DIFFERENTIAL GENE EXPRESSION IN OIL PALM VARIETIES SUSCEPTIBLE AND TOLERANT TO *GANODERMA*

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Abstract

Basal stem root (BSR) due to *Ganoderma* infection is the most important disease in oil palm plantations. Even though, various attempts have been made to overcome the disease, significant results are yet to be seen. Economic losses continue to increase annually during the production phase of oil palm. In molecular aspect, differential expressed genes leading to tolerance of *Ganoderma* have been extensively studied. Most of the works were carried out using artificial infection although *Ganoderma* -tolerant lines have been reported previously. Thus, it can possibly lead to misinterpretation of oil palm tolerance towards *Ganoderma* . Therefore, the objective of this study was to determine a better overview of differentially expressed genes in *Ganoderma* susceptible and tolerant plants. Transcript sequences of tested genes have been collected from open access database and manually annotated. Twenty one pairs of specific primers encoding twelve genes related to response to *Ganoderma* have been designed. *In silico* analysis has predicted the isoform of each gene. The PCR amplification resulted in fragments ranged from 181 to 220bp. The expression of these selected genes was compared between DxP MT Gano (moderate tolerant) and DxPYangambi (susceptible) oil palm varieties, respectively, by RT-qPCR. Sixteen out of 21 genes were differentially regulated between DxP MT Gano and DxPYangambi. Six upregulated genes (*EgCHI1, EgVIR-1, EgVIR-2, EgIFR-2, EgMT-1,* and *EgSPI-2*) in root can be considered as potential positive biomarkers for moderate tolerant oil palm varieties. Our results suggest distinct molecular regulation between moderate tolerant and susceptible oil palm varieties to *Ganoderma* .

Keywords: *oil palm, Ganoderma, differential gene expression, Quantitative RT-PCR, tissue*

1. Introduction

Basal stem root (BSR) caused by the white rot fungi *Ganoderma* sp. is the most important disease in oil palm plantations. This disease causes significant lost up to 80% of oil palm production, including in South-East Asia region, over repeated planting cycles [1]. Althoughearly *Ganoderma* out break spread at 1% of the plantation area, potential loss could reach USD 256 million per year [2].Currently, economic losses continue to increase annually during the production phase of oil palm due to the extent of *Ganoderma* infections not only in mature but also in juvenile oil palm trees. The colonization of *Ganoderma* sp.in oil palm roots is the key to devastating characteristic of white rot disease [3, 4]. White rot fungi produces hydrolytic and oxidative enzymes degrading tissues in basal stems[5, 6].Various attempts have been made to overcome this disease, but until now a real impact declining *Ganoderma* attack is yet to be seen.Thus, early detection or preventive measures are an important step to be taken to minimize the occurrence of *Ganoderma* infection. Molecular response of oil palm in relation to *Ganoderma* is essential for the development of diagnostic tools for disease control [7].

Gene expression analysis has been widelyused to explore biological processes related to plantdevelopmental and environmental cues. Theaccumulation of messenger RNA (mRNA) ismeasured using a rapid, sensitive and reliablemethod. Currently, a fluorescence based-PCR suchas Reverse Transcriptase quantitative PCR (RT-qPCR) is one of powerful techniques to detectlow abundance of mRNA in the cell[8,

9].Differential expression studies of gene transcripts leading to tolerance of *Ganoderma* have been extensively studiedin oil palm [10-15]. Several potential biomarkers toward tolerance to *Ganoderma* including those encoding chitinase class II, DEFICIENS 1, early methionine-labeled polypeptide 1, isoflavonereductase, metallothionein-like protein, and pathogenesis-related protein have been identified [10-16]. Nevertheless, most of the works were carried out using artificial *Ganoderma* infection, although *Ganoderma* -tolerant lines have been reported previously [17,18]. Even though the results are accurate, it can still possibly lead to misinterpretation of oil palm tolerance towards *Ganoderma* .

Therefore, the objective of this study was to determine a better overview of differentially expressed genes in *Ganoderma* susceptible and tolerant oil palm varieties. Gene expression profiles of target genes potentially related to *Ganoderma* tolerance in root and leaf of susceptible and moderate tolerant oil palm varieties were analyzed using Reverse Transcriptase Quantitative PCR (RT-qPCR). Twenty one pairs of specific primers encoding twelve genes related to response to *Ganoderma* have been designed. *In silico* analysis was coupled with primer design to ensure the specificity of each pair of oligos. Several potential biomarkers for moderate tolerant oil palm varieties to *Ganoderma* were obtained. Taken together, the results provide a better understanding about molecular regulation of *Ganoderma* -response genes in moderate tolerant and susceptible oil palm varieties.

2. Methods

Plant material

Leaf and root tissues were collected from 4-year-old mature oil palm trees of DxPSocfindoYangambi (susceptible) and DxPSocfindo MT Gano (moderate tolerant) varieties to *Ganoderma* at the Socfindo Seed Production and Laboratories (SSPL), Kebun Bangun Bandar, Desa Martebing, Kecamatan Dolok Masihul, Kabupaten Serdang Bedagai, North Sumatera, Indonesia (Latitude 3.32796; Longitude 99.04026). Both varieties were cultivated following standard operation procedure (SOP) of PT Socfindo.

Total RNA isolation

The method used for RNA extraction from leaf and root tissues is a modification of a protocol described earlier for pine trees [19]. One gram of tissue, mixed with 1.5% polyvinylpyrrolidone was ground by hand using a pestle and mortar in the presence of liquid nitrogen. The powder was transferred to a 50 ml tube, resuspended in 15 ml of preheated (65ºC) extraction buffer (100 mMTris-HCl pH 8.2, 1.4 M NaCl, 20 mM EDTA, 2% (w/v) CTAB, and 75 µl of β-mercaptoethanol) and homogenized in a warring blender at maximum speed for two minutes. Next, the homogenate was incubated at 65°C for 1h, while mixing by gentle vortexing every 15 min. The tubes were kept at room temperature for 5 min and then an equal volume of chloroform: isoamyl alcohol (24:1) was added. The mixture was shaken vigorously until an emulsion was formed, and then centrifuged at 12,000 g for 15 min at room temperature. The upper aqueous phase containing nucleic acids and polysaccharides was carefully transferred to a new tube, and extracted subsequently with phenol, phenol: chloroform: isoamyl alcohol (25:24:1), and chloroform: isoamyl alcohol (24:1), each time followed by centrifugation at 12,000 g for 15 min. The aqueous phases were transferred to new tubes and 8 M LiCl was added to a final concentration of 2 M. The RNA was allowed to precipitate overnight at 4°C and then recovered by centrifugation at 17,000 g at 4°C for 30 min. The pellet was dissolved in milliQ grade water and was extracted with phenol, phenol: chloroform: isoamyl alcohol (25:24:1), and chloroform: isoamylalcohol (24:1), respectively each time followed by centrifugation 12,000 g for 15 min at 4ºC. The supernatant was transferred to a new tube and RNA was precipitated with 0.1 volumes of 3 M sodium acetate pH 5.8 and 3 volumes of 100% ethanol at –70°C for 4 hrs to overnight. The RNA was recovered by centrifugation at 17,000 g in a microfuge at 4°C for 30 min. The pellet was washed with an equal volume of 70% cold ethanol, the ethanol was allowed to evaporate at room temperature for 15 min, and the purified RNA pellet was then resuspended in an appropriate volume of nuclease-free water. The purity and concentration of the RNA was determined spectrophotometrically at 230, 260, and 280 nm. The integrity of total RNA was checked by electrophoresis.

Primer design and validation

Twelve sequences (Acc. nb.AF322914, JN203288, GU301271, HQ831445, AY739700, EL695076, EL690340, EL684283, EL690964, EL690627, KJ789862, and KJ427025) from European Nucleotide Archive

(http://www.ebi.ac.uk/ena) were selected as template to primer design. Based on bibliographical analysis, these sequences encode genes related to potential *Ganoderma* tolerance. Primers were designed at the 3' side of each sequence using the Primer 3 module of Geneious (Biomatters Ltd., New Zealand) [20, 21]. *In silico* validation of each pair of oligos was carried out byMEGA-BLASTn short against *Elaeisguineensis*genome data published by Singh et al. [22] using GALAXY platform (http://galaxy.southgreen.fr/galaxy/). Real-time PCR amplification and the fusion curve were carried out using a mix of cDNAs in order to check the specificity of each pair of primers.

cDNA synthesis and Reverse Transcriptase Quantitative PCR (RT-qPCR) setup

A DNase treatment to eliminate gDNA contamination was done for each RNA sample according to the manufacturer's instructions of DNase I kit (Sigma-Aldrich, USA). cDNAs were synthesized from 1µg of total RNA to the final 20 µL reaction mixture using a Bioneer Accu Power Cycle Script RT PreMix according to the manufacturer's instructions (Bioneer, Korea). Full-length cDNA synthesis was checked on each cDNA sample by PCR amplification of the *EgActin*cDNA using primers at the cDNA ends. Reverse Transcriptase quantitative PCR was finally carried out using a real-time PCR StepOne Plus (Applied BioSystem, UK). The RT-qPCRreaction mixtures consisted of 1 µL RT product cDNA, 0.625 µL of 5 µM of each primer, 4 µL NFW and 3 µL 2×SYBR green select master mix (Bio SM, USA) in a 20-µL volume. PCR cycling conditions comprised one denaturation cycle at 95°C for 5 min, followed by 45 amplification cycles (95°C for 20s, 60°C for 15s, and 72°C for 20s).Real-Time PCR was done at 96-well plate.The transcript abundance level for each gene was relatively quantified by normalization with the transcript abundance of endogenous gene encoding oil palm actin *EgActin*as described by Tan et al.[15]. All the normalized ratios corresponding to transcript accumulation were calculated automatically by StepOne Software v2.3 provided by the manufacturer using the following calculation: Normalized Ratio = $2^{-\Delta(\text{Cp target-CpEgActin})}$.

Data analysis

Expression data were transformed into heat map presentation using Microsoft Excel 2010 (Microsoft, USA). No statistical analysis was done. To ensure the validity of the data, a stringent threshold of ratio value was applied. Upregulated genes were shown in bright red for a threshold value ≥5; dark red for value <5, and down-regulated genes were shown in bright green for a threshold value ≤0.2; dark green for value >0.2. The non-significant genes are shown in dark color [20, 21].

3. Results and Discussion

In silico primer design and analysis

Based on twelve template sequences from European Nucleotide Archive, 21 oligopairs of target genes and 1 pair of housekeeping gene were designed (Table 1). Primers were designed at the 3' side of each sequence in order to reduce the risk of error due to short cDNA synthesis often occurred in PCR reaction [20, 21]. The length of each primer was ranged from 19 to 22 bp with expected PCR product length from 181 to 220 bp. All pairs of primers matched 100% with potential targeted genes in Singh's *Elaeisguineensis* genome database. A MEGA BLAST-N short has predicted that these 21 pairs of primers encode potentially 12 known-genes in oil palm. Several of these genes were known to encode proteins induced by *Ganoderma* treatment in oil palm such as L-ascorbate peroxidase, Chitinase 10, MADS-box transcription factor 16 (DEF1), Em protein H2-like, Isoflavonereductase-like protein, Metallothionein-like protein type 2, and Pathogenesis-related protein PR-1 [14, 15].

Transcript variants, also known as transcript isoforms, are differentially expressed transcripts across different tissue/cell types, developmental stages and disease conditions [23]. Different transcript isoforms can lead to different protein isoforms with distinctive functions. Therefore, an *in silico*analysis of gene potential isoform is necessary before confirming a primer design. In this experiment, one potential isoform for each pair of primers was predicted, except for *EgDEF1-1* and *EgDEF1-2*having 4 and 3 potential isoforms, respectively. This result has shown that most of the designed primers were specific or at least encoding one gene (Table 1).

Validation for integrity of cDNAs

The quality and quantity of RNA determines largely the quality and quantity of synthesized cDNA[24, 25]. In this work, total RNA was isolated from each 2 samples of each tissue (root and leaf) and each plant material (DxPYangambi and DxP MTGano), respectively, counting in total 8 samples. The RNA

quantification using Nanoquant showed that its concentration and purity met the requirement for cDNA synthesis (data not shown). A experiment to validate cDNAs used in this work was carried out by observing melting curve of *EgActin* for each used tissue sample for each oil palm variety (DxPYangambi and DxP MT Gano) (Figure 1). The melting temperature for *EgActin* was ranged from 83.78 to 83.92. A single amplification of melt curve showed the specificity of*EgActin* primers and correspondingly the quality of cDNA used. These results confirmed that the cDNAs synthesized in this experiment has reached the requirement for differential gene analysis using RT-qPCR.

Basal expression of tested genes in root and leaf of DxPYangambi (susceptible) and DxP MT Gano (moderate tolerant) oil palm varieties

The transcript accumulation of 21 *Elaeisguineensis* genes potentially involved in tolerance to *Ganoderma* was measured in DxPYangambi (susceptible) and DxP MT Gano (moderate tolerant) oil palm varieties (Figure 2). In this work, fourteen genes were differentially expressed at basal level across two tissues and two oil palm varieties. Nine genes (*EgAD1-2, EgCAPX-1, EgCAPX-2, EgDEF1-1, EgDEF1-2, EgEMLP1-1, EgIFR-2, EgVIR-2,* and *EgWRI1*) had higher transcript accumulation in leaf as opposed to five genes (*EgCHI1, EgEMLP1-2, EgMT-1, EgSPI-1,* and *EgVIR-1*) with higher transcript accumulation in

Table 1. Primer list for tested genes of differential gene expression in this work. Primers were designed using Primer3 tool of Geneious software. All primer sequences were blasted against Singh's genomic database [22]to ensure specificity

Name	Forward (5'-3')	Reverse (5'-3')	MEGA BLAST-N short	Identity%	Potential isoform	Product (bp)
EgAD1-1	ACCTGCGAGTCTCAAAGCCACA	GCATGAAAGCTACGGGCAACCA	Defensin EGAD1 Mrna	100	1	200
EgAD1-2	AAGGACCTGCGAGTCTCAAA	CCACATAACCCTCACCATCC	Defensin EGAD1 Mrna	100	1	185
EgCAPX-1	AGGCCGTCGACAAGTGCAAGAA	AATAGCCTCACGGCGATGTCCA	L-ascorbate peroxidase, cytosolic (LOC105044666)	100	1	202
EgCAPX-2	GCCATCTGACAAGGCTCTTC	CGCCATCGTCTGCTTCTAAC	L-ascorbate peroxidase, cytosolic (LOC105044666)	100	1	208
EgCHI1	TGTGGTCAGGGCTACATTGA	CCATTTATTTGTGGCGTCCT	Chitinase-like protein 1 (LOC105049526)	100	1	220
EgCHI2	AACGGCACCGGATGGTCCTTAT	ACCATCAAATCCCAAGGCCCGT	Chitinase 10 (LOC105035527)	100	1	193
EgDEF1-1	TGTTCTCCAGCACCGGCAAGTT	ATCTTCACCCATCCGCTGCCTT	MADS-box transcription factor 16 (DEF1)	100	4	197
EgDEF1-2	GGCTTCCCACATGTATGCTT	GCGGATAGAGAGGCTTACCA	MADS-box transcription factor 16 (DEF1)	100	3	183
EgEMLP1-1	AGAGCGTTTGGCTGAAGGT	AGAACTGCGCGCTCTAAGAC	Em protein H2-like (LOC105057111), transcript variant X2	100	1	188
EgEMLP1-2	TCAGCACCATGGACGAGTT	AAACAAACCCGTCACCAAAC	Em protein H2-like (LOC105057111), transcript variant X2	100	1	182
EgIFR-1	GATCATCGCCGCAATAAAAG	ATGGGAGGAAGTAACCAGCA	Isoflavonereductase-like protein (LOC105037680)	100	1	203
EgIFR-2	ACCGGGTACATCGGAAAGTT	GGAGATCACCGTGTCCACTT	Isoflavonereductase-like protein (LOC105037680)	100	1	210
EgMT-1	AGGCAAATGTGGCTGTGGCGTT	ACTTGCAGTTGCAGCCTCCGTT	Metallothionein-like protein type 2 (LOC105044410)	100	1	191
EgMT-2	GGGCACTATGAAGGGTTTGA	TTGGATGCTTGGAAGGAGAC	Metallothionein-like protein type 2 (LOC105044410)	100	1	182
EgPR1-1	ACCCAGATCGTCTGGAAGAG	GGCACACCACCAATATGACA	Pathogenesis-related protein PR-1 (LOC105039610)	100	1	207
EgPR1-2	CATTATTCTGGGACCCAAGG	GAGTTGGCCGTGTAGGAGTAGT	Pathogenesis-related protein PR-1 (LOC105039610)	100	1	208
EgSPI-1	ATGGCCTTGCTGCAATAGGTGC	AAATAGCTTCTCGGCGGCGACT	Bowman-Birk type trypsin inhibitor-like (LOC105045610)	100	1	204
EgSPI-2	AAAGAATGCGTTCGATCACC	ACCCTCCTCCAAGAAAGCAT	Bowman-Birk type trypsin inhibitor-like (LOC105045610)	100	1	212
EgVIR-1	ATTTGGAATCGGAGGCTTG	CAAGGTCTCGTTTCCTGTCC	Virescens R2R3-MYB gene	100	1	216
EgVIR-2	TGCCGACTACGGTGGTTGAACT	TTGCCCAGGTGAGTGTTCCAGT	Virescens R2R3-MYB gene	100	1	185
EgWRI1.1	ATGCACCCCTTTCTTCTCCT	TTGCCTGCCTTTCTTGTTCT	Ethylene-responsive WRI1-like (LOC105046121)	100	1	212
EgActin	CCCACCTGAACGGAAATACA	CGGATGGCACCTCAGTCTTA	Actin-101-like (LOC105032827)	100	1	181

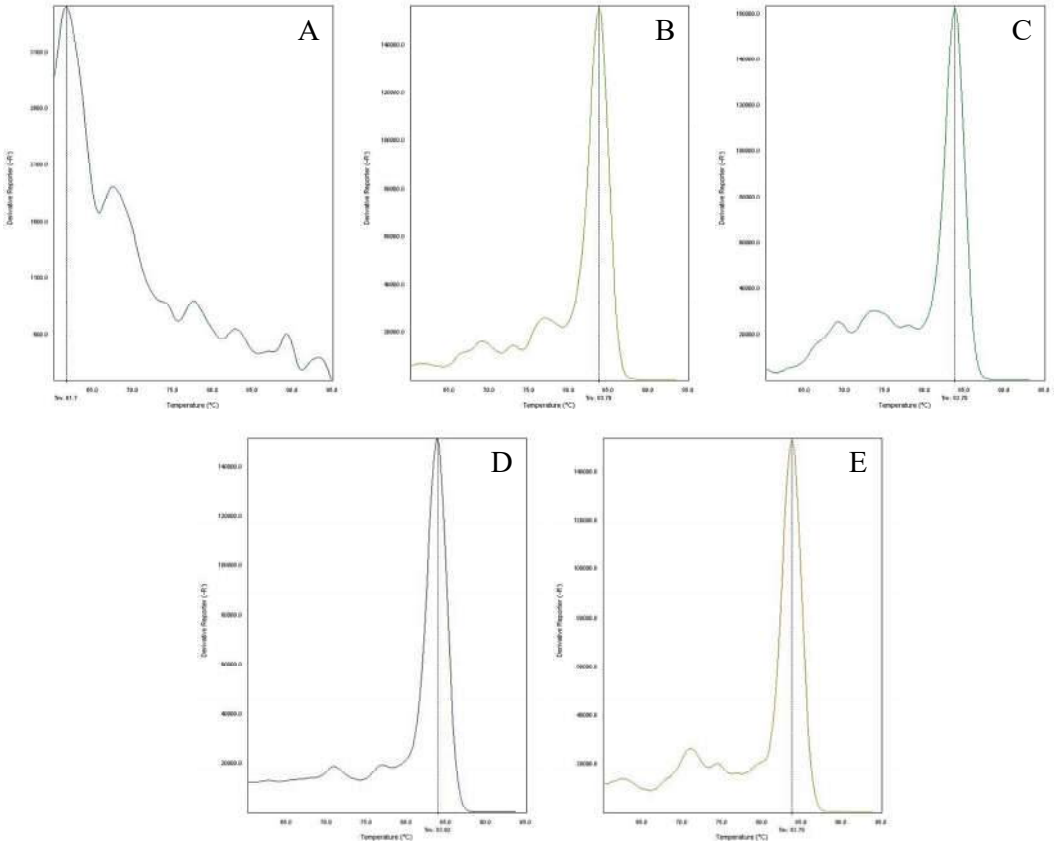

Figure 1.cDNA profile of leaf and root RNA samples from DxPYangambi and DxP MT Gano oil palm varieties. The integrity of the cDNAs was illustrated using *EgActin* amplification in a real-time PCR StepOne Plus. The data shown was the melting curve for each sample. The melt curve value was mean of two biological replicates. (A) Negative control H_2O, (B) Root from DxPYangambi, (C) Root from DxP MT Gano, (D) Leaf from DxPYangambi, (E) Leaf from DxP MT Gano.

root. Alongside with these genes, three genes (*EgCAPX-1, EgDEF1-2*, and *EgEMLP1-2*) had relatively strong basal expression in specific cases. The gene *EgCAPX-1* showed a high transcript accumulation in root and leaf in either oil palm varieties with values ranged from 1.90E-01 to 4.05E+00, respectively. The high basal expression of *EgCAPX-1* was potentially related to the effort of cell homeostasis during oxidative stress [14]. Based on the reference, the gene *EgDEF1* was highly expressed in flower tissue [26, 27]. In this work, the gene *EgDEF1-2* had relatively high transcript accumulation (4.22E-01) in leaf tissue of both oil palm varieties. Meanwhile, the gene *EgEMLP1-2* was the only gene with highest expression value reaching 1.13E+02 in root tissue of DxPYangambi variety. The same trend has been achieved by Tan et al. [15] using DxP GH500 oil palm variety. This result revealed that *EgEMLP1-2* could have an important role in basal regulation of secondary metabolism in root of oil palm.

Regulation of tested genes potentially involved in tolerance to *Ganoderma*

The ratios between the relative abundance of transcripts in the same tissue between susceptible and moderate-tolerant oil palm varieties showed that 16 out of 21 genes were differentially regulated between DxP MT Gano (moderate

tolerant) and DxPYangambi (susceptible) oil palm varieties (Figure 3). In root, six genes (*EgCHI1, EgVIR-1, EgVIR-2, EgIFR-2, EgMT-1*, and*EgSPI-2*) were upregulated as opposed to eight downregulated genes (*EgCAPX-1, EgCHI2, EgDEF1-1, EgIFR-1, EgSPI-1, EgAD1-1, EgEMLP1-2*, and*EgAD1-2*) in DxP MT Gano. The fold change of two upregulated genes (*EgCHI1*and *EgVIR-1*) in root reached more than five times in DxP MT Gano in comparison to DxPYangambi. In leaf, twelve genes (*EgIFR-2, EgMT-1, EgSPI-2, EgEMLP1-1, EgWRI1, EgCAPX-1, EgCHI2, EgDEF1-1, EgIFR-1, EgSPI-1, EgAD1-1,* and*EgEMLP1-2*) were upregulated as opposed to four downregulated genes (*EgCHI1, EgVIR-1, EgVIR-2,* and*EgAD1-2*) in DxP MT Gano. The expression marker genes differed between root and leaf except for three genes (*EgIFR-2, EgMT-1,* and *EgSPI-2*). This suggests that tissue-specific regulation of genes occurred between DxP MT Gano and DxPYangambi.

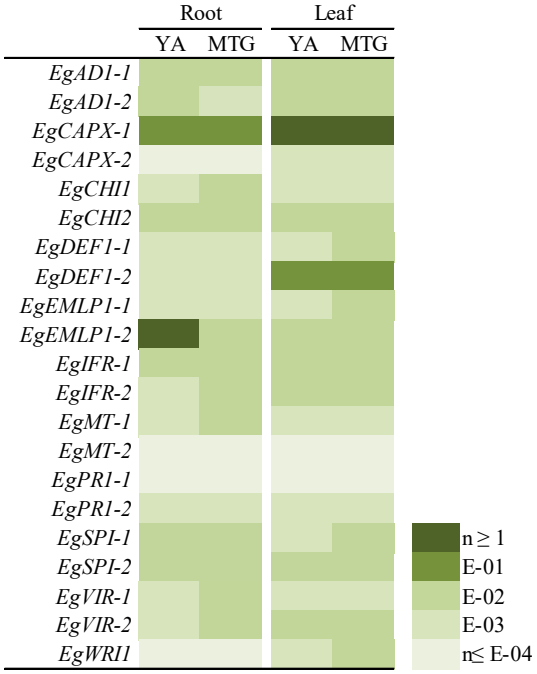

Figure 2. Basal expression of 21 tested genes of *Elaeisguineensis* potentially involved in tolerance to *Ganoderma* . The relative transcript abundance was measured by a real-time PCR StepOne Plus in root and leaf of DxPYangambi (susceptible) and DxP MT Gano (moderate tolerant) oil palm varieties. Values were mean of 2 replicates for each tissue. No statistical analysis was carried out. Values are represented by colors ranging from ≥1 to ≤10−4.

In order to accumulate known information about differential gene expression in oil palm, especially in relation to *Ganoderma* , current work results has been combined with previous studies (Table 2). Even though the table might not be exhaustive regarding that the expression data was generated from *Ganoderma* -treated plants and the inoculation of *Ganoderma* in the moderate tolerant varieties was not carried out in this work, the summarized information itself can be useful as an added value. The twelve target genes encode known function in oil palm. Firstly, in the current work, the target genes *EgCAPX*, *EgCHI2,* and *EgEMLP* have shown contrasting results with known previous studies. These genes were downregulated in comparison to upregulation in root tissue of previous studies (Table 2). For *Chitinase* genes, Naheret al. has demonstrated that the gene expression varied depending on tissue and type of treatment[11]. This statement has been correspondingly confirmed by the inversed result for *EgCHI*genes. A potential diverse regulation of ascorbate peroxidase (APX) and Early methionine-labeled polypeptide 1 (EMLP) between previous studies can be taken into account due to the use of different oil palm varieties in the experiment [14, 15]. Secondly, three target genes (*EgIFR, EgMT,* and *EgSP1*) had analogous results to previous studies. This suggests that the regulation of isoflavonereductase, metallothionein-like protein, and serine protease inhibitor were consistent across various oil palm varieties. Thirdly, *EgPR1* gene showed no expression difference between DxP MT Gano and DxPYangambi. Meanwhile, several authors showed an upregulation of *EgPR1* in root of *Ganoderma* -treated oil palm [13-15]. This result suggests that *EgPR1* might be controlled in relatively similar way in DxP MT Gano and DxPYangambi.

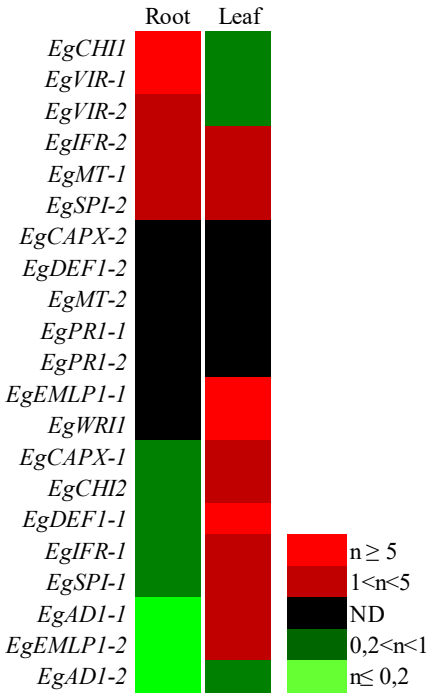

Figure 3. Fold change profile of 21 genes tested genes of *Elaeisguineensis* potentially involved in tolerance to *Ganoderma* . Values are ratio between susceptible and tolerant plants. Values of relative transcript abundances were calculated using StepOne Real-Time PCR software. No statistical analysis was done. Upregulated genes were shown in bright red for a threshold value ≥5; dark red for value <5, and down-regulated genes were shown in bright green for a threshold value ≤0.2; dark green for value >0.2. The non-significant genes are shown in dark color.

In regard to root as an important tissue related to direct contact during the attack of *Ganoderma* , the six upregulated genes genes (*EgCHI1, EgVIR-1, EgVIR-2, EgIFR-2, EgMT-1,* and *EgSPI-2*) can be considered as potential positive biomarkers for moderate tolerant oil palm varieties. The expression of *EgCHI1* has been studied by Naheret al. and Yeohet al. [10-12]. These studies have come up with a conclusion that the upregulation of chitinases was likely a universal response of host plant to fungal attack, including *Ganoderma* . The higher expression of *EgCHI1* in DxP MT Gano compared to DxPYangambi showed that the moderate tolerant oil palm varieties have potential higher tolerance to fungal attack. The function of *EgVIR-1* and *EgVIR-2* genes in oil palm fruit exocarp pigmentation has been demonstrated by Singh et al. [26]. The upregulation of *EgVIR-1* inDxP MT Gano suggests that the ripening of fruit bunch in moderate tolerant varieties to *Ganoderma* could be dissimilar from susceptible varieties. The upregulation of *EgIFR-2* inDxP MT Gano revealed an important role of this gene in moderate tolerant oil palm varieties. In matter of fact, Ho et al. has hypothesized that the fungal elicitor was considered as a suppressing agent of JA and JA-induced genes, including isoflavonereductase, during the attack of *Ganoderma* [14]. Thus, the upregulation of *EgIFR-2* in moderate tolerant was potentially an activated phytoalexin biosynthesis pathway related to defense against pathogen infection. The *EgMT*gene encoding early methionine protein has been demonstrated by Tan et al. to be a potential biomarker for early detection of *G. boninense* infection. The gene was highly induced in *Ganoderma* -treated plants compared with untreated ones [15]. In this work, similar upregulation was obtained in DxP MT Gano. This result suggests that an early defense mechanism against pathogen was constitutively activated in moderate tolerant oil palm varieties. In addition, the *EgSP1* gene encoding a serine protease inhibitor was highly induced in DxP MT Gano.

Previous studies have confirmed the positive regulation of serine protease inhibitors in *Ganoderma* -treated oil palm [13-15]. Protease inhibitors, a pathogenesis-related (PR) protein, are included in downstream oil palm defense responses. These are the secondary response of pathogen attack in plant. This result suggests that a secondary defense mechanism against pathogen was also constitutively activated in moderate tolerant oil palm varieties. Taken together, the results of this work highlighted a potential better defense mechanism against biotic stress in moderate tolerant oil palm varieties to *Ganoderma* .

Table 2. Summary of target genes for previous and current differential gene expressions in oil palm (*Elaeisguineensis*). The accession numbers were accessed at European Nucleotide Archive (http://www.ebi.ac.uk/ena). The specific expression in tissue comprises leaf (L), flower (F), fruit (Fr) and root (R). The title for expression data were upregulated (Up), downregulated (Down), and non-regulated (NoReg). The unknown data was shown as Unk

Gene name	Protein coded	Accession number	Known Expression Data*		This work		Function	Reference
			Tissue	G-treated	R	L		
EgAD1	DEFENSIN 1 protein	AF322914	F	Unk	Down	Up	Flowering abnormalities	[27]
EgCAPX	Cytosolic ascorbate peroxidase	JN203288	R	Up	Down	Up	Peroxidase activity	[14, 28]
EgCHI1	Chitinase class I	GU301271	L, R	NoReg	Up	Down	Pathogen defence	[10-12]
EgCHI2	Chitinase class II	HQ831445	L, R	Up	Down	Up	Pathogen defence	[10-12]
EgDEF1	DEFICIENS 1 protein	AY739700	F	Unk	Down	Up	Flowering phenotype	[29-31]
EgEMLP	Early methionine-labeled polypeptide	EL695076	R	Up	Down	Up	Secondary metabolism	[13, 15]
EgIFR	Isoflavonereductase	EL690340	R	Down	Down	Up	Isoflavonoid biosynthesis	[13-15]
EgMT	Metallothionein-like protein	EL684283	R	Up	Up	Up	Redox homeostasis	[13, 15]
EgPR1	Pathogenesis-related protein	EL690964	R	Up	NoReg	NoReg	Pathogen defence	[13-15]
EgSPI	Serine protease inhibitor	EL690627	R	Up	Up	Up	Proteolytic activity	[13-15]
EgVIR	VIRESCENS protein	KJ789862	Fr	Unk	Up	Down	Fruit color pigmentation	[26]
EgWR1.1	WRINKLED1 protein	KJ427025	Unk	Unk	NoReg	Up	Seed oil accumulation	[32]

*Expression value was considered from all transcript analyses (reads count, qPCR, etc). Thus it might not be an exhaustive data. All the works were done using *Ganoderma* -treated plants (G-treated).

4. Conclusion

Our findings showed that differential gene expression analysis is a powerful tool to determine a better overview of expressed genes in *Ganoderma* –susceptible and –tolerant oil palms. In summary, the results obtained in this work suggest distinct molecular regulation between moderate tolerant and susceptible oil palm varieties to *Ganoderma* . Sixteen out of 21 genes were differentially regulated between DxP MT Gano (moderate tolerant) and DxPYangambi (susceptible) oil palm varieties. Six upregulated genes genes (*EgCHI1, EgVIR-1, EgVIR-2, EgIFR-2, EgMT-1,* and *EgSPI-2*) in root can be considered

as potential positive biomarkers for moderate tolerant oil palm varieties.

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