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DIFFERENTIALLY EXPRESSED PLANT DEFENSE PROTEIN EgPR10 AND EgLTP GENES IN RESPONSE TO INFECTION OF *GANODERMA* IN *Elaeis guineensis* (OIL PALM) SEEDLINGS

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Basal stem rot caused by *Ganoderma boninense* is the most serious disease of oil palm in virtually all growing regions in South East Asia and listed as among the key challenges for this major oil crop. Plant pathogenesis related proteins and in particular PR peptides are an integral part of the activation of plant defense responses against fungal infection in plant cells and PR10 and Lipid Transfer Protein LTP are two such proteins whose expressions are affected by exposure to different stress conditions including fungal infection. Degenerate primer pair combinations for conserved amino acid regions based on *LTP* and *PR10* gene sequences from model plants were used to amplify Oil Palm LTP sequences (*EgLTP*) showing similarities of up to 100% with rice, while for *EgPR10* the similarities were similarly up to 100% with wheat. Significantly, the expression of both *EgPR10* and *EgLTP* genes measured using Q-PCR in infected oil palm were temporally down regulated during the early stages of infection suggesting that down regulation of the both are related to the establishment of infection. Consequently these genes could be used as early indicators of invasion before the onset of symptoms and in breeding programmes for early selection of the elusive resistant palms.

Keywords: LTPs, lipid transfer proteins, PRs, pathogenesis-related proteins, basal stem rot, *Ganoderma boninense, Elaeis guineensis*, oil palm.

Abbreviations: BSR, Basal stem rot; EgLTP, *Elaies guineensis* Lipid transfer protein; LTP, Lipid transfer protein; CTAB, cetyl trimethylammonium bromide; BLAST, basic local alignment search tool; EDTA, ethylenediaminetetraacetic acid; TE, tris EDTA; DEPC, diethylpyrocarbonate; PCR, polymerase chain reaction; PVP, polyvinyl pyrrolidone; Q-PCR, Quantitative PCR

INTRODUCTION

The plant defense response is related to a number of early and late actions which correspond to the beginning of stress, whether biotic or abiotic, in plant cells. Plant pathogenesis related proteins and in particular PR proteins are an integral part of the activation of such responses against fungal infection in plant cells. PR proteins are induced specifically in pathological or related situations (Sels *et al*., 2008). They have been classified into 17 groups and are most often associated with plant defense response (Hoffmann-Sommergruber, 2002; van Loon *et al*., 2006). PR10 and LTP are two such proteins whose production is affected by exposure to different stress conditions including fungal infection.

PR10 proteins have been implicated in fungal disease resistance in some plant species. However its transcriptional regulation is not well understood so far. The PR10 subfamily of this group includes a number of low molecular weight proteins ranging from 15 to 20 kDa, with different biochemical characteristics. In general PR10 proteins exhibit allergenic, anti-fungal and ribonuclease activities (Chadha and Das, 2006; Liu *et al*., 2006; Srivastava *et al*., 2006). Putative plasma membrane localized receptors of PR10 inducers are suggested, and secondary signals recognized as inducers of PR10 expression include salicylic acid (SA), jasmonic acid and ethylene. As with other PR proteins, PR10 is induced by both biotic (Koistinen *et al*., 2002) and abiotic stress (Hashimoto *et al*., 2004) such as drought (Dubos and Plomion, 2001), wounding and cold-hardening (Liu *et al*., 2003), and freezing tolerance (Ukaji *et al*., 2004). While PR10 genes were originally identified in peas expressing resistance to fungi (Riggleman *et al*., 1985) it has also been described as responding to stress and abscisic acid, as a pollen allergen and has also been shown to be constitutively expressed in roots (Tiwari *et al*., 2003). The PR10 proteins

are regulated developmentally and environmentally and they are widely distributed in seed plants.

The second group of proteins, the plant lipid transfer proteins (LTPs) are a homogeneous class of small (9–10 kDa), abundant, ubiquitous and mostly basic proteins containing eight cysteine residues with four conserved disulfide bridges (Sarowar *et al.*, 2009). LTP Membrane biogenesis involves lipid movement from its synthesis site, specifically the endoplasmic reticulum, to other organelles, such as chloroplasts and mitochondria. Initially their major role was proposed to be for the transport of phospholipid molecules synthesised in the endoplasmic reticulum either to other cell membrane compartments or between membranes (Arondel *et al.*, 2000). The role of LTPs has now been expanded to include many other different biological functions. LTP possess a signal peptide indicative of secretary proteins and are additionally observed mostly in the cell walls and cuticles thus its primary role is now believed to be for the assembly of cutin and wax in the surface layer (Segura *et al*., 1993; Cameron *et al*., 2006). LTPs may also be responsive to environmental stress, as well as having a protective role against fungal and viral infections (Garcia-Olmedo *et al*., 1995; Kristensen *et al*., 2000; Guiderdoni *et al*., 2002; Park *et al*., 2002). The antibiotic-like properties of LTP were discovered during screening of plant proteins for their ability to inhibit the growth of fungal and bacterial pathogens (Terras *et al*., 1992). LTPs are also involved in long-distance signalling during systematically acquired resistance in *Arabidopsis thaliana* (Maldonado *et al*., 2002). A wheat LTP competes with elicitin for a receptor site on plasma membrane that participates in controlling plant defense response indicating a role for LTP as a signal mediator (Buhot *et al*., 2001). LTPs are also thought to play a critical role in establishing pathogen defense mechanism in palms. This hypothesis may be useful in the development of diagnostic tools and the identification of resistant or less susceptible oil palm varieties.

The understanding of the oil palm's response to *Ganoderma boninense* is a continuing challenge as the basal stem rot caused by this fungus remains a serious disease of oil palm in virtually all oil palm growing regions and continues to be listed as among the key challenges for this oil crop (Murphy, 2007; Rees *et al*., 2007). As yet there are no resistant palms in commercial cultivation. Reports on the pathogenesis of the fungus has shown that there are still uncertainties on the events leading to the differential disease symptoms manifested in the palms under different physiological and environmental conditions (Rees *et al*., 2007). In the continued search for a sustainable method of managing this disease, it has become increasingly clear that basic information on the molecular events that accompany *Ganoderma* infection has to be generated. In the present work we have cloned both a *PR10 (EgPR10)* and a *LTP (EgLTP)* sequence from oil palm (*Elaies guineensis*) and monitored the quantitative changes in their transcript levels over a period of two months after an induced infection by the fungus *Ganoderma boninense*.

MATERIALS AND METHODS

***Plant material and fungal bioassay*:** All the seedlings used in this study were from normal uninfected seedlings of D x P (*Dura x pisifera* seedlings) obtained from Sime Darby Plantations following grown under standard nursery practices. The root tissues were washed soil-free under running tap water, briefly dried then frozen in liquid nitrogen prior to direct DNA and RNA extraction. The seedlings were germinated in poly bags for 1 month then artificially inoculated as described in the Protocol of Ganoderma Artificial Inoculation (Nur and Abdullah, 2008). The seedlings were removed from the poly bags and the root of each seedling exposed directly to a *Ganoderma* inoculum block, the poly-bag refilled with soil and the seedlings irrigated twice a day (Al-Obaidi *et al*., 2010). The inoculated oil palm seeds are monitored for 2 months while after every 2 weeks, destructive sampling was done at two weeks intervals to assess for *Ganoderma* lesions.

***DNA analysis*:** Genomic DNA extraction was performed using a CTAB method as described by Doyle and Doyle (1990) with some modifications (2% CTAB,100mM tris-HCl, pH 8, 2 mM NaCl, 20 mM EDTA,2% PVP 40,2%ß-mercaptoethanol), RNA was removed using RNaseA (20 µg/µl) followed by 30 minutes incubation at 37ºC. The DNA pellet was finally dissolved in 30 µl of TE solution, pH 8.0, or ultra pure dH_2O. Primers used in this work were designed based on known sequences of *LTP* genes from other plants available at GenBank. DNA and amino acid sequence alignment was performed using CLUSTALX (*http://align.jp*). Their amino acid sequences were aligned to allow the identification of possible conserved regions within the gene. For *LTP Oryza sativa* (Accession # U77295), *Zea mays* (Accession # NM-001112535), *Hordeum vulgare* (Accession # X96979), *Triticum aestivum* (Accession # EF432573) (full sequence see Apendix E) used to design the primer LTPf (5′ gcc cgt gac agt tgg tgt tg ′3) and LTPr (5′ tgc gag cga ctg cca tag tag 3′). For *PR10 Oryza sativa* (Accession # AB127580), *Triticum aestivum* (Accession # EU908212), *Rheum austral* (Accession # EU931221) and *Lycopersicon chilense* (Accession # AY899198) (full sequence see Apendix F) were used to design PR10f (5′acc tca gcc atg ccc ttc agc c3′) and PR10r (5′ tgg ccg tga cag act cct tgg c 3′). PCR was carried out to confirm the presence of the LTP and PR10 sequences in the oil palm genome using the designed degenerate primers. PCR conditions were: 1 cycle at 95°C for 2 min, 30 cycles at 95°C for 30 s, 60°C for 1 min, 72°C for 1 min, and a final step at 72°C for 5 min. Gel extraction of the genomic DNA using Qaigen kit then

cloned using *pGEMT* easy Promega cloning vector system I (cat#A1360). Plasmid was purified from recombinant bacterial clones (*E coli* JM109) using the protocol by Sambrook (Sambrook *et al*., 2001). Sequencing reactions were performed using the "ABI PRISM dye terminator cycle sequencing ready reaction" kit and DNA sequences were resolved with the 3130 xI genetic analyzer (Applied Biosystems). Sequence analyses were performed using Chromas software (*http://www.flu.org.cn/en/download-49.html*) and ClustalW algorithm for multiple alignments (*http://www.ebi.ac.uk/Tools/clustalw2/index.html*). BLAST searches at TIGR (http://www.tigr.org/tdb/tgi/plant.shtml) and at the International Nucleotide Sequence Databases (*http://www.ncbi.nlm.nih.gov/*) databases were performed with programs BLASTx with some changes in the default parameters (McGinnis and Madden, 2004).

***RNA extraction, cDNA synthesis and Q-PCR*:** Total RNA was extracted using conventional modified CTAB method (Al-Obaidi *et al*., 2010), using CTAB extraction buffer (2% CTAB, 100mM tris pH 8, 20 mM EDTA,1.4 M NaCl, 0.2 of 2-mercaptoethanol), Chloroform two times washing rather than chloroform:isoamylalcohol (24:1), absolute ethanol precipitation for 4-7 days then nucleic acid precipitation, 70% ethanol washing ,draying the pellet followed by dissolving using 20 µl DEPC treated water. Contaminating DNA was removed using Invitrogen amplification grade I Dnase treatment kit according to the provided protocol. High capacity cDNA Reverse Transcription Kit with RNAse inhibitor (Applied Biosystems, USA) were used for cDNA synthesis. q–PCR experiments were performed by using Power SYBR® Green PCR Master. Each q–PCR was performed in a total volume of 12 µl using 100 ng/reaction of total RNA and 50 ng of each of the two primers. The specific primers for q-PCR (LTPrtf 5´-acg ccg ccg tgg cca tca cc-3´, LTPrtr 5´-aag gct cct cac gcc gct gca g-3´ & PR10rtf5´-gca ccg cca tcg aga cga cca c-3´, PR10rtr5´ctc atc gtt tac ctc cac gcc c-3´) were designed using Primer Express Software for Real time for both PR10 and LTP.

The expression level (RQ values) of Actin generally does not vary significantly between the control and treated samples when the same amount of template was used for real time PCR, hence Actin was used as an endogenous control in this experiment (Lim *et al*., 2010).

The gene-specific primer set (forward, 5'- ctc cac ccg aac gga agt att c -3' and reverse, 5'- ccc ggc aac cct aca tga ctt g -3') for the actin gene was designed from the nucleotide sequence information for oil palm actin cDNA (GenBank accession no. AY550991.1).

The specificity of the primers were assessed in separate PCR experiments using, as a template, recombinant plasmid DNA containing the appropriate LTP or PR10 clone. PCR assays using up to 200 ng of plasmid DNA confirmed that each oligonucleotide pair amplified specifically the correct *LTP* or *PR10* gene (data not shown). Q-PCR analysis was performed for RNA samples from uninfected root (control) and infected roots from samples 0, 2, 4, 6 and 8 weeks after infection.

RESULTS

The *EgLTP* and the *EgPR10* genes were successfully amplified from the genomic DNA by PCR. The fragments were gel purified and cloned into *pGEMT* easy vectors in *E. coli* (JM109). Fig. 1A & 1B shows the results from colony PCR amplification with the putatively cloned *EgLTP* and *EgPR10* fragment (both ~500 bp). Sequence alignment of the deduced amino acid of both *EgLTP* and *EgPR10* using BLAST at NCBI (http:// www.ncbi.nlm.nih.gov/ blast/BLAST.cgi) indicated similarities to *LTP* and *PR10* amino acid sequences of other monocotyledons.

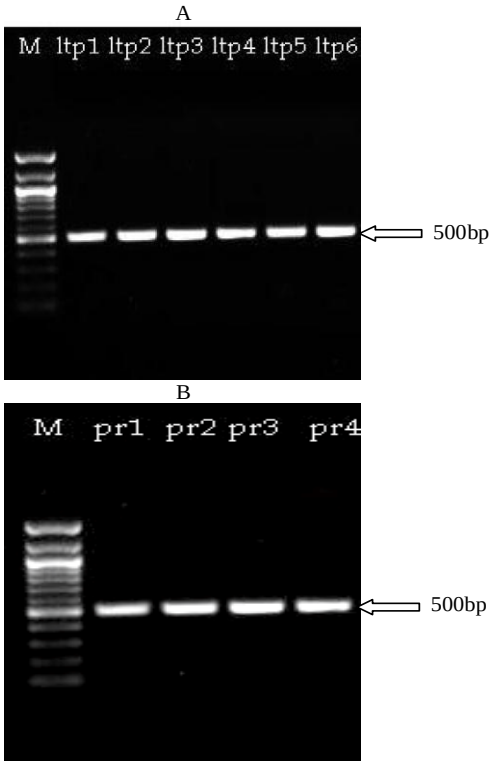


Figure 1. Agarose gel of PCR colony product fragments, A: wells labeled ltp1-ltp6 represent PCR colony for *EgLTP* fragment, 500bp (250bp of *EgLTP* fragment plus ~250bp DNA between the M13 forward and reverse priming sites) , B: wells labeled pr1-pr4 represent PCR colony for *EgPR10*, 500bp (250bp of *EgPR10* fragment plus ~250bp DNA between the M13 forward and reverse priming sites), lane labeled M: 1 kb DNA ladder (Promega). The gel was run at 120 volts until the dye front reached the bottom of the gel (approximately 30 minutes).

Table 1. Comparison of the oil palm *EgLTP* gene with other monocots (actual deduced amino acid alignment is shown in Figure 2)

Monocots	Positives%*	Bits§	Gaps	Similarity%
Oryza sativa	100	132	0	100
Sorghum bicolor	82	92	1	68
Zea diploperennis	77	84	1	64
Zea mays	82	84	1	66

Table 2. Comparison of the oil palm *EgPR10* gene with other monocots (actual deduced amino acid alignment is shown in Figure 3)

Monocots	Positives%*	Bits§	Gaps	Similarity%
Oryza sativa	83	493	0	93
Triticum aestivum	100	563	0	100
Brachypodium distachyon	94	489	0	90
Zea mays	94	454	0	82

*Positives represent fractions of residues that are either identical or similar,

§Bits represent measurement of the similarity between the two sequences: the higher the better (matches below 50 bits are very unreliable) (Claverie and Notredame, 2007).

The *EgLTP* (accession no. HQ436524) sequences were highly conserved in all the clones (up to 100% identity with rice) and showed very high homology to the sequences of other *LTP* genes in GenBank (Table 1, Fig. 2). *EgPR10* (accession no. HQ436525) sequence also showed high homology (up to 100% identity with that of *Triticum aestivum*) to the sequences of other *PR10* in GenBank (Table 2, Fig. 3).

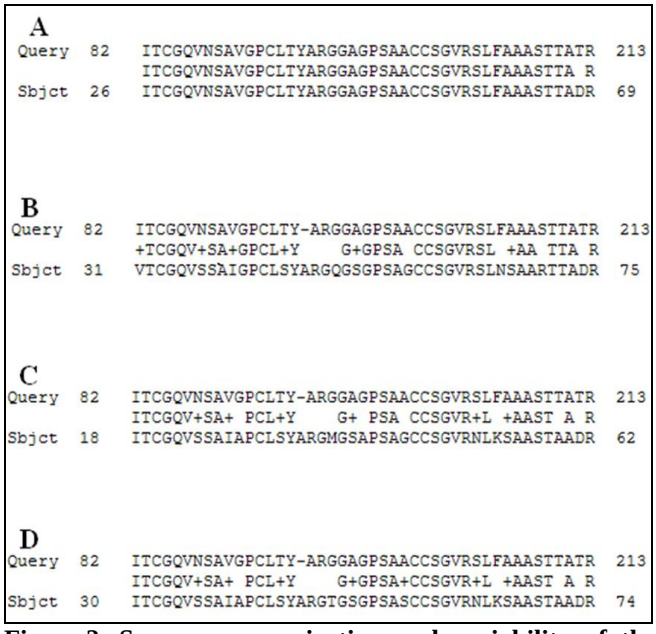


Figure 2. Sequence organization and variability of the query represent *EgLTP*. Regions (A), (B), (C) and (D) show similarities with *Oryza sativa*, *Sorghum bicolor*, *Zea diploperennis*,and *Zea mays*, respectively.

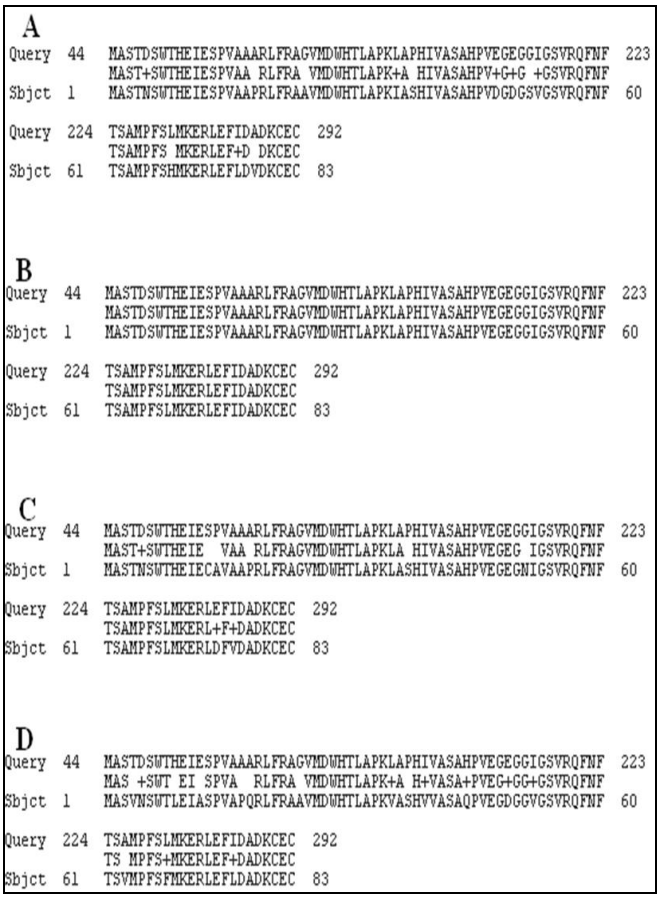


Figure 3. Sequence organization and variability of the query represented *EgPR10*. Regions (A), (B), (C) and (D) show similarities with *Oryza sativa*, *Triticum aestivum*, *Brachypodium distachyon*, and *Zea mays*, respectively.

Pure, high quality RNA was obtained using the modified CTAB method used as shown in Fig. 4A and 4B after DNAse digestion.

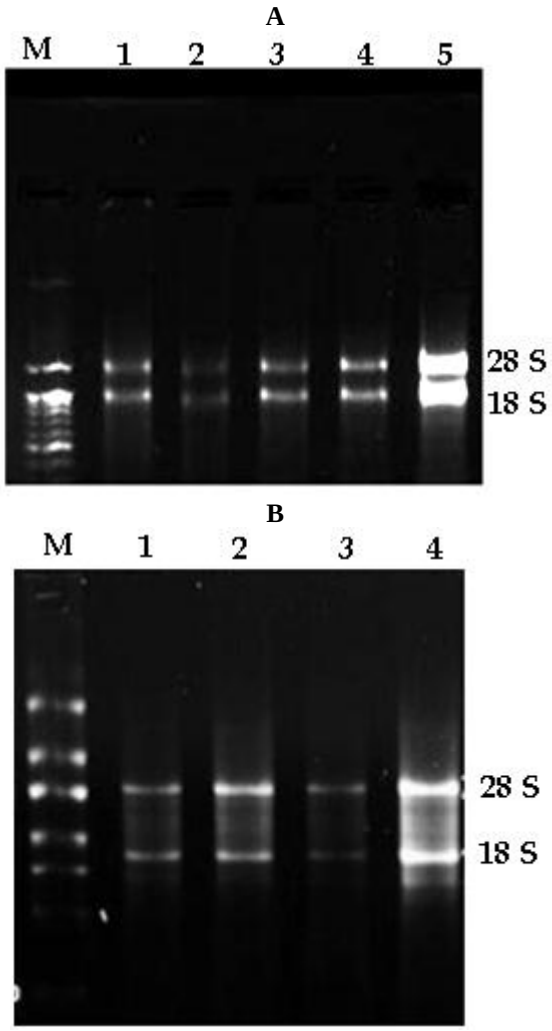


Figure 4. Ethidium bromide-stained 1.0% (w/v) agarose gel after DNase treatment (A) for infected samples and (B) for the non-infected root sample. Lane M: Low range RNA marker (Fermentas).

These were used to produce cDNA for quantitative Q-PCR analysis of expression. Amplification of the *EgLTP* with primers, LTPrtf and LTPrtr resulted in the expected 120 bp amplicon whereas amplification of *EgPR10* with primers PR10f and PR10r resulted in similar sized amplicons (Fig. 5 & 6). Primers for the Oil Palm actin gene was used as the amplification control in all the experiments. The results confirmed that the primers used were effective and the target genes were expressed in plants under the conditions studied as shown by the dissociation curves for amplifications using both primers for actin as well as for the target genes *EgLTP* (Fig. 7) and *EgPR10* (Fig. 8).

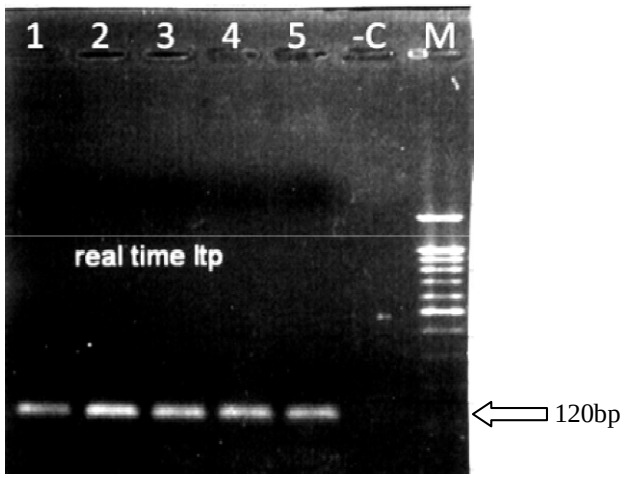


Figure 5. Ethidium bromide-stained 1.0% (w/v) agarose gel of PCR (real time condition) products, using oil palm cDNA as template and real time specific primers (*LTP*rtf and *LTP*rtr). With exception of the negative control (well –C), positive amplification showing presence of LTP cDNA fragments (~120bp) was obtained for all wells. Lane M: 100bp DNA ladder (Promega).

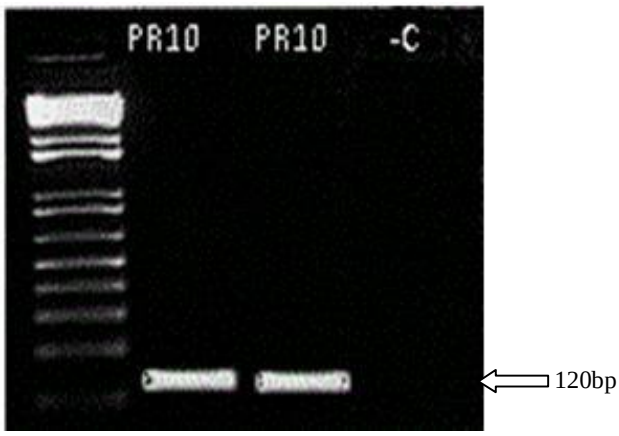


Figure 6. Ethidium bromide-stained 1.0% (w/v) agarose gel of PCR (real time condition) products, using oil palm cDNA as template and real time specific primers (*PR10*rtf and *PR10*rtr). With exception of the negative control (well –C), positive amplification showing presence of PR10 cDNA fragments (~120bp) was obtained for all wells. Lane M: 100bp DNA ladder (Promega).

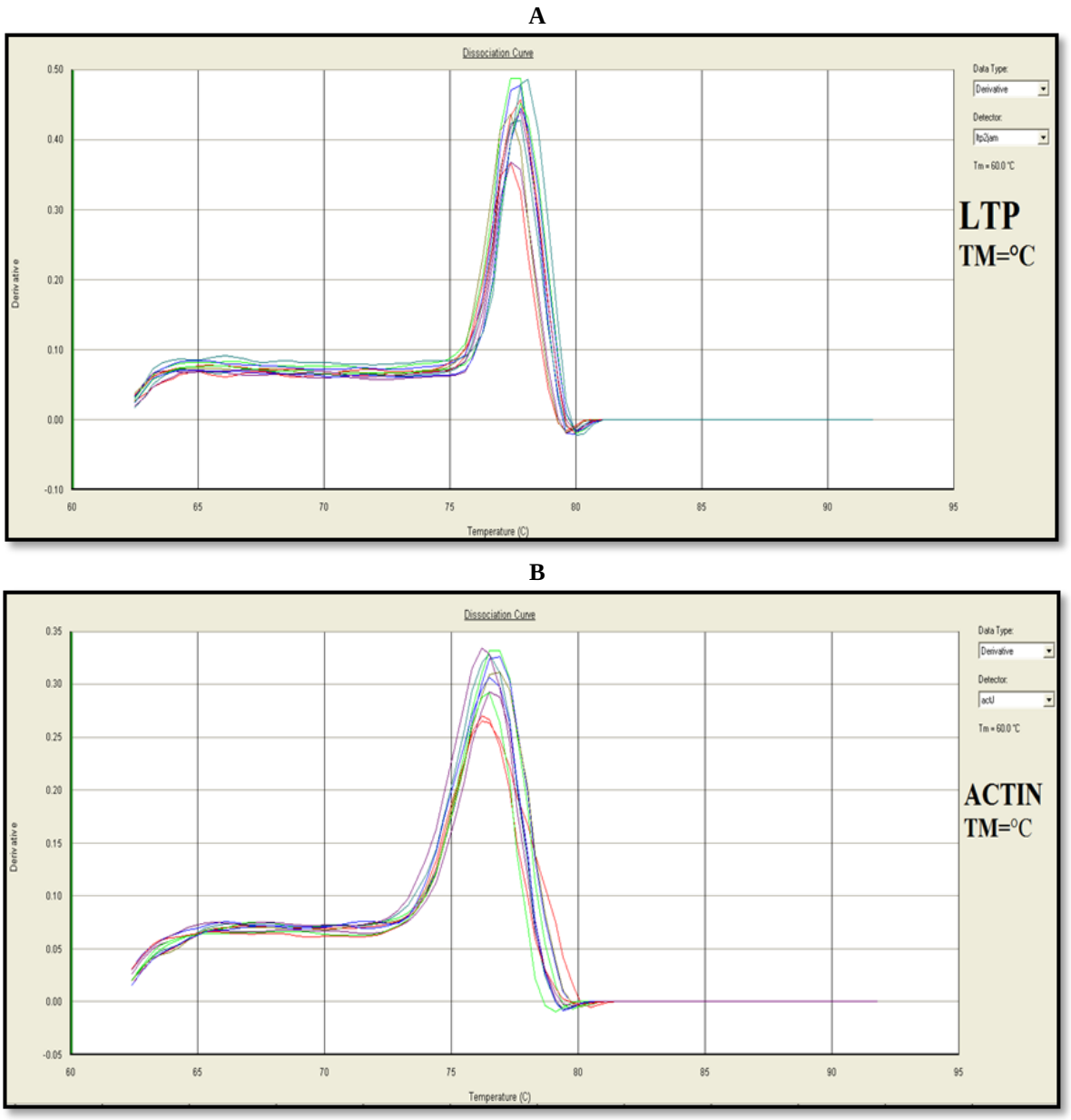
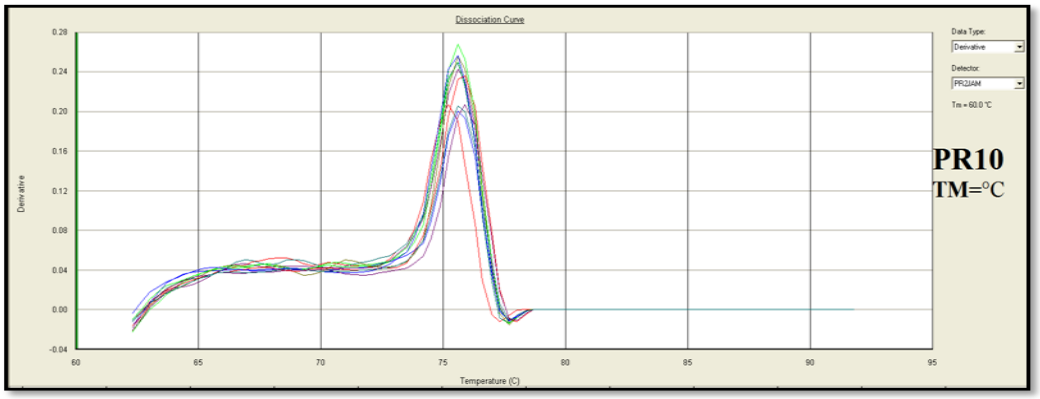


Figure 7. Dissociation curve for target gene *EgLTP* and endogenous control *β-actin*. (A) The dissociation curve for target gene *EgLTP*, showing the primer efficacy for specific product, amplicon size 120bp. (B) The dissociation curve for endogenous control *β-actin*, showing the primer efficacy for specific product, amplicon size 80bp. The single peak at 77.5°C for target gene and 77°C for *β-actin* gene verify that the primers used were efficient and effective for both infected and non-infected sample.

Figures 9 and 10 show the amplification plots for the real time analysis of post infection expression of *EgLTP* and *EgPR10*, respectively. The expression levels of the genes were assayed at 0, 2, 4, 6 and 8 weeks post-inoculation with *G. boninese*. The Q-PCR results indicated that the level of expression of *EgLTP* was significantly down-regulated when assayed 2 weeks post infection in comparison to the control (Fig. 11). The results for *EgPR10* also showed significant down-regulation of its level of expression (Fig. 12) when assayed 2 weeks post infection. The suppression of expression for both genes remained even after 8 weeks post infection. All p values for the five post-inoculation time points stages assayed were less than 0.05 (statistically significant).

A



B

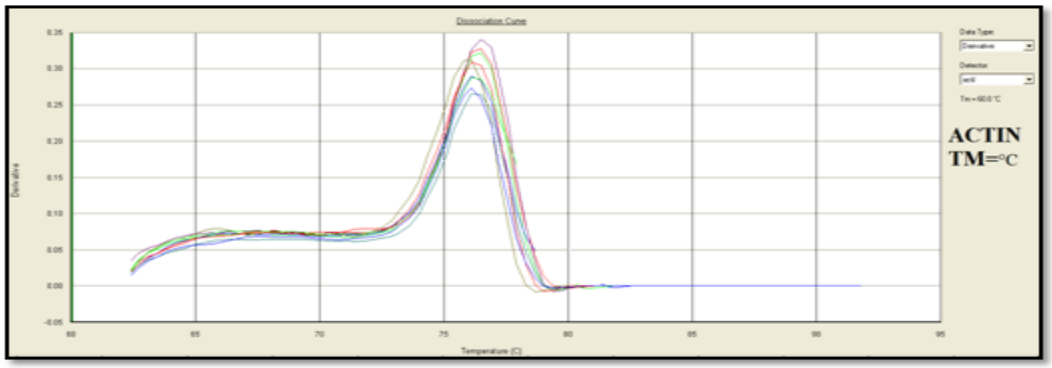


Figure 8. Dissociation curve for target gene *EgPR10* and endogenous control *β-actin*. (A) The dissociation curve for target gene *EgPR10*, showing the primer efficacy for specific product, amplicon size 120bp. (B) The dissociation curve for endogenous control *β-actin*, showing the primer efficacy for specific product, amplicon size 80bp. The single peak at 77.5°C for target gene and 77°C for *β-actin* gene verify that the primers used were efficient and effective for both infected and non-infected sample.

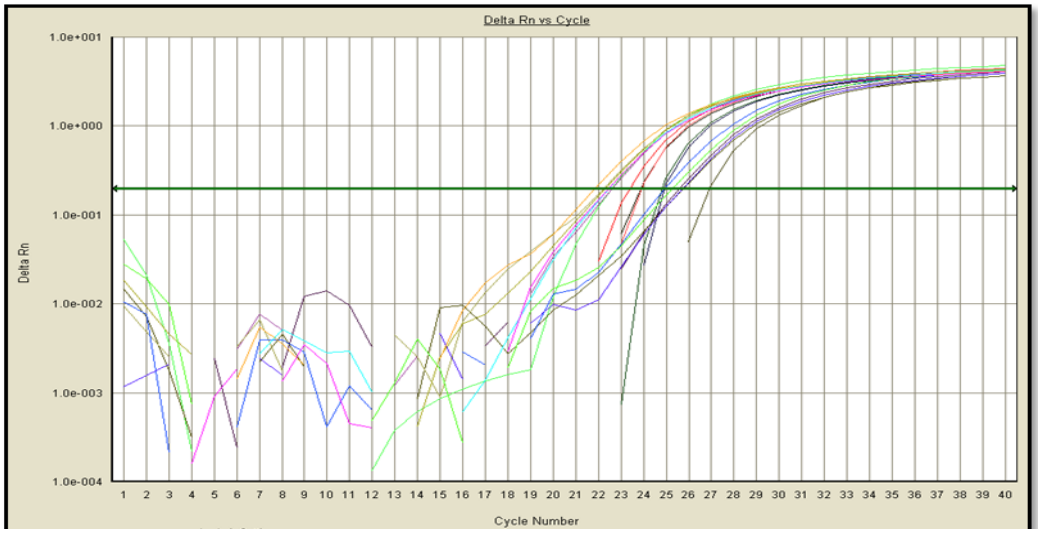


Figure 9. Amplification plot for the Real Time-PCR reaction. The Fig. shows the fluorescence signal versus cycle number for both target *EgLTP* and endogenous control *β-actin* genes using oil palm cDNA as a template at 0, 2, 4, 6 and 8 weeks for both infected and non-infected sample.

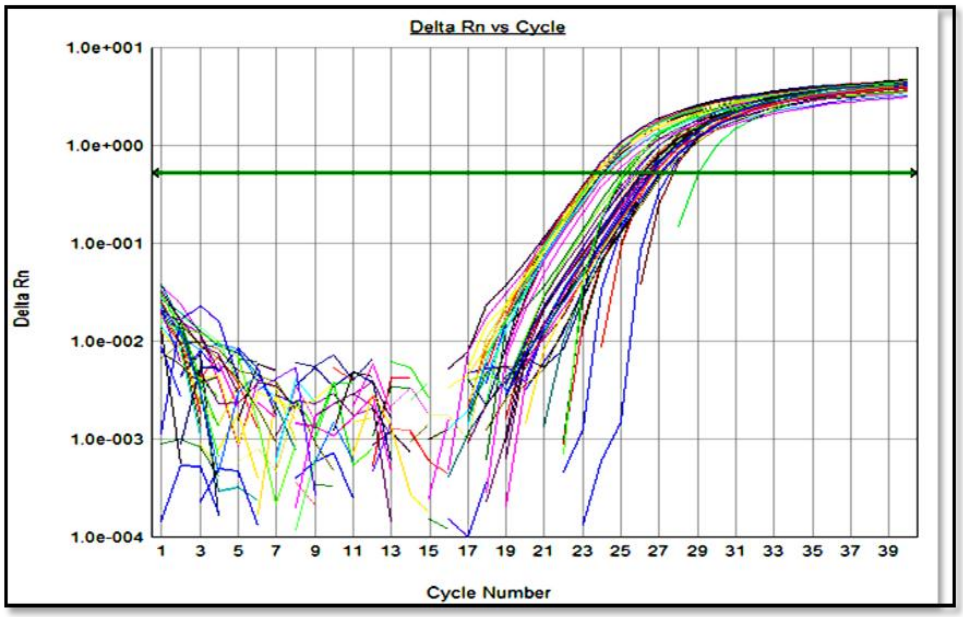


Figure 10. Amplification plot for the Real Time-PCR reaction. The Fig. shows the fluorescence signal versus cycle number for both target *EgPR10* and endogenous control *β-actin* genes using oil palm cDNA as a template at 0, 2, 4, 6 and 8 weeks for both infected and non-infected sample.

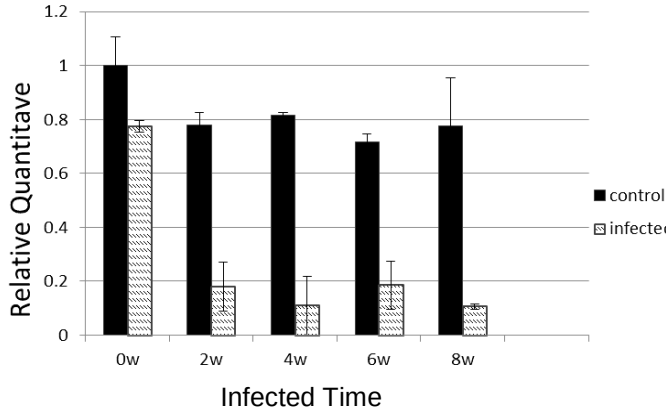


Figure 11. Q-PCR analysis of *LTP* gene expression in oil palm inoculated with *G. boninense*. Size of the amplicon of *EgLTP* gene transcripts and *β-actin* gene transcript were 120bp and 80bp, respectively. The latter was used as the constitutive control. Bars in the graph shows RQ values for expression of the gene at 0, 2, 4, 6 and 8 weeks of post-inoculation and the error bar shows the standard deviation of the mean of four biological replicates. All *p* values for the five post-inoculation time points stages were less than 0.05 (statistically significant).

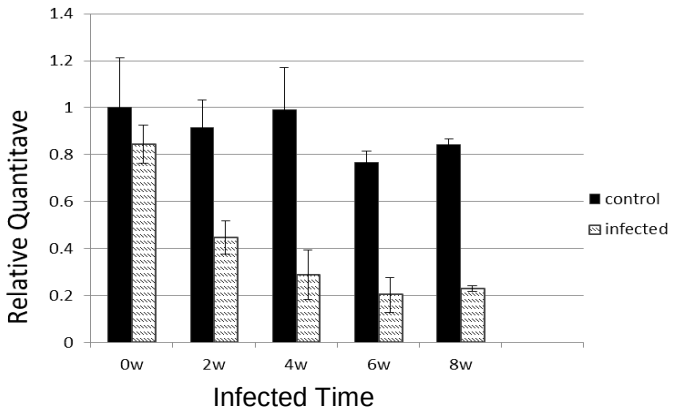


Figure 12. Q-PCR analysis of *PR10* gene expression in oil palm inoculated with *G. boninense*. The sizes of the amplicon of *PR10* gene transcripts and *β-actin* gene transcript were 120bp and 80bp, respectively. The latter was used as the constitutive control. Bars in the graph shows RQ values for expression of the gene at 0, 2, 4, 6 and 8 weeks of post-inoculation and the error bar shows the standard deviation of the mean of four biological replicates. All *p* values for the five post-inoculation time points stages were less than 0.05 (statistically significant).

DISCUSSION

The study of the expression of genes proposed to be involved in the early interaction with the basal stem rot fungus is a useful and practical approach to further understand the early response of oil palm cells during attack by *Ganoderma boninense* (Al-Obaidi *et al*., 2010). Pathogenesis related proteins PR10 and Lipid Transfer Protein LTP are two proteins whose expressions are known to be affected by exposure to different stress conditions including fungal infection. In oil palm, a simple profile of the changes in the expression of these genes during the early stages of infection might give valuable insights into the stage of the infection and the behaviour of the fungus in the root cells, since *G. boninense* rapidly degrades starch, lignin and cellulose and causes extensive breakdown of root cortical cell walls during infection. The expression profile of these proteins in the oil palm in response to *G. boninense* infection at the early stages of attack (within 2 months post infection) was investigated in this study.

A partial sequence of *EgLTP* was successfully identified and isolated from oil palm based on its similarity with other monocots. The expression of *LTP* is known to be regulated during development and in response to several stimuli (Krishnaswamy *et al*., 2008). In this study the Q-PCR results showed that the *EgLTP* level of expression was observed to be reduced after a week post-infection in comparison to the control (Fig. 11). These suggests that the oil palm response is similar with previously published reports on the down-regulation of the *LTP* gene after time course microbial infection such as seen in susceptible wheat (Gaudet *et al*., 2010). Other reports also supports the idea that root LTPs are down-regulated when the root is exposed to different stress conditions (Maghuly *et al*., 2009). In contrast LTPs are induced and will increase after fungal infection in hosts exhibiting resistance to the disease (Gottwald *et al*., 2012). The general consensus is that the lipid signal is essential for the activation of plant defense responses, but downstream components and regulation of the signalling pathways are still poorly defined.

LTPs exhibit broad antimicrobial activity *in vitro* (Garcia-Olmeda *et al*., 1995), and because of their high isoelectric point, they may act as membrane permeabilizing agents. The lipid signal is essential for the activation of plant defense responses, but the downstream components of the signalling pathway are still poorly defined. The biosynthesis of LTPs is induced in response to an infection by a pathogen (Van Loon and Van Strien, 1999). Purified LTPs have strong antifungal activities (Ge *et al*., 2003). One of the suggested mechanisms for LTP antifungal activity is that the LTP could possibly form pores when inserted into the fungal cell membrane, allowing low molecular weight compounds, such as nucleotides and coenzymes to leak, but the results show that these activity was inhibited by an unknown mechanism and caused a reduction of LTP starting from 2 weeks time after inoculation. LTPs can interact with receptors located on plant plasma membrane, which were then identified as the elicitin receptors (Buhot *et al*., 2001). The binding of elicitins to their receptors is known to induce disease resistance in some hosts. The LTPs are apparently required in the activation of the receptor-ligand complex (Maldonado *et al*., 2002). The down-regulated expression of LTPs after being infected with *G. boninense* may thus have affected the elicitin-induced response and hence allowed the pathogen to escape from host recognition (Schlink, 2010). This may have helped the pathogen to complete its life cycle and enter the necrotrophic growth stage, which causes extensive loss of roots (Liu *et al*., 2006).

Alternatively, the LTP antifungal mode of action may have occurred through the formation of pores in the fungal membranes (Wong *et al*., 2010). This results in an efflux of intracellular ions, which culminates in cell death and may offer an alternative explanation of our observed results.

The second defense related gene that was investigated in the present study, PR10, has also been well associated with pathogen infection, particularly those of the fungus (Hashimoto *et al*., 2004). However, it is still difficult to assign a causative role of the PR10 proteins in plant resistance to the pathogens (Edreva, 2005). The reason for this is that the numerous data on PRs as disease resistance factor are mostly of correlative character. Apart from being present in the primary and secondary cell walls of infected plants, PRs are also found in cell wall appositions (papillae), deposited at the inner side of the cell wall in response to a fungal attack (Jeun, 2000). This has also been shown using sensitive immunological techniques, which allowed the detection of PRs in roots of tobacco and tomato plants inoculated with the fungal pathogens *Chalaraelegans* and *Fusarium oxysporum*, respectively (Benhamou *et al*., 1991), as well as in lupine and birch roots exposed to abiotic stress (Przymusinski *et al*., 2004). The results of the present study showed the significant down-regulation of *EgPR10* gene after infection with *G. boninense*. This was similarly observed in the European peach (*Fagus sylvatica*; Schlink, 2010) when the plant was infected with the fungal-like pathogen, *Phytophthora citricola* and by Hyun *et al*., (2011) in their study of *Capsicum. annuum* var. Yeoju infected by *Colletotrichum acutatum*.

Pathogen recognition evokes a strong signal and coordinated defense reaction in all infected plants during incompatible interactions while the lack of proper recognition in compatible interactions leads to more individualized and less concerted responses among infected plants. The suppression of defense activation during earlier stages of the interaction observed in this study demonstrates how well the pathogen controls the host response and is an important factor for success of this hemibiotrophic pathogen. There are growing indications that some of the effectors are targeted to the host

nucleus and perhaps interfere with transcription of defense genes (Schlink, 2010). This opens up further opportunities for studies concerning the genes involved in energy metabolism during infection in order to support this theory.

***Conclusion*:** The expression profile of the two defense related genes suggest that *G.boninense* was able to deactivate or modulate the antifungal activities associated with LTP and PR10 proteins in such a way that leads to the reduction of their expression levels. Future studies on these and other defense related Oil Palm genes will involve approaches that are able to elucidate the mechanisms of the binding process of lipids on *LTP* and of the interactions of *LTP and PR10* with membranes that may lead to a better understanding of the early fungal pathogenic mechanisms that leads to the establishment of BSR infection (Fig. 13). The recent release of the draft genome of the Oil Palm (Singh *et al*., 2013) will certainly facilitate future functional studies on these genes.

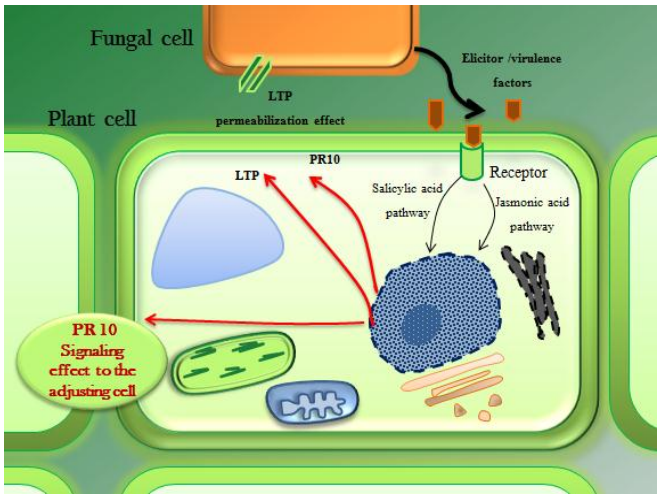


Figure 13. Predicted schematic representation of the respond of oil palm cell towards the *Ganoderma* infection. Red arrows represent the affected pathogen related proteins expression during basal stem rot.

The results also suggest that these genes may have the potential to be developed as early biomarkers of BSR disease and more importantly for the identification of *G. boninense* tolerant oil palms. This approach would involve the selection of palms showing up-regulation of the genes during challenge with the fungus and could easily be incorporated into breeding programmes in tandem with other recently reported indicators such as syringic acid (Chong *et al*., 2012). The profiling of these indicators in different oil palm cultivars could present another useful criteria in relation to phenological characteristics already described for many commercial cultivars used in breeding programmes (Forero *et al*., 2011).

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REFERENCES

Al-Obaidi, J.R., Y. Mohd-Yusuf, T. Chin-Chong, N. Mhd-Noh and R.Y. Othman. 2010. Identification of a partial oil palm polygalacturonase-inhibiting protein (EgPGIP) gene and its expression during basal stem rot infection caused by *Ganoderma boninense*. Afr. J. Biotechnol. 9:7788-7797.

Arondel, V.V., C. Vergnolle, C. Cantrel and J. Kader. 2000. Lipid transfer proteins are encoded by a small multigene family in *Arabidopsis thaliana*. Plant Sci. 157:1-12.

Benhamou, N., J. Grenier and A. Asselin. 1991. Immunogold localization of pathogenesis-related protein p14 in tomato root-cells infected by *Fusarium oxysporum* F. Sp. *Radicis-Lycopersici*. Physiol. Mol. Plant 38:237-253.

Buhot, N., J.P. Douliez, A. Jacquemard, D. Marion, V. Tran, B.F. Maume, M.L. Milat, M. Ponchet, V. Mikes, J.C. Kader and J.P. Blein 2001. A lipid transfer protein binds to a receptor involved in the control of plant defense responses. FEBS Lett. 509:27-30.

Cameron, K.D., M.A. Teece and L.B. Smart. 2006. Increased accumulation of cuticular wax and expression of lipid transfer protein in response to periodic drying events in leaves of tree tobacco. Plant Physiol. 140:176-183.

Chadha, P. and R.H. Das. 2006. A pathogenesis related protein, AhPR10 from peanut: an insight of its mode of antifungal activity. Planta 225:213-222

Chong, K.P., M. Atong and S. Rossall. 2012. The role of syringic acid in the interaction between oil palm and *Ganoderma boninense*, the causal agent of basal stem rot. Plant Pathol. 61:953-963.

Claverie, J.M. and C. Notredame. 2007. Bioinformatics for dummies. 2nd ed. Wiley, Hoboken, NJ.

Doyle, J.J. and J.L. Doyle. 1990. Isolation of plant DNA from fresh tissue. Focus 12:13-15.

Dubos, C. and C. Plomion. 2001. Drought differentially affects expression of a PR-10 protein, in needles of maritime pine (*Pinus pinaster* Ait.) seedlings. J. Exp. Bot. 52:1143-1144.

Edreva, A. 2005. Pathogenesis-related proteins: research progress in the last 15 years. Gen. Appl. Plant Physiol. 31:105-124.

Forero, D.C., P. Hormaza and H.M. Romero. 2012. Phenological growth stages of African oil palm (*Elaeis guineensis*). Ann. Appl. Biol. 160:56-65.

Garcia-Olmedo, F., A. Molina, A. Segura and M. Moreno. 1995. The defensive role of nonspecific lipid-transfer proteins in plants. Trends Microbiol. 3:72-74.

Gaudet, D.A., Y. Wang, C. Penniket, Z.X. Lu, G. Bakkeren and A. Laroche. 2010. Morphological and molecular analyses of host and non host interactions involving barley and wheat and the covered smut pathogen *Ustilago hordei*. Mol. Plant Microbe Interact. 23:1619-1634.

Ge, X., J. Chen, N. Li, Y. Lin, C. Sun and K. Cao. 2003. Resistance function of rice lipid transfer protein LTP110. J. Biochem. Mol. Biol. 36:603-607.

Gottwald, S., B. Samans., S. Luck and W. Friedt. 2012. Jasmonate and ethylene dependent defense gene expression and suppression of fungal virulence factors: two essential mechanisms of Fusarium head blight resistance in wheat? BMC Genomics 13:369.

Guiderdoni, E., M.J. Cordero, F. Vignols, J.M. Garcia-Garrido, M. Lescot, D. Tharreau, D. Meynard, N. Ferriere, J.L. Notteghem and M. Delseny. 2002. Inducibility by pathogen attack and developmental regulation of the rice Ltp1 gene. Plant. Mol. Biol. 49: 683-699.

Hashimoto, M., L. Kisseleva, S. Sawa, T. Furukawa, S. Komatsu and T. Koshiba. 2004. A novel rice PR10 protein, RSOsPR10, specifically induced in roots by biotic and abiotic stresses, possibly via the jasmonic acid signaling pathway. Plant Cell Physiol. 45:550-559.

Hoffmann-Sommergruber, K. 2002. Pathogenesis-related (PR)-proteins identified as allergens. Biochem. Soc. Trans. 30:930-935.

Hyun, T.K., M.N. Uddin, Y. Rim and J.Y. Kim. 2011. Cell-to-cell trafficking of RNA and RNA silencing through plasmodesmata. Protoplasma 248:101-116.

Jeun, Y.C. 2000. Immunolocalization of PR-protein P14 in leaves of tomato plants exhibiting systemic acquired resistance against Phytophthora infestans induced by pretreatment with 3-aminobutryic acid and preinoculation with Tobacco necrosis virus. J. Plant Dis. Prot. 107: 352-367.

Koistinen, K.M., V.H. Hassinen, P.A.M. Gynther, S.J. Lehesranta, S.I. Keinänen, H.I. Kokko, E.J. Oksanen, A.I. Tervahauta, S. Auriola and S.O. Kärenlampi. 2002. Birch PR-10c is induced by factors causing oxidative stress but appears not to confer tolerance to these agents. New Phytol. 155:381-391.

Krishnaswamy, S., S. Srivastava, M. Mohammadi, M. Rahman, M. Deyholos and N. Kav. 2008. Transcriptional profiling of pea ABR17 mediated changes in gene expression in *Arabidopsis thaliana*. BMC Plant Biol. 8:91.

Kristensen, A.K., J. Brunstedt, K.K. Nielsen, P. Roepstorff and J.D. Mikkelsen. 2000. Characterization of a new antifungal non-specific lipid transfer protein (nsLTP) from sugar beet leaves. Plant Sci. 155:31-40

Lim K.A., Z.H. Shamsuddin and C.L. Ho. 2010. Transcriptomic changes in the root of oil palm (*Elaeis guineensis* Jacq.) upon inoculation with *Bacillus sphaericus* UPMB10. Tree Genet. Genomes 6:793-800.

Liu, Jun-Jun, A.K.M. Ekramoddoullah and Y. Xueshu. 2003. Differential expression of multiple PR10 proteins in western white pine following wounding, fungal infection and cold-hardening. Physiol. Plant. 119:544-553.

Liu, X., B. Huang, J. Lin, J. Fei, Z. Chen, Y. Pang, X. Sun and K. Tang. 2006. A novel pathogenesis-related protein (SsPR10) from *Solanum surattense* with ribonucleolytic and antimicrobial activity is stress-and pathogen-inducible. J. Plant Physiol. 163:546-556.

Maghuly, F., E.G. Borroto-Fernandez, M.A. Khan, A. Herndl, G. Marzban and M. Laimer. 2009. Expression of calmodulin and lipid transfer protein genes in Prunus incisa x serrula under different stress conditions. Tree Physiol. 29:437-444.

Maldonado, A.M., P. Doerner, R.A. Dixon, C.J. Lamb and R.K. Cameron. 2002. A putative lipid transfer protein involved in systemic resistance signalling in Arabidopsis. Nature 419:399-403.

McGinnis, S. and T.L. Madden. 2004. BLAST: at the core of a powerful and diverse set of sequence analysis tools. Nucl. Acids Res. 32:W20-W25.

Murphy, D.J. 2007. Future prospects for oil palm in the 21st century: Biological and related challenges. Eur. J. Lipid Sci. Technol. 109:296-306

Nur Ain Izzati, M.Z. and F. Abdullah. 2008. Disease suppression in *Ganoderma*-infected oil palm seedlings treated with *Trichoderma harzianum*. Plant Protec. Sci. 44:101-107.

Park, C.J., R. Shin, J.M. Park, G.J. Lee, J.S. You and K.H. Paek. 2002. Induction of pepper cDNA encoding a lipid transfer protein during the resistance response to tobacco mosaic virus. Plant Mol. Biol. 48:243-254.

Przymusinski, R., R. Rucinska and E.A. Gwozdz. 2004. Increased accumulation of pathogenesis-related proteins in response of lupine roots to various abiotic stresses. Environ. Exp. Bot. 52:53-61.

Rees, R.W., J. Flood, Y. Hasan and R.M. Cooper. 2007. Effects of inoculum potential, shading and soil temperature on root infection of oil palm seedlings by the basal stem rot pathogen *Ganoderma boninense*. Plant Pathol. 56:862-870.

Riggleman, R.C., B. Fristensky and L.A. Hadwiger 1985. The disease resistance response in pea is associated with increased levels of specific mRNAs. Plant Mol. Biol. 4:81-86.

Sambrook, J. and D.W. Russell. 2001. Molecular cloning: A laboratory manual. 3rd ed., Cold Spring Harbor Laboratory Press, New York.

Sarowar, S., Y.J. Kim, K.D. Kim, B.K. Hwang, S.H. Ok and J.S. Shin. 2009. Overexpression of lipid transfer protein (LTP) genes enhances resistance to plant pathogens and LTP functions in long-distance systemic signaling in tobacco. Plant Cell Rep. 28:419-427.

Schlink, K. 2010. Down-regulation of defense genes and resource allocation into infected roots as factors for compatibility between *Fagus sylvatica* and *Phytophthora citricola*. Funct. Integr. Genomics 10: 253-264.

Segura, A., M. Moreno and F. Garcia-Olmedo. 1993. Purification and antipathogenic activity of lipid transfer proteins (LTPs) from the leaves of Arabidopsis and spinach. FEBS Lett. 332:243-246.

Sels, J., J. Mathys, B.M. De Coninck, B.P. Cammue and M.F. De Bolle. 2008. Plant pathogenesis-related (PR) proteins: a focus on PR peptides. Plant Physiol. Biochem. 46:941-950.

Singh, R., M. Ong-Abdullah, E.T. Low, M.A. Manaf, R. Rosli, R. Nookiah, L.C. Ooi, S.E. Ooi, K.L. Chan, M.A. Halim, N. Azizi, J. Nagappan, B. Bacher, N. Lakey, S.W. Smith, D. He, M. Hogan, M.A. Budiman, E.K. Lee, R. DeSalle, D. Kudrna, J.L. Goicoechea, R.A. Wing, R.K. Wilson, R.S. Fulton, J.M. Ordway, R.A. Martienssen and R. Sambanthamurthi. 2013. Oil palm genome sequence reveals divergence of interfertile species in Old and New worlds. Nature 500:335-339.

Srivastava, S., R.J.N. Emery, L.V. Kurepin, D.M. Reid, B. Fristensky and N.N.V. Kav. 2006. Pea PR 10.1 is a ribonuclease and its transgenic expression elevates cytokinin levels. Plant Growth Regul. 49:17-25.

Terras, F.R., I.J. Goderis, F.V. Leuven, J. Vanderleyden, B.P. Cammue and W.F. Broekaert. 1992. *In vitro* antifungal activity of a radish (*Raphanus sativus* L.) seed protein homologous to nonspecific lipid transfer proteins. Plant Physiol. 100:1055-1058.

Tiwari, S.B., G. Hagen and T. Guilfoyle. 2003. The roles of auxin response factor domains in auxin-responsive transcription. Plant Cell 15:533-543.

Ukaji, N., C. Kuwabara, D. Takezawa, K. Arakawa and S. Fujikawa. 2004. Accumulation of pathogenesis-related (PR) 10/Bet v 1 protein homologues in mulberry (*Morus bombycis* Koidz.) tree during winter. Plant Cell Environ. 27:1112-1121.

Van Loon, L.C. and E.A. Van Strien. 1999. The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. Physiol. Mol. Biol. Plant 55 (2):85-97.

Van Loon, L.C., M. Rep and C.M. Pieterse. 2006. Significance of inducible defense-related proteins in infected plants. Annu. Rev. Phytopathol. 44:135-162.

Wong, J.H., T.B. Ng, R.C. Cheung, X.J. Ye, H.X. Wang, S.K. Lam, P. Lin, Y.S. Chan, E.F. Fang, P.H. Ngai, L.X. Xia, X.Y. Ye, Y. Jiang and F. Liu. 2010. Proteins with antifungal properties and other medicinal applications from plants and mushrooms. Appl. Microbiol. Biotechnol. 87:1221-1235.