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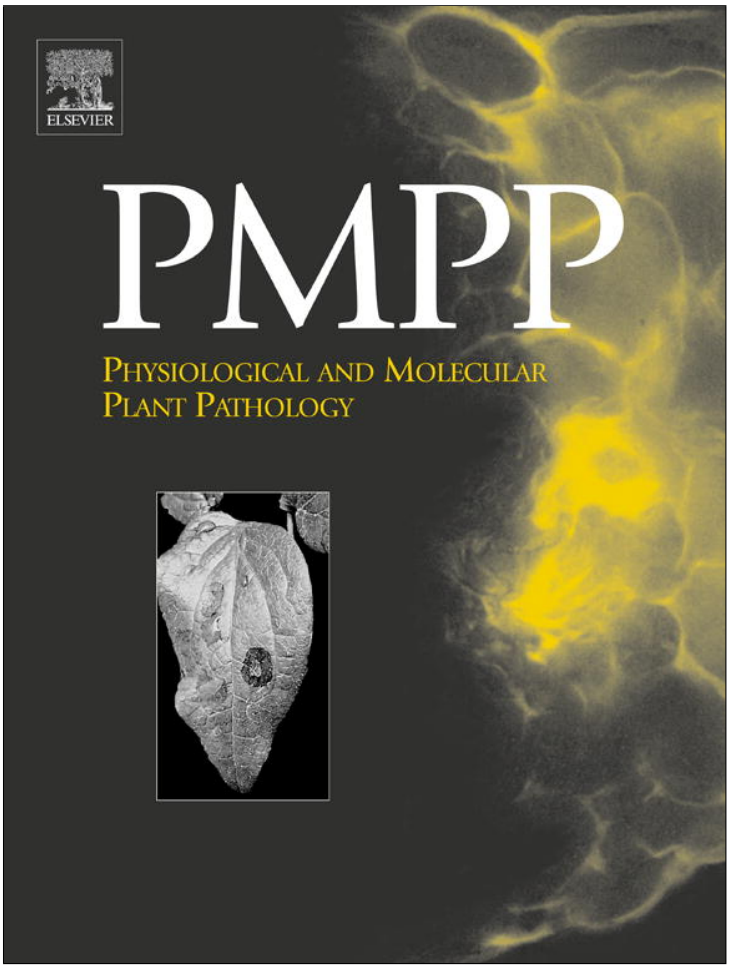

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Differentially expressed genes in incompatible interactions of *Pratylenchus coffeae* with *Musa* using suppression subtractive hybridization

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ABSTRACT

A cDNA library enriched with differentially expressed transcripts was constructed from *Pratylenchus coffeae* challenged resistant banana cultivar (Karthobiumtham-ABB) through suppression subtractive hybridization (SSH) technique to elucidate the nematode resistance mechanisms. A total of 256 unique genes of this SSH library were sorted into eight functional categories. Of these, 26.8% were unigenes involved in defense and/or signal transduction including resistant gene homologues, disease resistance response proteins and transcripts associated with disease related proteins and protein kinase signaling. These findings imply that invasion of nematode triggers multiple signaling pathways both through tissue damage caused by nematode invasion and recognition of nematode elicitors by R genes. Confirmation of defense specific representative genes through semi quantitative RT-PCR revealed their increased expression levels in resistant than in susceptible cultivar and the resistant cultivar triggered response much earlier than the susceptible cultivar.

1. Introduction

The migratory endoparasitic nematode, *Pratylenchus coffeae*, is a necrotrophic root lesion nematode that causes severe root and corm damage in banana. Due to their migratory feeding nature, initial dark purplish-red lesions appear on the root surface and as the disease progresses large necrotic areas are formed that can potentially affect the primary, secondary and tertiary roots. Additionally this damage acts as a food base and assists in the easy invasion of fungal pathogens of banana resulting in multiple infections [1]. For example, the necrotic damage caused by *P. coffeae* and the secondary infection of Fusarium wilt pathogen (*Fusarium oxysporum* fsp. *Cubense*) together can devastate banana plantations sometimes to the tune of 44–60% [2]. Intense *P. coffeae* root damages lead to the reduction of root system, decreased bunch weight, lengthy production cycle, weak anchorage and even root death resulting in toppling of plants and drastic crop losses at the major banana production zones [3]. The lesion nematode disease symptoms go unrecognized at the early stages as the infection occurs in belowground plant parts. It becomes hard and too late for the banana grower to realize the presence of these microscopic pests in order to take any pest control measures. To overcome this problem, sources of nematode resistance have been identified through intensive pot culture [2] and field screening studies [4], but heritability of the resistance has not been conclusively established. This hinders the banana improvement program [5].

Dominant nematode resistant genes (R-genes) have been identified mapped and integrated into breeding strategies for some crops but for banana owing to its parthenocarpy and sterile nature, mapping of resistant genes is a troublesome process [6]. In such cases knowledge on molecular mechanisms of plant responses to nematode infections helps in developing strategies to breed resistant varieties. There are extensive reports compiled on the molecular events following infection with various nematode species across the contrasting parents [7,8]. Over the past decade considerable attention has been given to study nematode resistance mechanism of banana against other nematodes like *Meloidogyne incognita* and *Radopholus similis*, but biochemical, molecular and cellular events in *Musa* following infection of root lesion nematodes have been rarely investigated. Although mechanism of resistance has been studied through biochemical [9] and histological methods [10] for the burrowing nematode *R. similis*, the same may not be applicable for the phylogenetically close *P. coffeae* as the culitvars

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resistant to *R. similis* are susceptible to *P. coffeae* [11]. The variation in the degree of resistance exhibited by the host plant may be due to the variation in the defense stimulating elicitors. The presence of a particular pathogen race or a particular host plant cultivar, or both may be the reason for specific resistance and accordingly a cocktail of defense mechanisms will be deployed during the infection process [12]. The underlying molecular basis of such defense mechanisms varies according to the genetic makeup of both host plant and the infecting pathogen. The most common mechanism for pathogen specific resistance are race-specific elicitors and host selective toxins [13]. This reinforces that resistant mechanism of banana would be nematode specific suggesting a need for the perceptive knowledge on *Musa–P. coffeae* interaction, but the information on this interaction is very meager. Unraveling the molecular mechanism of interaction between nematode and resistant cultivar and isolation of host genes related to resistant mechanism will be helpful in designing novel environmentally safe and sound strategies to manage this devastating pest and also to develop *P. coffeae* resistant banana varieties.

In this study, efforts were taken to understand the molecular mechanism of *Musa–P. coffeae* interactions in a resistant cultivar using subtractive suppression hybridization (SSH). The simple library construction procedure and decreased sequencing cost makes SSH an efficient functional genomics approach [14]. This technique suppresses the amplification of common transcripts and enriches the differentially expressed transcripts. It has been successfully employed to isolate differentially expressed genes from *Musa*–pathogen interaction such as Fusarium wilt [15], *Mycosphaerella eumusa* [16] and *Mycosphaerella fijiensis* [17]. We identified differentially expressed genes and particularly focused on a series of genes to assemble the molecular basis of the *Musa–P. coffeae* resistance mechanism.

2. Materials and methods

2.1. Plant materials and nematode inoculation

In our previous work, based on pot culture and biochemical studies, cvs. Karthobiumtham (ABB) and Nendran (AAB) were identified as resistant and susceptible to *P. coffeae* respectively [18]. Hence in the present study uniform size suckers of cv. Karthobiumtham were collected from National Research Centre for Banana farm, Trichy, Tamil Nadu, treated with nematicide and were planted in cement pots containing sterilized mixture of soil, sand and farm yard manure (2:1:1). *P. coffeae* was collected from root lesion infested roots of cv. Nendran, which is a highly susceptible, and multiplied in axenic culture maintained on carrot discs in 1% bactoagar medium. *P. coffeae* was extracted from this culture and used for inoculation. Inoculations were carried out following standard protocol of INIBAP [19]. Each cup was inoculated with 5000 active root lesion nematodes and ten plants were not inoculated with *P. coffeae*. A total of three replications with ten plants per replication were used for the study.

2.2. RNA and mRNA isolation

The root samples were taken at 1, 2, 3, 4, 5, 6, 7 and 8 days after inoculation (DAI) from nematode inoculated cup and un-inoculated plants. The time points were based on the previous time course expression studies [20]. The root samples were treated with 1% diethylpyrocarbonate (DEPC) and all the samples were immediately frozen by using liquid nitrogen and kept in −80 °C. Total RNA was extracted using the modified protocol of [21]. The RNA yield and quality were determined using bioanalyzer 2100 (Agilent Technologies). High quality RNA alone was used for mRNA isolation. Poly (A)+ RNA was purified from the total RNA using Qiagen kit following manufacturer's recommendations. The same procedure was also followed to obtain RNA samples from resistant and susceptible cultivars for semi-quantitative RT-PCR purposes.

2.3. SSH library construction

Total RNA isolated from root samples of cv. Karthobiumtham collected at different time intervals were equally pooled and used as tester. Similarly total RNA isolated from un-inoculated/mock root samples were used as driver. mRNA was isolated from tester and driver RNA and 300 ng of each mRNA was used for synthesizing the double stranded cDNA using subtraction kit (Clontech, U.S.A). Two rounds of subtractive hybridization and PCR amplification were performed according to manufacturer's instructions. The final PCR products were cloned into pGEM-T easy cloning vector and transformed into *E. coli* JM109 competent cells, which were then cultured in LB medium with IPTG and X-Gal. Individual white clones were picked and grown overnight at 37 °C. Well grown cultures were added in 15% Glycerol and stored at −80 °C.

2.4. DNA sequencing and data analysis

Plasmids of positive clones were isolated from overnight grown cultures using a HiYield™ plasmid Mini kit (Real Biotech Corporation, UK/USA) and were further validated by colony PCR using nested PCR primers provided in the kit. The positive clones were subjected to direct sequencing with M13 primers. The sequences were trimmed to eliminate the vector (http://www.ncbi.nlm.nih.gov/VecScreen) and the Poly A tails and adapter by using Trimest (http://emboss.bioinformatics.nl/cgi-bin/emboss/trimest). After trimming, sequences were assembled by using CAP3 (http://pbil.univ-lyon1.fr/cap3.php). The consensus sequences of contigs and singletons were annotated using BLAST2GO (http://www.blast2go.com).

2.5. Semi-quantitative PCR

Total RNA of resistant (cv. Karthobiumtham) and susceptible (cv. Nendran) cultivars were isolated from root samples of 0, 2, 4, 6, 7 and 8 DAI and purified as mRNA using Qiagen kit. First strand cDNA was synthesized from mRNA by reverse transcription with oligo-(dT) primers according to manufacturer's protocol (Promega). The first strand cDNA was diluted (1:10) with water and was used as template for PCR. RT-PCR primer sets were designed from the selected representative unigenes by using Primer3 software (Table 1).

Musa 28S rRNA was used as endogenous control. Semi-quantitative RT-PCR was carried out with three biological replicates and normalized with 28S rRNA. The PCR cycling profile consisted of denaturation at 94 °C for 1 min, annealing at 56–64 °C (depends upon the primer) for 1 min and an extension at 72 °C for 1 min for 30 cycles. The PCR reaction products were resolved on agarose gel (1%) visualized by ethidium bromide staining under UV and the image was captured using Alpha Imager. The intensity of bands was determined by densitometric analysis of gels using AlphaEase FC software.

3. Results

3.1. Construction of subtracted library

Root samples taken at time points, 1, 2, 3, 4, 5, 6, 7 and 8 DAI, were used to identify differentially expressed genes in *P. coffeae* inoculated and uninoculated resistant banana cultivar. In this study

Table 1
Primers used for semi-quantitative RT-PCR analysis.

Gene	Forward primer	Reverse primer
Metallothionein	5′GGTCAACTCTGAGACCTGA	5′-CCGAGGTACAGGTA GAACAT-3′
1,3 Glucanase	5′GGATGAGACTCTACGATCC	5′-GCCTGATCAAGTTCT GGTTG-3′
Chitinase	5′AGTCAAGGTGATGCTCTCCATC	5′-TCCGGCGATGTTGAAGT CTATG-3′
Lipoxygenase	5′TCCACCAGCTCATCAACCAC	5′-TCAGCAGCTTGAAGAT GGGG-3′
Cytochrome p450	5′AGAGCGACTCACAGACTCGAC	5′-CCGGGCAGGTACTTG TAGG-3′
Peroxidase	5′TATGCTCACCATTGCTGCTC	5′-TGATTACCATTGCGAGG ACA-3′
25S rRNA	5′ACATTGTCAGGTGGGGAGTT	5′-CCTTTTGTTCCACACGA GATT-3′

subtractive cDNA library was constructed from the cDNAs of *P. coffeae* inoculated root samples (tester) and uninoculated root samples (driver) of resistant cultivar Karthobiumtham. The subtraction method overcomes the problem of differences in mRNA abundance through normalization thereby enriching the less abundant genes. The subtraction efficiency was confirmed by evaluating the expression of the 28S rRNA between subtracted and unsubtracted cDNAs. The amplification of 28S rRNA could be detected in agarose gel only at 28 PCR cycles in subtracted sample whereas in the unsubtracted template the amplification was observed even at 15 PCR cycles. The reduction in expression level suggested that the subtraction procedure was successful. A total of 600 positive clones were randomly picked from the SSH library for plasmid isolations. All the plasmids were digested with *Eco*RI to eliminate false positive clones. Based on the digestion, it was observed that nearly 520 clones were having the inserts and these clones alone were sequenced. Out of 520 clones sequenced 428 readable sequences were obtained. All the readable sequences were assembled through CAP3 analysis which resulted in the construction of a unigene set of 256 ESTs (56.14%). Among them 188 were singletons and the remaining sequences were grouped into 68 contiguous sequences (contigs).

3.2. Annotation of differentially expressed cDNA sequences

Gene ontology analysis was carried out for all the unigenes using the blast2go program. Sequences were classified into three categories: biological process, molecular function and cellular component. The biological process category was subdivided into twelve groups according to the biological process level such as cellular process (33.3%), metabolic process (23.5%), response to stimulus (11.11%), localization (7.4%), cellular component (3.08%), biological regulation (3.08%), signaling (2.47%), reproduction (2.12%) developmental process (1.85%), multi-cellular organismal process (2.46%) and death (0.62%) (Fig. 1). The remaining 29 ESTs were identified as unknown function or appeared to have no significant similarity with known proteins/genes or hypothetical protein. In the classification of cellular component, transcripts related to organelle group were present at high percentage followed by transcripts of cell and membrane enclosed lumen. In the molecular function category, more than 50% of the transcripts are related to molecular binding followed by catalytic activity category and others are grouped in the enzyme regulator activity, structural molecular activity, transporter activity, molecular transduction activity and transcription regulator activity. All these unigenes were subjected to similarity search with the existing sequences in Gen Bank using BLASTX. Sequences that had significant matches (E value $<10^{-2}$) alone were categorized into eight groups namely, disease resistance/defense energy and metabolism, transcription, protein synthesis, folding and stabilization, signal transduction and intracellular traffic signaling, growth/division, unclassified or unknown function (Table 2). Nearly, 12.8% of the unigene set were found to hit with no functional characterization and included in hypothetical and predicted proteins.

3.3. RT-PCR analysis of differentially expressed genes in resistant and susceptible cultivars

Six defense related unigenes, chitinase, peroxidase, 1,3-endoglucanse, lipoxygenase, metallothionein and cytochrome p450, that are having high query coverage and high homology percentage, were selected to study their expression in resistant and susceptible cultivars during *P. coffeae* infection. Transcript level of these genes was investigated in both resistant and susceptible cultivar at 0, 2, 4, 6, 7 and 8 DAI of *P. coffeae*. All the genes were expressed in the uninfected (0 DAI) root tissues of both cultivars, but the level of expression varied between the resistant and susceptible cultivars (Fig. 2). In general, the expression of all the genes was comparatively higher in resistant than in susceptible cultivar. It was also observed that all the genes were upregulated in both resistant and susceptible cultivars, but the expression pattern of these genes was different among nematode infected resistant and susceptible cultivars (Fig. 2). Moreover, the expression of these genes was relatively high in nematode infected resistant cultivar when compared to susceptible one. The expression level of peroxidase, chitinase, 1,3-glucanase, was consistently lower in susceptible cultivar compared with resistant cultivar. The transcript level of chitinase started increasing drastically and reached maximum at 4DAI in resistant cultivar but in the susceptible cultivar there was no significant change in expression. The expression level of lipoxygenase, metallothionein and chitinase was maximum at 4DAI in the resistant cultivar. The expression of cytochrome p450 and peroxidase reached maximum level at 6DAI in the resistant cultivar, while it was at 7DAI for endoglucanase. Altogether this expression study showed that defense related gene mechanism peaked between 4DAI and 7DAI.

4. Discussion

We sequenced cDNA in SSH library to analyze the transcriptional diversity during *Musa–P. coffeae* interaction in the resistant cultivar. SSH is a powerful technique to identify genes with differential expression allowing the identification of both abundant and rarely expressed transcripts [22]. In this study, 256 differentially expressed unigenes were obtained during *Musa–P. coffeae* interaction in the resistant cultivar through SSH technique. Functional annotation of SSH derived ESTs showed that nearly 87% of the genes hit with known biological function categories (Fig. 1). Our goal was to mainly focus on the genes that are involved in defense response and to characterize the resistance pathways to *P. coffeae* in banana. In resistant cultivars within 8DAI we found that transcripts encoded defense-related proteins, involved in disease resistance, programmed cell death, cell wall strengthening, signaling activity and resistance related transcription factors while genes involved in secondary metabolism, were less. Our analysis revealed that the up-regulated ESTs are involved in the nematode recognition, signal transduction, protection mechanism and hypersensitive response (HR). The remaining 12.8% of ESTs with unknown function suggested that the responses to nematode infestation are rather complex and multigenic as reporter earlier [10]. Upregulation of genes involving in functions other than resistance/defense suggested that the gene expression is modified upon stress [23].

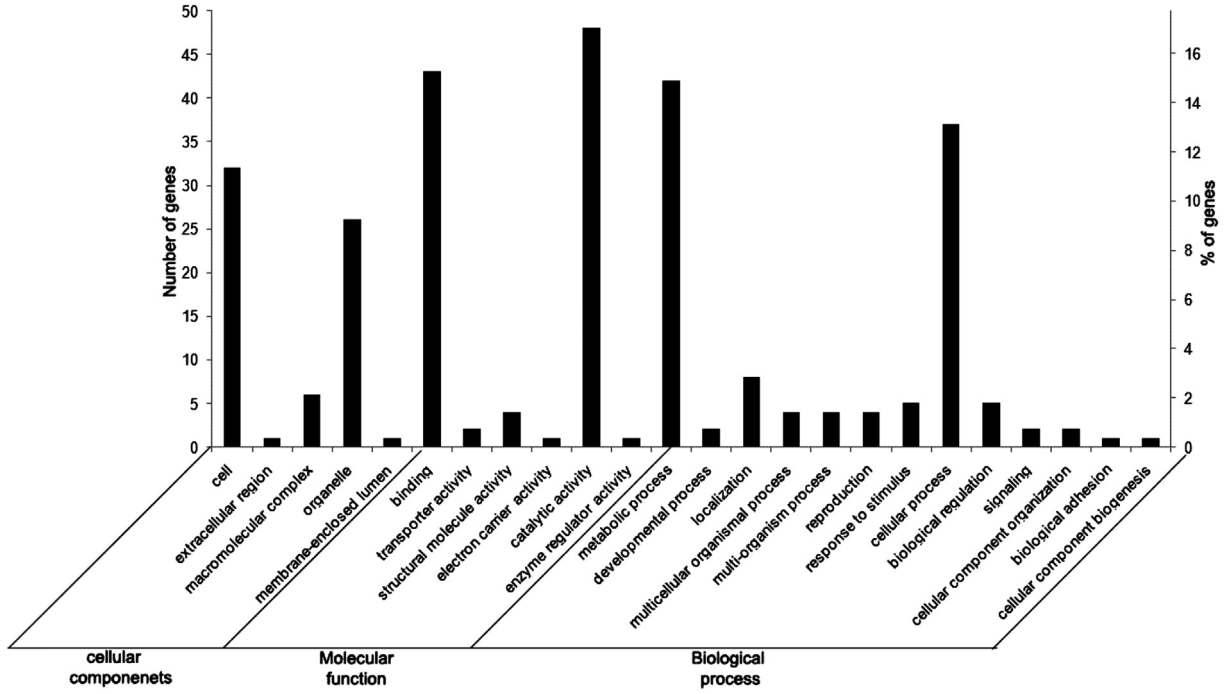

Fig. 1. Gene ontology (GO) annotations of unigenes obtained from suppression subtractive hybridization derived from *P. coffeae* challenged resistant *Musa* cultivar Karthobiumtham.

The SSH results were validated using semi quantitative RT-PCR with six defense specific representative genes including peroxidase and 1,3-glucanase (Fig. 2). Our study clearly demonstrated the difference between resistant and susceptible cultivars in transcript diversity with respect to timing and the level of gene expression in stress response to *P. coffeae*. The degree of resistance depended not only upon qualitative differences in the activated defense genes, but also on differences in the timing and magnitude of their expression [24]. For example, the hydrolytic enzyme 1,3-glucanase was up-regulated in both resistant and susceptible cultivars during *P. coffeae* infection, but the level of glucanase was abundant and two fold higher than the susceptible cultivar at 7DAI. A similar kind of expression profile was observed for peroxidase gene in which the resistant cultivar expression level was more than two fold when compared to the susceptible one and also reached the maximum expression level on 6DAI, two days earlier than the susceptible plant (Fig. 2C). Earlier studies also confirmed that the cell wall strengthening enzymes like peroxidase and polyphenol oxidase activities were high in resistant than in susceptible cultivar during *P. coffeae* infection [20,25]. Much quicker and amplified defense responses by the resistant cultivar indicate the need for the early evasion of *P. coffeae* that has a life cycle of less than 30 days [3].

Invasion of these nematodes trigger multiple signaling pathways through wounding caused by the penetrating action of stylet and the recognition of nematode presence [26]. Banana plants are able to recognize it by sensing the compounds on cuticle or secretions made by nematode or both mediated by the *R* genes triggered defense response. Although it is not completely understood about the nematode compounds that activate the R genes, few studies are available on the nematode effectors eliciting defense response such as the amphidial protein MAP-1 recognized by *Mi*-1 tomato resistance gene [27]. Chitin that is found in nematode egg shells and possibly in the stylet is speculated to be an effector. However, chitin is not found in the cuticle and surface coat that protects the nematode [26]. Such nematode effector molecules are recognized by *R* genes that encode proteins with transmembrane and extracellular leucine-rich repeat (LRR) domains and protein receptors. R gene products like serine threonine kinase, NBS containing resistance like protein, pathogen induced transcription factor NAC1 etc. are clearly shown in this study, thus supporting the hypothesis. Binding of the effectors to such receptors kick in immediate downstream of pathogen recognition processes, initiating an array of defense responses such as lignin deposition, programmed cell death, production and accumulation of hydrolytic enzymes that attack the cell wall of the nematode. Pathogen recognition and disease resistance signaling are also assisted by host plant proteases [28,29]. The expression of aspartic protease, ATP dependent protease, and cathepsin B-like cysteine protease during *P. coffeae* infection showed the importance of protease in resistance mechanism (Table 2). The role of cysteine protease in R-mediated resistance against pathogens and aspartic protease in plant defense response were demonstrated previously in Arabidopsis [29,30]. The host plant also generates some additional protective (peroxidase, cytochrome P450, metallothionein etc.), and adaptive mechanisms (chaperones, ATP synthase etc.) that help to recover/protect the host from damage caused by the nematode invasion. We name them as 'defense accessories' and are thought to provide a smooth functioning environment/platform for the defense mechanisms to occur.

Unlike other nematodes, *P. coffeae* do not form syncytia or giant cells but migrate along the root and feed on cells until they lyse to form cavities [1]. The first line of defense to prevent the pest entry and movement within the root is the cell wall mediated resistance. In plants, cytochrome P450 plays a major role in the synthesis of lignin intermediates and other secondary metabolites via phenylpropanoid biosynthesis pathway [31,32]. Upregulation of cytochrome P450 observed up to 6DAI in resistant cultivar indicated that plants overhaul the cell wall rigidification processes by lignification of feeding cell walls (Fig. 2E). Peroxidases also involve in cell wall building, cross linking of cell wall polymers leading to suberization and lignifications, provide additional wall strength [33]. Lignin polymers serve as barriers, walling off the nematode from the rest of the root and physically blocking its spread. An assortment of hydrolytic enzymes consisting of chitinase,

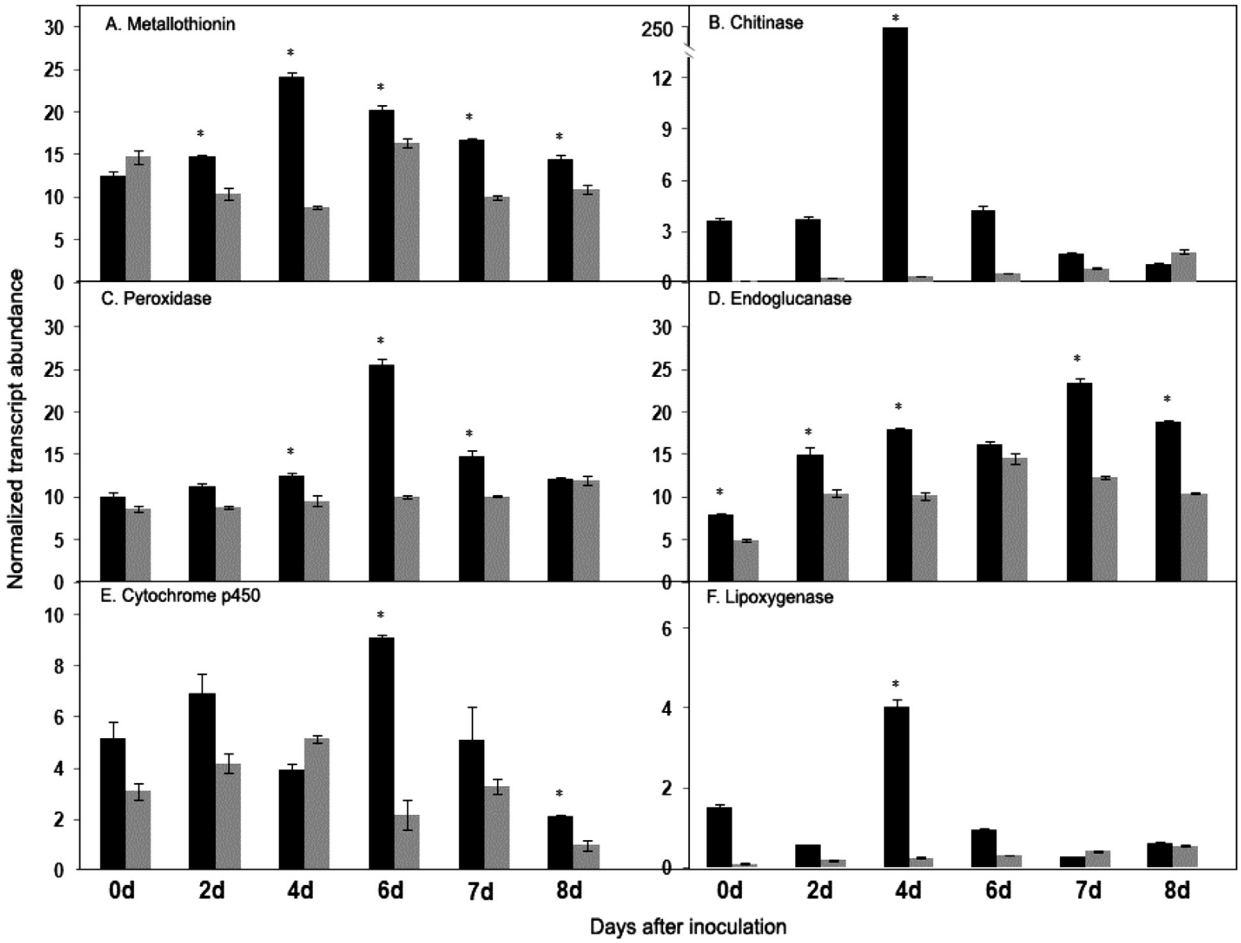

Fig. 2. Transcript accumulation measured after 0, 2, 4, 6, 7 and 8 DAI on Metallothionin (A), Chitinase (B), Peroxidase (C), Endoglucanase (D), Cytochrome p 450 (E), Lipoxygenase (F). Gene expression is shown as PCR/25S rRNA product. Data was normalized. All experiments have been performed with at least three biological replicates. Designation of treatments is as follows: Black bars, Karthombiumtham (resistant cv); Gray bars, Nendran (susceptible cv). Means ± SE, $n = 3$, *Tukey's test was used for proof of significance ($P < 0.05$) compared with the corresponding susceptible or resistant cultivar.

endoglucanse and protease that attack the nematode cell wall are produced (Fig. 2, Protease data not shown). Activation of oxidation-related genes such as peroxidases and type 2 metallothionine that function as scavengers of reactive oxygen species (ROS) are involved in blocking oxidant-mediated programmed cell death by converting H_2O_2 into hypochlorite (HOCl) and hydroxyl radicals (OH') [34]. Metallothionins also protect against ROS-induced DNA degradation [35]. Peroxidase and metallothionin upregulation in resistant cultivar supported the hypothesis that a complex balance of oxide concentration is maintained to resist the infection [36]. Resistance induced transcription factors like NAC1 are found to be present in resistant cultivar during the infection (Table 2). The NAC domain family of plant specific transcriptional regulators is involved in developmental processes, as well as in hormonal control and stress defenses [37]. However, the function of NAC1 transcription factor in banana defense response is unclear. Secondary metabolites like isoprenoids are also produced and can be speculated as nematocides (Table 2). Many isoprenoids were found to have antifeedant properties and nematocidal activity [35].

Lipoxygenase (LOX) activity observed during nematode infection is an evidence for the activation of induced defense responses in banana. LOXs add molecular oxygen to the fatty acids such as α-linolenic acid (18:3) that are derived from membrane lipids possibly during the stylet penetration to subsequently biosynthesize lipid-derived signaling molecules called oxylipins [38]. There is genetic evidence available in maize showing that LOX pathway and its oxylipin are involved in plant–nematode interactions [39].

Induction of proteins such as heat shock proteins (HSP), cyclophilins, Histidine-containing phosphotransfer protein, ADP-ribosylation factors 1 (ARF1) and MAPK suggest that they may be involved in maintaining a suitable functioning environment for the myriad of defense responses to take place. The role of HSP was evidenced in *Mi* gene mediated resistance against root knot nematode in tomato [40,41], R-gene mediated resistance (RPS2) against *P. syringae* in Arabidopsis [42], RPM1mediated resistance to *P. syringae* [43] and Pto-mediated resistance to *P. syringae* [44]. Also HSP family members act as molecular chaperones to stabilize or facilitate refolding of proteins that have been denatured during stress events [45]. Similarly, cyclophilins (Cyp) that are involved in *in vivo* protein folding were observed in resistant cultivar. Marivet J et al. [46] found that the transcription of a bean cyclophilin gene was up-regulated due to environmental or pathogenic stresses, indicating this gene may be important for signal transduction or protein folding under these stresses. Drastic induction of the Cyp in resistant cultivar was observed in pepper–*Xanthomonas campestris* pv. *vesicatoria* [47] and peanut–*Phaeoisariopsis personata* interaction [48]. However, the precise role of Cyp in this interaction is unclear. The over expression of Histidine-containing phosphotransfer protein that involves cytokinin pathway and gibbrellin 20 oxidase suggested that nematode infection leads to altering various components

Table 2
List of selected ESTs obtained by suppression subtractive hybridization between *P. coffeae* inoculated and un-inoculated banana root samples of resistant cultivar Karthobiumtham.

Clone no.	Size bp	Sequence homology	Accession no.	*E*-value	Sequence identity
Energy and metabolism					
S-9	421	ADP-ribosylation factor 1	NM00115348	3e-15	74%
S-3	310	ATP-dependent protease subunit (clpP) gene	EF590357.1	4e-97	85%
S127	184	Cytochrome p450 sterol C22 desaturase	AM497812.1	2e-12	90%
S594	383	Glyceraldehyde 3 phosphate dehydrogenase mRNA	BT131063.1	2e-102	88%
S148	559	Glucose-6-phosphate-1-dehydrogenase	AM497810.1	5e-12	100%
S201	326	Phosphoglycerate kinase	AM497801.1	2e-7	96%
S332	487	Aminocyclopropane 1 carboxylate synthase	D89732.1	2e-10	100%
Transcription					
S-75	381	Putative splicing factor Prp8	DQ122784.1	5e-13	86%
S121	159	Single stranded nucleic acid binding R3H	AM748509.1	4e-12	100%
S-61	243	RNA polymerase beta subunit (rpoC2) gene	EU017044.1	1e-100	99%
S369	388	Transcription factor	AM293617.1	2e-4	100%
S-66	179	Zinc finger protein 521	EF445043.1	5e-10	81%
S556	676	Ethylene signal transcription factor	AB266320.1	6e-8	86%
S236	503	TATA binding protein associated factor	AB192883.1	4e-6	91%
S-61	243	RNA polymerase beta subunit (rpoC2) gene	EU017044.1	1e-100	99%
S826	319	DEAD/DEAH box helicase mRNA	AF462218.1	1e-82	84%
S179	231	Nuclear acid binding protein	XM_002510220.1	1e-30	76%
Disease resistance/defence					
S-276	140	NBS containing resistance like protein gene	EF653105.1	1e-87	90%
S-354	673	Disease resistance related gene	DQ372580.1	3e-18	83%
S-107	345	Plant disease resistance response protein family	AM748435.1	9e-09	88%
S359	390	Type 2 metallothionein	DQ317317.1	3e-117	88%
S336	168	Peroxidase	DQ317315.1	2e-08	86%
S-38	914	Class III acidic chitinase gene	KC489086	2e-54	75%
S-108	103	Cold-inducible unknown mRNA	DQ078775.1	2e-17	88%
S827	375	Isoprenoid biosynthesis like protein	DQ663589.1	3e-160	97%
S593	182	Pathogen induced transcription factor NAC1 like mRNA	EU294352.1	4e-2	100%
S-13	276	Manganese superoxide dismutase	AF061514.1	2e-2	100%
S-105	463	Lipoxygenase	FM164378.1	2e-43	89%
S 12	168	Endoglucanase (cel5 gene)	AJ293762.1	0.0	100%
S-115	104	(E,E)-alpha-farnesene synthase	AB370228.1	7e-3	96%
S 106	191	Inorganic phosphate transporter	AJ534339.1	3e-04	100%
S-110	716	3-carene synthase gene	FJ609174.2	3e-19	75%
Protein synthesis, folding and stabilization					
S123	248	60S ribosomal protein	EU284930.1	8e-46	91%
S-36	398	Ribosomal protein	AY293037.1	2e-137	91%
S-42	246	Ribosomal protein S16 (rps16) gene	FJ428139.1	4e-100	99%
S842	484	26S proteasome regulatory complex, ATPase RPT4	XM_001761096.1	2e-51	74%
S-71	345	Cyclophilin	AK228231.1	3e-09	78%
S770	788	Elongation factor 1 alpha	DQ057979.1	5e-55	89%
S-5	122	Translation initiation factor 5A	EU195534.1	3e-18	85%
S-21	304	Beta-galactosamide alpha-2,6-sialyltranferase 1	BC117603.1	3e-08	91%
S855	319	Translation initiation factor 4A	Z21510.1	3e-71	81%
Signal transduction and intracellular traffic Signaling					
S-49	800	Rho GTPase activator/Rho GTPase binding/phosphoinositide binding	NM_118591.5	1e-31	83%
S-7	128	ROP family GTPase	AY168618.2	7e-36	97%
S555	345	MAP3K alpha protein kinase	AJ010090.1	3e-3	100%
S-16	180	Inorganic phosphate transporter	AJ534339.1	4e-3	100%
S838	360	Membrane protein	EU962248.1	2e-83	83%
S-18	298	Synaptobrevin	AY072422.1	6e-67	83%
S290	226	Sulfurase gene	EU673449.1	6e-2	81%
S-73	348	ADP-ribosylation factor-like protein	FJ607303.1	2e-113	88%
S-112	161	Aspartyl protease (asp gene)	AM497798.1	43-2	100%
S-37	109	Toll/interleukin-1 receptor-like protein 3	AF051151.1	0.002	96%
S-78	870	Serine/threonine kinase gene	DQ375116.1	2e-22	82%
S598	235	Proline rich protein APG precursor	AB182103.1	2e-3	96%
Unclassified or unknown function					
S-51	649	Phosphoinositide 3-kinase	Y11312.1	3e-11	91%
S-59	323	Hypothetical protein	AM706411.1	8e-22	80%
S-65	332	Calcium binding protein	AF069772.1	5e-12	73%
S-52	148	Sulfotransferase	AB284983.1	2e-4	100%
S-54	306	3-methyl-2-oxobutanoate hydroxymethyltransferse	AY923838.1	7e-3	79%
S 79	225	Allatotropin	AJ5089071	4e-24	85%
S-104	426	Histidine-containing phosphotransfer protein 2	AJ841794.1	5e-76	95%

involved in the complex interactions between hormone-regulated defense signaling pathways. Rivero RM et al. [49] indicated that elevated cytokinin levels maintain high cellular redox potentials during stress, thereby reducing irreversible damage from reactive oxygen species to induce tolerance in tobacco. ARF1 plays an important role in intracellular vesicular trafficking and expression in nematode infected banana roots hint that it might be a component of various plant defense signaling pathways in inducing the

expression of a subset of PR genes [50]. MAPKs are important intracellular mediators of information in early defense signaling through protein phosphorylation of downstream signaling components and target several transcription factors [51] and have been previously shown to be involved in ethylene signaling [52].

In summary the plants recognize the effectors secreted by the esophageal glands and trigger lignin production, ROS burst and induced defense signaling events like ethylene response. Although this study does not fully address the detailed defense signaling events, it has offered an insight into the broad mechanistic picture with the limited varieties of disease resistance genes that we obtained in *Musa–P. coffeae* interaction. An in-depth analysis of expression of various defense genes will help to clarify the defense signaling network and currently work is in progress for an in-depth transcriptional profiling using next generation sequencing method. Since *R* genes appear to be a major player in this root infecting pathogen response a further detailed study on molecular characterization of the *R* genes involved in this interaction is necessary.

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