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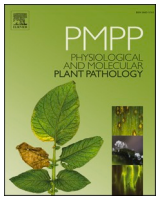

Gene expression of transcripts encoding putative secreted proteins from an oil palm fungal pathogen *Ganoderma boninense*

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ABSTRACT

Ganoderma boninense is a white rot fungus which causes basal stem rot (BSR) disease in oil palm and economic loss to the oil palm industry in Southeast Asia. The existing solutions for the disease are ineffective and the genetic determinants responsible for the disease occurrence are understudied. This study reported the characterisation of four transcript sequences encoding metalloproteinase (*U14090*), aspartic protease (*U42611*), lipase (*U56931*), and polysaccharide deacetylase (*U128397*) from *G. boninense*. The candidate transcripts were cloned and confirmed by sequencing. The gene expression of *U56931* was up-regulated in mycelial cultures at 21- and 28-days after inoculation (dai) whereas the expression of *U42611* was down-regulated in mycelial cultures at 28-dai, in comparison to that at 7-dai. Salicylic acid which is involved in plant biotic stress response was shown to be able to down-regulate the gene expression of *U56931*. The gene expression of *U14090* was up-regulated in *G. boninense* in contact with oil palm roots for 24- and 48-h post inoculation (hpi) compared to that in the un-inoculated control. The findings of this study may facilitate the design of future functional studies and help to prioritise candidate fungal genes for gene editing.

1. Introduction

The African oil palm (*Elaeis guineensis* Jacq.) is a highly productive oil crop, which produces approximately 3.3 tonnes of oils per hectare [1]. It plays an important role in meeting the global demand for edible vegetable oils. For the marketing year 2024/25, palm oil is forecasted to constitute 35 % (80.03 million metric tons) and 55 % (46.57 million metric tons) of the global major vegetable oil production and exports, respectively [2]. The palm oil production in Southeast Asia is threatened by basal stem rot disease (BSR). The BSR causes a drastic 50–80 % reduction in oil yield, and eventual plant death [3]. *Ganoderma boninense* is a hemibiotrophic fungal pathogen which causes the BSR in young and mature palms. The infected young palms usually exhibit one-sided yellowing, mottling and necrosis of lower fronds, shorter and chlorotic unfolded leaves, pale, and retarded palm growth as well as unopened spear leaves; whereas the diseased mature palms show similar symptoms with drooping dead fronds which form a skirting appearance [4]. The current measures for *G. boninense* disease control are not effective to contain BSR [5] partly due to a late onset of aboveground disease symptoms which delays the initiation of disease control efforts. In addition, soil which is pre-infested with *Ganoderma* inoculum could increase the BSR disease incidence in young oil palms [6].

Host-pathogen interactions may lead to partial host resistance or susceptibility [7]. An in-depth understanding of the molecular events occurring during the plant-pathogen interaction before the emergence of the symptoms is of great importance. An increasing number of studies were conducted on *E. guineensis-G. boninense* interaction using next-generation sequencing technologies [8–10]. The oil palm host may employ chitin-triggered immunity and salicylic acid (SA)-mediated defence against *G. boninense* infection [11,12] besides modulating its resources or suppress other biological processes to prioritise its defence response against *G. boninense* [9]. On the other hand, the *G. boninense* interacting with the host was found to have an increased abundance of transcripts encoding for lignin degrading and modification enzymes, and a reduced abundance of transcripts responsible for fungal respiration, chitin degradation, and pathogenesis [9]. The pathogen may

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modify fungal chitin surface to avoid recognition and degradation by plant chitinase [13]. The cloning and functional characterisation of fungal secreted proteins from *G. boninense* are still lacking.

During a plant-pathogen interaction, pathogens may secrete effectors, hydrolytic enzymes, organic acids, secondary metabolites, or small RNA molecules that suppress host defence and facilitate host cell wall invasion as well as nutrient acquisition [14–18]. Putative effectors from *G. boninense* were predicted [10] and several genes or proteins from *G. boninense* including cyclophilins [19], hydrophobin [20], pheromone receptor [21], necrosis and ethylene inducing protein (NEP)-like proteins [22], laccases [23], manganese peroxidases [23], non-ribosomal peptide synthetase [24], lanosterol 14α-demethylase [25], and cerato-platanins [26] have been characterised. Of which, the recombinant NEP-like protein (originated from *G. boninense*) was shown to cause necrosis in tobacco and tomato [22] while the RNAi-silencing of lanosterol 14α-demethylase in *G. boninense* was reported to cause a lower mycelial growth and ergosterol level in the pathogen [25].

Secreted proteases and lipases often contribute to microbial pathogenicity. The transcripts encoding metalloproteinase, aspartic protease, polysaccharide deacetylase, and lipase from *G. boninense* were previously reported to be over-expressed in a *G. boninense*-treated oil palm root transcriptome [8]. Metalloproteinase and aspartic protease could degrade plant chitinase (a component of plant defence) [27,28], while polysaccharide deacetylase may suppress chitin-induced defence [29]. Lipase was reported to be involved in cell wall degradation [30], plant cell wall adhesion and penetration [31]. The GDSL-lipase was reported in the modification of host innate immunity-related fatty acid [32].

The current work presents the cloning and sequencing of the complete open reading frames encoding metalloproteinase (*U14090*), aspartic protease (*U42611*), lipase (*U56931*), and polysaccharide deacetylase (*U128397*) from *G. boninense* which were not previously available or annotated in the database. Since the proteins encoded by these transcripts may play a role in breaching the oil palm defence through suppression or avoidance of chitin-triggered immunity, precise nucleotide sequences and gene expression information of these proteins are important for future experiments on gene silencing and protein inhibition.

2. Materials and methods

2.1. Fungal culture

Ganoderma boninense BLSM5B was provided by the Sarawak Tropical Peat Research Institute, Kota Samarahan, Sarawak. It was collected from Balingian, Sarawak (GPS coordinate: N02°57′51.7″, E112°30′28.8″) [33,34] and maintained in sterile tap water to preserve its pathogenicity [35]. Its pathogenicity was confirmed through reinoculation of oil palm seedlings [34]. For gene isolation, the fungal culture was grown in 30 mL malt extract broth (MEB) (Difco, US) at 30 °C in the dark at static condition for 14 days before the mycelia were harvested and immediately frozen in liquid nitrogen before being kept at −80 °C.

2.2. Sample preparation for gene expression studies

For gene expression analysis, *G. boninense* BLSM5B was grown in 30 mL potato dextrose broth (PDB) (HiMedia, India) at 30 °C in the dark at static condition. To understand if the selected genes were involved in regulating fungal growth, the fungal mycelia were harvested at 7-(control), 14-, 21-, and 28-days after inoculation (dai).

To study the gene expression of fungal samples that were in contact with host plants, five-month-old oil palm seedlings of FGV Yangambi ML161 (Deli x Yangambi ML161) were purchased from a Code of Good Nursery Practice for Oil Palm Nurseries (CoPN)-certified nursery premise, Sarjoh Plantation Nursery Sdn. Bhd. (Kuching, Sarawak). The seedlings were carefully removed from the planting medium and washed under running tap water to remove all the soil residues. The roots were rinsed in 0.5 % (v/v) sodium hypochlorite for 10 min twice and subsequently in 70 % (v/v) ethanol for 5 min. The rinsing was repeated twice. Then, the seedlings were left overnight in sterilised ultrapure water (UPW) before a final rinsing using sterilised UPW on the day of the experiment. The roots of each seedling were placed in contact with a seven-day-old *G. boninense* BLSM5B liquid culture in a conical flask at room temperature. The *G. boninense* samples that were in contact with the oil palm roots and a control (*G. boninense* culture without the oil palm roots) were collected at 12-, 24-, and 48-h post inoculation (hpi), respectively. The experiment was repeated twice (designated as Experiment 1 and Experiment 2, respectively; each with three biological replicates with three technical replicates for qPCR).

To study the gene expression of fungal samples upon phytohormone and elicitor treatments, the seven-day-old *G. boninense* BLSM5B was treated with salicylic acid (SA) (Sigma-Aldrich, US), jasmonic acid (JA) (Duchefa Biochemie, Netherlands), or hydrogen peroxide (H_2O_2) (Sigma-Aldrich, US) at a final concentration of 1 mM, as mentioned in Ho et al. [23] for 1 day at 30 °C. Untreated fungal culture was used as control.

2.3. Primer design

Four fungal transcripts encoding metalloproteinase, aspartic protease, polysaccharide deacetylase and lipase were selected from a previous study by Ho et al. [8] with the following criteria: i. presence in the *G. boninense*-treated oil palm root transcriptomes (but absent in untreated oil palm root transcriptome); ii. presence in the whole genome sequence of *G. boninense*; iii. encode for secreted proteins, and iv. have homologs involved in plant pathogenesis. Gene-specific primers were designed to amplify the coding sequence of four partial transcripts that were present in *G. boninense*-treated oil palm root transcriptome [8]. The primer sequences were examined for specificity using Primer3 version 0.4.0 [36]. Primers for qPCR were designed using PrimerQuest™ Tool [37]. The details of the primers are included in Supplementary Data: Table S1.

2.4. Total RNA extraction and cDNA cloning

Total RNA was extracted from *G. boninense* mycelia using a protocol adapted from Wang et al. [38] with minor modifications, followed by DNase I treatment (NEB, UK). The first-strand cDNA was synthesised using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA) according to the manufacturer's protocol. PCR amplification was conducted using KAPA HiFi Kit (KAPA Biosystems, US). The thermal cycling program was 30 cycles of 98 °C for 20 s, respective annealing temperature (Supplementary Data: Table S1) for 15 s, and 72 °C for 90 s. The amplified PCR products were analysed by 1 % (w/v) agarose gel electrophoresis and gel-purified using QIAquick Gel Extraction Kit (QIAGEN, Germany). The purified PCR products were adenylated, ligated to T&A cloning vector (Yeastern Biotech, Taiwan), and transformed into *Escherichia coli* One Shot TOP10/P3 (Invitrogen, US). The putative transformants were confirmed by colony PCR and restriction enzyme analysis (*Hind*III, from NEB, UK) before Sanger sequencing.

2.5. Sequence analysis

The identity of the sequences was verified using BLASTx [39]. The translated amino acid sequences were analysed using SignalP Version 5.0 [40], WoLF PSORT [41], and DeepTMHMM [42]. The top five sequence homologs were aligned to the transcript using Clustal Omega [43], and the motifs present in the sequence were annotated according to the Conserved Domain Database at default setting [44]. Phylogenetic analysis was conducted using Geneious Prime® version 2025.1.2 using the Jukes-Cantor genetic distance model, neighbour-joining method, with Ascomycota as an outgroup with 1000 bootstrap resampling, and a threshold at 70 % [45].

Table 1
Sequence details of the isolated candidates.

Unigene	Number of nucleotides	Conserved domains	Homologs in *G. sinense*		
			E-value	Identity	Accession
U14090 Metalloproteinase	2481 bp	pfam07504: fungalysin/thermolysin propeptide (FTP), pfam02128: fungalysin metallopeptidase (M36), cd09596: peptidase M36 family	0.0	94 %	PIL32564.1
U42611 Aspartic protease	1251 bp	cd05471: pepsin-like aspartic protease, pfam00026: eukaryotic aspartyl protease, PTZ00013: plasmepsin 4 (PM4)	0.0	92 %	PIL25289.1
U56931 Lipase	939 bp	COG3240: phospholipase/lecithinase/hemolysin, cd01846: fatty acyltransferase-like subfamily of the SGNH hydrolases, pfam00657: GDSL-like lipase/acylhydrolase, PRK15381: type III secretion system effector	1e-141	86 %	PIL23397.1
U128397 Polysaccharide deacetylase	1413 bp	pfam01522: polysaccharide deacetylase, COG0726: peptidoglycan/xylan/chitin deacetylase, PgdA/CDA1 family, cd10952: catalytic NodB homology domain	0.0	94 %	PIL29657.1

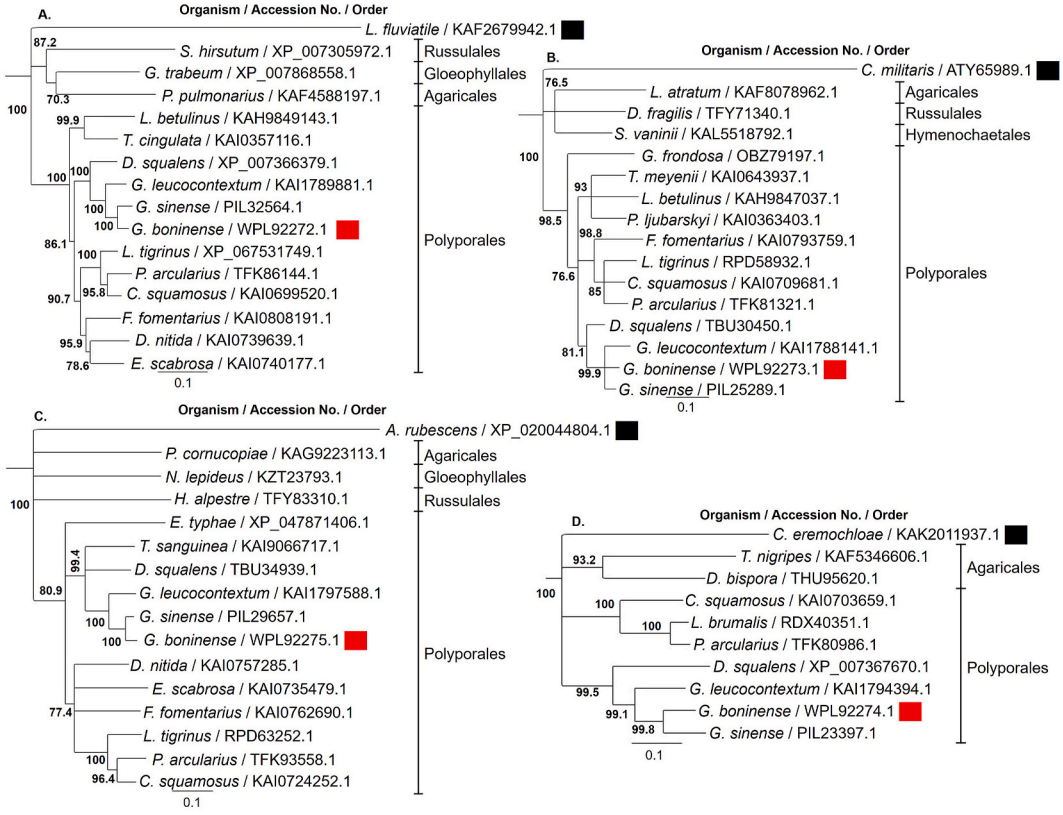

Fig. 1. Phylogenetic tree inferred from amino acid sequence using the neighbour-joining method. A. *U14090*, B. *U42611*, C. *U128397*, and D. *U56931*. The bootstrap support % based on 1000 replicates is indicated next to the node and the scale bar corresponds to 0.1 substitutions per site. The outgroup from Ascomycota division is labelled with ■. The sequence obtained from this study is labelled with ■, otherwise were retrieved from GenBank.

2.6. Gene expression analysis by qPCR

Real-time PCR was conducted using QuantStudio™ 6 Pro Real-Time PCR System (Applied Biosystems, US). The qPCR reaction (10 μL) was prepared in triplicates, each containing 20 ng cDNA, 1X PowerUp™ SYBR™ Green Master Mix (Applied Biosystems, US), 0.5 mM forward primer, 0.5 mM reverse primer (Supplementary Data: Table S1), and RT-PCR Grade Water (Invitrogen, US). A no-template-control (NTC) was also prepared in parallel for each primer pair. The thermal cycling program was set at 50 °C for 2 min, 95 °C for 2 min; 40 cycles of 95 °C for 15 s, 60 °C for 1 min; followed by a melt curve analysis from 60 °C to 95 °C. Data analysis was performed using Design & Analysis Software version 2.4.3 [46]. Reference genes were adapted from Lim et al. [47] with elongation factor 2 (eef) and α-tubulin genes for normalisation based on the $2_T^{-\Delta\Delta C}$ method [48].

For gene expression analysis, the transcript abundance of *G. boninense* harvested at 14-, 21-, and 28-dai was compared to that at 7-dai; while the transcript abundance of *G. boninense* treated with SA, JA, or H_2O_2 was compared to that of untreated *G. boninense*; and the transcript abundance of fungal samples that were grown together with the oil palm (12-, 24-, and 48-hpi) was compared to that of the control (fungal samples that were grown in a flask without oil palm) at respective time-points. T-test was conducted on the Cq values at a p-value < 0.05. The transcript abundance was expressed as RQ (calculated relative gene expression level of treated sample compared to control in fold change). A gene is considered to be up-regulated when RQ ≥ 2; and

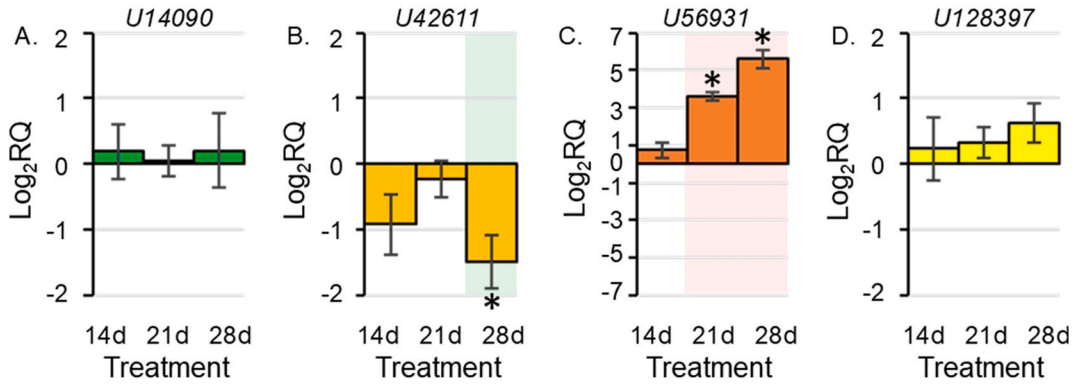

Fig. 2. The normalised transcripts expression for candidate transcripts in fungal samples during vegetative growth measured at different time-points. A. *U14090*; B. *U42611*; C. *U56931*; D. *U128397*. Asterisk (*) represents significant expression ($|\log_2 RQ| \geq 1$, *t*-test with $p < 0.05$). Error bar was derived from standard deviation of three technical replicates.

a gene is considered to be down-regulated when RQ ≤ 0.5, in comparison to the controls [23]. This RQ threshold was only applied if the gene expression differences were tested to be significant by *t*-test at p-value < 0.05.

3. Results and discussion

3.1. Cloning of candidate transcripts

In this study, the coding sequences of four fungal proteins were isolated from *G. boninense* BLSM5B. These candidates have the highest sequence similarity to their homologs from *G. sinense* (Table 1). The deduced amino acid sequence of these candidates was closely related to their fungal homologs from the Polyporales order (Fig. 1). They were predicted to have a signal peptide, localised extracellularly and without transmembrane domains, which are typical for secreted proteins. Secreted proteins are often reported to be involved in plant pathogenesis. Secreted enzymes may overcome the host barrier by degrading the structural component of the host cell wall [49]. Based on the conservation of sequence and signature domain of the candidates (Table 1), they may play the same role as their homologs (Supplementary Data: Fig. S1–S4). However, gene silencing experiments are necessary to verify this in the future.

3.2. Gene expression of fungal samples harvested at different time-points

The transcript abundance was measured at 14-, 21-, and 28-dai in relative to that of the actively growing *G. boninense* BLSM5B fungal mycelia at 7-dai, to understand if the selected genes were differentially expressed at different growth stages or involved in regulating fungal growth. According to the growth curve reported earlier [50], *G. boninense* was at its actively growing stages at 7-dai and 14-dai, and at its declining growth stages at 21-dai and 28-dai. Santiago et al. [51] also observed a lower metabolism of *G. boninense* when the cell growth decreased at 21-dai onwards.

The gene expression of *U42611* (which encodes an aspartic protease) was down-regulated at 28-dai (0.36-fold), whereas the gene expression of *U56931* (which encodes a lipase) was significantly up-regulated at 21-dai (12.1-fold) and 28-dai (48.4-fold), compared to that of the *G. boninense* harvested at 7-dai (Fig. 2). Aspartic protease was important for conidial production and mycelial proliferation in *F. proliferatum* [52], whereas lipase was responsible for fungal growth and production of aerial hyphae in *F. graminearum* [53]. The down-regulation of secreted *U42611* in *G. boninense* culture at 28-dai could be due to suppression of fungal biofilm and hyphal formation [54] under nutrient starvation which may happen during prolonged incubation. The up-regulation of *U56931* in *G. boninense* culture at 21- and 28-dai may be required by the fungus for nutrient assimilation when the nutrients depleted during prolonged incubation. Fungus could use its storage lipids as an energy source by degrading aged mycelial cells during starvation [55].

According to Nawawi and Ho [50], the pH of the culture medium turned acidic (pH 3.8) during active fungal growth (18-dai) and increased to 7.3 when the mycelial growth decreased at 27-dai. A similar pattern of pH changes was observed in our experiment, where the pH decreased from 4.5 to 3.8 at 7-dai and further declined to 3.5 at 14-dai, before it increased to 6.9 at 28-dai (Supplementary Data: Fig. S5). The pH fluctuation during active fungal growth could be attributed to a higher nutrient consumption rate leading to higher organic acid production, which lowered the pH of the medium at 7- and 14-dai [56]. A significantly higher gene expression of aspartic protease was previously reported in *Paracoccidioides brasiliensis* under acidic conditions [57]. Despite that the *U42611* was not being up-regulated at 7- and 14-dai when the pH of the culture medium was acidic as reported in Silva et al. [57], we observed the down-regulation of *U42611* at 28-dai when the pH increased. Following an active growth stage, the pH of the growth medium may increase due to nutrient depletion at 21- and 28-dai. Since the recombinant GDSL-like lipase from *Pleurotus sapidus* (a white rot fungus) was found to function optimally at pH 8 [58], it is not unusual that we found the up-regulation of *U56931* at 21-dai and 28-dai when the pH turned neutral (pH 6.9). In summary, *U56931* and *U42611* may play a role in fungal survival under an unfavourable environment when the nutrients in the culture medium depleted and the pH became neutral during prolonged incubation [50].

3.3. Gene expression in fungal samples in contact with host plants

To examine whether the gene expression of these four candidates was affected by plant-pathogen interaction, their transcript abundance was analysed in fungal samples in contact with oil palm roots at 12-, 24-, and 48-hpi in comparison to that in fungal samples in the absence of a host. In a previous study, Nusaibah et al. [59] reported that oil palm cell wall degradation could happen as early as 24-hpi of *G. boninense* inoculation. Besides, the fungal basidiospores were reported to adhere to cut oil palm roots at 24-hpi, followed by the penetration and degradation of root epidermis by *G. boninense* at 72–96-hpi [60]. However, the fungal proteins expressed during different stages of oil palm-*G. boninense* interaction remain unknown. In other plant-pathosystem, *Phytophthora infestans* was recognised by the tomato host at 24-hpi [61], whereas the rice blast fungus *Magnaporthe oryzae* was found to invade the host tissue at 24–38-hpi [62]. Therefore, we postulated that *G. boninense* may start to secrete extracellular enzymes as early as 24-hpi upon exposure to the oil palm hence the gene expression analysis was conducted on mycelial samples in contact with oil palm roots at 12-, 24-, and 48-hpi.

The gene expression patterns obtained from Experiment 1 were

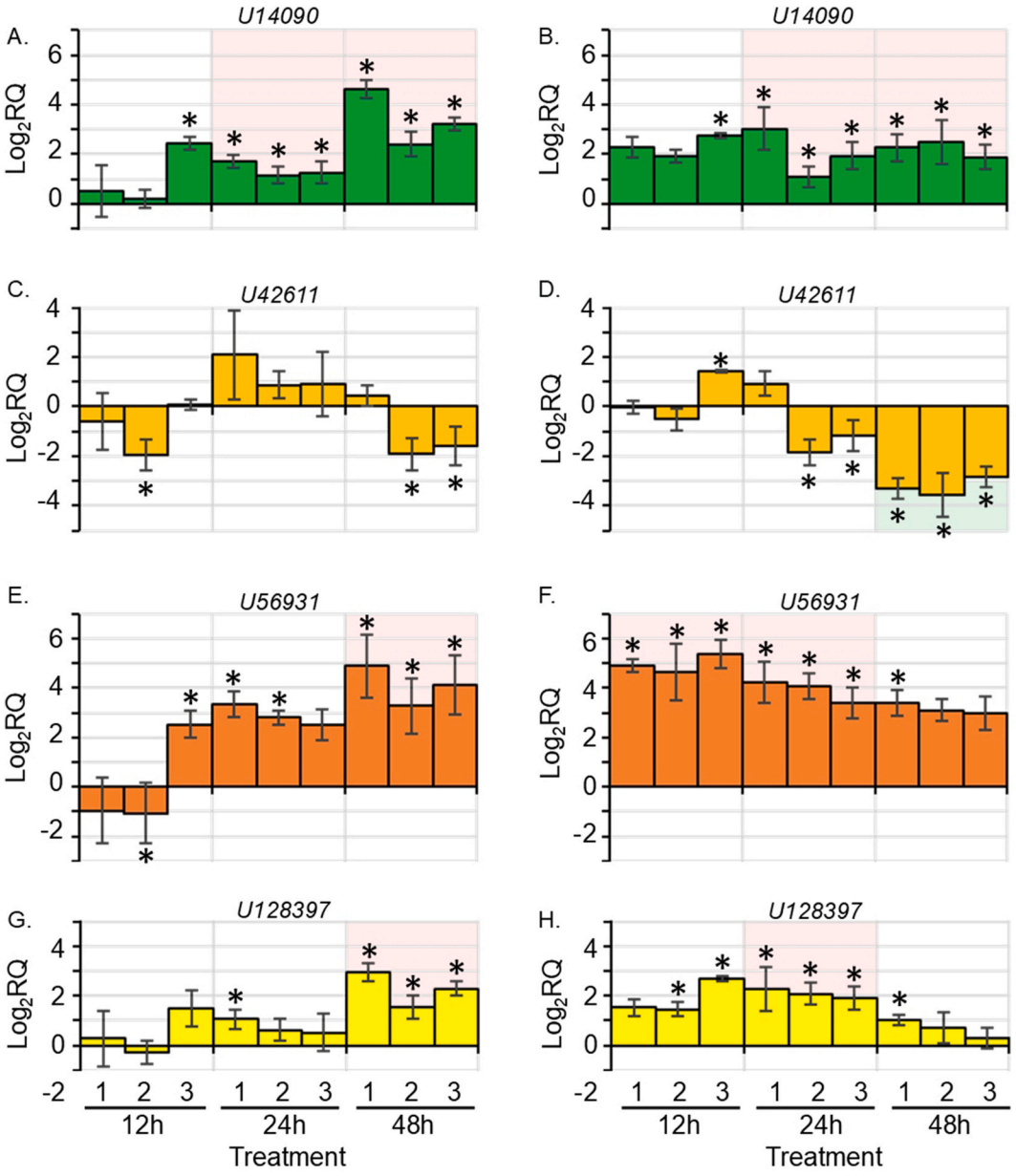

Fig. 3. The normalised transcripts expression for candidate transcripts in fungal samples in contact with host plants. A–B: *U14090*; C–D: *U42611*; E–F: *U56931*; G–H: *U128397*. A,C,E,G: Experiment 1, and B,D,F,H: Experiment 2. 1–3: biological replicates. Asterisk (*) represents significant expression ($|\log_2 RQ| \geq 1$, *t*-test with $p < 0.05$). Error bar was derived from standard deviation of the three technical replicates from each biological replicate.

slightly different compared to that in Experiment 2, possibly due to (i) slight differences of temperature and air humidity in the culture environment, and (ii) different levels of resistance or susceptibility of individual oil palm seedlings towards *G. boninense*. The oil palm seedlings used in these two experiments may have different genotypes hence different levels of fungal tolerance towards the pathogen. Due to that, the expression of the candidate fungal genes may also vary in *G. boninense* samples that were in contact with host plants that have different degrees of fungal tolerance. The consistency of experiments could be improved if a controlled growth chamber and oil palm clones having identical genotypes were used. Herein, we only discussed significant gene expression changes that were consistent in all biological replicates from the two experiments.

The expression of *U14090* (which encodes a putative metalloproteinase) was consistently up-regulated in all three biological replicates of *G. boninense* in contact with oil palm compared to the *G. boninense* without a host at 24-hpi and 48-hpi for both Experiment 1 and Experiment 2. In Experiment 1, *U14090* was up-regulated 2.23–3.26-fold and 5.32–24.7-fold, at 24-hpi and 48-hpi, respectively (Fig. 3A). In Experiment 2, *U14090* was up-regulated 2.14–8.21-fold and 3.71–5.65-fold, at 24-hpi and 48-hpi, respectively (Fig. 3B). Fungal metalloproteinase was reported to be responsible for host colonisation and disease progression [63]. The production of metalloproteinase which is a putative hydrolytic enzyme may be necessary for a pathogen to degrade the host cell wall proteins during host colonisation and nutrient uptake [64]. Hydrolytic enzymes or their products may diffuse through the plant cell wall to the plasma membrane before the establishment of direct contact [65]. M36 metalloproteinase was reported to breach plant defence through the degradation of host chitinase, hence protecting the fungal cell wall from chitinase hydrolysis and prevented the release of chitin oligomers (elicitors) from the fungal cell wall or chitin-triggered immunity in the host [27]. Recently, fungalysin (also known as M36 metalloproteinase) which could induce a hypersensitive response in tobacco leaves was found in a 14-day-old culture filtrate of

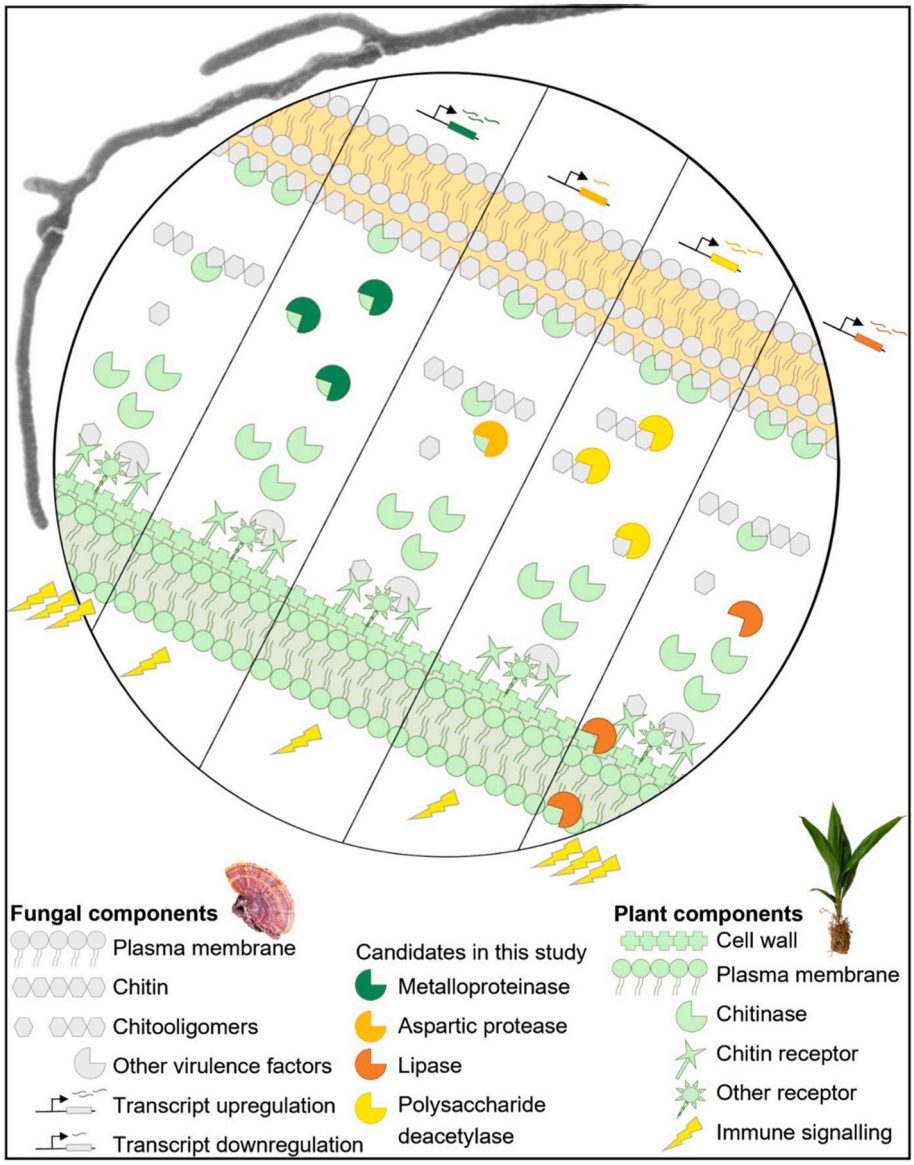

Fig. 4. Proposed roles of candidate genes in oil palm-*G. boninense* interaction. *G. boninense* may secrete the candidates extracellularly to overcome host defence. *U14090*, *U42611*, and *U128397* may interfere the chitin-triggered immunity in oil palm, whereas *U56931* may involve in host cell adhesion, penetration, and degradation.

G. boninense [26]. Several studies have confirmed the presence of chitinase and its encoding genes in the oil palm host during *G. boninense* infection [66,67]. Metalloproteinase with chitinase-degrading activity may assist *G. boninense* to avoid recognition by oil palm during the early interaction stage. However, the presence of chitinase-degrading activity in *U14090* may need further investigation.

The transcript abundance of *U128397* increased > 2-fold at 48-hpi in Experiment 1 (Fig. 3G) and at 24-hpi in Experiment 2 (Fig. 3H). The *U128397* encodes a polysaccharide deacetylase which may have chitin deacetylase activity. Chitin deacetylase causes the hydrolysis and conversion of chitin oligomers (elicitors) into chitosan, which may not be recognised by the pattern recognition receptors in plant host, hence preventing reactive oxygen species generation and chitin-triggered immunity by the host plant [68]. In addition, chitin deacetylase was also reported to be involved in the formation of fungal fruiting body and in fungal invasion [69,70]. The gene expression of *U42611* was down-regulated 0.08–0.14-fold at 48-hpi in Experiment 2 (Fig. 3D). The *U42611* which encodes an aspartic protease may degrade host proteins. The aspartic protease from grape was found to be able to degrade chitinase [28]. By degrading chitinase which is a host pathogenesis-related protein, *U42611* may help *G. boninense* to escape from the host defence.

The transcript abundance of *U56931* was increased 8.74–29.49-fold at 48-hpi in oil palm-treated *G. boninense* in relative to the untreated *G. boninense* (control) in Experiment 1 (Fig. 3E). However, the up-regulation of *U56931* was detected at earlier time-points in the treated *G. boninense* in relative to control in Experiment 2, i.e., 30.02–41.52-fold and 10.62–18.39-fold at 12-hpi and 24-hpi, respectively (Fig. 3F). The up-regulation of *U56931* which encodes a putative lipase may be necessary during early host exposure. Lipase was reported to be induced by plant host and play a role in plant cell wall adhesion and penetration [31,71]. The importance of GDSL-lipase in the utilisation and modification of host innate immunity-related fatty acid has been reported [32]. The loss of lipase activity often resulted in reduced virulence [72]. The proposed roles of the candidate transcripts during the *G. boninense* pathogenesis are illustrated in Fig. 4.

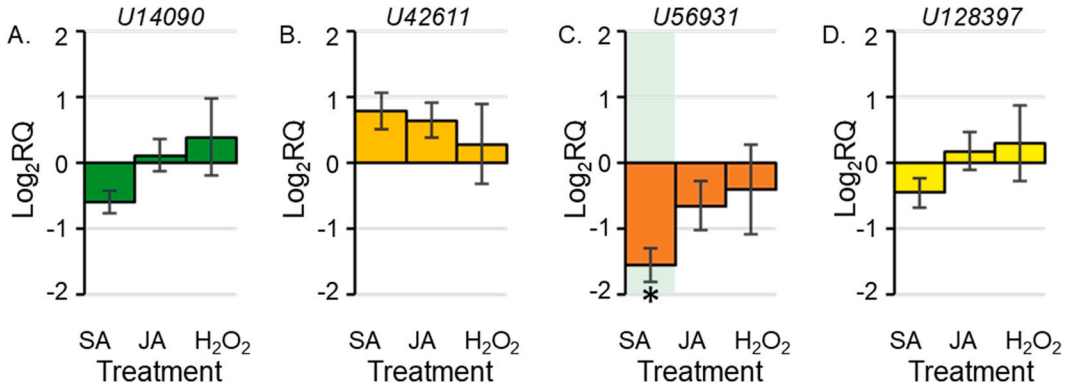

Fig. 5. The normalised transcripts expression for candidate transcripts in fungal samples upon phytohormone and elicitor treatment. A. *U14090*, B. *U42611*, C. *U56931*, D. *U128397*. Asterisk (*) represents significant expression ($|\log_2 RQ| \geq 1$, *t*-test with $p < 0.05$). Error bar was derived from standard deviation of three technical replicates.

3.4. Gene expression upon phytohormone and elicitor treatments

SA, JA, and H_2O_2 are important components in plant immune signalling. They were hypothesised to mediate oil palm defence at different stages of *G. boninense* infection [8]. Previously, SA in the range of 0.36–1.45 mM was shown to be sufficient to inhibit the growth of *G. boninense* (equivalent to 50 ppm, 100 ppm, 150 ppm, and 200 ppm of SA, respectively) with a 50 % effective concentration (EC_{50}) of SA at 50 ppm (0.36 mM) [73]. In addition, Ong et al. [74] found that 1.09 mM of SA (equivalent to 150 ppm) could inhibit aggressive *Ganoderma* strains. On the other hand, 0.95 mM JA was able to inhibit 86 % of the spore germination of rice blast fungus (equivalent to 200 ppm), with an ED_{50} of JA at 0.71 mM (150 ppm) [75]. Several studies demonstrated that 1 mM H_2O_2 could cause partial translational repression in *Cryptococcus neoformans* [76] and induced apoptosis in *Colletotrichum trifolii* [77]. In our current study, the application of 1 mM of SA on *G. boninense* cultures was sufficient to down-regulate *U56931* encoding a putative lipase, which could be necessary for fungal growth.

Under phytohormone/elicitor treatment, only *U56931* which encodes a putative lipase was significantly down-regulated by SA (0.34-fold), as compared to the untreated control. The SA treatment in this study may mimic the oil palm host defence action against the *G. boninense*. The role of SA in mediating early defence response in asymptomatic oil palms infected by *G. boninense* was reported [78]. The SA, JA, and H_2O_2 treatments did not cause significant changes in the expression of other candidate genes (Fig. 5).

The putative functions and roles of candidates in this study are based on our gene expression analysis and inferred from available information of their homologs that are involved in other pathosystems. The actual roles of these candidates in oil palm-*G. boninense* pathosystem awaits further validation. In addition, the candidate genes can be silenced in pathogenic *G. boninense* for pathogenicity test. Gene-knockout using CRISPR/Cas9 has only been demonstrated in *Ganoderma* species recently [79]. RNAi-based silencing of the lanosterol 14α-demethylase encoding gene (*GbERG11*) in *G. boninense* was reported to be able to reduce the ergosterol content and pathogenicity of the pathogen [25]. The same technique can be implemented for functional analysis of our candidate genes in *G. boninense*.

4. Conclusions

In this study, we have cloned and verified the complete coding sequence of four candidates from *G. boninense* by sequencing. This presents the first cloning of the transcripts for metalloproteinase, aspartic protease, polysaccharide deacetylase, and lipase from the *Ganoderma* species. These sequences are necessary for future work, such as gene silencing and recombinant protein expression which requires accurate gene and transcript sequences. The differential gene expression of *U56931* (encoding a putative lipase) and *U42611* (encoding a putative aspartic protease) in 28-day culture suggested that these genes may involve in nutrient acquisition and response towards environmental stress in *G. boninense*. The *U14090* (encoding a putative metalloproteinase), *U56931*, and *U128397* (encoding a putative polysaccharide deacetylase) were up-regulated in fungal culture in contact with oil palm suggesting their roles in host-pathogen interactions, possibly during early host recognition. These candidates may help the pathogen to evade host defence surveillance through the suppression of chitin-induced defence in oil palm. The suppression of *U56931* by SA suggests its regulation by this phytohormone in the host plant. The roles of these four candidate genes in oil palm-*G. boninense* pathosystem can be confirmed by the virulence test of recombinant proteins on the host plants, RNAi-based silencing or gene-knockout using CRISPR/Cas9.

CRediT authorship contribution statement

Mui Sie Jee: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis. **Chai-Ling Ho:** Writing – review & editing, Writing – original draft, Supervision, Formal analysis, Conceptualization. **Mohd Termizi Yusof:** Writing – review & editing, Supervision. **Sharon Yu Ling Lau:** Writing – review & editing, Supervision, Funding acquisition. **Frazer Midot:** Writing – review & editing. **Mei Lieng Lo:** Writing – review & editing. **Mei-Yee Chin:** Writing – review & editing. **Lulie Melling:** Writing – review & editing, Supervision, Funding acquisition.

Data availability

All data generated or analysed during this study are included in this published article. Transcript sequence information is deposited in GenBank with the following accession numbers: ON922904 (*U14090*), ON922905 (*U42611*), ON922906 (*U56931*), and ON922907 (*U128397*).

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Declaration of competing interest

None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pmpp.2025.102715.

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