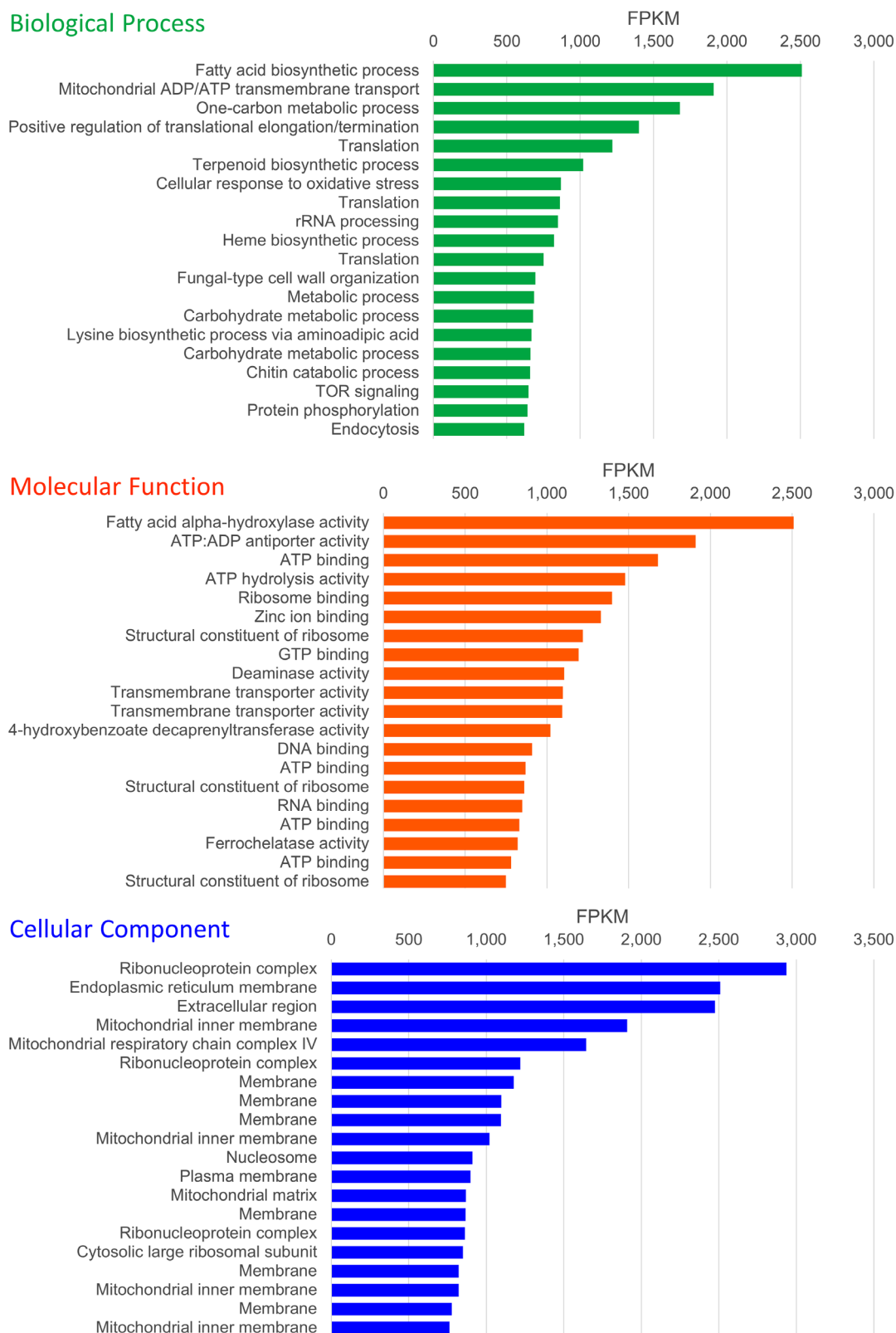


Fig. 1. Summary of Top 20 functional annotation using Gene Ontology (GO) terms, which comprising with three aspects, biological process, molecular function, and cellular component of *Dryadomyces quercus-mongolicae*. FPKM, fragments per kilobase of transcript per million fragments mapped.

Among the 4,368 annotated transcripts, Table 2 shows the top 20 most highly expressed transcripts that encoded putative proteins based on the matched sequences. Mean values derived from the three culture media were applied to select the top 20 highly expressed transcripts. The 40S ribosomal protein S25, ceramide very long chain fatty acid hydroxylase, Epl1 protein, ADP/ATP translocase, and dihydrofolate synthetase fol3 were determined to be highly expressed in *D. quercus-mongolicae* (Table 2). Ribosomes play a well-known crucial role in protein production and are vital for a wide variety of organisms, from bacteria to animals (Karsi et al., 2002). The eukaryotic 80S ribosome consists of a small subunit (40S) and a large subunit (60S), with the 40S subunit containing the 18S rRNA and 32 ribosomal proteins (Wool, 1979). Ribosomal protein genes are responsible for coding the structural elements of ribosomes, which are the cellular machinery for the protein synthesis process (Mullis et al., 2020). Because ribosomal proteins are essential for fungal growth and survival, it is expected that the 40S ribosomal protein S25 would be highly expressed in *D. quercus-mongolicae*. Very long chain fatty acids (VLCFAs) are fatty acids that have chain lengths more than 18 carbon atoms (Batsale et al., 2023). VLCFAs serve as essential components for creating more complex molecules and play important roles in various cellular functions, such as energy storage, biochemical signaling, and membrane structure as various types of lipids, such as triacylglycerols, sphingolipids, and phosphoglycerolipids (Haslam and Kunst, 2013). In numerous organisms, the VLCFAs are also fundamental in forming an extracellular structure known as the cuticle, which covers the outside of these organisms and acts as the main physical barrier against the environment (Raffaele et al., 2009). Although VLCFAs compose a small fraction of the total fatty acids in cells, their importance is exemplified by the fact that plants cannot survive without VLCFAs (Beaudoin et al., 2009); however, further investigation is warranted to understand the precise role of VLCFAs in *D. quercus-mongolicae*. Ceramide hydroxylase plays a role in the

hydroxylation process of VLCFAs that are associated with sphingolipids (Chen et al., 2008). Higher expression levels of ceramide VLCFA hydroxylase-encoded transcripts indicates that this protein is likely important for survival and growth of this fungus. Eliciting plant response (Epl) 1 protein-encoded transcripts were highly expressed in *D. quercus-mongolicae*. The Epl1 protein, which is reported as a plant-resistance elicitor, is crucial for fungal adaptation to environmental conditions, particularly in *Trichoderma* and other interactions among fungal pathogens and plants (Gomes et al., 2015). In addition, ADP/ATP translocase, dihydrofolate synthetase, cytochrome c oxidase, woronin body major protein, An1-type zinc finger protein, and ABM domain-containing protein-encoded transcripts were also greatly enriched in transcriptome of *D. quercus-mongolicae* (Table 2).

Gene annotation based on Gene Ontology Functional Classification System (GO terms) was applied to functional annotation of transcripts from *D. quercus-mongolicae*, and the results were shown in Fig. 1. The three GO aspects provided by the GO database comprised biological process (BP), molecular function (MF), and cellular component (CC). Results from this study indicated that 39.4% (1,722 out of 4,368 transcripts), 61.2% (2,675 out of 4,368 transcripts), and 43.3% (1,890 out of 4,368 transcripts) were successfully annotated to BP, MF, and CC aspects, respectively. The expressed transcripts within the BP aspect were annotated to fatty acid biosynthetic process (GO:0006633), mitochondrial ADP/ATP transmembrane transport (GO:0140021, GO:1990544), one-carbon metabolic process (GO:0006730), positive regulation of translational elongation/termination (GO:0045901, GO:0045905), and translation (GO:0006412) (Fig. 1). Meanwhile, the MF aspect comprised expressed transcripts encoding fatty acid α -hydroxylase activity (GO:0080132), ATP:ADP antiporter activity (GO:0005471), ATP binding (GO:0005524), ATP hydrolysis activity (GO:0016887), and ribosome binding (GO:0043022). Within the transcriptome of *D. quercus-mongolicae*, genes with higher expression that

Table 3. List of annotated pathway to expressed transcripts of *Dryadomyces quercus-mongolicae*

Locus	FPKM	Protein name	Pathway
NIPS01000010.1:777871–780111	2,508.836	Ceramide very long chain fatty acid hydroxylase	Lipid metabolism; sphingolipid metabolism
NIPS01000003.1:641266–650297	124.857	Tryptophan synthase	Amino-acid biosynthesis; L-tryptophan biosynthesis; L-tryptophan from chorismate
NIPS01000001.1:195805–197525	3.0194	Enolase	Carbohydrate degradation; glycolysis; pyruvate from D-glyceraldehyde 3-phosphate

FPKM, fragments per kilobase of transcript per million fragments mapped.

Table 4. List of information of expressed transcripts which assigned as CAZymes including locus, levels of transcripts (FPKM values), assigned enzyme family (terms), substrates, and encoded protein names from *Dryadomyces quercus-mongolicae*

Locus	FPKM	Term	Substrate	Protein name
NIPS01000037.1:1241745–1247077	1,177.308	AA3	Cellulose/lignin	Heat shock protein 30
NIPS01000041.1:493212–496011	678.28	GH78	Pectin	Alpha-l-rhamnosidase
NIPS01000002.1:454711–459781	661.6523	GH18	Chitin	Class 3 chitinase 2
NIPS01000006.1:1641133–1646046	657.5897	GH18	Chitin	Chitinase (EC 3.2.1.14)
NIPS01000042.1:2004324–2009417	389.8305	GH47	α -glucoside	-
NIPS01000010.1:1897558–1902128	331.9	AA7/GT31	Cellulose	D-lactate dehydrogenase [cytochrome], mitochondrial
NIPS01000043.1:4064087–4067375	219.709	GT3	Glycogen_syn	Glycogen [starch] synthase (EC 2.4.1.11)
NIPS01000037.1:1268103–1271104	183.1702	CBM24	Binding	Alpha-1,3-glucanase/mutanase
NIPS01000005.1:316276–319423	170.0813	GH32	Sucrose	Glycoside hydrolase family 32 protein
NIPS01000005.1:1064591–1068891	164.4062	CBM21	Binding	Protein phosphatase regulator

CAZymes, carbohydrate-active enzymes; FPKM, fragments per kilobase of transcript per million fragments mapped.

were annotated to the CC aspect included ribonucleoprotein complex (GO:1990904), endoplasmic reticulum membrane (GO:0005789), extracellular region (GO:0005576), mitochondrial inner membrane (GO:0005743), and mitochondrial respiratory chain complex IV (GO:0005751) (Fig. 1).

Based on the results of pathway annotation, three different pathways were annotated, which included sphingolipid metabolism (lipid metabolism), L-tryptophan biosynthesis (amino acid biosynthesis), and glycolysis (carbohydrate degradation) (Table 3). Sphingolipids are critical components of the fungal plasma membrane, contributing to the stability and integrity of the cellular structure (Fabri et al., 2020), and these components play an essential role in regulating membrane fluidity, which enables fungi to adapt altering environmental conditions (Usmani et al., 2023). Furthermore, sphingolipids have been shown to be important for the modulation of signal transduction pathways correlated with cell growth and stress responses (Dickson et al., 2006). Moreover, the sphingolipids also contribute to fungal colonization and infection processes (Usmani et al., 2023). Based on our results, sphingolipid metabolism is also likely important for growth of *D. quercus-mongolicae*. The important roles of fungi-specific tryptophan synthases gene (*TRP1* gene) in fungal survival through the tryptophan biosynthesis are well demonstrated in previous studies (Ireland et al., 2008; Sen et al., 2016; Staats et al., 2004). In our study, the transcripts for the tryptophan synthase pathway and its biosynthesis metabolism were highly expressed in *D. quercus-mongolicae*, which suggests a critical role for this metabolic pathway.

Many fungi produce various enzymes to degrade plant

cell-wall components, such as cellulose, hemicellulose, and pectin, or plant carbohydrates, such as starch, sucrose, and mannose (Ibarra Caballero et al., 2019). These enzyme families, which are known as CAZymes, comprise several groups, including glycoside hydrolases (GHs), glycosyl-transferases, polysaccharide lyases, carbohydrate esterases, auxiliary activities, and carbohydrate-binding modules (Drula et al., 2022). Among all of the expressed transcripts in *D. quercus-mongolicae*, 10 transcripts were annotated as CAZymes (Table 4) including heat shock protein 30 and D-lactate dehydrogenase with cellulose as the likely substrate. Annotated CAZymes also included α -l-rhamnosidase and GH family 32 proteins that likely use pectin and sucrose as substrates, respectively. Furthermore, annotated transcripts included a class 3 chitinase 2.

The function of a protein is highly dependent on its localization and prediction of its subcellular location can provide key information about its functionality and possible interactions with other proteins (Gillani and Pollastri, 2024). In this study, we attempted to localize encoded proteins. After identifying the locations of each protein with the DeepLoc model, a total of 515 proteins were localized in the extracellular region, and these proteins were considered as putative secreted proteins from *D. quercus-mongolicae*. Among the 515 proteins, 100 (19.4%) were identified as predicted effector proteins, and 415 (80.6%) were identified as non-effector proteins. Of the predicted 100 effector proteins, 57 (11.1%) were predicted cytoplasmic effectors and 43 (8.3%) were predicted apoplastic effectors. Subsequently, a 0.75 cut-off value of prediction score was implemented to select proteins with a higher probability of being an effector protein (Table 5). Of these higher prob-

Table 5. Candidate cytoplasmic and apoplastic effectors (prediction score > 0.75) of encoded proteins from *Dryadomyces quercus-mongolicae*

Locus	Cytoplasmic	Apoplastic	Protein name
NIPS01000043.1:17659–20044	-	0.933	Non-hemolytic phospholipase C
NIPS01000037.1:1250846–1251960	-	0.92	Uncharacterized protein
NIPS01000042.1:3976259–3977142	-	0.895	Chitinase
NIPS01000001.1:137720–138557	-	0.882	Chitinase
NIPS01000002.1:736812–738568	-	0.879	DNase I protein
NIPS01000037.1:1265869–1267801	-	0.866	Uncharacterized protein
NIPS01000005.1:1061641–1063394	-	0.831	Uncharacterized protein
NIPS01000042.1:4034412–4036902	-	0.819	Amine oxidase
NIPS01000042.1:3258285–3260727	-	0.796	Glycosyl hydrolase
NIPS01000037.1:113538–116040	-	0.794	Ribosomal protein S17
NIPS01000043.1:2279544–2280499	-	0.784	Uncharacterized protein
NIPS01000010.1:2835881–2836668	-	0.763	Uncharacterized protein
NIPS01000010.1:868341–868876	0.822	0.775	-
NIPS01000005.1:1434676–1436501	0.925	-	DUF895 domain membrane protein
NIPS01000039.1:499470–500337	0.854	-	Amine oxidase
NIPS01000005.1:435141–435849	0.836	-	Uncharacterized protein
NIPS01000041.1:707113–709193	0.821	-	Major facilitator superfamily transporter
NIPS01000010.1:981730–983942	0.818	-	-
NIPS01000010.1:2367137–2367679	0.813	-	-
NIPS01000040.1:716910–719402	0.799	-	Uncharacterized protein
NIPS01000001.1:187365–188357	0.798	-	Uncharacterized protein
NIPS01000038.1:359456–360244	0.787	-	Nanos-type domain-containing protein
NIPS01000040.1:845430–848174	0.784	-	DNA repair protein RAD14
NIPS01000005.1:265010–265883	0.779	-	24-Dienoyl-CoA reductase
NIPS01000038.1:202171–202560	0.774	-	-
NIPS01000010.1:2184954–2186663	0.76	-	Altered inheritance of mitochondria protein 6
NIPS01000002.1:43624–44867	0.752	-	-

ability effector proteins, 13% (13 out of 100) and 15% (15 out of 100) of effectors were localized to the apoplast and cytoplasm, respectively. Because apoplastic effectors directly interact with host plant tissues on the outside of plant cell, including the cell wall and intercellular spaces (Rocafort et al., 2020), we focused on these apoplastic effectors. In this study, apoplastic effectors identified in *D. quercus-mongolicae* included non-hemolytic phospholipase C, chitinase, DNase I protein, amine oxidase, GH, ribosomal protein S17, and uncharacterized protein. Phospholipids and proteins constitute the primary chemical components of the plant cell membrane (Colin and Jaillais, 2020). Consequently, enzymes that can hydrolyze these plant cell membrane components, such as phospholipases and proteinases, are likely to play a significant role in the processes

of membrane disruption that occur during the infection and invasion of plant host cells (Schmiel and Miller, 1999). The action of phospholipases, which cleave phospholipids, leads to membrane destabilization and ultimately results in host cell lysis (Ghannoum, 2000). Furthermore, fungal chitinases may serve a fundamental role in the plasticization of the cell wall, or they may function more specifically in processes such as cell separation, the acquisition of nutritional chitin, or competitive interactions with other fungal species (Langner and Göhre, 2016). A fungal GH, which is also a CAZyme class, was also identified as an apoplastic effector in the *D. quercus-mongolicae*. This enzyme can hydrolyze glycosidic bonds between carbohydrate molecules of fungi (Valadares et al., 2016). Additionally, previous studies provide substantial evidence for the significant role various

GH protein families play in the virulence of phytopathogenic fungi (Bradley et al., 2022; Vu et al., 2012). As such, various apoplastic effectors, non-hemolytic phospholipase C, chitinase, and GH, can be considered to have potential roles in the virulence of *D. quercus-mongolicae*.

In summary, this study has identified 4,368 significant transcripts of *D. quercus-mongolicae*, many of which encode proteins essential for the fungal survival and growth. Within *in vitro* grown *D. quercus-mongolicae*, these highly expressed proteins include 40S ribosomal protein S25, ceramide very long chain fatty acid hydroxylase, Epl1 protein, and ADP/ATP translocase. These proteins are particularly noteworthy due to their critical roles in the metabolism and environmental adaptation of fungi. Key metabolic pathways, including sphingolipid metabolism, L-tryptophan biosynthesis, and glycolysis, which have important implications for the physiological functions of *D. quercus-mongolicae*. Furthermore, the phospholipase C, chitinase, and GH were identified apoplastic effectors, which could be associated with virulence of *D. quercus-mongolicae*. As a consequence, this baseline transcriptomic study provides useful background information for future studies aimed at better understanding the physiological mechanisms and biological functions of *D. quercus-mongolicae*.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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Electronic Supplementary Material

Supplementary materials are available at The Plant Pathology Journal website (<http://www.ppjonline.org/>).

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