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Research Article
Cloning and Expression Analysis of *HbPR-1b* and *HbPR-3* in *Hevea brasiliensis* During Inoculation with *Rigidoporus microporus*

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Abstract

Background and Objective: Pathogenesis-related (PR) proteins are dramatically accumulated after pathogen infection. Innate defense response through increasing PR-proteins is important for rubber rootstock selection that is tolerant to the white root disease caused by *Rigidoporus microporus*. This study was aimed to investigate the expression levels of *PR-1* and *PR-3* genes in tolerant (PB5/51) and susceptible (BPM24 and RRIM600) rubber clones after *R. microporus* infection. **Materials and Methods:** The mRNA of *HbPR-1b* and *HbPR-3* was isolated and characterized from rubber leaves. Gene expression levels of *HbPR-1b* and *HbPR-3* were compared among three rubber clones (PB5/51, BPM24 and RRIM600) after *R. microporus* infection at 0, 12, 24, 48, 72 and 96 h using quantitative real-time PCR. The relative transcript abundances between inoculated and control plants were compared using the means of gene expression between time points and by Tukey's HSD test. A probability value ($p \leq 0.05$) was used to determine the significance of difference between time points. **Results:** The open reading frame of *HbPR-1b* is 492 bp with deduced 163 amino acid residues and the phylogenetic analysis showed it shared significant evolutionary history and clustering into group I of PR-protein. Moreover, the partial *HbPR-3* was isolated with 390 bp. Gene expression levels of *HbPR-1b* and *HbPR-3* showed marked differences in both transcripts depending on the rubber clones. Two genes demonstrated up-regulation of both tolerance and susceptibility in response to attack by *R. microporus*. The highest expression levels were found in seedlings of PB5/51 after inoculation. In RRIM600, low expression levels of *HbPR-1b* and *HbPR-3* were initially observed but gradually increased at 24 h post inoculation. The transcription profile of *HbPR-1b* was stable expression in BPM24. **Conclusion:** The results demonstrated that the level of *HbPR-1b* and *HbPR-3* transcription can distinguish between tolerant and susceptible clones. The candidate defense genes to the white root disease were observed in PB5/51 seedlings, particularly *HbPR-1b*.

Key words: *Rigidoporus microporus*, *Hevea brasiliensis*, pathogenesis-related protein, white root disease, gene expression

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Hevea is one of the major crops for smallholders in Thailand and is also an industrially important crop in Southeast Asia. It is planted for natural latex production and rubber wood is a secondary product. Thailand is a leading producer of rubber and has exported the highest quantities of natural rubber in the world since 1991[1]. At present, Thailand is still the leading exporter of rubber. However, rubber plants are susceptible to several pathogenic microorganisms during their growth, such as bacteria[2], viruses[3] and especially fungi[4].

Fungi are more complicated microorganisms than others and are causing agents for most plant diseases including white root disease in rubber tree, incited by *Rigidoporus microporus*. White root disease involves the host-parasite interactions attacking the tap root of the rubber tree and is distributed in tropical and sub-tropical regions[5]. *Rigidoporus microporus* can grow and extend in the deep soil and infect trees through root contact by producing rhizomorphs that attach to wood debris[6]. In order to defend themselves from the pathogen, plants have developed a broad variety of defense responses with structural and biochemical changes[7,8]. They produce plant proteins which possess the antimicrobial function[9]. These plant proteins are called PR-proteins. The PR-proteins are a set of plant defense protein induced by the plants as a defense response system in stress conditions[10] and induced in response to pathogen attack during compatible and incompatible interactions with the pathogen[11]. The PR-proteins have been widely reviewed. They have been basically divided into 17 families based on their properties and functions[12]. The *PR-1* and *PR-3* are the antifungal proteins that have been shown to inhibit fungal growth[13]. The *PR-1* is a constitutive protein and presents in different tissues. The *PR-1* transcript showed a high accumulation in plants after fungal infection and it was consequently used as a marker for defensive responses[14-16]. Besides, *PR-3* belongs to the chitinases and was produced by host plants and thereby able to degrade fungal cell walls which consist of chitin as a major structural component[12]. In order to improve tomato disease resistance to early blight disease, potato plants was pre-inoculated by Arbuscular Mycorrhizal Fungi (AMF), *Funneliformis mosseae* and the causal pathogen of the early blight disease (*Alternaria solani*), was inoculated. Pre-inoculation with AMF enhanced four enzyme activities, including β-1,3-glucanase, chitinase, phenylalanine ammonia-lyase and lipoxygenase in the leaves of tomato after inoculation with *A. solani*. A set of *PR* genes have higher expression, including *PR-1* and *PR-3*. However, the AMF inoculation alone did not affect the transcripts of most of defense-related genes tested. The study demonstrated that AMF inoculation influenced tomato disease resistance to the early blight disease[17]. Moreover, *PR-1* and *PR-3* were found correlate with the defence-related mechanism in cucumber seedlings after *Fusarium oxysporum* CS-20 inoculation. The pathogen-mediated resistance response was regulated by both genes[18]. Accordingly, the abundances of *PR-1* and *PR-3* mRNA transcripts could then be used as indicators of tolerance rubber clones. Therefore, it is interesting to investigate the correlation between white root disease tolerant trait and gene expression levels of *PR-1* and *PR-3* in rubber tree. The characteristics of their cDNA and amino acid sequences were analyzed by the bioinformatic tools. Meanwhile, the expression profile of *PR-1* and *PR-3* genes in *Hevea* after inoculation at the seedling stage were investigated using quantitative real-time PCR (qPCR). The results obtained from this study are beneficial for early selection of rubber tree for the white root disease tolerance breeding programs. Analysis of genetic linkage among markers and identification of the genetic locations of desirable phenotypes would further improve the selection accuracy to help accelerate the future improvement of this economically important crop. It will facilitate and accelerate the genetic improvement of *H. brasiliensis* through molecular breeding and exploitation of genetic resources.

MATERIALS AND METHODS

Plant materials: In this study, PB5/51 was used as a tolerant clone. The RRIM600 and BPM24 were included as the susceptible clones. The seeds of these clones were collected from Songkhla and Trang provinces. The plants were grown in a growth chamber under controlled conditions of temperature (25±2°C) in January, 2016. Samples were arranged in a Completely Randomized Design (CRD). Seedlings with approximately the same height were grown for inoculation studies after 3 months.

Preparation of fungal inoculum: A highly virulent *R. microporus* derived from Department of Pest Management, Prince of Songkla University was used. The generative hypha was collected from the diseased rubber tree clone and cultivated on potato dextrose agar for 7 days at room temperature.

Treatment of rubber plants with *R. microporus* and RNA extraction: The seeds of rubber clones were germinated in the growth chamber. Three months seedlings were inoculated with *R. microporus*. Three biological replications

Table 1: Nucleotide sequence primers used for molecular cloning

Genes	Directions	Sequences (5′→3′)
HbPR-1b	Forward	TCTTGTGCATTCCAGCAATC
	Reverse	CACTTCACCTTAGCACATCCT
HbPR-3	Forward	ATGGGCTACTGCACCAGACGGA
	Reverse	CCACCGTTRATGATGTTYGT

were inoculated. Inoculation was implemented by creating wounds on stem section close to roots. The surface area for inoculation was disinfected by the 70% ethanol (Molecular biological grade). A sterile surgical scalpel was used to create a wound around the bark of trunk. The agar plug (0.5 cm in diameter) of active fungal mycelia was placed close to the created wounds. Mock inoculation with the uncontaminated agar was also designated as the control. The leaves of all inoculated plants were collected at 0, 12, 24, 48, 72 and 96 h post inoculation (hpi) for RNA extraction and gene expression checked by qPCR. For RNA extraction, leaf samples (0.1 g) were used by extraction buffer pH 9.0 (100 mM Tris-HCl, 10 mM EDTA, 100 mM LiCl, 2% SDS) and phenol:chloroform:Isoamyl (25:24:1, v/v) with 500 µL (molecular biological grade). A mixture was mixed the sample by vigorously shaking by briefly vortexing. The tube with sample was centrifuged for 15 min at 4200 rpm at 4°C. The upper aqueous phase was transferred to a new microcentrifuge tube. Subsequently, phenol:Chloroform:Isoamyl was added to a tube, mixed by hand and centrifuged for 15 min at 4200 rpm at 4°C. The upper aqueous phase was transferred to a new microcentrifuge tube and chloroform:Isoamyl (24:1; v/v, 0.5 mL) was added of. The sample was mixed by hand and centrifuged at 4200 rpm, 4°C for 15 min. The upper aqueous phase was moved to a new microcentrifuge tube. Total RNA was precipitated overnight at 4°C in 8 M LiCl and centrifuged at 13,000 rpm, 4°C for 20 min. The pellet was washed twice with cold 70% ethanol, dried at room temperature, suspended in 20 µL RNase-free water and treated with RNase-free RQ1 DNase (promega) to eliminate DNA. The RNA quantification was carried out by BioDrop DUO UV/VIS Spectrophotometer.

Molecular cloning of *PR-1* and *PR-3*: The cDNA was synthesized with Maxima H Minus First Strand cDNA Synthesis kit in accordance with the manufacturer's instruction (Thermo Scienti c, USA). The cDNA templates coding for *PR-1* and *PR-3* were produced by RT-PCR amplification with their degenerate specific primers (Table 1). The PCR thermal cycling program began at 95°C for 4 min, followed by 35 cycles of three steps of amplification process including degeneration step at 94°C for 1 min, annealing step at 55°C for 1 min and extension step at 72°C for 1 min, respectively. To examine the PCR product, the gel electrophoresis was performed using 1.5% agarose gel and stained with ethidium bromide. The PCR product band was excised from the agarose gel. The obtained DNA was cloned into RBCTA-cloning vector kit from RBC Bioscience (Taiwan). Recombinant clones were sequenced using the automated sequencing facility at BigDye® Terminator v3.1 cycle sequencing kit at the First Base DNA Sequencing Services, Malaysia. A full-length cDNA of rubber tree PR-genes was obtained from a pair of specific primers (forward primer: 5′-AAGGTTCTCCCCGTAAGGAC-3′, reverse primer: 5′-CAACTCAGTTCGCCTAGGATGT-3′) designed using premier PRIMER 5.0 software. The 3' and 5' rapid amplification of cDNA ends (RACE) was performed using terminal deoxynucleotidyl transferase (Thermo Scientific, USA).

Bioinformatic analysis: All of nucleotide and protein sequences of PRs in this study were obtained from GenBank databases via the National Center for Biotechnology Information (NCBI). The DNA comparison and predicted amino acid sequences were performed by BLAST analysis. The Open Reading Frame (ORF) was predicted using ORF Finder. The theoretical molecular weight (MW) and isoelectronic point (pI) were calculated using Compute pI/MW tool at ExPASy website. Multiple sequence alignments were performed using the ClustalX1.81 program[19] and GENEDOC program[20]. A phylogenetic tree was constructed using molecular evolutionary genetics analysis 4 (MEGA4)[21]. The evolutionary history was inferred using a neighbor-joining method[21,22].

Gene expression analysis of PRs: Primers were designed based on the analogous *H. brasiliensis* coding sequences that were gotten from gene cloning results shown in Table 2. The gene expression was determined using qPCR system in a Light Cycler 480 SYBR Green I Master (Roche). The PCR thermal cycling program composed of denaturation step at 95°C for 10 min, followed by 35 two-step cycles of PCR, including denaturation step at 95°C for 15 sec and annealing and polymerization at 59°C for 1 min. Finally at the end of each run, melting curve analysis was carried out following

Table 2: Nucleotide sequence primers used for qPCR

Genes	Directions	Sequences (5′→3′)	Amplicon size (bp)
HbPR-1b	Forward	GCCTGCTTAACCCTACCCTT	
	Reverse	CCGTAAGGACGATTGCTGGA	190
HbPR-3	Forward	TACGGTAGAGGTCCCATCCAA	
	Reverse	CCACCGTTGATGATGTTCGT	269

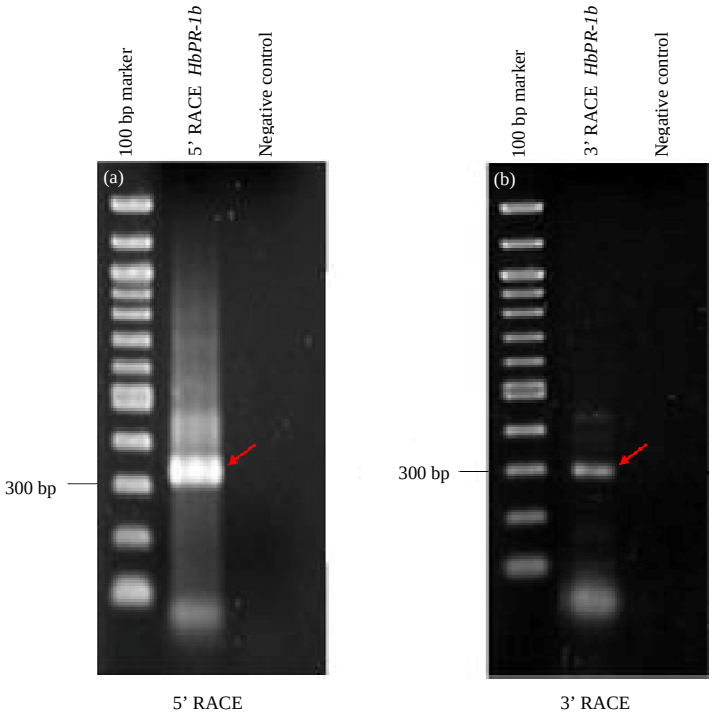

Fig. 1(a-b): PCR products generated by 5' and 3' rapid amplification of cDNA ends (RACE) (a) 3′ RACE 246 bp and (b) 5′ RACE 336 bp (indicated by arrows)

instruments related instructions, to ensure the specificity of primer and the purity of the amplified product. The cycle threshold (Ct) values was used for calculating relative gene expression by the $2^{-\Delta\Delta Ct}$ method[23]. The 18S rRNA validated for normalization in gene expression in the rubber tissues was used as the internal reference gene for normalization[24]. Each relative transcript abundance value was the mean of three biological replicates.

Statistical analysis: Statistical analysis was carried out after logarithmic transformation of raw data. The relative transcript abundances between inoculated and control plants were compared using the means of gene expression between time points and Tukey's HSD test[25]. The p-value equal or less than 0.05 was used to determine the significance of difference between time points.

Tissue-specific of *HbPR-1b* and *HbPR-3* expression in healthy rubber tree: In order to determine the organ-specific expression of *PR* genes in rubber tree, the total RNA was extracted from young leaf, mature leaf, bark, pre-mature seeds and mature seeds. The cDNA templates coding for *HbPR-1* and *HbPR-3* were produced by RT-PCR amplification with their specific primers. They are shown in Table 1. Thermal conditions were: 95°C for 4 min followed by 35 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min, with a final 72°C for 10 min. The PCR product was checked on 1.5% agarose gel with the ethidium bromide staining. Three samples from each organ were analyzed in duplicate.

RESULTS AND DISCUSSION

Molecular cloning of *HbPR-1b* and *HbPR-3*: This study presented the detailed cloning and characterization of *HbPR-1b* and *HbPR-3* in the rubber tree in order to confirm the nucleotide sequences of these genes. *HbPR-1b* and *HbPR-3* were produced by RT-PCR amplification. In order to obtain a full-length cDNA of *HbPR-1b*, the pair of specific primers was generated. The 3' and 5' rapid amplification of cDNA ends (RACE) were carried out from the partial gene of *HbPR-1b* using terminal deoxynucleotidyl transferase. The 3' and 5' RACE analysis, two fragments were obtained (Fig. 1). Furthermore, their fragments were assembled into 492bp consensus sequence including poly-A tail signal region. The ORF finder predicted the ending extended sequence contained an intact ORF, encoding *HbPR-1b* similar to *Ricinus communis* 82%, *Jatropha curcas* 79%, *Populus trichocarpa* 78%, *Populus euphratica* 77% and *Gossypium raimondii* 73%. The putative initiation ATG codon was in the context of AAAATGG, satisfying the Kozak consensus A/GXXATGG. A

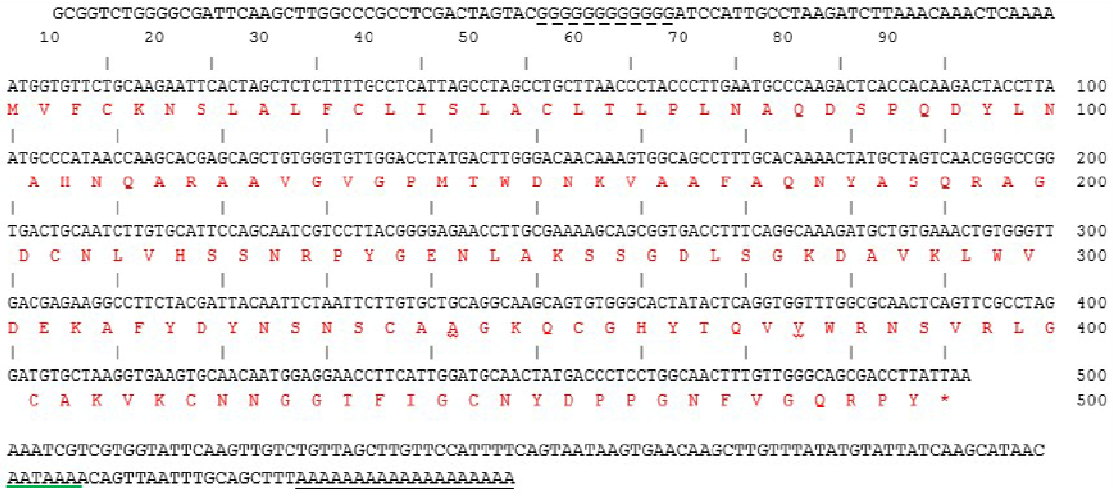

Fig. 2: Coding region and deduced amino acid sequence of *HbPR-1b*

putative polyadenyl signal, AAUAAA, was presented in the 3' untranslated region of *HbPR-1b* shown in Fig. 1. In addition to the partial PCR product of *HbPR-3* is 390 bp. The BLASTX analysis demonstrated the sequence shared high similarity (99%) to class I chitinase in *H. brasiliensis* (GenBank accession No. CAD24068.1). The *HbPR-1b* encoded a deduced protein of 163 amino acids with 17.69 kDa. The *HbPR-1b* with the isoelectric point (pI) 8.57 was grouped into the basic type of *PR-1* protein[26]. The basic type of *PR-1* protein significantly ($p \leq 0.05$) enhances the antifungal resistance[27,28]. Six conserved cysteine residues forming disulphide bridges and two conserved domains of CRISPs were found in *HbPR-1b*. Comparison of the cDNA sequence with other plants, the data showed that the *HbPR-1b* of rubber tree had high similarity to the basic tobacco PR-1 protein (*prb-lb*)[29] and the basic PR-1 protein of pepper (*CABPR1*)[30].

Sequence analysis: The *HbPR-1b* gene (Accession No. KT334160) encoded a predicted protein of 163 amino acids (Accession No. ALS87256.1) (Fig. 2), with a calculated molecular mass of 17.69 kDa and pI of 8.57, which is similar to most *PR-1* proteins. To clarify the relationships between *HbPR-1b* and other PR proteins, the phylogenetic tree was generated by a Neighbor-Joining (NJ) phylogram based on the deduced sequence of *HbPR-1b* that contained other members of the antifungal proteins (*PR-1* to *PR-5*). The result indicated that *HbPR-1b* might share a common ancestor and display similar functions with group 1 of PR (Fig. 3). The mature protein contained six conserved cysteine residues forming disulphide bridges and two conserved domains of the cysteine-rich secretory proteins (CRISPs) were found namely CRISP1and CRISP2 (Fig. 4). The BLASTP analysis of *HbPR-1b* protein showed SCP_PR-1-like conserve (Fig. 5a). It is SCP-like extracellular protein domain. The SCP-like extracellular protein domain was found plant *PR-1* protein from plants, which accumulated after pathogenic infections and involved in cell wall loosening. The SCP-like extracellular protein domain was also included CRISPs conserved region. The partial gene of *HbPR-3* was translated to the partial protein. The BLASTP analysis of the partial *HbPR-3* showed high similarity (98-99%) to class I chitinase (Accession No. AJ431363.1, AJ238579.1, KF648872.1 and KF648873.1). The BLASTP analysis found chitinase_ glyco_ hydro_19 domain (Fig. 5b). Family 19 chitinases in plant are thought to be part of a defense mechanism against fungal pathogens and some plant chitinases exhibit antifungal activity[31].

Tissue-specific of *HbPR-1b* and *HbPR-3*: In order to speculate the expression of *HbPR-1b* and *HbPR-3* with particular tissues with in healthy rubber tree, the expression profiles were analyzed using the different rubber tree tissues. The total RNA was isolated from young leaf, mature leaf, bark, pre-mature seed and mature seed. The transcripts of two genes were detected in all tissues examined. As shown in Fig. 6a, b, the expression of the *HbPR-1b* and *HbPR-3* showed differential tissue-specific expression. The lowest expression of both genes was observed in pre-mature seed. They were highly expressed in bark, mature leaf, young leaf and mature seed, respectively. These results indicated that expression profiles of the *HbPR-1b* and *HbPR-3* genes vary throughout a healthy rubber plant in a developmental stage and specific

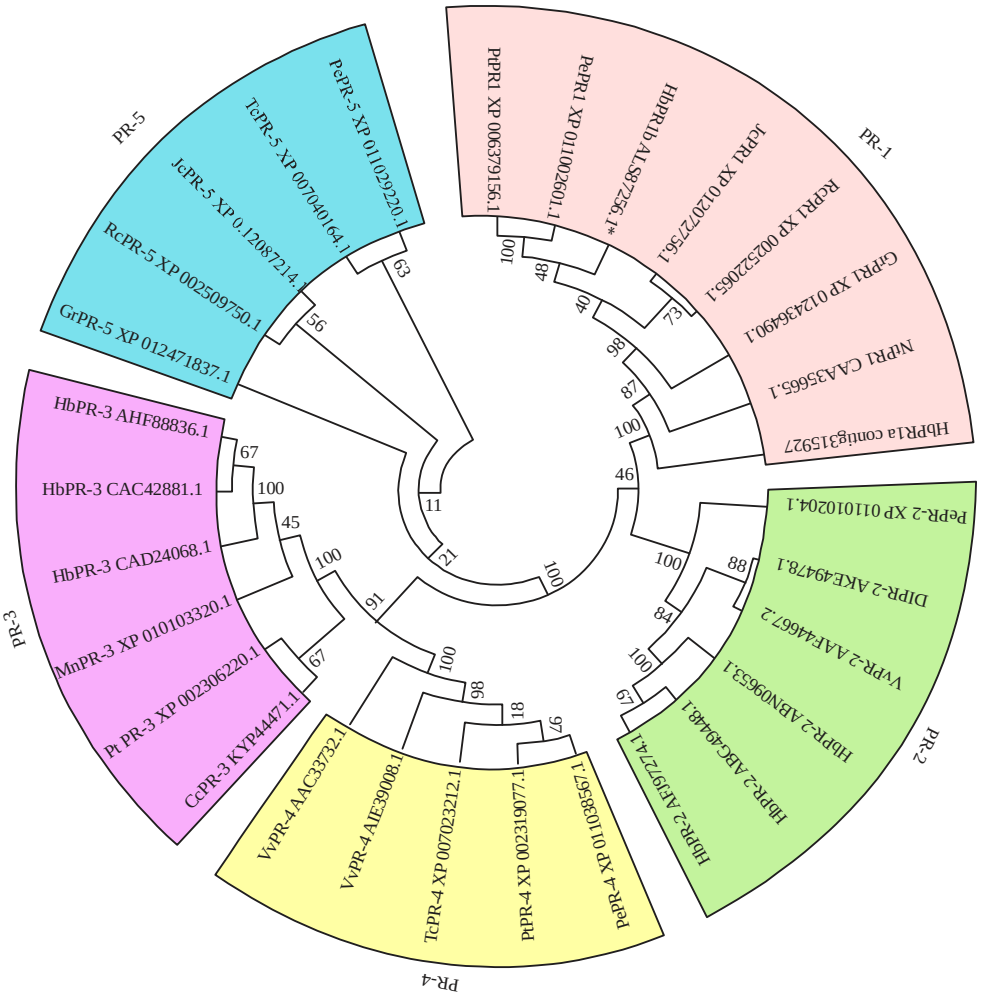

Fig. 3: Phylogenetic tree of *HbPR-1b* with 29 other PR-proteins

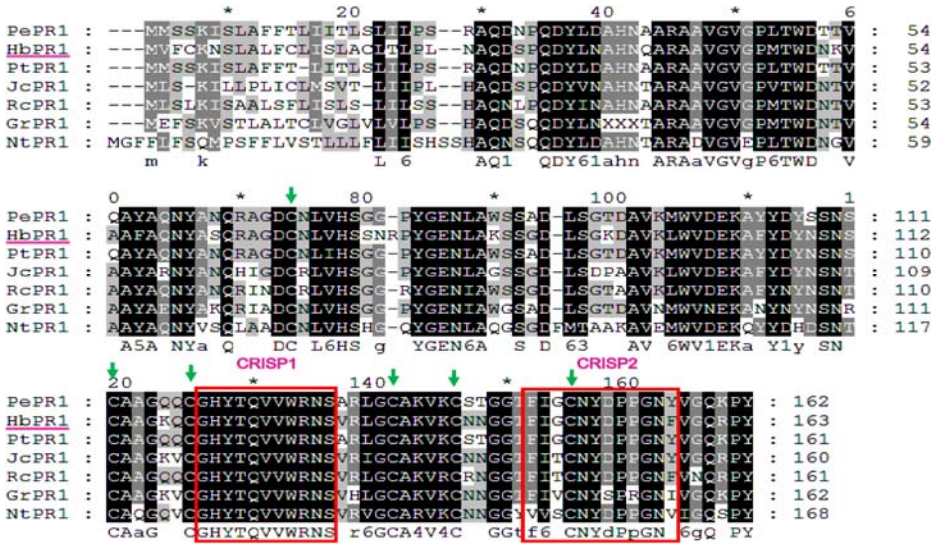

Fig. 4: Multiple alignments of amino acid sequence of *HbPR-1* gene by *H. brasiliensis* compared with other plants

The consensus amino acid sequences are highlighted in black. Amino acid substitutions are marked in gray

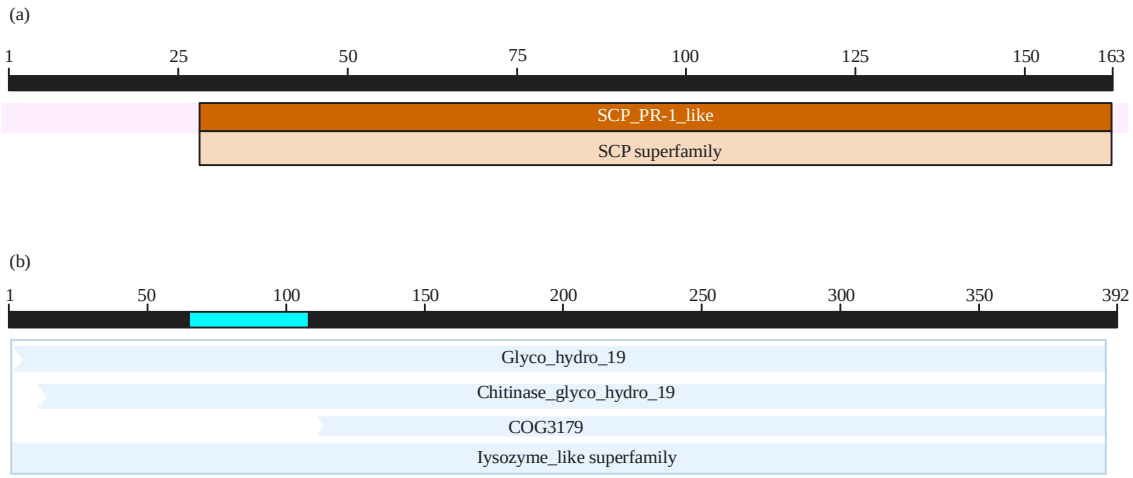

Fig. 5(a-b): Conserved domain analysis of the deduced *HbPR-1b* and *HbPR-3* protein (a) SCP_PR-1_like domain of *HbPR-1b* and (b) Chitinase_glyco_hydro_19 domain of *HbPR-3*

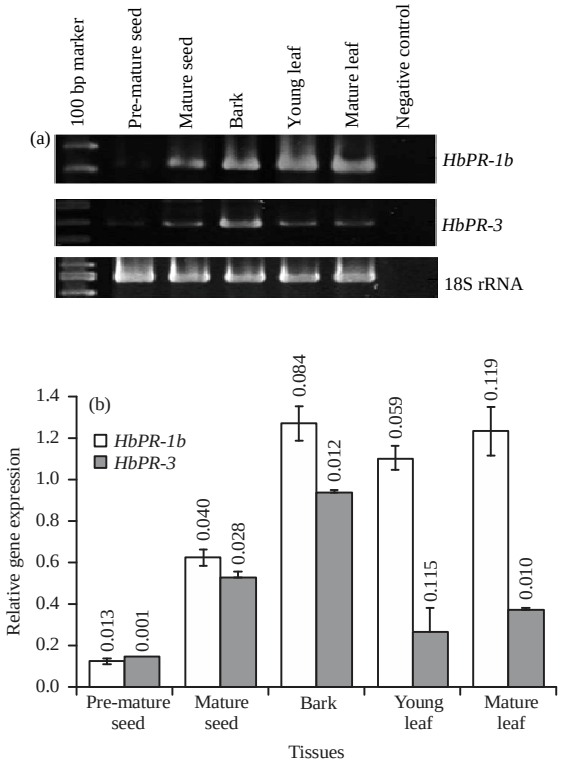

Fig. 6(a-b): Tissue-specific expression study of *HbPR-1b* and *HbPR-3* genes (a) Semi-quantitative gene expressions and (b) *HbPR-1b* and *HbPR-3* expressions in various tissues
Vertical bars indicate the values of SEM from three independent experiments

organs. Moreover, expression of *HbPR-3* in all organs was lower than that of *HbPR-1b* gene.

The PR-proteins are exhibited in many plant organs such as root, stem, leave, flower and seed[32-34]. The mRNA distribution in healthy potato tissues was studied. The results showed that low amounts of *PR-1b* were identified in old leaves, roots, carpels of flowers and tubers, whereas it was not detectable in young leaf and stem. In leaves, *PR-1b* is strongly accumulated and protein occurred in response to infection by the oomycete pathogen (*P. infestans* or *Pseudomonas syringae* pv. *Maculicola*)[35]. It is similar to our study that *HbPR-1b* is strongly accumulated in mature leaf. However, in young leaf, low amounts of *HbPR-1b* are also detectable. Additionally, the expression profile analysis in different citrus organs (root, stem, bark, leaf, flower, seed and fruit peel) was performed to speculate the association between preferential expression of some *PR*-gene families and particular organs. The result showed that the *PR-3* gene was accumulated in all of the studied citrus organs. However, The *PR-1* gene families were not expressed in citrus fruit peel and seed[36]. In 2006, the *PR-3* gene family was reported that it was synthesized in some organs of healthy rice, including leaf, sheath, root and meristem. These results suggested that *PR-3* proteins encoded by the genes have considerable biological effects[37]. Accordingly, PR-protein synthesis can be not only activated by environmental/external cues but triggered by internal plant developmental stimuli[38]. Moreover, a set of PR-proteins was formed in leaves of healthy tobacco plant as they reached the flowering and senescing stage as well as the relation of *PR-3* to seed germination[39,40]. Alternatively, plant organ specificity of PR-proteins indicated that these PR-proteins may have functions other than defense responses[30]. The results of the

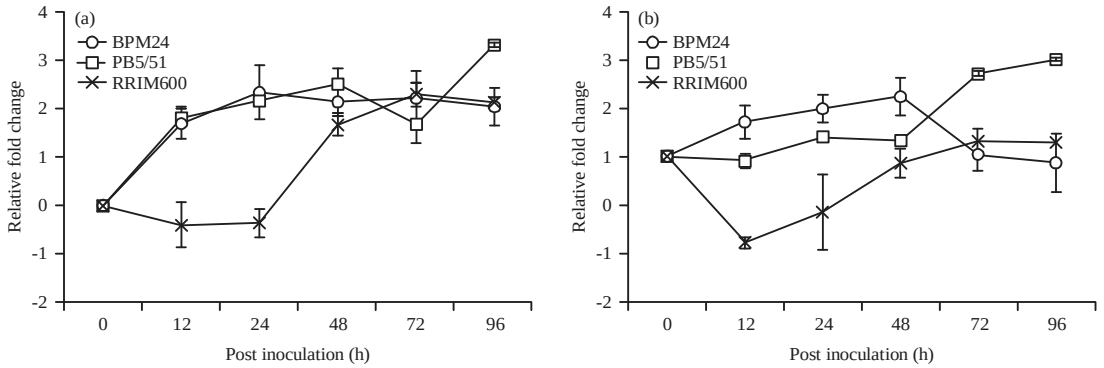

Fig. 7(a-b): qPCR analyses of the expression profile of *HbPR-1b* and *HbPR-3* in PB5/51, RRIM600 and BPM24 after inoculation with *R. microporus* compared with mock inoculation (a) Expression profile of *HbPR-1b* and (b) Expression profile of *HbPR-3*

present study suggested that the rate of *HbPR-1b* and *HbPR-3* genes activation in leaves is much more important than that in bark or seed.

Gene expression profiles of *HbPR-1b* and *HbPR-3*: Genes belonging to the pathogenesis related proteins have been strongly implicated with the resistance mechanism of plants against fungal pathogens[13]. In this study, the white root disease tolerant rubber clone (PB5/51) and susceptible clones (RRIM600 and BPM24) were used to examine the expression profiles of *HbPR-1b* and *HbPR-3* after inoculation with *R. microporus*. The transcript patterns of putatively encoding HbPR-1b and HbPR-3 proteins in all *Hevea* clones were investigated at 0, 12, 24, 48, 72 and 96 hpi. Expression of *HbPR-1b* after inoculation was highly up-regulated in PB5/51, BPM24 and RRIM600, respectively. Although RRIM600 showed low expression at 0-24 hpi, it showed high expression at 48-96 hpi as shown in Fig. 7. Transcription level of *HbPR-3* demonstrated continuous up-regulation and showed the highest expression level at 96 hpi in PB5/51. The RRIM600 showed down-regulated level at early stage, but after that showed up-regulated level. However, their expression levels were not higher than PB5/51. In contrast to BPM24, the *HbPR-3* exhibited high expression level at early stage and subsequently down-regulation at the final stage. When consider the transcript levels of these genes (*HbPR-1b* and *HbPR-3*) among three studied clones; the data showed that only PB5/51 was up-regulated especially at 96 hpi (Fig. 8a). In the RRIM600 clone, *HbPR-1b* and *HbPR-3* expression rapidly increased during the final stages of infection at 48 hpi and remained relatively unchanged during the latter stages of infection (Fig. 8b). On the other hand, BPM24 tends to be down-regulation in both genes after 48 hpi (Fig. 8c). Our results demonstrated that all examined clones showed different expression profiles. The trigger timing and magnitude varied between the clones. They were observed the induction of *HbPR-1b* and *HbPR-3* in both tolerant and susceptible rubber clones. However, their expression levels were lower in susceptible clones. In accordance with Mishra *et al.*[41] who studied pathogenesis-related genes expression of resistant and susceptible chilli pepper inoculated with *Collectotrichum truncatum.* The results demonstrated that *PR1* and *PR3* were highly expressed in resistant chili pepper cultivar compared with susceptible cultivar[41]. Further, a correlation in the expressions of *PR1* and *PR3* has been reported in *H. brasiliensis*. Four-years-old tolerant clone and susceptible clone of rubber trees were used to examine the relationship to tolerance against *Phytophthora meadii* infections. The PR-proteins were produced 24 hpi in tolerant clones but 48 hpi in susceptible clones. In inoculated tolerant plants, PR-protein expression levels were also higher than that of susceptible clones[42]. Moreover, the first set of defensive gene expression patterns was provided in the host plant-pathogen interaction of the rubber trees and white root disease. Gene expression levels of defense-related genes, including *PR-1*, *PR-3*, *PR-5*, *PR-8* and *PR-9*, in two *Hevea* clones (RRIM 612 and PR107) after inoculation with *R. microporus* exhibited the variability in defense responses. The transcript of *PR-1* was down-regulated in RRIM612 (highly susceptible) but no significant difference with PR107 (least susceptible). In the other hand, the transcript of *PR-3* was highly up-regulated in both clones. This result suggested that the genetic characteristics of *Hevea* clones may play an important role in defense gene expression[43]. Besides, the gene expression levels of two *PR*-genes (*PR-2* and *PR-3*) extracted from leaves

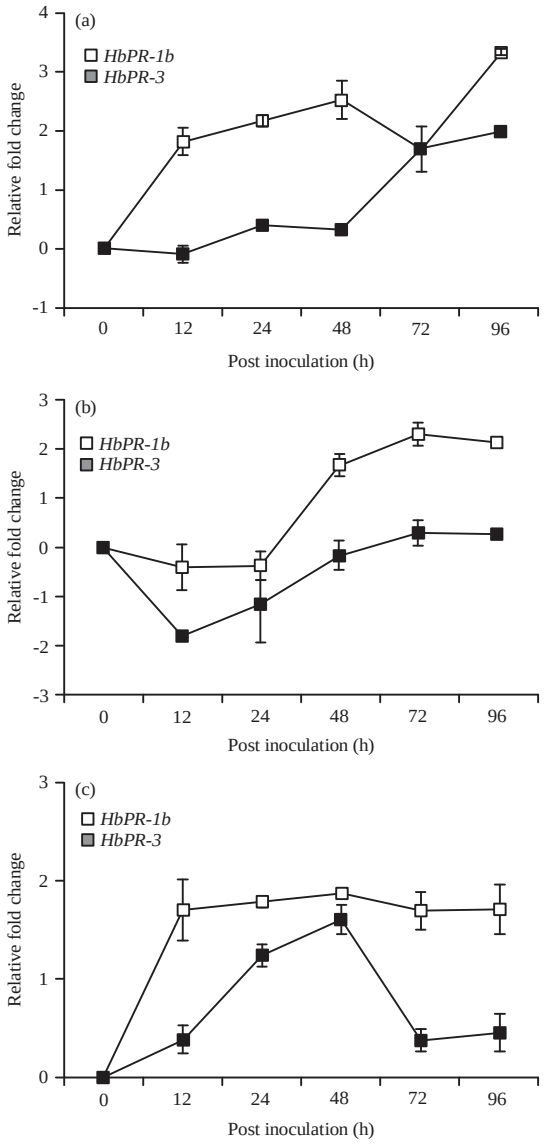

Fig. 8(a-c): Expression profile of *HbPR-1b* and *HbPR-3* after inoculation with *R. microporus* compare with mock inoculation (a) PB5/51, (b) RRIM600 and (c) BPM24

of two rubber clones, RRIM 2002 (tolerant clone) and PB 350 (susceptible clone) were detected after inoculation with *Neofusicoccum ribis* at four-period intervals using semi-quantitative RT-PCR. The expression patterns of these genes were expressed by up and down-regulations and varied with time. The inoculation trial indicated that the gene expressions were higher in RRIM 2002 than in PB 350[44]. PR-proteins are therefore important tools for understanding the molecular markers of plant response to fungal attack.

CONCLUSION

The expressional variability of two *PR*-genes was demonstrated that contribute to the establishment of white root disease tolerant in the rubber tree. The *HbPR-1b* and *HbPR-3* can distinguish between tolerant and susceptible clones and exhibited more transcript levels in PB5/51 than the others. Therefore, accumulations of *HbPR-1b* and *HbPR-3* may be considered as a promising criteria to assess the tolerance of new rubber clones against *R. microporus*.

SIGNIFICANCE STATEMENTS

This study discovers the positive regulation of *HbPR-1b* and *HbPR-3* genes under tolerant rubber clones, particularly *HbPR-1b*. The result from this study could be used as the early selection of rubber rootstock for white root disease tolerance.

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