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TRANSCRIPTIONAL REGULATORY MECHANISM OF EARLY DEFENSE RESPONSE OF OIL PALM AGAINST *GANODERMA BONINENSE*

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Abstract: Basal stem rot (BSR) caused by *Ganoderma boninense,* a white-rot fungus is one of the major threats in oil palm industry that accounted for huge economic losses. Furthermore, the defense mechanism of oil palm against *G. boninense* infection is poorly understood. Elucidation of the molecular changes in defense response in oil palm at early stages of the fungal colonization where symptoms have not yet appeared will promise further insights for prevention measures. Whole transcriptome RNA sequencing (RNA-Seq) was performed on *G. boninense*-infected oil palm seedlings at 3, 7, and 11 days post inoculation (d.p.i.). Analysis on differentially expressed genes (DEGs) revealed strengthening of the plant cell wall as well as degradation of fungal cell wall through chitinase activity. Flavonoids were suggested to be synthesized at later stage (7 and 11 d.p.i). Besides, 1-aminocyclopropane-1-carboxylate synthase (involves in ethylene biosynthesis) and MYC2 transcription factor genes were observed significantly induced at from 3 – 11 d.p.i. Both of the genes presumably work in concert at early interactions between oil palm and *G. boninense*. This study further clarifies the roles of defense response genes in oil palm at early stages of interaction with *G. boninense*.

Keywords: *Elaeis guineensis, Ganoderma boninense*, necrotrophic, phytohormones, transcription factors.

INTRODUCTION

G. boninense is the most prevalent fungal species associated with oil palm BSR cases in Malaysia and Indonesia (Ommelna et al., 2012; Razi et al., 2010). The total losses due to BSR was estimated as 500 million USD per year (Ommelna et al., 2012; Arif et al., 2011). BSR will decompose the oil palm roots and subsequently impair the uptake of water and nutrient of the plant. These will bring about frond wilting and unopen spear leaf, and ended with stand collapse (Chung, 2011). Regrettably, these symptoms do not appear during early infection of *G. boninense* on oil palm unless the BSR has progressed up to 60-70% (Chong et al., 2017). Pattern recognition receptors, pathogenesis-related proteins, and phytohormones are important plant antifungal agents in defense against fungal attacks for their survival. Pathogenesis-related 1 (PR-1) protein is recognised as ubiquitous defense mediator in plants towards offending factors by the regulation of jasmonate and partially salicylate, and regularly being utilised as marker for systemic acquired resistant (Buxdorf et al., 2013; van Loon & van Strien, 1999). Necrotrophic pathogen, *Verticillium dahlia* was reported to interact with *Arabidopsis thaliana* at 12 hrs after infection (Tischner et al., 2010). In addition, a study conducted by our group reported on the production of various kinds of secondary metabolites including pyridine, benzo[H]quinoline, fucosterol, and α- and β-tocopherol in oil palm

infected by *G. boninense* within 6 to 168 hrs (Nusaibah et al., 2016). However, the earlier reports on gene expression analysis of defense mechanism in plants against necrotroph attacks are limited to a small number of transcripts and unable to depict the full story of defense responses. RNA-seq is becoming a useful tool in comprehensive gene expression analysis. Analysis on sequenced transcripts of *G. boninense*-infected oil palm demonstrated significant differential expression of phytohormone biosynthetic genes, PR proteins and transcription factors at three weeks after infection which explained the complex regulatory systems in oil palm defense (Ho et al., 2016). However, it is ambiguous whether the differential gene expression occurred during early plant-pathogen interaction. Hence, our project aims to elucidate the differential gene expression in oil palm at early stages of interactions with the necrotroph, *G. boninense*.

METHODS

Four month-old oil palm (*Elaeis guineensis* Jacq. Dura x Pisifera) seedlings were adopted as test plants. Sterile rubber wood blocks (RWBs) that are6 x 6 x 6 cm were covered with malt extract agar (MEA) and inoculated with *G. boninense* inoculum for one month. The artificial infection method of *G. boninense* on the oil palm seedlings was carried out according to Nusaibah et al.(2016). Treated plants were harvested at 3, 7, and 11 d. p. i, while control plants were harvested at time 0. The experiment consists of two biological replicates for control and each time point where six plants were pooled for each replicate. Total RNA of samples were isolated according to Prescott and Martin (1986) with a few modifications. Total yield and RNA Integrity Number (RIN) of each RNA library were determined prior to sequencing with Illumina HiSeq 2000 system. The quality of sequenced paired-end raw reads were confirmed with FastQC software. The raw reads were aligned to *Elaeis guineensis* (African Oil Palm) reference genome using Geneious software v9.1.5 to generate transcripts. The software were also used to calculate gene abundance in each sample and compare between treated and control samples to determine the differential gene expression. Differentially expressed genes (DEG) were determined according to [Number of reads count of treated oil palm seedlings/ Number of reads count of untreated oil palm seedlings] ≥ |1.0| (corresponding to 2-fold or more upregulation/downregulation) with P-value ≤ 0.01. Annotation was applied to the DEGs to find the functional characteristics of each genes using Blast2GO software. Subsequently, Gene Set Enrichment Analysis (GSEA) were performed to define the enriched pathways governed by the DEGs.

RESULTS AND DISCUSSION

The sequenced RNA library produced 113 million and 313 million of paired-end reads from control and treated samples, respectively. The mean of quality score of each bases of the 101 bp reads waswithin the high quality range (28-39) where the threshhold value is 20. Based on determination of DEGs, 15,380 genes were downregulated while 9,215 genes were upregulated.

The annotation procedure successfully retrieved Gene Ontology (GO) terms of each DEG and they were clustered into biological process, molecular function and cellular component. During early interactions between *E. guineensis* and *G. boninense*, among the most upregulated genes were related to transport, organonitrogen compound metabolic process, oxidation-reduction process, cellular macromolecule biosynthetic process and protein phosphorylation. Whereas, the highest downregulated genes were related to stimulus, single-organism biosynthetic process and oxidation-reduction process.

Based on enrichment analysis, GO terms including chitinase activity, chitin binding, amino sugar catabolic process, and glucosamine-containing compound metabolic/catabolic process were the most enriched pathways in upregulated genes at 3 d.p.i. However, the expression of these genes were subsequently suppressed as these GO terms were enriched in downregulated genes at 7 and 11 d.p.i. Flavonoid biosynthetic process was enriched in upregulated genes at 7 and 11 d.p.i. On the other hand, aminoglycan catabolic process, inorganic anion transport, and modification-dependent protein catabolic process were the most enriched pathways in downregulated genes at 7 and 11 d.p.i. Isomerase activity, proteolysis involved in cellular protein catabolic process, and cellular macromolecule catabolic process were the most enriched pathways in downregulated genes at all time points.

Enriched GO terms in upregulated genes from 3 d.p.i. to 7 d.p.i suggested that the oil palm presumably strengthened its physical barrier as frontline defense against *G. boninense* through cell wall biogenesis, as well as attack against fungal colonization via chitinase activity. Fabrication of phytoalexins were suggested to be initiated at later stages as evidenced by enriched flavonoid biosynthetic process at 7 and 11 d.p.i. The enriched pathways in downregulated genes at all time points speculated that primary metabolism is utilized to hold up the cellular energy during plant-pathogen interactions (Kangasjarvi et al., 2012). It turns to be a fitness cost where the metabolic pathway genes in plants is downregulated to pay for the upregulation of defense response genes (Kempel et al., 2011; Meldau et al., 2012).

1-Aminocyclopropane-1-carboxylate synthase (*ACS*) and *MYC2* transcription factor (*MYC2*) were observed to be induced in *G. boninense*-infected oil palm (Figure 1). Both of these genes were significantly induced as early as 72 hrs after treatment and maintained at high level of expression until 11 d.p.i . *ACS* is the first committed step and rate-limiting enzyme in ethylene biosynthesis, while *MYC2* transcription factor is a transcriptional activator activated by the actions of jasmonate (JA) signaling. Recently, ethylene-stabilized ETHYLENE INSENSITIVE3 (EIN3) and JA-activated MYC2 were shown to interact with each other in *Arabidopsis thaliana* and is crucial in modulating antagonistic mechanism between ET and JA in defense response such as towards necrotroph attacks (Song et al., 2014).
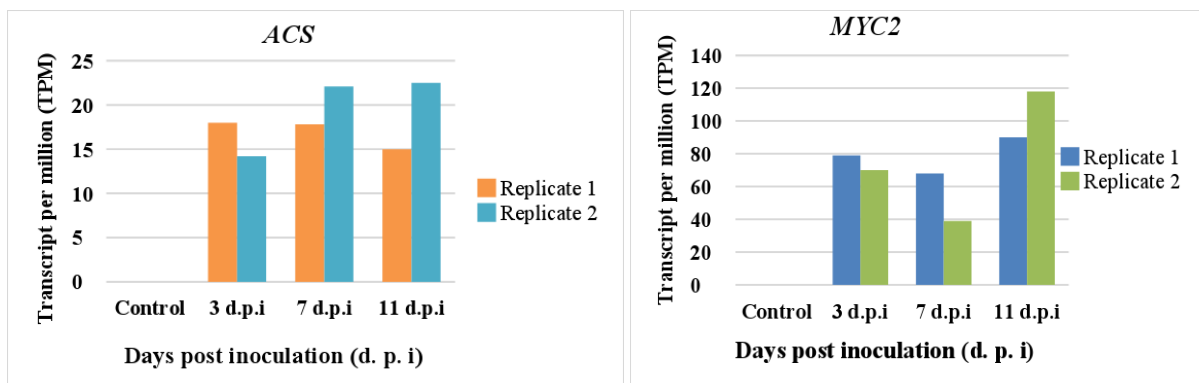

Figure 1: Differentially expressed genes (DEGs) analysis of 1-aminocyclopropane-1-carboxylate synthase (*ACS*) and MYC2 transcription factor (*MYC2*) in oil palm seedlings treated with *Ganoderma boninense*.

CONCLUSIONS

In this study, artificial infection of oil palm seedlings with *G. boninense* demonstrated defense related gene expressions in the oil palm. Significantly expressed genes were observed as early as 72 hrs post infections. Phytohormone related genes were suggested to play crucial roles in defense response against fungal attacks. Nonetheless, the sequencing data needs further confirmations. Besides, the interactions between *ACS* and *MYC2* in plant defense responses towards necrotroph attacks have to be elucidated in great details for further clarification of their mechanism of actions.

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