

# Molecular Characterization of $\beta$ -1,3-Glucanase Gene in Pathogenic Fungi Tolerant and Susceptible Rubber Tree Clones

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## ABSTRACT

$\beta$ -1,3-glucanase is a major lutoidic protein in lactiferous cells of rubber tree. It belongs to a pathogenesis-related (PR) protein family and is classified in PR2 group. It has been reported that the expression of  $\beta$ -1,3-glucanase gene was induced by pathogenic fungi and wounding. In this research, to understand the relation of  $\beta$ -1,3-glucanase gene and pathogenic fungi tolerance in rubber tree, the expression pattern of  $\beta$ -1,3-glucanase gene was analyzed in non-infected plants of pathogenic fungi tolerance rubber tree clone (BPM24) and susceptible one (RRIM600) using quantitative real time polymerase chain reaction (qRT-PCR). In addition, the promoter of  $\beta$ -1,3-glucanase gene obtained from whole genome shotgun contigs in the National Center for Biotechnology Information (NCBI) database of BPM24 and RRIM600 rubber tree clones were analyzed *in silico*. The results showed that the expression of  $\beta$ -1,3-glucanase gene was significantly higher in BPM24 than RRIM600. Many *cis*-acting regulatory elements responsive to several hormones, biotic and abiotic stresses were found on  $\beta$ -1,3-glucanase promoters. Higher number of defense responsive elements was found on the  $\beta$ -1,3-glucanase promoter of BPM24 than RRIM600. The knowledge obtained from this study leads to a better understanding of the expression and regulation of  $\beta$ -1,3-glucanase gene in rubber tree which is beneficial for the improvement of rubber tree clone through pathogenic fungi resistance breeding program.

**Keywords:**  $\beta$ -1,3-glucanase, Pathogenesis-related proteins, Rubber tree

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## Introduction

Rubber tree (*Hevea brasiliensis*) is an important economic crop of Thailand. It is a major commercial source of natural rubber which is obtained from latex after tapping the rubber tree trunk. One of the incidences affecting latex yield of rubber tree is pathogen infection which results in significant economic loss [1]. The pathogenesis-related (PR) proteins were induced in plant in order to defend against pathogenic infection. Nowadays, 17 different families of PR proteins are classified according to their properties and functions [2]. For example, the activity of  $\beta$ -1,3-glucanase, a protein belonging to the PR2 family, was increased after infection of potato leaves by *Phytophthora infestans* [3].  $\beta$ -1,3-glucanase is a hydrolytic enzyme that catalyzes the cleavage of 1,3- $\beta$ -D glycosidic linkages in  $\beta$ -1,3-glucans present in the cell wall of many pathogenic fungi [4]. In rubber tree,  $\beta$ -1,3-glucanase is a luteoidic protein that played roles in coagulation process of rubber particles and defense mechanism against pathogen infection [5]. This protein was induced by pathogen infection and wounding. Many rubber tree clones were reported to be resistant to pathogen infection. It was reported that  $\beta$ -1,3-glucanase was highly accumulated in pathogenic fungi tolerant rubber tree clones, such as BPM24 [6], PB5/51 [6], RRII105 [7], RRIM2002 [8] and RRIT251 [9], after pathogen infection. Havanapan et al. (2016) [10] performed the comparative proteome of rubber tree latex serum from non-infected plants of pathogenic fungi tolerant BPM24 and susceptible RRIM600 clones. One of the proteins higher accumulated in BPM24 (a tolerant clone) than in RRIM600 (a susceptible clone) was  $\beta$ -1,3-glucanase [10]. This study was performed to verify the expression level of  $\beta$ -1,3-glucanase gene in BPM24 and RRIM600 rubber tree clones under non-infected condition through qRT-PCR technique in order to understand the relation of this gene and pathogenic fungi tolerance of rubber tree. In addition, to understand the transcriptional regulation, the promoters of  $\beta$ -1,3-glucanase gene from BPM24 and RRIM600 were also analyzed *in silico*. The information obtained from this study will lead to a better understanding of the expression and regulation of  $\beta$ -1,3-glucanase gene in rubber tree and be beneficial for improvement of pathogenic resistant rubber tree clone in rubber tree breeding program.

## Materials and Methods

### Plant materials

The latex samples were obtained from 10-year-old of BPM24 and RRIM600 rubber tree clones planted in the same plot at Chiang Rai Horticultural Research Center, Thailand. All of rubber trees used in the experiment were non-infected. Three trees of each clone were selected for their homogeneous girth and latex yield. The tapping system is half spiral downward cut, at

one day tapped and one day rest (S/2 d2). After discarding the first 20 drops, 6 ml of latex from each tree were collected in 6 ml of 2X fixation/extraction buffer (50 mM Tris-HCl, 300 mM LiCl, 10 mM EDTA, 10% SDS, pH 9.0). The latex samples were immediately deep-frozen in liquid nitrogen and then stored at  $-80^{\circ}\text{C}$  before RNA extraction.

#### **Phylogenetic tree construction**

The phylogenetic tree was constructed based on the  $\beta$ -1,3-glucanase amino acid sequences from *H. brasiliensis* and various plants retrieved from the NCBI database. The phylogenetic tree was constructed by MEGA7.0.26 using Maximum Likelihood method and Jones-Taylor-Thornton (JTT) model [11]. Support for the tree obtained was assessed using the bootstrap method with 1000 replications. The phylogenetic tree was edited by FigTree version 1.3.1 and Microsoft PowerPoint 2013.

#### **RNA extraction and cDNA synthesis**

Total RNA was extracted using LiCl precipitation method adapted from Pujade-Renaud et al. (1994) [12] and developed by Kongsawadworakul et al. (2004) [13]. The amount and purity of RNA were determined by Nano drop<sup>®</sup> ND-1000 UV-Vis Spectrophotometer at the wavelength of 260 and 280 nm. Total RNA was treated with DNase I (DNA-free<sup>™</sup>, Ambion) to remove contaminating DNA from RNA preparation. The first-strand cDNA synthesis was performed using SuperScript<sup>™</sup> III First-Strand Synthesis System for RT-PCR (Invitrogen). For every sample, the first-strand cDNA synthesis and qRT-PCR amplifications were performed in triplicate.

#### **Gene expression analysis by qRT-PCR**

The qRT-PCR was performed with the ABI-7500 fast real-time PCR system (Applied Biosystems, Foster City, CA, USA). The amplifications were performed using 2  $\mu\text{l}$  of the 1:25 diluted cDNA, Platinum<sup>®</sup> Taq DNA Polymerase (Invitrogen, Life Technologies) and specific primers. The specific primers of  $\beta$ -1,3-glucanase gene were designed from the GenBank (<http://www.ncbi.nlm.nih.gov>) nucleotide sequence accession number HBU22147 using Primer3 Plus and OligoAnalyzer 3.1 programs (Table 1). Polymerase chain reaction (PCR) was performed for 40 cycles. Two-step PCR included denaturation at  $95^{\circ}\text{C}$  for 15 sec and annealing as well as polymerization at  $60^{\circ}\text{C}$  for 1 min. The relative quantity of target gene transcript, compared to a reference of housekeeping gene *actin*, was automatically calculated as  $2^{-\Delta\text{Ct}}$  where  $\Delta\text{Ct} = \text{Ct of target gene} - \text{Ct of reference gene}$ .

**Table 1. Primers used for qRT-PCR.**

Genes	Forward (5' → 3')	Reverse (5' → 3')
<i>β-1,3-glucanase</i>	GCC TAA CAG AGC TAT AGA GAC T	CAT GGA TAC ATG CAT ACA TTG G
<i>Actin</i>	AGT GTG ATG TGG ATA TCA GG	GGG ATG CAA GGA TAG ATC

### Promoter analysis

The promoter regions were acquired from whole genome shotgun contigs of *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. (taxid:3981) in the NCBI database. To investigate the cis-acting responsive elements, the promoter region was analyzed by NewPLACE (<https://sogo.dna.affrc.go.jp>).

### Statistical analysis

The significant difference of *β-1,3-glucanase* gene expression, obtained from qRT-PCR, between different rubber tree clones was analyzed by Independent-Samples T Test with 99% confidence interval. Statistical analysis was conducted by IBM SPSS Statistics 23 and Microsoft Excel 2013.

## Results and Discussion

### Phylogenetic analysis of *β-1,3-glucanase*

A phylogenetic tree based on amino acid sequence of Hb *β-1,3-glucanase* with other homologs reported from different plants was classified into 2 major clades including dicotyledons and monocotyledons. The Hb-*β-1,3-glucanase* (AAA87456.1) was classified into dicotyledons clade and malpighiales sub-clade with *β-1,3-glucanase* from *Manihot esculenta*, *Linum usitatissimum* and *Populus euphratica*. Amino acid sequence of Hb *β-1,3-glucanase* showed closely related to cassava (*M. esculenta*) Me *β-1,3-glucanase* with 79.7% similarity. Both rubber tree and cassava belongs to the Euphorbiaceae family. The function of *β-1,3-glucanases* from these plants might be conserved. *β-1,3-glucanases* hydrolyze *β-1,3-glucan*, a major component of the surface structure and cell wall of many pathogenic fungal and insects [14]. Me *β-1,3-glucanase* gene was significantly up-regulated during the hypersensitive reaction (HR) of leaves in response to an incompatible *Pseudomonas syringae* pv. tomato which indicated that this protein might be one of the candidate gene to assist cassava breeding program [15].

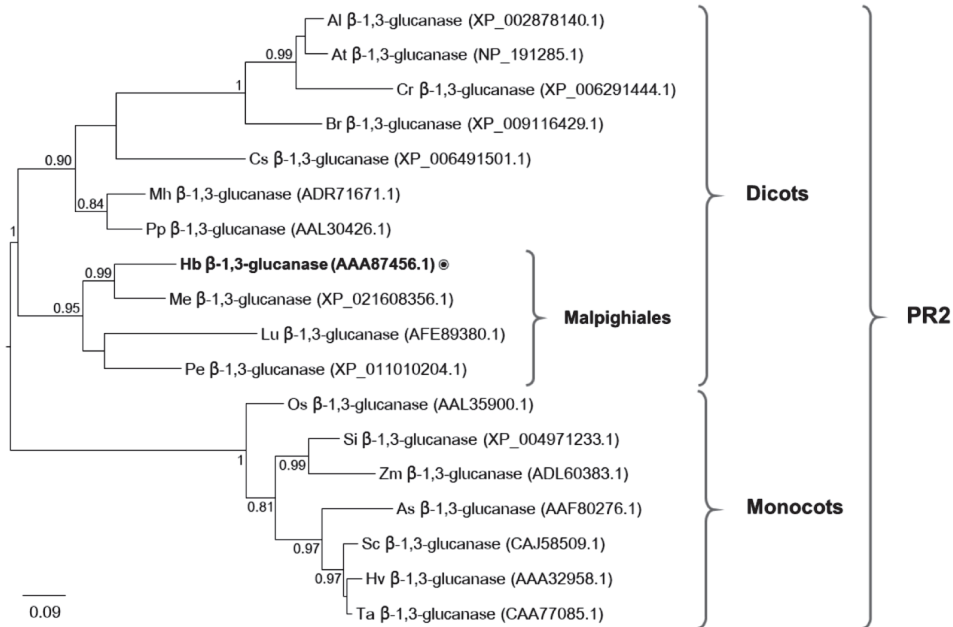
### Expression pattern of *β-1,3-glucanase* gene

The qRT-PCR was performed to investigate the differential expression of *β-1,3-glucanase* gene in BPM24 and RRIM600 rubber tree clones. Interestingly, the expression level of *β-1,3-glucanase* gene was significantly higher in pathogenic fungi tolerant clone BPM24 than

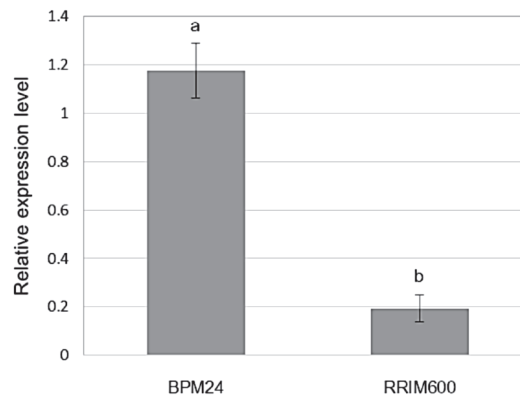
susceptible clone RRIM600 (Fig. 2). Our results were in agreement with the previous study [10] which showed that  $\beta$ -1,3-glucanase protein was highly abundant in tolerant BPM24 clone comparing to the susceptible RRIM600. It has also been reported that the expression of  $\beta$ -1,3-glucanase gene in the tolerant clone (RRII105) was higher in the intensity and longer for the duration of response to *Phytophthora meadii* than the susceptible clone (RRIM600) [7]. In addition, after infection with *Neofusicoccum ribis*, the expression of  $\beta$ -1,3-glucanase was significantly higher in tolerant clone (RRIM2002) than in the susceptible one (PB350) [7]. In 2017, Woraathasin et al. [6] studied the expression of *PR2* ( $\beta$ -1,3-glucanase), *PR4* (low molecular mass chitinase) and *PR5* (thaumatin) genes in roots of tolerant (PB5/51) and susceptible (BPM24 and RRIM600) rubber tree clones infected with *Rigidoporus microporus*. The expression results showed that *PR2* gene was up-regulated in PB5/51 and RRIM600 but down-regulated in BPM24. *PR4* gene was up-regulated in PB5/51 and BPM24 but down-regulated in RRIM600. However, *PR5* gene was significantly up-regulated in all clones [6]. This evidence confirmed the expression level of PR genes in tolerant clone and pathogen resistance in rubber tree may not be dependent on single gene expression. The co-expression of multiple PR gene families in plant defense mechanism against pathogenic fungi was more effective than a single gene [6]. Besides the rubber tree, the expression of  $\beta$ -1,3-glucanase was increased after infection with various pathogens in many host plants including *Manihot esculenta* [15], *Triticum aestivum* [16], *Hordeum vulgare* [17], *Populus euphratica* [18] and *Linum usitatissimum* [19]. Moreover, the expression of  $\beta$ -1,3-glucanase was also increased by salicylic acid, methyl jasmonate, ethylene, gibberellin and wounding [20, 21].

#### Promoter analysis of $\beta$ -1,3-glucanase gene

$\beta$ -1,3-glucanase promoters of BPM24 and RRIM600 rubber tree clones were obtained from whole genome shotgun contigs of *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. (taxid:3981) in the NCBI database. The promoter sequences of  $\beta$ -1,3-glucanase in BPM24 and RRIM600 were 3,000 bp and 2,129 bp respectively. The *cis*-acting regulatory elements (CAREs) on  $\beta$ -1,3-glucanase promoters were analyzed by NewPLACE and classified into eight groups consisting of promoter consensus, hormone response, defense response, mineral response, organ-specific expression, light response, water stress response and miscellany. The percentage of each CAREs group was shown in Figure 3. The results showed that the expression of  $\beta$ -1,3-glucanase gene was not only induced by pathogen infection but also by several hormones, salt stress and wounding [21, 22]. Due to the function of  $\beta$ -1,3-glucanase as PR protein, this study focused on CAREs involving in defense response. The percentage of defense responsive CAREs on  $\beta$ -1,3-glucanase promoters was about 4% in both of BPM24 and RRIM600 (Figure 3 A, B).

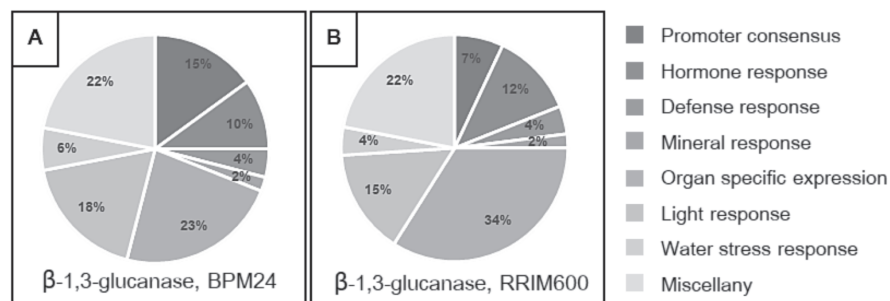


**Figure 1** Phylogenetic tree based on the  $\beta$ -1,3-glucanase amino acid sequence from *H. brasiliensis* (Hb) and various plants. The phylogenetic tree was constructed by MEGA7.0.26 using Maximum Likelihood method and Jones-Taylor-Thornton (JTT) model [11]. The various plants are as follows: Al (*Arabidopsis lyrata*), As (*Avena sativa*), At (*Arabidopsis thaliana*), Br (*Brassica rapa*), Cr (*Capsella rubella*), Cs (*Citrus sinensis*), Hb (*Hevea brasiliensis*), Hv (*Hordeum vulgare*), Lu (*Linum usitatissimum*), Me (*Manihot esculenta*), Mh (*Malus hupehensis*), Os (*Oryza sativa*), Pe (*Populus euphratica*), Pp (*Prunus persica*), Sc (*Secale cereal*), Si (*Setaria italica*), Ta (*Triticum aestivum*), Zm (*Zea mays*).

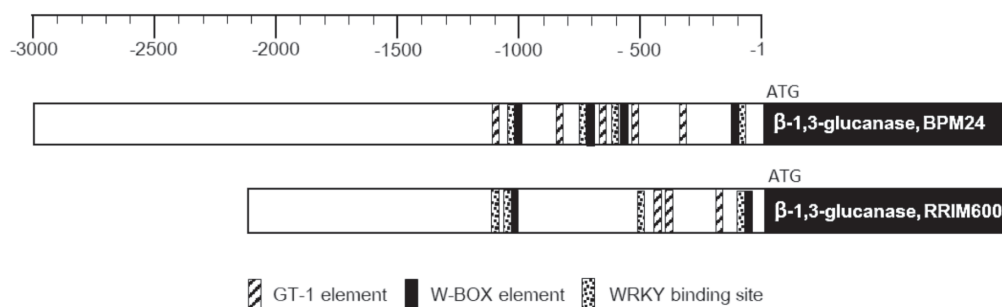


**Figure 2** Relative expression of  $\beta$ -1,3-glucanase gene in BPM24 and RRIM600 rubber tree clones. Data corresponded to the mean of nine independent replicates performed in triplicate and standard error ( $n=27$ ). Different letters indicated the significant difference of transcript levels within rubber tree clones (Independent-Samples T-Test,  $P < 0.01$ ).

Several sites of defense responsive elements on  $\beta$ -1,3-glucanase promoters of BPM24 and RRIM600 were shown in Figure 4. The higher number of defense responsive elements was found on the  $\beta$ -1,3-glucanase promoters of BPM24 than RRIM600. The defense responsive CAREs, including GT-1 (GAAAAA) element, W-box (TGACY), and WRKY (TGAC) binding site, were found differentially on the promoters of  $\beta$ -1,3-glucanase gene in BPM24 and RRIM600 rubber tree clones. GT-1 element played a role in the cellular responses to pathogen and NaCl-induction. SCaM-4 promoter deletion in *Arabidopsis* showed the increasing of GUS activity in construct containing GT-1 cis-element after treatment with pathogen and NaCl [22]. W-box element was reported to be the binding site of WRKY transcription factor. In parsley, WRKY bound specifically on the PR1-1 and PR1-2 promoters to induce plant defense resistance [23]. Interestingly, on the minus strand of  $\beta$ -1,3-glucanase promoter of RRIM600, the site of silencing element binding factor (SEBF) was found. The overexpression of SEBF in potato protoplasts repressed the activity of PR-10 suggesting the role of SEBF as transcriptional repressor [24].



**Figure 3** The pie charts reveal percentage of each CAREs group available on  $\beta$ -1,3-glucanase promoter of BPM24 (A) and RRIM600 (B).



**Figure 4** Defense responsive elements found on  $\beta$ -1,3-glucanase promoter.

## Conclusion

$\beta$ -1,3-glucanase was classified as PR2 protein induced by pathogen infection and wounding. From the result, the expression of Hb $\beta$ -1,3-glucanase gene in pathogenic fungi tolerant clone (BPM24) was higher than in pathogenic fungi susceptible clone (RRIM600). Considering for the result of Havanapan et al. (2016) [10], this showed the correlation of higher  $\beta$ -1,3-glucanase gene and protein in pathogenic fungi tolerant clone than in the susceptible one. The cis-acting regulatory elements (CAREs) on  $\beta$ -1,3-glucanase promoters of rubber tree clones BPM24 and RRIM600 were analyzed. The verification of CAREs within the  $\beta$ -1,3-glucanase promoter sequences leads to a better understanding of transcriptional regulation of  $\beta$ -1,3-glucanase gene. Concerning to the function as PR protein,  $\beta$ -1,3-glucanase may be one of the candidate genes for genetic transformation of rubber tree to produce disease resistant clones. However, the plant defense mechanism against pathogenic fungi infection required multiple PR genes. The expression of other PR genes should be further investigated to provide a better understanding of pathogenic fungi tolerant mechanism in rubber tree. The integration of results such as these with field-based and molecular genetic analysis of disease resistance should shorten rubber tree breeding program by assisting breeders to select or modify potentially resistant cultivars.

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## References

1. Viswanathan, P. K., Kadavil, T. G., & Jacob, C. K. (2005). Crop loss in rubber due to abnormal leaf fall: an analysis on the economic feasibility of plant protection measures in India. *Journal of Plant Protection Research*, 45(4), 235-248.
2. Moosa, A., Farzand, A., Sahi, S. T., & Khan, S. A. (2018). Transgenic expression of antifungal pathogenesis-related proteins against phytopathogenic fungi—15 years of success. *Israel Journal of Plant Sciences*, 65(1-2), 38-54.



3. Kombrink, E., Schröder, M., & Hahlbrock, K. (1988). Several “pathogenesis-related” proteins in potato are 1,3- $\beta$ -glucanases and chitinases. *Proceedings of the National Academy of Sciences*, 85(3), 782-786.
4. Simmons, C. R. (1994). The physiology and molecular biology of plant 1, 3- $\beta$ -D-glucanases and 1,3; 1,4- $\beta$ -D-glucanases. *Critical Reviews in Plant Sciences*, 13(4), 325-387.
5. Subroto, T., van Koningsveld, G. A., Schreuder, H. A., Soedjanaatmadja, U. M., & Beintema, J. J. (1996). Chitinase and  $\beta$ -1, 3-glucanase in the lutoid-body fraction of *Hevea* latex. *Phytochemistry*, 43(1), 29-37.
6. Woraathasin, N., Nualsri, C., & Nakkanong, K. (2017). Expression responses of pathogenesis-related proteins in tolerant and susceptible *Hevea brasiliensis* clones to the white root disease. *Pakistan Journal of Biotechnology*, 14(2), 141-148.
7. Thanseem, I., Joseph, A., & Thulaseedharan, A. (2005). Induction and differential expression of  $\beta$ -1,3-glucanase mRNAs in tolerant and susceptible Hevea clones in response to infection by *Phytophthora meadii*. *Tree Physiology*, 25(11), 1361-1368.
8. Ngobisa, A. N., Ntsomboh-Ntsefong, G., Yun, W. M., Dzarifah, M. Z., & Ndongo, P. O. (2016). Transcriptional expression of three putative pathogenesis-related proteins in leaves of rubber tree (*Hevea brasiliensis*) inoculated with *Neofusicoccum ribis*. *Journal of Applied Biology & Biotechnology*, 4(2), 11-15.
9. Sunpapao, A., & Pornsuruya, C. (2015). Overexpression of  $\beta$ -1, 3-glucanase gene in response to *Phytophthora palmivora* infection in leaves of *Hevea brasiliensis* clones. *Walailak Journal of Science and Technology*, 13(1), 35-43.
10. Havanapan, P. O., Bourchookarn, A., Ketterman, A. J., & Krittanai, C. (2016). Comparative proteome analysis of rubber latex serum from pathogenic fungi tolerant and susceptible rubber tree (*Hevea brasiliensis*). *Journal of Proteomics*, 131, 82-92.
11. Jones, D. T., Taylor, W. R., & Thornton, J. M. (1992). The rapid generation of mutation data matrices from protein sequences. *Bioinformatics*, 8(3), 275-282.
12. Pujade-Renaud, V., Clement, A., Perrot-Rechenmann, C., PrevÔt, J. C., Chrestin, H., Jacob, J. L., & Guern, J. (1994). Ethylene-induced increase in glutamine synthetase activity and mRNA levels in *Hevea brasiliensis* latex cells. *Plant Physiology*, 105(1), 127-132.
13. Kongsawadworakul, P., Peret, B., Pellegrin, F., Nandris, D., & Chrestin, H. (2004). The ethylene/cyanide metabolic crossroad in *Hevea* bark and latex. In *Proceedings of IRRDB Seminar on Hevea Physiology and Breeding* (pp. 331-345). Kunming, China.
14. Bowles, D. J. (1990). Defense-related proteins in higher plants. *Annual Review of Biochemistry*, 59(1), 873-907.

15. Kemp, B. P., Beeching, J. R., & Cooper, R. M. (2005). cDNA-AFLP reveals genes differentially expressed during the hypersensitive response of cassava. *Molecular Plant Pathology*, 6(2), 113-123.
16. Münch-Garhoff, S., Neuhaus, J. M., Boller, T., Kemmerling, B., & Kogel, K. H. (1997). Expression of  $\beta$ -1,3-glucanase and chitinase in healthy, stem-rust-affected and elicitor-treated near-isogenic wheat lines showing *Sr5*-or *Sr24*-specified race-specific rust resistance. *Planta*, 201(2), 235-244.
17. Jutidamrongphan, W., Andersen, J. B., Mackinnon, G., Manners, J. M., Simpson, R. S., & Scott, K. J. (1991). Induction of beta-1,3-glucanase in barley in response to infection by fungal pathogens. *Mol Plant Microbe Interact*, 4, 234-238.
18. Zemanek, A. B., Ko, T. S., Thimmapuram, J., Hammerschlag, F. A., & Korban, S. S. (2002). Changes in  $\beta$ -1,3-glucanase mRNA levels in peach in response to treatment with pathogen culture filtrates, wounding, and other elicitors. *Journal of plant physiology*, 159(8), 877-889.
19. Wróbel-Kwiatkowska, M., Lorenc-Kukula, K., Starzycki, M., Oszrński, J., Kepczyńska, E., & Szopa, J. (2004). Expression of  $\beta$ -1, 3-glucanase in flax causes increased resistance to fungi. *Physiological and Molecular Plant Pathology*, 65(5), 245-256.
20. Strompen, G., Grüner, R., & Pfitzner, U. M. (1998). An as-1-like motif controls the level of expression of the gene for the pathogenesis-related protein 1a from tobacco. *Plant Molecular Biology*, 37(5), 871-883.
21. Wu, C. T., & Bradford, K. J. (2003). Class I chitinase and  $\beta$ -1,3-glucanase are differentially regulated by wounding, methyl jasmonate, ethylene, and gibberellin in tomato seeds and leaves. *Plant Physiology*, 133(1), 263-273.
22. Park, H. C., Kim, M. L., Kang, Y. H., Jeon, J. M., Yoo, J. H., Kim, M. C., ... & Yoon, H. W. (2004). Pathogen-and NaCl-induced expression of the *SCaM-4* promoter is mediated in part by a GT-1 box that interacts with a GT-1-like transcription factor. *Plant Physiology*, 135(4), 2150-2161
23. Rushton, P. J., Torres, J. T., Parniske, M., Wernert, P., Hahlbrock, K., & Somssich, I. E. (1996). Interaction of elicitor-induced DNA-binding proteins with elicitor response elements in the promoters of parsley PR1 genes. *The EMBO Journal*, 15(20), 5690-5700.
24. Boyle, B., & Brisson, N. (2001). Repression of the defense gene *PR-10a* by the single-stranded DNA binding protein SEBF. *The Plant Cell*, 13(11), 2525-2537.