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Research

Genome-Wide Identification of β-1,3-Glucanase Genes in *Hevea brasiliensis*

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ABSTRACT

β-1,3-glucanase is one of the pathogenesis-related proteins well-known for their antifungal properties which can be abundantly found in *Hevea brasiliensis*. Utilization of β-1,3-glucanase in the genetic improvement of *H. brasiliensis* is very important as the high susceptibility to various fungal infections has challenged the current natural rubber industry. A few nucleotide sequences for β-1,3-glucanase have been reported and their role in biotic stress management has been demonstrated. Being a multigene family, it is necessary to identify and characterize more isoforms of β-1,3-glucanase to select the most suitable isoform to be utilized in genetic improvement. In the current study, we conducted a genome-wide identification of β-1,3-glucanases in *H. brasiliensis*, their classification based on the functional domains and phylogenetic analysis, using different bioinformatics tools. All publicly available nucleotide sequences were collected and curated by eliminating sequences that lack glycoside hydrolase family 17 (GH 17) domain as well as the partial and closely identical sequences and obtained 14 full-length sequences. The sequences were categorized into 4 distinct classes (I-IV) based on their functional domains and C-terminal extension. Class III and IV which lack the carbohydrate-binding C-terminal X8 domain are the largest classes identified with 5 β-1,3-glucanase each while 4 β-1,3-glucanase contain a variable C-terminal X8 domain. Phylogenetic analysis showed the clustering of β-1,3-glucanases into six major clades (I-VI) based on the domains. Clades I and II were identified as the largest clades with 4 β-1,3-glucanase in each. Several paralogous clusters have been observed for *H. brasiliensis* indicating the gene family expansion within the species or in the immediate ancestors with possible species-specific function. Further functional characterization is necessary to select the suitable gene to be utilized in genetic improvement and the present study provides a platform for it.

Key words: β-1,3-glucanase, comparative genomics, disease resistance, *Hevea brasiliensis*, pathogenesis-related proteins

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INTRODUCTION

Rubber can be classified into two types based on its source: synthetic rubber and natural rubber. Natural rubber (NR) is a biopolymer derived from rubber-producing plants, whereas synthetic rubber (SR) is a polymer of alkenes or dienes derived from the petrochemical industry (Men *et al*., 2019). NR is utilized in manufacturing sectors such as motor, electrical, and medical industries (Nair, 2021). It has been massively used to produce heavy-duty tires, high-performance engineering components, and surgical gloves (Lau *et al*., 2016; Men *et al*., 2019). NR is irreplaceable in many industrial applications because of its distinct properties which synthetic rubber lacks: resilience, elasticity, abrasion and impact resistance, efficient heat dispersion, and malleability at cold (Cataldo, 2000; Cornish, 2001).

Although NR can be harvested from around 2500 rubber-producing plants from diverse taxa, most plants have unfavorable characteristics, such as poor rubber production and low polymer molecular weight (Van Beilen & Poirier, 2007). Within the genus *Hevea*, *Hevea brasiliensis* is the most exclusive species for producing an economically viable amount of NR, accounting for the majority of the world's NR production (99%) (Clément-Demange *et al*., 2007; Men *et al*., 2019). As a monoecious tropical tree belonging to the family Euphorbiaceae and native to South America's Amazon basin, it is now mainly grown in tropical Southeast Asia countries, such as Malaysia, Indonesia, India, Sri Lanka, and Thailand (Archer & Audley, 1987; Clément-Demange *et al*., 2007; Lau *et al*., 2016; Priyadarshan, 2017). The NR industry of *H. brasiliensis* has faced challenges due to its specific growth environments, high susceptibility to

various fungal infections, laborious harvesting, and allergic response to the latex proteins (Mooibroek & Cornish, 2000; Prakash *et al*., 2004).

The susceptibility of *H. brasiliensis* to fungal diseases is determined by the climatic conditions and cultural techniques used (Nair, 2021). Pathogenesis-related (PR) proteins can be induced in plants upon pathogenic infection and release of exogenous chemicals (Radhakrishnan *et al*., 2021). It can involve systemic acquired resistance (SAR), which can offer a broad spectrum and long-lasting resistance to secondary infections by generating multiple defense signals such as salicylic acid (SA) (Gao *et al*., 2014). The production of PR protein is also known as inducible expression systems as PR proteins are produced upon pathogen attack and promoters of the genes encoding PR proteins are highly regulated by the signals produced by the pathogens (Radhakrishnan *et al*., 2021).

β-1,3-glucanases (E.C. 3.2.1.39) are PR proteins belonging to the PR-2 family (Leubner-Metzger & Meins, 1999). They can be commonly found in bacteria, fungi, viruses, and various plant species, including *Arabidopsis*, rice, tobacco, and soybean (Xu *et al*., 2016). Other than their essential involvement in plant physiological and developmental processes, β-1,3-glucanases are also known for their antifungal activity and significant role in the pathogen defense mechanism (Doxey *et al*., 2007). There are two strategies used to defend themselves against pathogen invasion: constitutive and induced defense mechanisms. During the constitutive defense, β-1,3-glucanases function as a catalyst in the hydrolysis of 1,3-β-D-glycosidic linkages in β-1,3-glucans, a major structural component of fungal cell walls (Legentil *et al*., 2015; Xu *et al*., 2016). During the induced defense, it promotes the release of cell wall-associated immune elicitors, further stimulating defense reactions and indirectly evoking hypersensitive responses (HR) (Ebel & Scheel, 1992; Boiler, 1995; Leubner-Metzger & Meins, 1999). Due to their diverse physiological roles, β-1,3-glucanases can be found in multiple structural isoforms with different sizes, iso-electric points, primary structures, cellular localization, and regulation patterns (Radhakrishnan *et al*., 2021). Each β-1,3-glucanase comprises an N-terminal signal peptide (NTS), a C-terminal domain, and a glycoside hydrolase family 17 (GH-17) functional domain. Based on the diversity of its C-terminal domain, β-1,3-glucanase has been further classified into different categories (Doxey *et al*., 2007). It has been reported that β-1,3-glucanase express differentially in susceptible and tolerant clones on exposure to *Phytophthora meadii*. Despite the β-1,3-glucanase genes being present in both tolerant and susceptible clones, only the tolerant clones showed prolonged gene expressions. The transcript levels of β-1,3-glucanase showed a marked increase in the leaves of both clones 48 hr after the inoculation. However, it dropped drastically at 96 hr in the susceptible clone while it remained high in the tolerant clone (Thanseem *et al*., 2005).

Although *H. brasiliensis* is the exclusive species that supply the most promising yield of NR, its high susceptibility to various fungal diseases has caused a considerable amount of NR yield losses yearly (Radhakrishnan *et al*., 2021; Yeang *et al*., 2002). Present-day, β-1,3-glucanases has gained interest in the research field due to their antifungal properties. Therefore, the complete characterization of β-1,3-glucanases is vital to develop novel *H. brasiliensis* clones with enhanced disease resistance. As a multigene family, identification, and characterization of the complete set of β-1,3-glucanase genes are necessary to find the suitable isoforms to be utilized for developing *H. brasiliensis* clones with better disease resistance. Recent advancements in next-generation sequencing and the availability of whole genome assemblies for *H. brasiliensis* provide a good opportunity to mine more isoforms. In the present study, we performed a genome-wide identification of β-1,3-glucanase in *H. brasiliensis* and a comprehensive analysis of sequence identity, functional domains, and phylogeny using various bioinformatics tools.

MATERIALS AND METHODS

Identification and curation of β-1,3-glucanases genes in the *H. brasiliensis* genome

The nucleotide sequences of β-1,3-glucanases genes were obtained from the draft genome of *H. brasiliensis* clone RRIM 600 (Rahman *et al*., 2013) (Rubber Genome Browser. http://bioinfo.ccbusm.edu.my/cgi-bin/gb2/ gbrowse/Rubber/), nucleotide browser and genome assembly of clone Reyan 7-33-97 (https://www.ncbi.nlm.nih.gov/genome/gdv/browser/genome/?id=GCF_001654055.1), Rubber Genome and Transcriptome Database USM-RIKEN (http://ricefox.psc.riken.jp/rubber/search.html) and Nucleotide collection in National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/nuccore/?term=%CE%B2-1%2C3-glucanases+). Using all the sequences obtained as queries, more nucleotide sequences were obtained from NCBI nucleotide collection (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome) and various *H. brasiliensis* assembly databases such as ASM165405v1 (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch&PROG_DEF=blastn&BLAST_SPEC=Assembly&UID=20322323),ASM1045892v1 (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch&PROG_DEF=blastn&BLAST_SPEC=Assembly&UID=118663904), HbrasiliensisBPM24_01 (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch&PROG_DEF=blastn&BLAST_SPEC=Assembly&UID=19747773) and ASM190799v1 (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch&PROG_DEF=blastn&BLAST_SPEC=Assembly&UID=19313073) by Basic Local Alignment Search Tool (BLAST) tool. No particular E-value was set as a cut-off point to detect a maximum number of sequences. Accession numbers were manually checked and duplicated sequences were eliminated.

The nucleotide sequences were translated into amino acid sequences using the ExPASy translate tool (https://web.expasy.org/translate/) and were subjected to domain analysis using NCBI Conserved Domain Database (CDD) to identify the presence of glycoside hydrolase family 17 (GH-17) domain. The sequences which lack the GH-17 domain were eliminated. Identity between the sequences was determined by local blast using CLC Genomics Workbench (CLC bio, Denmark). Sequences with E-value < 10-5 and percentage identity ≥ 90% were grouped and thus the sequences were classified into different clusters. The sequences in each cluster were aligned using Clustal W (Thompson *et al*., 1994) multiple sequence alignment and pairwise sequence alignment in BioEdit version 7.2 (Hall, 1999). Among the sequences with very high identity (99%), only one sequence which is full length and has duplicates in different databases or shows the highest identity to other sequences was retained and others were eliminated.

Sequence characterization and classification of β-1,3-glucanases genes in *H. brasiliensis*

Multiple sequence alignment of the selected nucleotide sequences, as well as amino acid sequences, was conducted using Clustal W (Thompson *et al*., 1994) tool in BioEdit version 7.2 (Hall, 1999), and the identity between the sequences was determined. The functional domains in the amino acid sequences were identified using NCBI CDD and Simple Modular Architecture Research Tool (SMART) Protein Analysis (http://smart.embl-heidelberg.de/index2.cgi). The sequences were classified based on the presence of domains additional to GH-17.

Phylogenetic analysis of β-1,3-glucanase genes

To conduct a phylogenetic analysis of β-1,3-glucanase genes, a total of 60 amino acid sequences of β-1,3-glucanase genes from *H. brasiliensis* and other species representing different taxonomic groups (*Arabidopsis thaliana*, *Nicotiana tabacum*, *Populus szechuanica*, *Populus tremula x Populus alba*, *Prunus persica*, *Manihot esculenta*, *Brassica rapa* subsp. *Chinensis*, *Brassica oleracea* and *Medicago sativa*) were aligned using Clustal W multiple sequence alignment in BioEdit version 7.2. The amino acid sequences from other plants were obtained from NCBI protein sequence collection. The aligned sequences were exported to MEGA v11.0.13 software (Tamura *et al*., 2021) and a phylogenetic tree was generated using the Neighbor-Joining method (Saitou & Nei, 1987) with 1000 bootstrap resamplings (Felsenstein, 1985). The p-distance method was adapted to compute the evolutionary distance (Nei & Kumar, 2000). All gaps and missing data were removed with a final number of 117 positions in the phylogenetic tree.

RESULTS

Identification and curation of β-1,3-glucanases genes in the *H. brasiliensis* genome

To identify the full set of β-1,3-glucanase genes in the *H. brasiliensis* genome, nucleotide sequences of β-1,3-glucanase reported in 4 databases were obtained: draft genome sequence of RRIM 600, NCBI databases, a genome browser for clone Reyan 7-33-97 (ASM165405V1), Rubber Genome & Transcriptome Database USM-RIKEN. The draft genome sequence of RRIM 600 (Rahman *et al*., 2013) has reported 11 nucleotide sequences for β-1,3-glucanase from RRIM 600 while 56 sequences were reported from the same clone in Rubber Genome & Transcriptome Database USM-RIKEN. 28 and 18 sequences were obtained from NCBI nucleotide databases and the genome browser for clone Reyan 7-33-97 (ASM165405V1) respectively. Furthermore, sequences were obtained from the NCBI nucleotide database and different genome assemblies for *H. brasiliensis.* After curation 14 full-length sequences were obtained designated as Glucanase (GLU) 1-14. The number of sequences obtained from different databases and the number of full-length sequences obtained after curation is tabulated in Table 1.

Sequence characterization and classification of β-1,3-glucanases genes in *H. brasiliensis*

The manually curated sequences were translated into amino acid sequences. Both nucleotide and amino acid sequences were subjected to multiple alignments (Figure S1, Figure S2), and the identity between the sequences was determined (Table S1, Table S2). Glucanase 1 and Glucanase 2 showed the highest sequence identity at both nucleotide and amino acid levels, which are 96.1% and 93.3% respectively. Contrastingly, the lowest identity is between Glucanase 9 and Glucanase 10 at the nucleotide level (22.5%) while Glucanase 8 and Glucanase 10 have the lowest identity at the amino acid level (14.3%).

The β-1,3-glucanase sequences vary in length and structure. Among the 14 β-1,3-glucanase sequences, Glucanase 13 is the longest sequence with 495 amino acids whereas Glucanase 10 is the shortest sequence with 300 amino acids (Table 2). By using NCBI CDD and SMART Protein Analysis, functional domains of the sequences were identified and protein domain architectures of β-1,3-glucanases in *H. brasiliensis* were constructed as shown in Figure 1. β-1,3-glucanase genes were then categorized into 4 different classes as shown in Table 2 based on the presence/absence of functional domains and C-terminal extension.

Among the 14 full-length β-1,3-glucanase gene sequences, two sequences were identified as Class I, two as Class II, five as Class III, and five as Class IV Glucanase (Table 2). It was found that all sequences contain an N-terminal signal peptide (NTS) and a GH-17 domain. Class I Glucanase contains NTS, GH-17, X8 domain, and CTS while Class II Glucanase contains X8 domain without a CTS. Meanwhile, Class III Glucanase contains the NTS, GH-17, and CTS whereas Class IV comprises only NTS and GH-17 (Figure 1).

Table 1. The total number of a β-1,3-glucanase gene sequence in *H. brasiliensis*

Database	Number of β-1,3-glucanase sequences	Number of full-length sequences after curation
Draft genome sequence of RRIM 600 (Rahman *et al*., 2013)	11	14
NCBI nucleotide collection	28	
Genome browser for clone Reyan 7-33-97 (ASM165405V1)	18	
Rubber Genome & Transcriptome Database USM-RIKEN	56	

Table 2. Classification of β-1,3-glucanase gene sequences in *H. brasiliensis* based on protein domain

Class	Glucanase	Protein Length (aa)	Molecular Weight (kDa)	Isoelectric Point (pH)
I	Glucanase 12	484	52.69	5.14
	Glucanase 13	495	53.86	5.52
II	Glucanase 8	460	51.32	8.21
	Glucanase 9	466	51.92	8.01
III	Glucanase 1	374	41.16	7.61
	Glucanase 2	374	41.37	8.24
	Glucanase 3	373	40.86	9.03
	Glucanase 11	447	49.57	5.78
	Glucanase 14	382	42.27	8.7
IV	Glucanase 4	343	37.51	8
	Glucanase 5	345	37.9	4.78
	Glucanase 6	345	37.73	6.87
	Glucanase 7	347	38.17	8.64
	Glucanase 10	300	32.87	4.82

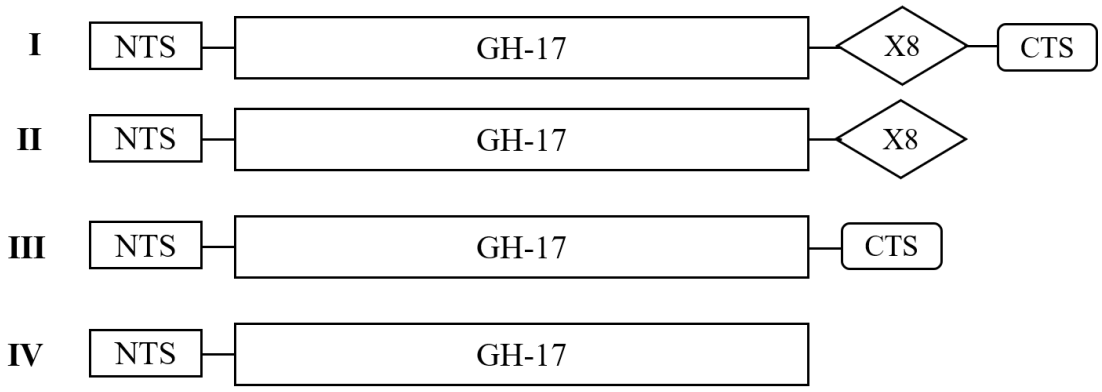

Fig. 1. Protein domain architectures of β-1,3-glucanase gene sequences in *H. brasiliensis*. I –IV: different classes of β-1,3-glucanase sequences based on the presence of functional domain. NTS: N-terminal sequence. GH-17: glycosyl hydrolase family 17 domain. X8: carbohydrate-binding domain. CTS: hydrophobic C-terminal sequence. These five architectural types are based on the presence/absence of the X8 domain and CTS in the C-terminal.

Phylogenetic analysis of β-1,3-glucanase genes in *Hevea brasiliensis*

A phylogenetic tree was constructed using 14 curated β-1,3-glucanase genes sequences from *H. brasiliensis* and 46 sequences from 7 other plant genera. The 14 β-1,3-glucanase sequences from *H. brasiliensis* were clustered along with the sequences from other plants into six major clades: Clade I, Clade II, Clade III, Clade IV, Clade V, and Clade VI as shown in Figure 2. Clade I contains GLU4, GLU5, GLU6, GLU7 (class IV); Clade II contains GLU1, GLU2, GLU3, GLU14 (class III); Clade III contains GLU10 (class IV); Clade IV contains GLU11 (class III); Clade V contains GLU8, GLU9 (class II); Clade VI contains GLU12, GLU13 (class I). The phylogenetic analysis reveals several paralogous groups among the β-1,3-glucanase genes in *H. brasiliensis* as GLU 4 and GLU 5; GLU 1, GLU 2, GLU 3 and GLU 14; GLU 8 and GLU 9; GLU 12 and GLU 13. This indicates the expansion of the β-1,3-glucanase gene family within the species or in an immediate ancestor.

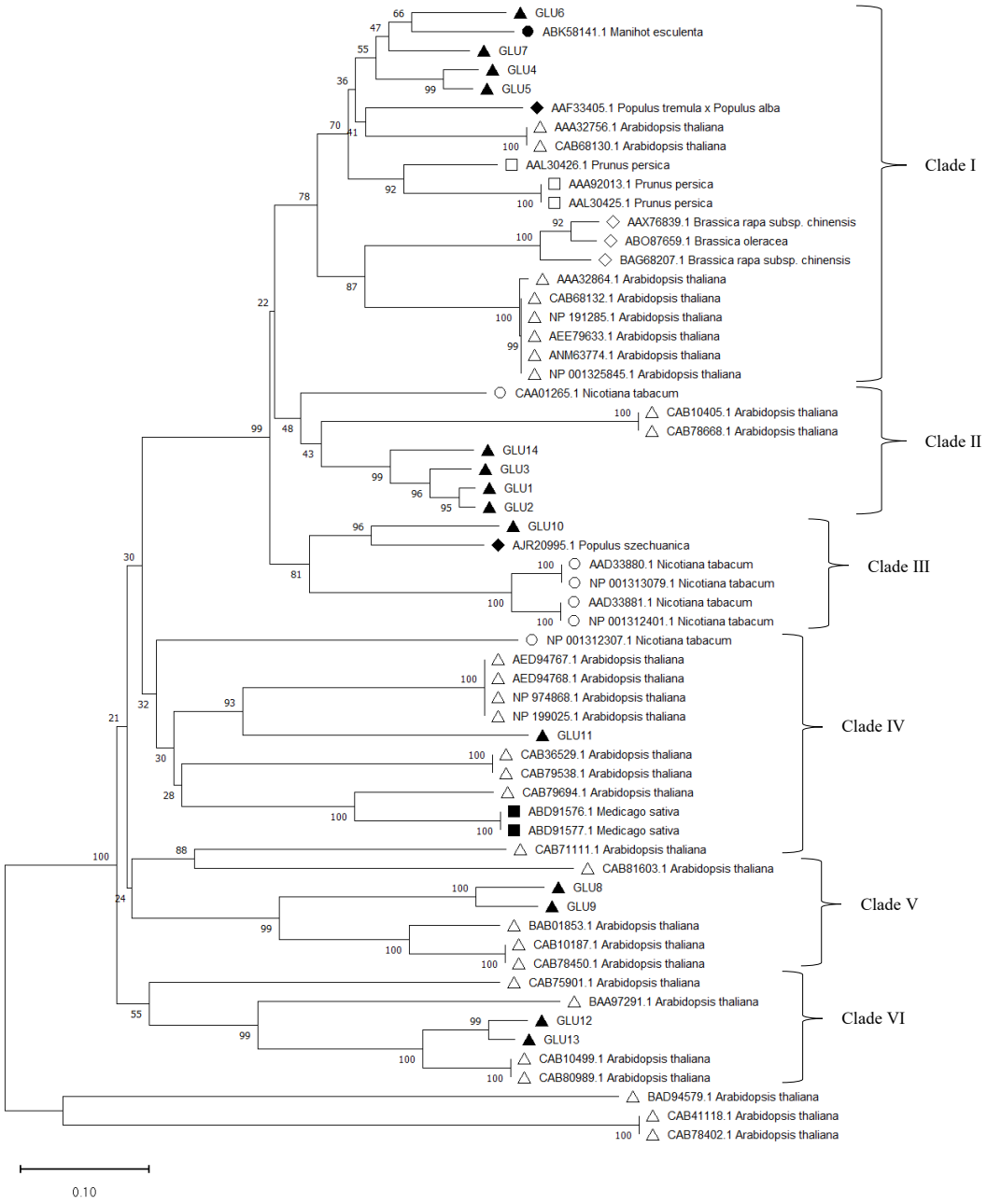

Fig. 2. Phylogenetic tree of β-1,3-glucanase genes built using Neighbor-Joining method with 1000 bootstrap resamplings in MEGA11. *Hevea* (▲); *Manihot* (●); *Populus* (♦); *Arabidopsis* (△); *Prunus* (□); *Brassisca* (◊); *Nicotiana* (○); *Medicago* (■).

DISCUSSION

ß-1,3-glucanases play a significant role in the defense mechanism against pathogenic disease due to their ability as a catalyst in the hydrolysis of 1,3-ß-D-glycosidic linkages in ß-1,3-glucans (Leubner-Metzger & Meins, 1999). Due to its cation-binding properties, glucanase has also assisted in regulating pH and ion homeostasis between the lutoids and the C-serum (Martin, 1991; Van Parijs *et al*., 1991; D'Auzac *et al*., 1995; Kanokwiroon *et al*., 2008). The findings of Wang *et al*., (2013), which many previous studies have supported, have shown that rubber particle aggregation (RPA) can be substantially induced by glucanase and hevein in a lectin-like manner synergistically, while chitinase has an inhibitory effect on RPA (Gidrol *et al*., 1994; Alenius *et al*., 1996; Subroto *et al*., 2001; Hagel *et al*., 2008; Wititsuwannakul *et al*., 2008). Moreover, as has been previously reported in many studies, β-1,3-glucanase with a molecular mass of 36 kDa, has been identified as an allergenic protein (IUIS code: Hev b 2) due to its specificity to IgE binding and skin reactivity *(Sunderasan et al*., 1995; Kurup *et al*., 2000; Yeang *et al*., 2002).

Several ß-1,3-glucanases sequences are available in the public domain from whole genome assemblies and individual depositions. However, the number of sequences obtained from different databases

varies. Moreover, many sequences were partial and lacked the GH-17 domain. Hence, the sequences obtained from various databases were manually curated and finally, 14 full-length sequences were identified.

The 14 full-length β-1,3-glucanases gene sequences were categorized into four classes based on the presence or absence of different domains. All sequences contain an NTS and a GH-17 domain as Doxey *et al*., (2007) reported. GH-17 domain is responsible for the catalytic activity in the hydrolysis of 1,3-ß-D-glucosidic linkages in ß-1,3-glucans a major structural component of fungal cell walls. Doxey *et al*., (2007) have reported 50 β-1,3-glucanases gene sequences from *Arabidopsis* and categorized them into five classes based on the diversity of the C-terminal region.

Class I and Class III Glucanase comprise a CTS that significantly encodes the transient transmembrane domain and vacuolar localization (Borner *et al*., 2002; Henrissat & Davies, 2000; Leubner-Metzger & Meins, 1999). On the contrary, Class II and Class IV lack CTS. In the present study, we observed the CTS in 7 out of the 14 sequences. In *H. brasiliensis*, two sequences were identified as Class I whereas according to Doxey *et al*., (2007) 25 out of 50 *Arabidopsis* β-1,3-glucanase genes belong to Class I which are basic proteins that comprise a CTS. Sequences with 2 CBM43 domain, which is designated as Class III in *Arabidopsis* (Doxey *et al*., 2007) and *Gossypium* (Xu *et al*., 2016) were not found in *H. brasiliensis*. A similar observation was found in *Theobroma cacao* and *Vitis vinifera* also (Xu *et al*., 2016). In the absence of a CTS, *Arabidopsis* Class II and III Glucanase are acidic proteins induced by pathogens and localized in the cell-extracellular spaces (Beffa *et al*., 1993; Leubner-Metzger & Meins, 1999; Doxey *et al*., 2007). Apart from that, Class IV and V are also acidic proteins but with distinct expression patterns compared to Class II and III proteins (Doxey *et al*., 2007). Tag1 is a novel class of *Arabidopsis* β-1,3-glucanase explicitly expressed in tobacco anthers (Bucciaglia & Smith, 1994). Similar to Class II and III, Tag1 is an acidic protein encoding an NTS that lacks the CTS (Leubner-Metzger & Meins, 1999).

In the current study, a variable C-terminal domain, known as the X8 domain has been identified in both Class I and Class II. This finding is to findings reported by (Doxey *et al*., 2007), in which the X8 domain (Henrissat & Davies, 2000) was pinpointed in 27 out of 50 sequences of *Arabidopsis* β-1,3-glucanase genes. X8 domain is a novel class of carbohydrate-binding modules (carbohydrate-binding modules family 43, CBM43), which is accountable for the binding of β-1,3-glucanases to β-1,3-glucan (Barral *et al*., 2005).

Xu *et al*., (2016) have reported 67 β-1,3-glucanase genes family in *Gossypium* which has been categorized into eight subfamilies based on their respective conserved protein domain architecture and intron/exon structure. Consistent with the finding of the β-1,3-glucanase gene family in *Arabidopsis*, there were five classes of β-1,3-glucanase in *Gossypium* and all 67 β-1,3-glucanase genes possess an NTS and a GH-17 domain (Doxey *et al*., 2007; Xu *et al*., 2016). Out of 67 β-1,3-glucanase genes, there were 39 β-1,3-glucanase genes (Class I, II, III) that contain CBM43 domain and 42 β-1,3-glucanase genes (Class I, IV) which contain CTS. Comparing the number of each class of β-1,3-glucanase in *Gossypium, A. thaliana*, *Theobroma cacao,* and *Vitis vinifera*, it was inferred that Class III was the smallest class, followed by Class V, IV, and II and I (Xu *et al*., 2016). However, in the present study, none of the sequences were identified to be in Class III, and Class I is the smallest group with 2 genes. Since the majority of β-1,3-glucanase genes are present in Class I, it was suggested that Class I with an NTS, a GH-17 domain, a CBM43 domain, and a CTS are the ancestral glucanase in plants. Doxey *et al*., (2007) suggested that the evolution of new β-1,3-glucanase was attributable to the loss of the C-terminal region and the alteration of expression patterns of duplicated genes due to the acquiring or losing of regulatory cis-elements during the evolutionary process.

In the current study, the phylogenetic tree was constructed with β-1,3-glucanase amino acid sequences from *H. brasiliensis* and other related species. The sequences from *H. brasiliensis* were clustered into six clades (I-VI). The clustering is depending on the presence of specific domains. All sequences in Clade II contain CTS whereas CTS is absent in Clade I. Furthermore, all sequences clustered in Clade V contain the X8 domain while it is absent in Clade I, II, III, and IV. Also, it was identified that all sequences in Clade VI contain both the X8 domain and CTS. This finding ties well with previous studies by Xu *et al*., (2016), wherein 123 β-1,3-glucanase genes from *G. raimondii*, *A. thaliana,* and tobacco were clustered into eight subfamilies and all sequences in subfamilies A, F, and H contained CTS, however, absent in subfamilies B and G. Besides, CBM43 domain was present in subfamilies B, D, F and G but absent in subfamilies E. Several paralogous clusters were noticed (GLU 4 & GLU 5; GLU 1, GLU 2, GLU 3 & GLU 14; GLU 8 & GLU 9; GLU 12 & GLU 13) which indicates the gene family expansion within the species or in an immediate ancestor. Further functional studies are necessary to understand the importance of gene family expansion.

CONCLUSION

A total of 14 β-1,3-glucanase gene sequences were identified from the *H. brasiliensis* genome. Based on the functional domains and C-terminal extension of the sequences, β-1,3-glucanase genes were categorized into 4 distinct classes. The largest class of β-1,3-glucanase in *H. brasiliensis* are Class III and IV with 5 sequences that contain the X8 domain, a novel class of carbohydrate-binding modules. According to the phylogenetic analysis, β-1,3-glucanase sequences can be clustered into six major clades (I-VI) based on the structural domains, with Clade I and II, as the largest clades. Paralogous clusters are observed in the tree indicating the expansion of the gene family within the species or in the immediate ancestor. The current study with the discovery of complete β-1,3-glucanase gene sequences in the *H. brasiliensis* genome provides a platform for further studies. In the future, studies can be extended to functional characterization and expression analysis of β-1,3-glucanase using different clones under various conditions, leading to the breeding of novel *H. brasiliensis* clones with enhanced disease resistance.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

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