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Genome-Wide Analysis and Differential Expression of Chitinases in Banana Against Root Lesion Nematode (*Pratylenchus coffeae*) and *Eumusa* Leaf Spot (*Mycosphaerella eumusae*) Pathogens

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Abstract Knowledge on structure and conserved domain of *Musa* chitinase isoforms and their responses to various biotic stresses will give a lead to select the suitable chitinase isoform for developing biotic stress-resistant genotypes. Hence, in this study, chitinase sequences available in the *Musa* genome hub were analyzed for their gene structure, conserved domain, as well as intron and exon regions. To identify the *Musa* chitinase isoforms involved in *Pratylenchus coffeae* (root lesion nematode) and *Mycosphaerella eumusae* (*eumusa* leaf spot) resistant mechanisms, differential gene expression analysis was carried out in *P. coffeae*- and *M. eumusae*-challenged resistant and susceptible banana genotypes. This study revealed that more number of chitinase isoforms (CIs) were responses upon *eumusa* leaf spot stress than nematode stress. The nematode challenge studies revealed that class II chitinase (GSMUA_Achr9G16770_001) was significantly overexpressed with 6.75-fold (with high fragments per kilobase of exon per million fragments mapped (FPKM)) in resistant genotype (Karthobiumtham-ABB) than susceptible (Nendran-AAB) genotype, whereas when *M. eumusae* was challenge inoculated, two class III CIs (GSMUA_Achr9G25580_001 and GSMUA_Achr8G27880_001) were overexpressed in resistant genotype (Manoranjitham-AAA) than the susceptible genotype (Grand Naine-AAA). However, none of the CIs were found to be commonly overexpressed under both stress conditions. This study reiterated that

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the chitinase genes are responding differently to different biotic stresses in their respective resistant genotypes.

Introduction

Banana is one of the most important tropical and subtropical fruit and staple crop grown in more than 130 countries in the world, but their production is constrained by biotic and abiotic stresses. Among biotic stresses, leaf spot diseases and nematodes are considered as important factors in reducing the crop yield. Yield loss due to leaf spot (*Mycosphaerella* spp.) and root lesion nematode (*Pratylenchus coffeae*) was estimated at 30 and 44 %, respectively [1, 2]. Although chemical control of leaf spot diseases and nematodes is effective, the use of chemicals of late widely related to loss of biodiversity and increased health hazards [3]. Under this circumstance, cultivation of high-yielding resistant cultivars is one of the important alternate strategies to manage these pest problems. However, developing resistance in ruling cultivar of banana through conventional breeding is a difficult task due to polyploidy nature and low fertility problem [4], and hence, genetic transformation of susceptible banana geno-types with resistant gene may be an alternative approach. Generally, plants are capable of expressing a range of enzymes that function by inhibiting invading pathogens. Cloning of such natural genes should be exploited for developing pathogen-resistant varieties. For this, under-standing of plant–pathogen interaction at molecular level is important for isolation and characterization of genes encoding resistance. Among different defense-related enzymes, the role of chitinases in hypersensitive reactions during induced host resistance in tissues followed by pathogen infection was demonstrated by several research workers [5]. Chitin-mediated resistance has been proved against fungal diseases in various crops such as rice, grapevine, and peanut [6–8] and nematode infection in rice and tomato [9]. Similarly in the case of banana, more accumulation of endochitinases and putative chitinase transcripts, which are involved in fungal cell wall degradation, was reported in *Mycosphaerella musicola*-challenged leaves [10]. Overexpression of chitinase was also observed in *P. coffeae*-challenged resistant banana genotype compared to susceptible genotype [11]. These findings emphasized the important role of chitinase for conferring resistance to various biotic stresses in banana.

On the contrary, expression of high level of chitinase in tobacco and rice transgenic plants has no detrimental effects on the growth of *Cercospora nicotianae* and *Spodoptera frugiperda*, respectively [12, 13]. Also, introgression of *Manduca sexta* chitinase in soybean [14] and *Trichoderma harzianum* chitinase in tobacco [15] did not provide enhanced resistance to nematodes. However, in general, induction of specific chitinase isoform in the incompatible host mostly proved to be effective against specific pathogens in several crops [16–18]. Therefore, it can be inferred that native and specific chitinase isoforms are highly effective to exert resistance in susceptible cultivar through transgenic approach [19]. These findings reinforces that success of developing transgenic plants in enhancing resistance to biotic stresses lies mainly on selection of specific chitinase isoforms, as each isoform may have different roles like antifungal and antifreeze activities [16, 20]. This type of selective activity is mainly because of variation in their sequences and three-dimensional structures of chitinase isoforms. Hence, knowledge on gene structure and on the conserved domains of the chitinase families and their expression profile under various pathogen invasions are important to develop transgenic plants with durable resistance. Therefore, the present study aimed at

identifying the most effective chitinase isoforms which are specific to pathogens like *Mycosphaerella eumusae* (*eumusa* leaf spot) and *P. coffeae* (root lesion nematode) resistance.

Materials and Methods

Nematode Challenging and Sample Collection for RNA Isolation

Previous screening studies for nematode resulted in the identification of Karthobiumtham (ABB) as resistant and Nendran (AAB) as susceptible genotypes [2]. Ten uniformly sized suckers of both cultivars each were collected from Research Farm, National Research Centre for Banana, Trichy, and planted in earthen pots containing sterilized mixture of soil, sand, and farm yard manure (2:1:1). Nematodes were extracted from infected Nendran roots through sieves and *P. coffeae* were confirmed through morphological characterization under microscope. *P. coffeae* nematodes were multiplied under carrot culture as per the protocol given by INIBAP. After 1 month, nematodes were extracted from axenic culture maintained on carrot disks in 1 % bactoagar medium and used for challenge inoculation. Individual healthy young roots of resistant and susceptible cultivars were selected from well-established plants and inserted into a small plastic cup through a convenient hole made at the lower half of the cup. The cup was filled with the same potting mixture mentioned above and inoculated with 3000 infective nematode juveniles. A control set was created but without nematode inoculation. The experiment was replicated three times. Root tissues were collected on 2, 3, 4, 5, 6, 7, and 8 days after inoculation from nematode-challenged and unchallenged plants of both cultivars and stored in −80 °C.

M. eumusae Challenging and Sample Collection for RNA Isolation

Two-month-old (four-leaf stage) secondary hardened tissue-cultured *eumusa* leaf spot resistant cv. Manoranjitham and susceptible cv. Grand Naine banana plants were kept under controlled condition with 95 % relative humidity and 22 °C. Then, fungal spores of 10^8/ml were inoculated by spraying on both surface of leaves, and leaf samples were collected from 6 to 260 h after pathogen inoculation at different time intervals. At the same time, leaf samples were also collected from unchallenged plants. Collected leaf samples were weighed, frozen with liquid nitrogen, and stored at −80 °C until used for RNA isolation.

Isolation of RNA, Construction of cDNA Library, and Illumina Deep Sequencing

Two grams of root tissues were used for extraction of total RNA using Agilent Plant RNA isolation mini kit (Agilent Technologies, Inc., USA) (product no. 5188–2780). RNA integrity was checked using Agilent's Bioanalyzer 2100 (Agilent Technologies, Inc., USA). Equal amount of RNA was taken from root tissues collected from nematode-challenged samples of resistant and susceptible cultivars independently and pooled together for the construction of challenged library for resistant and susceptible genotypes. Similar procedure was followed in unchallenged root samples for the construction unchallenged library. The same procedure was followed for *M. eumusae*-challenged and *M. eumusae*-unchallenged leaf samples of resistant and susceptible genotypes.

Transcriptome library for sequencing was constructed according to the IlluminaTruSeq RNA library protocol outlined in "TruSeq RNA Sample Preparation Guide" (part no. 15008136; Rev. A; November 2010). Briefly, the total RNA was denatured for 4 min at

elevated temperature (94 °C) in the presence of divalent cations and reverse-transcribed with Superscript II Reverse transcriptase by priming with random hexamers. Second-strand complementary DNA (cDNA) was synthesized in the presence of DNA polymerase I and RNaseH. The cDNA was cleaned up using AgencourtAmpure XP solid-phase reversible immobilization (SPRI) beads (Beckman Coulter). Illumina Adapters were ligated to the cDNA molecules after end repair and addition of "A"-base. SPRI cleanup was performed after ligation. The library was amplified using 11 cycles of PCR for enrichment of adapter-ligated fragments. The prepared library was quantified using Nanodrop and validated for quality by running an aliquot on High Sensitivity Bioanalyzer Chip (Agilent Technologies, Inc., USA). The libraries were made suitable for 100-bp paired end transcriptome sequencing in Illumina Hiseq platform with (Genotypic PVT. Ltd, Bangalore) insert size ranging from 160 to 340 bp.

DGE Analysis of *Musa* CIs

Banana RNA-Seq reads were mapped to the reference AA genome (*M. acuminata malacansis*) with TopHat2 (v2.0.4); transcripts were assembled; and their relative abundances were calculated using Cufflinks (v2.0.1) and then annotated independently. The summation of fragments per kilobase of exon per million fragments mapped (FPKM) values for every transcript associated with a particular gene gave the expression measurement. The details of the RNA-Seq reads are given in Supplementary Table S1. Transcripts were assembled, and their relative abundance was calculated using cufflinks. Based on the annotation of the reference genome, cufflinks of chitinase isoforms (CIs) alone were separated and used for comparative analysis. As a result four sets of cufflinks, CIs were derived for each biotic stress (*P. coffeae* and *M. eumusae*) from their respective resistant and susceptible genotypes and are referred as nematode-challenged resistant (NCR), nematode-unchallenged resistant (NUR), nematode-challenged susceptible (NCS), nematode-unchallenged susceptible (NUS), *M. eumusae*-challenged resistant (ECR), *M. eumusae*-unchallenged resistant (EUR), *M. eumusae*-challenged susceptible (ECS), and *M. eumusae*-unchallenged susceptible (EUS). The summation of number of FPKM genome sequence was determined to measure the expression level of each CI. From these FPKM values, differential expression of each CI was calculated based on fold value using the ratio of NUR/NCR, NCS/NCR, NUS/NCS, EUR/ECR, ECS/ECR, EUS/EUR, EUS/ECS, and EUR/ECR (Supplementary Tables S2 and S3). For the significance, differential gene expression (DGE) analysis was tested statistically and scores were corrected for multiple testing [21].

Musa Chitinase Sequence Analysis

The nucleotide and protein sequences of *Musa* chitinase were retrieved from *Musa* genome hub (http://banana-genome.cirad.fr/). Complete CD sequences were downloaded after removing the partial fragment sequences. The complete CDs were analyzed in NCBI conserved domain search (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) and were grouped into two classes GH 18 and GH 19. The GH18 class were further classified based on the individual domain predicted by SMART (http://smart.embl-heidelberg.de/), Pfam (http://pfam.sanger.ac.uk/) database. The GH 18 sequences were analyzed for the presence of its catalytic residues in scan prosite (http://prosite.expasy.org/scanprosite/). The exon–intron structural organization of the CIs was predicted in webscopio (http://www.webscipio.org) with both the coding sequences and genomic sequences. Protein subcellular localization was predicted in TargetP (http://www.cbs.dtu.dk/services/TargetP/), and signal peptide cleavage sites were predicted in signalP (http://www.cbs.dtu.dk/services/SignalP/). The chitinase protein

sequences of *Musa* sequences were loaded in molecular evolutionary genetics analysis (MEGA) 5 [22]. In MEGA, neighbor-joining method and distance algorithmic method were used to construct the phylogenetic tree and the bootstrap value was also adjusted to 1000 replicates.

Results and Discussion

Mining of Chitinase Genes in *Musa* Genome

The genome sequences of *Musa* available in the public domain (http://banana-genome.cirad.fr/) facilitated to identify the chitinase encoding genes by keyword search. A total of 26 chitinase sequences were downloaded from *Musa* AA genome. Of which only 14 sequences had complete ORF whereas the remaining 12 sequences were incomplete, partial CDs. The occurrence of partial CDs might be due to the presence of fragmented genes and/or sequencing gaps in the *Musa* whole genome sequences. The numbers of complete CDs obtained in *Musa* are lesser than other model crop species such as 24 genes in *Arabidopsis* and 37 genes in rice [9]. Based on the conserved domains and sequence similarity, these *Musa* chitinase CDs were grouped into three classes (I, II, and III) as against seven (I–VII) classes of chitinases observed in other plants [23, 24]. These chitinases belonged to glycoside hydrolase families 18 and 19 (GH 18 and GH 19) and each family contain seven sequences. Except IV, VI, and VII chitinase classes of GH 19 family others classes, namely I and II were observed in *Musa*. Similarly, though GH18 includes classes III and V, *Musa* genome was shown to devoid of class V chitinases. It was also revealed that other monocots such as rice and maize lack class V chitinase [25]. It is reported that glycoside hydrolase family 19 genes are almost exclusively present in plants, whereas glycoside hydrolase family 18 genes are distributed in various organisms, including animals, plants, fungi, and bacteria [26].

Chromosomal Position of CIs in *Musa* Genome

All the 26 complete/incomplete sequences were analyzed for their chromosomal position using banana genome hub (http://banana-genome.cirad.fr/). Among these, 24 sequences were positioned in linkage map (Fig. 1) whereas two sequences were grouped in unrandom chromosome. It was also observed that chitinases were distributed into the whole genome except four linkage groups 2, 4, 10, and 11. The nonlocalization of chitinase in some of the linkage groups indicated sparse dispersion in *Musa* compared to other model crops like Arabidopsis and rice [27]. Linkage groups 1 and 5 had minimum of one chitinase gene whereas linkage groups 3 and 8 had a maximum of seven chitinases. It was also reported that the highest number of chitinase sequences in the eight chromosome of maize suggested that this chromosomal segment may share the common ancestor [25]. The occurrence of maximum number of chitinases and their tight linkage is suggestive of the common ancestor of this chromosomal segment. It was also observed that complete CDs of CIs are distributed only in five linkage groups, namely 3 (four chitinases), 9 (three chitinases), 6 (two chitinases), 8 (three chitinases), as well as 7 and 1 (one chitinase) only. Of these, linkage groups 3 and 9 had both the chitinase families (GH 18 and GH 19), while linkage groups 7 and 8 had only GH 18 and linkage groups 1 and 6 had only GH 19 family.

It was observed that class I chitinases were distributed on linkage groups 3, 6, and 9 whereas class II chitinases were positioned on linkage groups 9 and 6. Similarly, class III genes were located on linkage groups 8, 3, 9, and 7. The clustering of class III chitinase gene (two genes) containing narbonin domain in linkage group 3 and three more class III CIs, which are
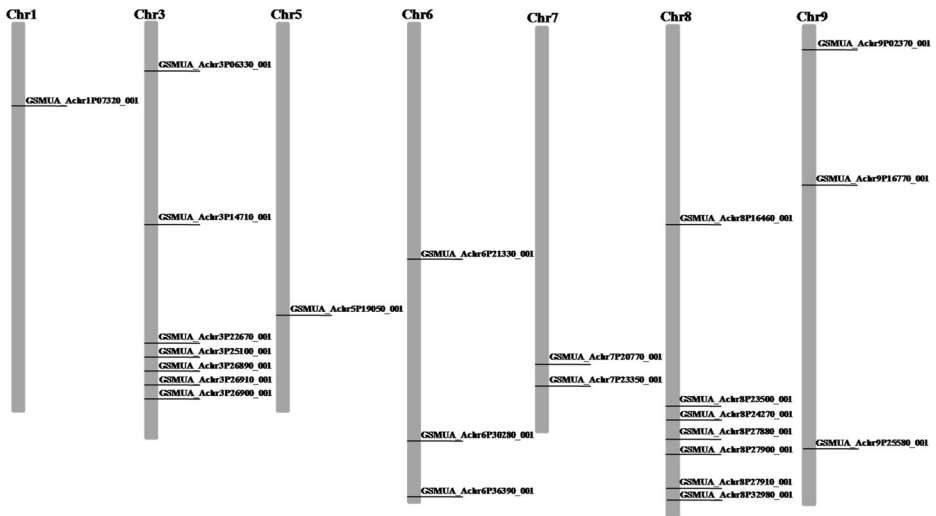
Fig. 1 Genomic localization of *Musa* CIs. The locus of each CI is marked in individual chromosomes with its CIRAD ID

having XIP domain, in linkage group 8 suggested that these clustering could be occurred due to tandem duplication.

Subcellular Localization and Signal Peptides of Chitinase

Our subcellular localization studies revealed that complete CDs of GH 18 family are located in secretory pathway except GSMUA_AChr 3P25100_001 which has mitochondrial location. In GH19, five sequences were located in secretory pathway and two sequences in mitochondria (Table 1). The results of subcellular localization of chitinases showed that majority of chitinases exist in secretory pathways, suggesting that these could be involved in the degradation of exo- or endo-chitin. Moeller et al. [28] also reported that proteins containing signal peptides are generally targeted to the secretory pathways. To determine the presence of signal peptide, chitinase protein sequences were analyzed in signalP program. In GH 18 family, except GSMUA_AChr 3P25100_001 and GSMUA_AChr 7P23350_001, all the sequences contained signal peptide cleavage sites. The location of cleavage site ranged from of 25 to 32. It was observed that XIP domain containing chitinases had signal peptide. Similarly, all the complete CDs of GH 19 family except for two GSMUA_AChr 3P26890_001 and GSMUA_AChr 6P36390_001 had signal peptide cleavage sites, and their cleavage position ranged from 20 to 36. The presence of the signal peptide in most of the chitinase genes irrespective of the GH family suggested that the protein is synthesized as a pre-protein, and cleavage occurs at the signal peptide cleavage site to form a mature protein that is targeted to the general secretary pathway of *Musa*.

Phylogeny of *Musa* Chitinases

The amino acid sequences of all the 14 complete CDs of chitinase were aligned and analyzed using the software MEGA. The analysis showed that these chitinase sequences were clustered into four well-supported subgroup. It was observed that all the classes (I and II) of GH 19 cluster under group I (Fig. 2). This is mainly because of all the family 18 chitinases have

Table 1 Details of domains, signal peptide region, and subcellular localization of *Musa* chitinase isoforms with complete CDs

CIRAD ID	Domain	Signal peptide region	Chromosomal position	Subcellular location
GSMUA_AChr 8P27880_001	Hevamine_XipI_class_III	Between 25 and 26	Chr8	Secretory pathway
GSMUA_AChr 3P25100_001	SI-CLP	–	Chr3	Mitochondrion
GSMUA_AChr 9P25580_001	Narbonin	Between 31 and 32	Chr9	Secretory pathway
GSMUA_AChr 8P27910_001	Hevamine_XipI_class_III	Between 25 and 26	Chr8	Secretory pathway
GSMUA_AChr 7P23350_001	Narbonin	–	Chr7	–
GSMUA_AChr 8P27900_001	Hevamine_XipI_class_III	Between 25 and 26	Chr8	Secretory pathway
GSMUA_AChr 3P22670_001	Hevamine_XipI_class III	Between 26 and 27	Chr3	Secretory pathway
GSMUA_AChr 3P26890_001	Hevein or type I chitin-binding domain	–	Chr3	Mitochondrion
GSMUA_AChr 6P36390_001	–	–	Chr6	Mitochondrion
GSMUA_AChr 9P16770_001	–	Between 35 and 36	Chr9	Secretory pathway
GSMUA_AChr 1P07320_001	–	Between 19 and 20	Chr1	Secretory pathway
GSMUA_AChr 3P26900_001	Hevein or type I chitin-binding domain	Between 19 and 20	Chr3	Secretory pathway
GSMUA_AChr 6P21330_001	Hevein or type I chitin-binding domain	Between 23 and 24	Chr6	Secretory pathway
GSMUA_AChr 9P02370_001	Hevein or type I chitin-binding domain	Between 21 and 22	Chr9	Secretory pathway

catalytic domains of triosephosphateisomerise (TIM barrel) fold with a conserved DxDxE motif [29] which catalyze the hydrolytic reaction by substrate-assisted mechanism [30], whereas family 19 chitinases have high percentage of alpha-helices and adopt the single displacement catalytic mechanism [31]. It is observed that class II is imbedded within the class I chitinases. It was also suggested that class I chitinase may arise from class II chitinase by insertion of chitin-binding domain [32].

All the chitinases of GH 18 family were clustered into three subgroups. The multiple sequence alignment (data not shown) also proved that these grouping were mainly based on the presence of catalytic domain and conserved domain. It was noticed that sequences containing the conserved catalytic motif GXDXDXE were grouped under group II and others under group III and IV. It was noticed that the entire hevamine or XIP domain containing class III chitinase of GH 18 family were clustered together (group II), whereas group III had one chitinase with stabilin-1 interacting chitinase-like protein (SI-CLP) domain and group IV had two chitnases which had narbonin domain.

Domains and Motifs of Chitinase

GH 18 family members of chitinases have a multidomain architecture with various combinations of signal peptide, catalytic domains, chitin-binding domain, and serine/threonine (S/T)
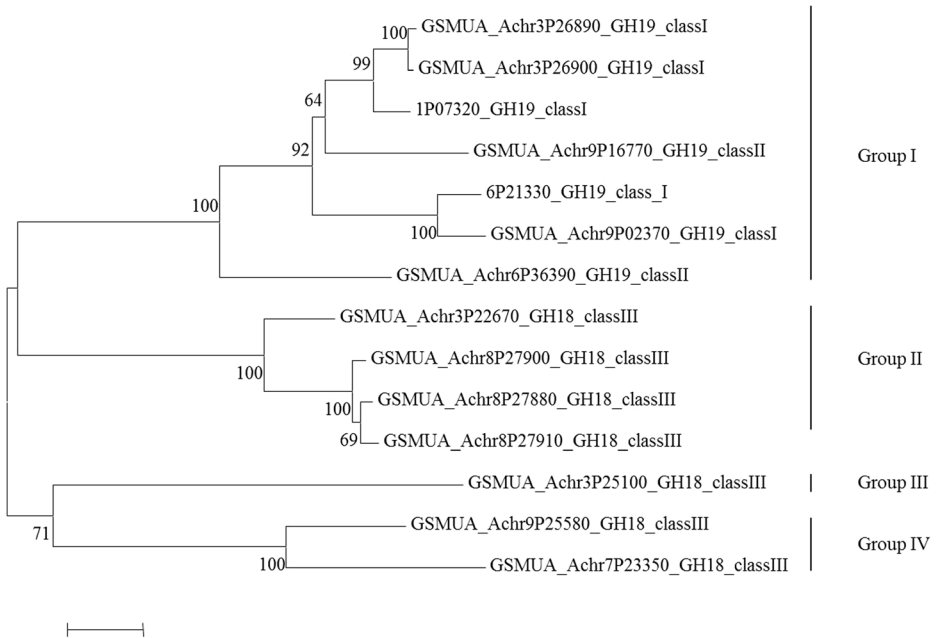
Fig. 2 Phylogenetic tree of *Musa* chitinase gene family that have complete CD sequences. Clustering pattern was indicated in groups (I, II, III, IV). The tree was constructed using the software MEGA 5.0 with bootstrap value of 1000 replicates. The bootstrap values are shown in the cladogram

rich linkers [33]. It was observed that all the members of GH18 family of *Musa* CIs contained three types of domains. Four out of seven GH 18 chitinases contained hevamine or XIP domain, and two sequences contained narbonin domain and one gene with SI-CLP domain (Table 1). Narbonin and SI-CLP domain containing chitinases found in plants are reported to have no enzymatic function [34]. It is a storage protein found in seeds and lack conserved catalytic residue [35]. SI-CLP also has no enzymatic activity. Only five out of seven chitinase genes of GH 18 family contained the conserved catalytic motif GXDXDXE. Out of seven members of GH19 family, only four had hevein or type I chitin-binding domain, and interestingly, none of the members of GH 19 contained any catalytic domain.

Exon and Intron Organization

The exon and intron distribution of all the complete CDs of chitinase revealed that the number of introns ranged from 1 to 4 (Fig. 3). Only four out of seven CIs of GH 18 family contained introns, whereas all the seven genes of GH 19 family had introns. Hence, based on the number of introns present, *Musa* chitinases were grouped into three: genes with no introns, genes with only one intron, and genes with two or more introns. The absence of intron region in three genes of GH 18 family, which belong to class III chitinases, exhibited high similarity with fungal chitinases (data not shown). Similar results were also observed by Jiang et al. [27] and reported that class III chitinases preceded the divergence between fungi and plants. Based on this theory, we could say that one of the class II genes and all the class I genes might have existed for a long time and then, intron loss occurred, and other members of class II and class IV genes appeared [36].
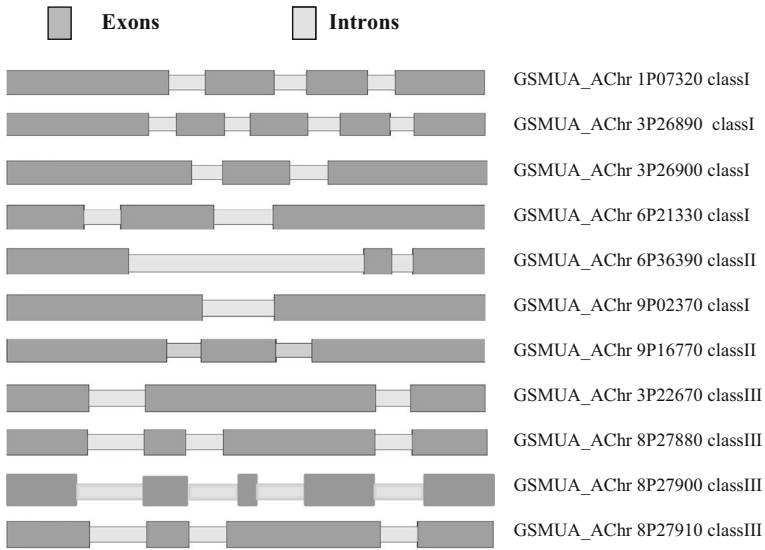
Fig. 3 Exon–intron structural organization of *Musa* CIs for the complete CD sequences. The exons are illustrated by *black boxes* and introns by *gray boxes*. The classes to which the CIs belongs are also marked along with its CIRAD ID

Expression Pattern of CIs in Nematode and *M. eumusae*-Challenged Contrasting *Musa* Genotypes

Generally, comparative transcriptome analysis of contrasting parents under stress conditions yield a novel candidate transcripts having association with tolerant/resistant mechanism [37]. Thus, in the present investigation, efforts were made to identify the differentially expressed CIs upon challenging with *P. coffeae* and *M. eumusae* by comparing the transcriptome profiling of their respective contrasting genotypes (Fig. 4). Pooled data of CI transcripts of each nematode-
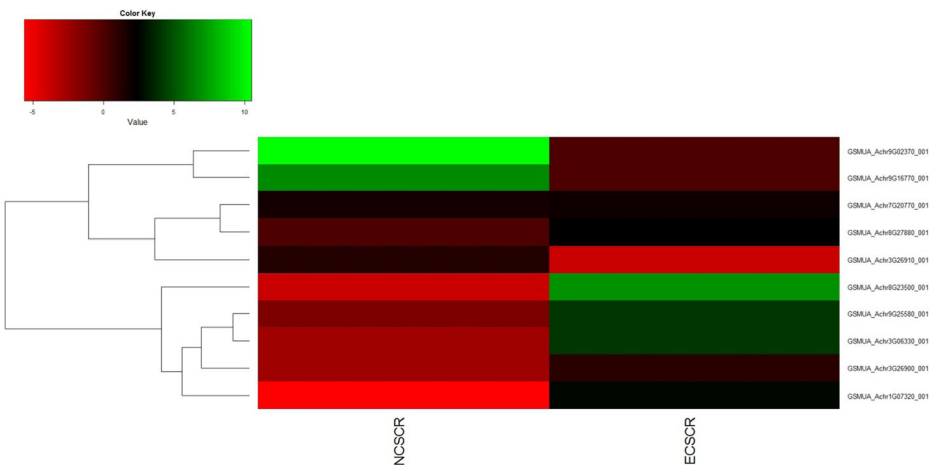

Fig. 4 Heat map expression profile of *Musa* CIs based on the ratio of normalized FPKM value between challenged susceptible and challenged resistant for nematode (*Pratylenchus coffeae*) and leaf spot (*Mycosphaerella eumusae*) stresses, respectively

challenged and nematode-unchallenged genotypes revealed that a total of 21 CIs were expressed in each resistant and susceptible genotypes. However, DGE analysis revealed that more number of CIs were overexpressed in NCS (15 CIs) as compared to NCR (12 CIs). Besides, expression of a unique CI was observed only in NCS. As less number of CIs were expressed in nematode-challenged resistant genotype when compared to susceptible one, it can be inferred that resistant pathways for this nematode may include not only chitin-induced pattern-triggered immunity (PTI) pathway but also other PTI pathways.

In the case of *M. eumusae*, pooled data of CI transcripts of contrasting genotypes to leaf spot resistance under *M. eumusae*-challenged and *M. eumusae*-unchallenged condition expressions of 19 CIs in each resistant and susceptible genotypes were observed (Table 2). However, more number of CIs was overexpressed in ECR (11) as compared to ECS (7). Similarly, the number of expression of unique CIs was more in resistant genotypes (six) than the susceptible one under *M. eumusae*-challenged condition. Thus, overexpression of more number of CIs (12) and the unique CIs (6) in ECR indicated the important role of chitin-induced PTI pathways for *eumusa* leaf spot resistance.

Stress Specificity of CIs in Banana

The specificity and diversity of the CIs were identified by comparing the expression of NCS/NCR and ECS/ECR ratios (Table 3) based on their significant ($P<0.05$) expression (>2-fold) (Table 3). In general, it was observed that only two classes of chitinases, namely class I (five) and class II (one), were significantly expressed in NCR, of which class II chitinase isoform (GSMUA_Achr9G16770_001) located in chromosome 9 was significantly overexpressed with 6.75-fold (with high FPKM value) in NCR as compared to NCS (Table 3). The comparison all the five CIs of class I based on the sequence similarity showed no similarity between overexpressed and underexpressed CIs of class I chitinase. Bagnaresi [19] also stated that identification of nematode-induced chitinase at the sequence level helps to narrow down the choice of chitinase isoforms for introgression into susceptible crops for developing resistance against nematodes. However, when comparison of was made between NUR and NCR for the expression of these two CIs, the CI of class I was overexpressed (2.01-fold) whereas CI of class II was underexpressed (−4.4-fold) in NCR. These findings indicate that the CI of class I located in chromosome 9 might be responsible for nematode resistance in resistant genotype. Hirao [38] also reported that expression level of class I chitinase was high in resistant than susceptible pine cultivar under nematode (*Bursaphelenchus xylophilus*) stress condition.

Table 2 Summary of expression of CIs under nematode and *M. eumusae* stress in their respective contrasting genotypes

Gene expression under various conditions	Nematode		M. eumusae		Common genes
	Resistant	Susceptible	Resistant	Susceptible	
Number of CIs expressed (under challenged and unchallenged condition)	21	21	19	19	19
Genes overexpressed under challenged condition	12	15	12	7	2
Genes expressed only after challenging	–	1	6	3	–
Genes downregulated under challenged condition	3	2	4	2	–
Genes expressed in challenged resistant and susceptible	21		19		17

Table 3 Transcript profile of differentially expressed *Musa* CIs under challenged with *Pratylenchus coffeae* and *Mycosphaerella eumusae* in their respective resistant and susceptible genotypes

CIRAD ID	Chromosome	Class	Nematode				M. eumusae			
			NCS (FPKM)	NCR (FPKM)	P value	Fold change	ECS (FPKM)	ECR (FPKM)	P value	Fold change
GSMUA_Achr1G07320_001[a]	1	Class I	12.15	2.16	0.00	−5.61	–	–	–	–
GSMUA_Achr3G26900_001[a]	3	Class I	80.43	31.43	0.02	−2.55	–	–	–	–
GSMUA_Achr9G02370_001[a]	9	Class I	0.09	1.04	0.01	10.49	–	–	–	–
GSMUA_Achr3G26910_001	3	Class I	–	–	–	–	61.53	16.62	0.00	−3.70
GSMUA_Achr7G20770_001	7	Class I	–	–	–	–	152.01	302.94	0.05	2.00
GSMUA_Achr8G23500_001	8	Class I	16.77	4.54	0.01	−3.69	1.25	8.67	0.00	6.92
GSMUA_Achr9G25580_001[a]	9	Class III	–	–	–	–	3.54	14.73	0.00	4.15
GSMUA_Achr3G06330_001	3	Class I	23.35	9.54	0.05	−2.44	1.27	5.43	0.00	4.26
GSMUA_Achr9G16770_001[a]	9	Class II	4.79	32.42	0.00	6.75	0	1.07	0.02	inf
GSMUA_Achr8G27880_001[a]	8	Class III	–	–	–	–	1.60	3.92	0.04	2.45

P values with less than 0.05 and ≥2-fold alone are shown in the table

NCS nematode-challenged susceptible genotype, *NCR* nematode-challenged resistant genotype, *ECS M. eumusae*-challenged susceptible genotype, *ECR M. eumusae*-challenged resistant genotype

[a] Indicates the complete CDS of *Musa* CIs

In *M. eumusae*-challenged resistant genotype, among six CIs expressed which belong to class I (four CIs) and class III (two CIs) chitinases, five CIs were overexpressed and one CI was underexpressed. Of the five overexpressed isoforms, three belonged to class I and two belonged to class III. Though three CIs of class I were overexpressed in ECR when compared to ECS, all these CIs were underexpressed in ECR as compared to EUR. These findings revealed that the high expression of class I CIs in EUR might be due to varietal character and may not have specific role in resistance mechanism against *M. eumusae*.

With regard to two CIs of class III which were overexpressed, one CI has hevamine or XIP domain belongs to acidic group and other CI has SI-CLP domain. The overexpression of acidic chitinase of class III CIs in ECR was also supported by Nielsen [39], who reported the potential involvement of acidic class III chitinase in response to invading pathogen (*Cercospora beticola*) in sugar beet. They also proved that overexpression of this chitinase was not observed in other plant pathogen interaction and emphasized that this chitinase is induced only upon specific pathogen invasion. Vasconcelos [40] proved that class III chitinase, which contains hevamine or XIP domain, act as xylanase inhibitors which inhibit the germination of fungal spores of *Phakopsora pachyrhizi* in soybean. On the other hand, the SI-CLP domain containing class III CI, which is a storage protein, was significantly overexpressed (4-fold) in ECR than ECS. The role of defense mechanism of storage protein of class III chitinase was also reported in *Medicago sativa* [41]. Thus, in the present study, the overexpression of two CIs of class III chitinase during banana–*M. eumusae* incompatible interaction suggested that these CIs might have a role in defense mechanism against *M. eumusae* in banana.

In conclusion, the present investigation has revealed that among the 21 *Musa* CIs identified, none of the CIs were commonly induced under both nematode- and *M. eumusae*-challenged conditions. However, it was observed that one CI of class I (GSMUA_Achr9G02370_001) and two CIs of class III (GSMUA_Achr9G25580_001 and GSMUA_Achr8G27880_001) chitinases were overexpressed in nematode and *M. eumusae*-challenged conditions, respectively, in their respective resistant genotypes. Thus, our study reiterated that different CIs are involved in different biotic stress-resistant mechanisms, and the differential expression of CIs under various biotic stresses emphasized that specific chitinase isoform, which is having resistant mechanism to specific stress, should be used for developing a resistance genotype for a specific stress through transgenic approaches.

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