Transcriptomic Analyses of Differential Host Responses to Red Rot Pathogen Colletotrichum Falcatum in Sugarcane Through Subtractive Library and NGS Approach

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Abstract

The fungal pathogen *Colletotrichum falcatum* causes the stalks, the economically important for sugar extraction. Although, disease management is achieved by cultivating resistant cultivars, the complex polyploidy of sugarcane genome complicates understanding the inheritance of disease resistance. Earlier attempts of using resistant and susceptible varieties to understand host-pathogen interaction resulted in cultivar specific expression of genes due to different genomic background of the varieties. To avoid host background variation in the interaction, suppression subtractive hybridization (SSH) based next generation sequencing technology was utilized in the same cv Co 7805 which behaves differently as incompatible and compatible to two different *C. falcatum* pathotypes. In the incompatible interaction (ICI) with *C. falcatum* pathotype CF87012 (Less virulent, LVir) 10,038 contigs were assembled from ~54,699,263 raw reads. In the compatible interaction (CI) to the *C. falcatum* pathotype CF94012 (Virulent, Vir) 4022 contigs were assembled from ~52,509,239 raw reads. The transcripts homologous to CEBiP receptor and transcripts involved in the signals ROS, Ca\(^{2+}\), BR, JA and ABA were exhibited in both the responses. Additionally, MAPK, ET, PI signals and JA amino conjugation related transcripts were found only in ICI. Finally, the temporal gene expression of a total number of 16 transcripts was monitored in qRT-PCR. Most of the transcripts exhibited highest induction in ICI in comparison with CI. Further, more than 17 transcripts specific to the pathogen were found only in CI, indicating that the pathogen colonizes the host tissue whereas it failed to do so in ICI. Overall, this study has identified for the first time, the differential responses of a single sugarcane host to two different *C. falcatum* pathotypes and PAMP triggered immunity (PTI) is exhibited in both the responses, but the more efficient effector triggered immunity (ETI) was found only in ICI at the molecular level.

Introduction

Plants, being sessile are continuously exposed to various biotic and abiotic stresses during their lifetime. Among them, biotic stresses caused by the invading pathogen often results in complete devastation of the diseased plant. To cope up with the pathogen stress, plants have evolved sophisticated defense strategies to recognize and restrict pathogen invasion by activating their immune responses. At the plant-pathogen interface, plant perceives pathogenicity determinants termed pathogen associated molecular patterns (PAMPs) such as chitin and glucans present in the pathogen cell wall by the cognate pattern recognition receptors (PRRs) located at the surface of plasma membrane and triggers defense responses termed PAMP triggered immunity (PTI). Successful pathogens evade this host detection by secreting effector proteins and make the host susceptible to pathogen invasion known as effector triggered susceptibility (ETS). Whereas, the effector proteins of pathogens are recognized by a set of resistance (R) genes present in the plant which further activate host defense termed as effector triggered immunity (ETI). This mode of defense is stronger and often termed as R-gene mediated defense or gene for gene interaction. PTI emerges as a basal defense to prevent further colonization of the pathogen. ETI leads to hypersensitive response mediated programmed cell death (HR-PCD) and enhanced resistance at the whole plant level which is long lasting (Hamdoun et al. 2013). Resistance is determined by a set of R
genes localized at the surface of the plant plasma membrane or the cytoplasm. Flor’s gene for gene hypothesis states that a specific R gene in the plant recognizes its cognate avirulence (Avr) gene in the pathogen. Specific recognition results in provoking defense gene expression in the plant system (Ali & Reddy, 2000; Beers & McDowell, 2001). During evolution, new resistant specificities are generated to cope with the newly evolved virulent strains of pathogens. Some R genes such as Hm1 and RPM1 are present as single copy in resistant plants and are absent in the susceptible plants (Flor, 1971). But most of the R genes are organized in complex loci containing an array of homologous genes. For example, Rp1, Rpp5, Xa21, Pto, Dm3, I2, N, M and Cf genes are localized in a cluster (Gao et al. 2000; Grant et al. 1995). In a crop with complex polyploidy and aneuploidy, the R genes must be organized in a complex locus. The polyploid nature and genetic complexity of sugarcane, makes it difficult to find a specific R gene for a particular disease/pathogen. So far, Bru1 is the only known resistant gene conferring resistance to brown rust and is found to segregate in a Mendelian pattern of 3:1 (Asnaghi et al. 2004).

Sugarcane (Saccharum spp. hybrid) is an economically important crop cultivated in tropical and subtropical regions of the world. India ranks second in sugarcane production next to Brazil (www.fao.org/corp/statistics/en/). Red rot caused by the ascomycete fungal pathogen Colletotrichum falcatum Went (Teleomorph: Glomerella tucumanensis [Speg.] Arx and Muller) is a serious threat for sugarcane cultivation in the tropical regions of the world (Viswanathan 2010, 2021a). Management of the disease depends solely on cultivating red rot resistant cultivars. However, during the past decades, severe disease epidemics have occurred that resulted in removal of many elite cultivars from cultivation. Frequent emergence of new variants of the pathogen C. falcatum contributes to the varietal breakdown (Viswanathan and Rao 2011). Hence, detailed studies were conducted on the molecular basis of the interaction between sugarcane and C. falcatum to understand the host resistance mechanism by our research group. Firstly, early and prominent induction of pathogenesis-related (PR) proteins was documented as a defense and induced defense response against C. falcatum (Viswanathan et al. 2003, 2005). In addition to induction of the PR proteins, accumulation of phytoalexins at the pathogen infection site was documented as marker for red rot resistance (Malathi et al. 2008, Kumar et al. 2015, Nandakumar et al. 2021a). The chitinase gene from sugarcane has been characterized as a class IV glycosyl hydrolase based on full gene sequence and in silico 3D structure prediction. Further, the differential expression of the chitinase gene in red rot resistant and susceptible sugarcane cultivars was monitored through qRT-PCR (Rahul et al. 2015). Differential display (DD)-RT-PCR was used to identify differential transcripts upregulated during pathogenesis of C. falcatum in resistant and susceptible cultivars of sugarcane (Pratima et al. 2013, Rahul et al. 2016).

The NGS based sequencing technology plays a vital role in exploring genes and genomes of an organism. The whole genome and transcriptome of C. falcatum were sequenced using Illumina Hi-Seq 2500 (Viswanathan et al. 2016, Prasanth et al. 2017). Earlier we adopted suppression subtractive hybridization (SSH) strategy to identify the time specific and initial defense responses of sugarcane during C. falcatum pathogenesis which hypothesized involvement of jasmonic acid (JA), ethylene (ET), reactive oxygen species (ROS), phosphoinositolite (PI) and calcium (Ca^{2+}) signals in disease
resistance (Sathyabhama et al. 2015, 2016). The previous transcriptomic studies, a set of sugarcane cultivars varying in red rot resistance, either a resistant or a susceptible host were used to determine differential transcripts upregulated during *C. falcatus* pathogenesis (Pratima et al. 2013, Sathyabhama et al. 2015, 2016). The variation in transcript accumulation between resistant and susceptible varieties we cannot rule out the changes in transcriptomes due to their genetic complexity and this may have a profound influence on identifying the genes/proteins involved in pathogen defense in sugarcane. In our experimental trials, certain cultivars of sugarcane, exhibiting differential host responses to different pathotypes of *C. falcatus* was established (Viswanathan et al. 2020). In this study, the sugarcane cv Co 7805 exhibiting incompatible (ICI) and compatible (CI) interactions to *Cf* 87012 and *Cf* 94012, respectively was used for SSH and subtracted transcriptome sequencing and to identify the initial defense responses exhibited in sugarcane against *C. falcatus* in fool-proof manner. Illumina HiSeq 2000 sequencing platform was used to sequence subtracted transcripts derived from the two responses. This study generated comparative transcriptomes of ICI and CI in sugarcane against *C. falcatus* and identified detailed information on resistance mechanism in sugarcane to *C. falcatus* for the first time.

**Materials And Methods**

**Plant material and pathogen culture**

A tropical sugarcane cv Co 7805 was planted in sugarcane field at ICAR-Sugarcane Breeding Institute (SBI) (ICAR), Coimbatore, India and the crop was raised following standard field practices for a tropical sugarcane. Two pathotypes of *C. falcatus* isolated from infected stalk tissue samples of sugarcane cv Co 87012 and Co 94012 named as pathotypes *Cf* 87012 and *Cf* 94012 respectively, maintained as part of *C. falcatus* culture collections, Plant Pathology lab, ICAR-SBI, Coimbatore were used for this study. The differential response of sugarcane cv Co 7805 to the two pathotypes *Cf* 87012 and *Cf* 94012 was assessed over a period of three years. The cultivar consistently showed incompatible response upon inoculation with the pathotype *Cf* 87012 and compatible response to the pathotype *Cf* 94012 (Fig. 1). Pathogen inoculation was performed by plug method in the third internode from the base of the cane following standard protocol for sugarcane cultivar as reported earlier (Mohanraj et al. 2012, Viswanathan et al. 2021). Separate sets of sugarcane stalks of the cv Co 7805 were inoculated in triplicates with the two *C. falcatus* pathotypes and mock inoculated samples served as controls for the experimental samples. Stalk tissue samples were collected in triplicates with their respective controls 36h post *C. falcatus* inoculation and stored in -80°C till RNA isolation.

**RNA extraction and suppression subtractive hybridization (SSH)**

Total RNA was extracted from all the samples with TRI reagent (Sigma-Aldrich, USA). The quality of RNA was checked in an agarose gel and quantified in a NanoDrop™ 1000 spectrophotometer (Thermo Scientific, USA). 1µg of purified RNA was used for cDNA synthesis following the manufacturer’s
instructions of Smarter™ PCR cDNA synthesis kit (Clontech, CA, USA). Forward and reverse subtractions for the cDNAs were done following the manufacturer’s instructions of PCR-Select™ cDNA subtraction kit (Clontech, CA, USA). Forward subtraction represents resistance response library (RRL), in which cv Co 7805 challenged with pathotype Cf87012 was used as tester, cv Co 7805 challenged with Cf94012 and mock sample of cv Co 7805 were used as the driver. In reverse subtraction representing susceptible response library (SRL), the cv Co 7805 challenged with Cf94012 was used as tester and cv Co 7805 challenged with the pathotype Cf87012 and mock sample of cv Co 7805 were used as driver.

**Illumina library construction and sequencing**

The cDNA pools of the subtracted two transcripts were sequenced by Illumina HiSeq 2000 paired end (PE) sequencing platform at Xcelris Genomics Pvt Ltd, Ahmadabad, Gujarat India. The two subtracted ds cDNAs were fragmented using Covaris S2 (Covers Inc., Massachusetts, USA). After fragmentation, Illumina indexing adapters were added to the blunt ends and size selected in the range of 300-600 bp in 2% agarose-Etbr gels. The two subtracted ds cDNAs were prepared for sequencing according to the Illumina TruSeq DNA sample preparation guide v2 (August 2011, rev. A) (Illumina Inc, San Diego, USA) for Illumina Paired-End (PE) Multiplexed sequencing. Cluster generation was carried out for the PE library by hybridization of template DNA molecules onto the oligonucleotide-coated surface of flow cell v3 (Illumina Inc., San Diego, USA). Immobilized DNA template copies were amplified by bridge amplification to generate clonal DNA clusters. The process of cluster generation was performed on cBOT using TruSeq PE Cluster kit v3-cBot-HS (Illumina Inc., San Diego, USA). TruSeq SBS v3-HS kits (Illumina Inc., San Diego, USA) were used to sequence DNA of each cluster on a flow cell using sequencing by synthesis technology on the Illumina HiSeq 2000 flow cell v3. Samples were sequenced using 100bp PE runs.

**Transcript assembly and annotation**

The raw reads were quality trimmed; adaptor sequences removed and the reads were size selected using Trimomatic v0.17. After adaptor trimming, high quality reads with mean quality scores more than 25 and sequence length longer than 50bp were selected for assembly. De novo assembly of the subtracted transcript assembled contigs were performed in a CLC genomics workbench v6.0. Functional annotation of the assembled transcript contigs were predicted with gene ontology (GO) terms through BLASTx analysis using BLAST2GO program. An e-value threshold of e⁻⁵ and a high scoring segment pair (hsp) filter of 33 were kept as default parameters for similarity search. Sequences less than 100 were filtered and removed.

**Pathway analysis by KEGG-KAAS**

The transcript assembled contigs that belong to the metabolic pathways that are expressed in the interaction were identified through mapping the assembled transcripts to Kyoto Encyclopedia for Genes and Genomes (KEGG) eukaryotic database using KEGG Automatic Annotation Server (KAAS). All the transcripts were compared against KEGG-KAAS database using BLASTx with default threshold bit-score value of 60.
Validation of gene expression through qRT-PCR

Temporal gene expression of 16 transcripts mapped to KEGG-KAAS database was studied in (Step One Plus™ Real-Time PCR Systems, Applied Biosystems Inc., Life Technologies, USA). Representative transcripts involved in recognition, signalling and defense were studied. The transcripts include chitin elicitor binding protein (CEBiP), mitogen activated protein kinase kinase kinase 1 (MAPKKK1), mitogen activated protein kinase kinase 1 (MAPKK1), Brassinosteroid signalling kinase (BRSK), disease resistant protein RPM1 (DRPRPM1), disease resistant protein RPS5 (DRPRPS5), cyclic nucleotide gated channel (CNGC), calcium dependent protein kinase (CDPK), calcium binding protein CML (CBPCML), superoxide dismutase Cu Zn (SOD Cu Zn), V type proton transporting ATPase subunit1 (VTPATPase), jasmonic acid aminoacid synthetase (JAAS), Abscisic acid responsive element binding factor (ABAREBF), ethylene receptor (ER), 14-3-3 protein epsilon (14-3-3 PE) and Chitinase. The sugarcane cv Co 7805 stalks inoculated with C. falcatum pathotypes Cf87012 and Cf94012 were collected at 12, 36 and 72h post C. falcatum inoculation. Total RNA was extracted by TRI reagent (Sigma-Aldrich, USA). 500 µg of total RNA was used for cDNA synthesis; rst strand cDNA was diluted further to 200ng and used as templates for qRT-PCR. SYBR green PCR mastermix (Applied Biosystems Inc., Life Technologies, USA) was used for the analysis. 25SrRNA was used as the internal control. The list of primers is represented in Table 1.

Results

Transcriptome sequencing and denovo assembly

The number of raw reads generated by Illumina HiSeq 2000 was 54,699,263 and 52,509,239 for RRL and SRL with respective the number of filtered reads was 41,025,151 and 42,001,812. Quality trimming, adaptor sequence removal and size selection of transcript reads resulted in a total of 10,038 and 4,022 high quality reads for RRL and SRL, respectively. Analyses of two sets of transcripts assigned the transcripts to the 7,849 and 2,899 transcripts for RRL and SRL through BLASTx. There was no homology for 2,189 and 1,123 for RRL and SRL, respectively and they are described as novel genes or hypothetical proteins. The transcripts were submitted in NCBI sequence read archive (SRA) database, with accession numbers SRR2992210 and SRR 2992249 for RRL and SRL respectively.

BLAST homology with other species and annotation

In RRL, the BLAST hits constituted to maximum homology of 34% with Sorghum bicolor followed by 32% with Zea mays and 4% with Oryza sativa. In case of SRL, the respective homologies were 42%, 27% and 5% for Sorghum bicolor, Zea mays and Oryza sativa. In RRL, 1% homology in Triticum urartu, Vitis vinifera, Glycine Max, Hordeum vulgare and Medicago truncatula species were found. In SRL, no homology was found with those species. Saccharum officinarum and Populus trichocarpa had 1% homology in SRL, and no homology was found in RRL. Both RRL and SRL had 1% homology with Saccharum hybrid cultivar (Suppl. Fig. 1a, 1b). In both RRL and SRL, many of the transcripts from BLAST
annotation were found to be hypothetical proteins or novel genes. So, to know the functional ontology of the differential transcripts, GO distribution and KEGG-KAAS functional categorization were done.

**Gene Ontology (GO) distribution**

The high-quality assembled transcripts were annotated with gene ontology (GO) terms. The GO terms were distributed as biological processes, molecular functions and cellular components (Fig. 2). In biological process, transcripts pertaining to aromatic amino acid family biosynthetic process, protein N-linked glycosylation, small GTPase mediated signal transduction, GPI anchor biosynthetic process, IMP biosynthetic process and so on were expressed differentially in RRL. In SRL, transcripts pertaining to sucrose biosynthetic process, negative regulation of peptidase activity and protein deubiquitination were present. In cellular component, transcripts pertaining to RNA polymerase complex, cis-golgi network, anaphase-promoting complex, transcription factor TFIID complex, photosystem I & II were present in RRL. In SRL, a single differential transcript pertaining to cullin-RING ubiquitin ligase complex was present. In molecular function, transcripts pertaining to shikimate kinase activity, cellulose synthase (UDP-forming) activity, P-P-bond-hydrolysis-driven protein transmembrane transporter activity, aspartic-type endopeptidase activity, serine-type endopeptidase activity and mannose-1-phosphate guanylyl transferase (GDP) activity were present in RRL. In SRL, serine-type endopeptidase inhibitor activity and quinone binding - oxidoreductase activity, acting on NADH or NADPH were present. The gene ontology of the 3 GO terms is presented in Table 2.

**KEGG-KAAS functional annotation of subtracted transcriptome**

High quality reads corresponding to 10,038 for RRL and 4022 for SRL were mapped in KEGG-KAAS database. The transcripts were mapped to 12 categories pertaining to carbohydrate metabolism, energy metabolism, lipid metabolism, nucleotide metabolism, amino acid metabolism, glycan metabolism and biosynthesis, metabolism of cofactors and vitamins, metabolism of terpenoids and polyketides, biosynthesis of other secondary metabolites, genetic information processing, environment information processing and plant pathogen interaction. Of the total transcripts mapped, 42% were found to be present in both the interactions or unchanged during *C. falcatum* pathogenesis, 47% of the transcripts were upregulated in RRL and 11% of the transcripts were found to be upregulated in SRL. In all the categories several transcripts were mapped in common i.e., those transcripts were present in both the responses or unchanged during the interaction (Fig 3). The representative transcripts are listed in Table 3.

**Differential transcripts from *Glomerella graminicola* from BLAST homology search**

From the BLAST homology search, a total number of 17 transcripts homologous to *Glomerella graminicola* were present only in SRL. The transcripts represented pathogenic determinants of *G. tucumanensis*, the perfect stage of *C. falcatum*. The transcripts were found to be involved in fungal morphogenesis (alanine glyoxylate aminotransferase), intra cellular signal transduction (Ras), translation (ribosomal proteins), glycolysis (hexokinase), RNA splicing and the E3 Ub liagase of the Ub-26S proteasome pathway (Table 4). The expression of transcripts corresponding to the pathogen even after
subtraction of cDNA suggested that the *C. falcatum* could colonize the host tissues in compatible interaction whereas transcripts related to *C. falcatum* colonization was not found in the incompatible interaction.

**Validation of gene expression through qRT-PCR**

The gene expression of the following transcripts CEBiP, MAPKK1, MAPKK1, DRPRPM1, DRPRPS5, CBPCML, JAAS and ABAREBF showed a gradual increase in their expression at 12h and 36h and a decline at 72h post *C. falcatum* inoculation in RR. Whereas, in SR, CEBiP, MAPKK1 and CBPCML exhibited an inconsistency in their expression at all the time intervals. MAPKK1 and JAAS showed gradual decrease from 12h to 72h. DRPRPM1 showed a similar response as RR but the transcript level was less than 2-fold. ABAREBF showed gradual raise from 12 to 72h and reached 2.5-fold at 72h in SR which is higher than the RR. The defense gene chitinase showed a gradual raise in both RR and SR from 12 to 72h. However, the transcript accumulation was found to be higher in RR and reached a maximum of 4-fold at 72h post *C. falcatum* inoculation. The transcripts CNGC and CDPK showed a gradual increase in RR and a gradual decrease in SR from 12 to 72h post *C. falcatum* inoculation. The transcripts 14-3-3 PE and SOD Cu Zn showed an increase in expression from 12 to 36h and a decline at 72h in the SR. In RR, there was an unstable expression. 5-fold expression was noticed in RRL for SOD Cu-Zn at 12h post *C. falcatum* inoculation. For, VTP ATPase, the expression in RR was unstable and SR showed a constant 6-fold expression at 36 and 72h post *C. falcatum* inoculation. The BR SK and ER showed an inconsistent expression in both the responses. At 12h, BR SK showed more than 5-fold expression in RR. At 36h ER showed more than 10-fold expression in SR. Overall, incompatible interaction revealed higher expression of different transcripts associated with host resistance to defense upon *C. falcatum* inoculation whereas in the compatible interaction, except for a few transcripts where the gene expression was not prominent.

**Discussion**

Red rot, caused by the fungal pathogen *Colletotrichum falcatum* is a devastating disease of sugarcane crop. The survival of a sugarcane cultivar in India is highly linked to red rot resistance in almost all parts of sugarcane cultivating regions of the country. Once, the popular cultivars are affected by red rot, they cannot be propagated in the field and has to be removed from cultivation. The pathogen infection causes complete devastation of the crop under field conditions. Hence, concerted efforts were given to identify red rot resistant cultivars in sugarcane varietal development programmes (Viswanathan, 2021b). During the last two decades, considerable efforts were made to understand defense strategies adopted by sugarcane in response to *C. falcatum*. Evidences of induction of PR-proteins and 3-deoxyanthocyanidin phytoalexins as biochemical defense responses during *C. falcatum* pathogenesis were found (Viswanathan et al. 2005; Malathi et al. 2008; Kumar et al. 2015). In recent years, through massively parallel next generation sequencing (NGS), a large number of transcripts at the interface of plant – pathogen interaction has been sequenced and identified many candidate genes responsible for resistance or susceptibility. In this study, forward and reverse subtracted transcriptomes captured during sugarcane – *C. falcatum* interaction were sequenced through an NGS-Illumina Hi-Seq 2000 sequencing
platform. The pathotype \(Cf87012\) (LVir) exhibited incompatible interaction (ICI) and the pathotype \(Cf94012\) (Vir) exhibited compatible interaction (CI) when inoculated on the sugarcane cv Co 7805 which exhibited a differential host interactions. A total of 10,038 and 4,022 transcripts were derived for ICI and CI respectively. In that, only 7,849 and 2,899 transcripts had BLAST homology for ICI and CI, respectively. The transcripts were mapped in KEGG-KAAS for functional categorization and biochemical pathway analysis (Fig. 3). Finally, a hypothetical model representing the probable occurrence of PTI in both ICI and CI, ETI in ICI and ETS in CI has been proposed in sugarcane for the first time.

In this study, a transcript homologous to chitin elicitor binding protein (CEBiP), and a S/T protein kinase were induced in both ICI and CI. In rice, the chitin molecule/elicitor of the fungal pathogen, \(Magnaporthe grisea\) is perceived by an extracellular LysM receptor containing chitin elicitor binding protein (CEBiP), a PRR (Kaku et al. 2006). PRRs recognize both pathogen-derived nonself PAMPs/MAMPs and plant-derived DAMPs, which triggers PTI. But the transcripts involved in downstream signalling namely MAPK and PI were present only in ICI. Even though, there is probable occurrence of PTI in both ICI and CI, the magnitude of signals activated by MAPK, JA and PI may be higher in ICI which is probably responsible for resistance than the CI (Fig. 4). The pathogen responsive MAPK activation is likely to promote the generation of ROS in chloroplasts and JA signalling, which plays an important role in execution of HR cell death in plants. Usually, the virulent pathogen secretes effectors to make the plant susceptible or to evade PTI termed ETS (Jones et al. 2006). In this study, from BLAST homology search, it is evident that, unlike the pathotype \(Cf87012\), \(Cf94012\) penetrated the host surface. The pathogenic transcripts homologous to \(G. graminicola\) involved in primary metabolites production and the transcript; alanine-glyoxylate amino transferase involved in fungal morphogenesis were upregulated only in CI (Table 4). The transcripts captured in CI homologous to \(G. graminicola\) must be indicating successful pathogenesis and the pathogen’s proliferating stage inside the susceptible host. Also, recent studies by Bhadauria et al (2012 a, b) have demonstrated the essential role of the enzyme alanine: glyoxylate aminotransferase (AGT1) in the rice blast pathogen \(Magnaporthe oryzae\). AGT may provide a means to maintain redox homeostasis in appressoria and contribute to the triglyceride mobilization from conidia to appressoria. Similarly, in the interaction between sugarcane and \(C. falcatum\), the role of AGT must be essential to transfer nutrients and enhance lipid mobilization which is very much needed for melanisation of appressorium utilizing the glycerol during pathogenesis.

In the present study, only in ICI, the transcripts homologous to disease resistance proteins RPM1, RPS2 and RPS5 were upregulated. qRT-PCR experiments carried out in ICI and CI, at three-time intervals post pathogen inoculation revealed the transcriptional gene expression of RPM1 and RPS5 in both the responses (ICI and CI). But the magnitude of expression was higher in ICI (Fig. 4). Also, RPS5 was found to be more than 40-fold in ICI, but a constant expression of 5-fold was noticed in CI throughout the period of study (Fig. 4). Probably, here, proteins involved in the decoy model of defense may exist and the pathogenic effectors secreted by \(C. falcatum\) may be recognized by guarded/decoy proteins and ETI gets activated. Whereas, in CI, the effector may have the ability to inactivate the R gene products and induce pathogenicity, which can be termed as effector triggered susceptibility (ETS) (Jones and Dangl 2006). The \(C. falcatum\) pathotype \(Cf94012\) has probably induced ETS in host cv Co 7805 by secreting effector
proteins. Recently, two probable molecular signatures from C. falcatum viz., CfEPL1 (eliciting plant response-like protein 1, a ceratoplatanin protein) and CfPDIP1 (plant defense inducing protein 1, a novel protein) were found and their Functional characterization of the respective genes revealed that they induce HR in tobacco and systemic resistance against C. falcatum in sugarcane. These studies have indicated that these PAMPs/Effectors of C. falcatum may govern PAMP-triggered immunity (PTI)/effector-triggered immunity (ETI) in sugarcane (Ashwin et al. 2017, 2018). The gene expression of three transcripts differentially regulated from RRL and SRL pertaining to Ca^{2+} signals, the CDPK, CNGC and calcium binding protein CML (CBP CML) were quantified in qRT-PCR in cv Co 7805 inoculated with two different C. falcatum pathotypes. For all the transcripts, the ICI showed upto 3.8-fold expression whereas in CI, less than 1.5-fold expression was noticed (Fig. 4). This proves the probable involvement of all the transcripts of Ca^{2+} signalling in host resistance to C. falcatum.

In this study, several transcripts involved in provoking defense responses like clathrin heavy chain, PCD 6 interacting protein and transcripts involved in PI signalling were found only in ICI. In addition, this study has documented the crucial role of secretory pathway and vesicle trafficking in HR-PCD. The presence of clathrin heavy chain, PCD 6 interacting protein and signal peptidase transcripts in ICI and its absence in CI is a convincing factor to determine that PCD takes place at a rapid phase only in ICI. In CI, PCD may not be a response which gives place for successful pathogenicity and disease spread. In this study, only few transcripts pertaining to secretory pathway were commonly expressed in both the interactions ICI and CI. But most of the important transcripts namely PCD6 interacting protein and signal peptidase were upregulated only in the ICI. This proves the involvement and possible role of secretory pathway in HR-PCD. In a typical R-Avr gene interaction, few rapidly elicited proteins like Avr9/Cf9 are reported and in sugarcane, the transcript was found upregulated in the resistant cultivar (Sathyabhama et al 2015). Avr9/Cf-9-induced F-Box1 (ACIF1; ACRE189) is an F-box protein with a leucine-rich-repeat domain found in a screen to identify proteins involved in Cf9-mediated ETI in N. benthamiana (Rowland et al. 2005). ACIF1 is widely conserved and is closely related to F-box proteins that regulate plant hormone signaling in Arabidopsis. Silencing of ACIF1 Arabidopsis homologs (VFBs) induced a subset of methyl jasmonate- and ABA-responsive genes, supporting a regulatory role of ACIF1/VFBs in hormone-mediated plant defense responses (van den Burg et al. 2008). Janjusevic et al. (2006) gave direct evidence on how the bacterial-secreted proteins act as an E3 ligase in plant cells and affect the host defense response. When the Pseudomonas syringae-type III effector AvrPtoB is delivered into tomato plants containing the Pto resistance gene, it elicits PCD that inhibits pathogen invasion. In contrast, AvrPtoB suppresses programmed cell death (PCD) in the Pto-absent plants, leading to rapid pathogen spread in the infected tissues. Structural analysis indicated that the C-terminal region of AvrPtoB is highly homologous to the U-box and RING-finger domain of eukaryotic E3 ligases. In vitro E3 ligase activity assay indicated that it has ubiquitin ligase activity. Mutation of the conserved residues in the U-box/RING-finger motif of AvrPtoB significantly compromised AvrPtoB’s anti-PCD activity in tomato leaves and dramatically reduced disease symptom on infected plants. These results clearly demonstrated that P. syringae use an E3 ligase effector protein to suppress plant PCD and probably other defense-related processes in the infected cells. Similarly, in this study, a few Ub-ligases are upregulated only in the ICI and a specific F-box and DNA
damage binding proteins were found only in the CI (Table 3). This could be because of the host modification strategies followed by the pathogen. Still this study provides a new insight on the involvement of UPS in sugarcane and C. falcatum interaction. It is novel information in this particular host pathogen interaction. Our recent studies suggest that micro(mi)RNAs regulate many target genes that are involved in inciting early responses to C. falcatum infection during the incompatible and compatible interactions in sugarcane against C. falcatum. We identified miRNA miR5568b involved in chloroplast and mitochondrial function, HR response, enhancing JA and SA accumulation, a fungal responsive miRNA miR169b.3p regulating phenylpropanoid biosynthesis, post-transcriptional gene regulation, inner membrane transporter by HR response and defense-related miRNA, miR166b.5p involved in increasing resistance by activating ETI, PTI, and ER stress in the host-pathogen interaction (Nandakumar et al. 2021b). The network of miRNAs identified in sugarcane - C. falcatum interaction me has validated the present findings in the role of signalling molecules and regulatory genes.

Conclusion

This study has provided new insights in to the molecular mechanisms of resistant and susceptible responses of sugarcane in response to C. falcatum through a detailed transcriptomic approach. This is the first report which indicates the association of signals likes MAPK, Ca^{2+}, JA, PI, ET, ROS, ABA and BR in an incompatible interaction, whereas in compatible interaction, the absence of MAPK, PI and ET signals indicated that the resistance mechanism is confined to MAPK, PI and ET signals. Also, in CI the pathogen has developed its dynamic nature to evade host detection. The upregulation of AGT in CI is a convincing factor to determine the pathogenesis of C. falcatum at the transcript level. Also, the involvement of chloroplastic photosystem proteins, the ubiquitin proteasome system and the differential expression of a CNGC protein in providing defense responses to this pathogen in sugarcane system are novel finding in this study. Further, this study has provided evidence on the essential role of pathogenic determinants of C. falcatum establishment inside the host tissue. The probable adaptive mechanisms exhibited by the pathogen and its ability to modify host defense mechanisms are reported for the first time. Further, the hypothesis developed by this study goes in parallel with the zig zag model of plant immunity, where in ICI based on the transcripts upregulated, the immune reaction is PTI-ETS+ETI exists. In CI, PTI-ETS+ETI exist (Fig. 5). This study has framed a new dimension to look into the enigmatic sugarcane – C. falcatum interaction and has proved its occurrence in a logical way.

Declarations

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Author Contribution Statement

RV conceived, designed and received funds for the research work. MS conducted the experiments. PM and ARS analyzed the data. MS and CNP analyzed NGS data. MS and RV wrote the manuscript. All authors read and approved the manuscript.

Compliance with Ethical Standards

The authors declare that they have no conflict of interest.

The present research did not involve human participants and/or animals

Informed consent was obtained from all individual participants included in the study

References

1. Ali GS, Reddy ASN (2000) Inhibition of fungal and bacterial plant pathogens by synthetic peptides: in vitro growth inhibition, interaction between peptides and inhibition of disease progression. Mole Plant-Microbe Interact 13:847–859

2. Ashwin NMR, Barnabas EL, Sundar AR, Malathi P, Viswanathan R, Masi A, Agrawal GK, Rakwal R (2017) Comparative secretome analysis of Colletotrichum falcatum identifies a cerato-platanin protein (EPL1) as a potential pathogen-associated molecular pattern (PAMP) inducing systemic resistance in sugarcane. J Proteomics 169:2–20. https://doi.org/10.1016/j.jprot.2017.05.020

3. Ashwin NMR, Barnabas EL, Sundar AR, Malathi P, Viswanathan R, Masi A, Agrawal GK, Rakwal R (2018) CfPDIP1, a novel secreted protein of Colletotrichum falcatum, elicits defense responses in sugarcane and triggers hypersensitive response in tobacco. Appl Microbiol Biotechnol 102:6001–6021. https://doi.org/10.1007/s00253-018-9009-2

4. Asnaghi C, Roques D, Ruffel S, Kaye C, Hoarau JY, Telismart H, D’Hont A (2004) Targeted mapping of a sugarcane rust resistance gene (Bru1) using bulked segregant analysis and AFLP markers. Theor Appl Gene 108:759–764

5. Beers E, McDowell J (2001) Regulation and execution of programmed cell death in response to pathogens, stress and developmental cues. Curr Opin Plant Biol 4:561–567

6. Bhadauria V, Banniza S, Vandenberg A, Selvaraj G, Wei Y (2012a) Peroxisomal alanine:glyoxylate aminotransferase AGT1 is indispensable for appressorium function of the rice blast pathogen, Magnaporthe oryzae. PLoS ONE 7(4):e36266

7. Bhadauria V, Banniza S, Vandenberg A, Selvaraj G, Wei Y (2012b) Alanine: Glyoxylate aminotransferase 1 is required for mobilization and utilization of triglycerides during infection process of the rice blast pathogen, Magnaporthe oryzae. Plant Signal Behav 7(9):1206–1208
8. Flor HH (1971) Current status of the gene-for-gene concept. Ann Rev Phytopathol 9:275–296. 
http://dx.doi.org/10.1146/annurev.py.09.090171.001423

9. Gao X, Neufeld TP, Pan D (2000) Drosophila PTEN regulates cell growth and proliferation through 
PI3K-dependent and -independent pathways. Dev Biol 221:404–

10. Grant G, More LJ, McKenzie NH, Dorward PM, Buchan WC, Telek L, Pusztai A (1995) Nutritional and 
haemagglutination properties of several tropical seeds. J Agric Sci 124:437–445

11. Hamdoun S, Liu Z, Gill M, Yao N, Lu H (2013) Dynamics of defense responses and cell fate change 
during Arabidopsis-Pseudomonas syringae interactions. PLoS ONE 8(12):e83219.
https://doi.org/10.1371/journal.pone.0083219

12. Janjusevic R, Abramovitch RB, Martin GB, Stebbins CE (2006) A bacterial inhibitor of host 
programmed cell death defenses is an E3 ubiquitin ligase. Science 311:222–226

13. Jones JD, Dangl JL (2006) The plant immune system. Nature 444:323–329

14. Kaku H, Nishizawa Y, Ishii-Minami N, Akimoto-Tomiyama C, Dohmae N, Takio K, Shibuya N (2006) 
Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. 
Proc Natl Acad Sci USA 103:11086–11091

15. Kumar VG, Viswanathan R, Malathi P, Nandakumar M, Sundar AR (2015) Differential Induction of 3- 
deoxyanthocyanidin phytoalexins in relation to Colletotrichum falcatum resistance in sugarcane. 
Sugar Tech 17:314–321

16. Malathi P, Viswanathan R, Padmanaban P, Mohanraj D, Kumar VG, Salin KP (2008) Differential 
accumulation of 3-deoxy anthocyanidin phytoalexins in sugarcane varieties varying in red rot 
resistance in response to Colletotrichum falcatum infection. Sugar Tech 10:154–157

17. Nandakumar M, Malathi P, Sundar AR, Rajadurai CP, Philip M, Viswanathan R (2021b) Role of 
microRNAs in the host–pathogen interaction between sugarcane and Colletotrichum falcatum, the red 
rot pathogen. Plant Cell Rept 40:851–870. DOI:10.1007/s12010-021-02682-9

18. Nandakumar M, Malathi P, Sundar AR, Viswanathan R (2021a) Host-pathogen interaction in 
sugarcane and red rot pathogen: Exploring expression of phytoalexin biosynthesis pathway genes. 
Indian Phytopathol 74:529–535. DOI: 10.1007/s42360-020-00306-y

19. Prasanth CN, Viswanathan R, Malathi P, Sundar AR, Tiwari T, Krishna N (2017) Unraveling the genetic 
complexities in gene set of sugarcane red rot pathogen Colletotrichum falcatum through 
transcriptomic approach. Sugar Tech 19:604–615. DOI: 10.1007/s12355-017-0529-3

20. Pratima PT, Raveendran M, Kumar KK, Rahul PR, Kumar VG, Viswanathan R, Sundar AR, Malathi P, 
Sudhakar D, Balasubramanian P (2013) Differential regulation of defense-related gene expression in 
response to red rot pathogen Colletotrichum falcatum infection in sugarcane. Appl Biochem 
Biotechnol 171:488–503. DOI 10.1007/s12010-013-0346-4

21. Rahul PR, Kumar VG, Sathyabahama M, Viswanathan R, Sundar AR, Malathi P (2015) 
Characterization and 3D structure prediction of chitinase induced in sugarcane during pathogenesis of 
Colletotrichum falcatum. J Plant Biochem Biotechnol 24:1–8. DOI: 10.1007/s13562-013-0226-6
22. Rahul PR, Kumar VG, Viswanathan R, Sundar AR, Malathi P, Prasanth CN, Pratima PT (2016) Defense transcriptome analysis of sugarcane and *Colletotrichum falcatum* interaction using host suspension cells and pathogen elicitor. *Sugar Tech* 18:16–28

23. Rowland O, Ludwig AA, Merrick CJ, Baillieul F, Tracy FE, Durrant WE, Jones JD (2005) Functional analysis of Avr9/Cf-9 rapidly elicited genes identifies a protein kinase, ACIK1, that is essential for full Cf-9–dependent disease resistance in tomato. *Plant Cell* 17:295–310

24. Sathyabhama M, Viswanathan R, Nandakumar M, Malathi P, Sundar AR (2015) Understanding sugarcane defence responses during the initial phase of *Colletotrichum falcum* pathogenesis by suppression subtractive hybridization (SSH). *Physiol Mol Plant Pathol* 91:131–140

25. Sathyabhama M, Viswanathan R, Malathi P, Sundar AR (2016) Identification of differentially expressed genes in sugarcane during pathogenesis of *Colletotrichum falcum* by suppression subtractive hybridization (SSH). *Sugar Tech* 18:176–183

26. Van den Burg HA, Tsitsigiannis DI, Rowland O, Lo J, Rallapalli G, MacLean D, Jones JD (2008) The F-box protein ACRE189/ACIF1 regulates cell death and defense responses activated during pathogen recognition in tobacco and tomato. *Plant Cell* 20:697–719

27. Viswanathan R (2010) Plant Disease: Red Rot of Sugarcane, Amol Publishers, New Delhi, India, p306

28. Viswanathan R (2021a) Red rot of sugarcane (*Colletotrichum falcatum* Went). *CAB Reviews* 16(023). doi: 10.1079/PAVSNNR202116023

29. Viswanathan R (2021b) Sustainable sugarcane cultivation in India through threats of red rot by varietal management. *Sugar Tech* 23:239–253. DOI: 10.1007/s12355-020-00882-3

30. Viswanathan R, Malathi P, Sundar AR, Aarthi S, Premkumari SM, Padmanaban P (2005) Differential induction of chitinases and thaumatin-like proteins in sugarcane in response to infection by *Colletotrichum falcum* causing red rot disease. *J Plant Dis Protect* 112:537–542

31. Viswanathan R, Nandakumar R, Samiyappan R (2003) Role of pathogenesis-related proteins in rhizobacteria-mediated induced systemic resistance against *Colletotrichum falcum* in sugarcane. *J Plant Dis Protect* 110:524–534

32. Viswanathan R, Padmanaban P, Selvakumar R (2020) Emergence of new pathogenic variants in *Colletotrichum falcum*, stalk infecting ascomycete in sugarcane: role of host varieties. *Sugar Tech* 22:473–484. DOI: 10.1007/s12355-019-00780-3

33. Viswanathan R, Prasanth CN, Malathi P, Sundar AR (2016) Draft genome sequence of *Colletotrichum falcum* – a prelude on screening of red rot pathogen in sugarcane. *J Genomics* 4:1–3. doi: 10.7150/jgen.13585

34. Viswanathan R, Rao GP (2011) Disease scenario and management of major sugarcane diseases in India. *Sugar Tech* 13:336–353

**Tables**
Table 1. Primer sequences of selected transcripts used in gene expression assays
| Transcript name                                      | Primer sequences 5’-3’ |
|-----------------------------------------------------|-----------------------|
| Chitin elicitor binding protein                      | F TGC CGGTCCAGGATG TGTG  |
|                                                     | R ATGACGGAGCTCCTCCACATGG |
| Mitogen activated protein kinase kinase1             | F GGTGCTGCTGATATACAGACAGGC |
|                                                     | R GGACCAAGACTG GCGT |
| Mitogen activated protein kinase kinase1             | F GGTGCTGCTGATATACAGACAGG |
|                                                     | R ACCAGACTTGGG GCGTGAAT |
| Brassinosteroid signaling kinase                     | F CAGCACACG GCCCGT TA  |
|                                                     | R CTGTGACGCA TTTT GGCC |
| Disease resistance protein RPM1                      | F GTGCAGGGTG GTCCTAAGCT |
|                                                     | R GGTGGAGTG CTCCTAAGGTACTG |
| Disease resistance protein RPS5                      | F GGCAGGGT GGAAGGACCAAG |
|                                                     | R CCATATTCTG GAAAAAGGTGCAG |
| Cyclic nucleotide gated channel                      | F CGCGATTGCCAACGACAG |
|                                                     | R TGCAGCAGT CTTGCAGGCA |
| Calcium dependent protein kinase                     | F GCACACCGGCT TGACAGACC |
|                                                     | R GTGGCTGTG CCAAAG TCTTGCC |
| Calcium binding protein CML                          | F ACACCAGTCA AAGCACACGCC |
|                                                     | R AGGATTCGA AAAGA GCTGGGT |
| Superoxide dismutase Cu-Zn family                   | F GCTGAGGGC GTAGCTGAGG |
|                                                     | R GCC AACAACACACATGCCAGT |
| V type proton transporting ATPase subunit1           | F GAGCTTGCG CATCAGAGCTG |
|                                                     | R GCAGTGCATG GAGAAGGCA |
| Jasmonic acid amino acid synthetase                  | F AGCCATTGGGCC CACTTGAG |
|                                                     | R GGTGGT GTTCTACTT ACTG |
| Abscisic acid responsive element binding factor      | F GGACTG GTACACTGCACTCTG |
|                                                     | R CGATGCCTG GAGAACATTTGA |
| Ethylene receptor                                   | F TCGTTTCTC CCGT |
|                                                     | R TGTGAGGCTGACTCCTT TGAGA |
| Protein              | F          | R          |
|----------------------|------------|------------|
| 14-3-3 protein epsilon | CGCATGCTCCCTCGCCA | CGTCAGGCTTGCTTGATCCC |
| Chitinase            | GCTGCAGATCTCGTGGAACGTCACAAC | GTGCACGTGTTCGCCAGAAG |
| 25SrRNA              | GCCAGCCAAGCGTTCATAGC | GGGTAAAAACTAATACCTGTCACGAC |

Table 2: Representative GO of RR and SR through BLAST homology search
| GO biological process | RRL | SRL |
|----------------------|-----|-----|
| 1. transcript_256    | aromatic amino acid family biosynthetic process; | transcript_277 | sucrase biosynthetic process; |
| 2. transcript_681    | protein N-linked glycosylation; | transcript_254 | negative regulation of peptidase activity; |
| 3. transcript_789    | small GTPase mediated signal transduction; | transcript_273 | spermine biosynthetic process; spermidine biosynthetic process; |
| 4. transcript_2602    | D-amino acid catabolic process; | transcript_649 | protein deubiquitination; post-translational protein modification; |
| 5. transcript_4835    | one-carbon metabolic process; | | |
| 6. transcript_2942    | 6PI anchor biosynthetic process; | | |
| 7. transcript_3315    | two-component signal transduction system (phosphorelay); peptidyl histidine phosphorylation; | | |
| 8. transcript_4821    | IMP biosynthetic process; | | |
| 9. transcript_4947    | small GTPase mediated signal transduction; | | |
| 10. transcript_5013   | mitotic chromosome segregation; | | |
| 11. transcript_6441   | oxidation-reduction process; terpenoid biosynthetic process; | | |
| 12. transcript_6937    | inositol triphosphate metabolism process; | | |
| **GO cellular component** | | | |
| 13. transcript_1862   | RNA polymerase complex; | transcript_2078 | E1-like RING ubiquitin ligase complex; |
| 14. transcript_2394    | cte-Celeb network; | | |
| 15. transcript_3744   | low-density lipoprotein particle; high-density lipoprotein particle; | | |
| 16. transcript_4014    | anaphase-promoting complex; | | |
| 17. transcript_4349    | cell junction; cell wall; | | |
| 18. transcript_7532    | transcription factor TFIID complex; | | |
| 19. transcript_7780    | photosystem II; chloroplast thylakoid membrane; | | |
| 20. transcript_7849    | photosystem II; integral to membrane; chloroplast thylakoid membrane; | | |
| **GO molecular function** | | | |
| 21. transcript_256    | thiamine kinase activity; ATP binding; magnesium ion binding; | transcript_254 | serine-type endopeptidase inhibitor activity; peptidase activity; |
| 22. transcript_555    | P-P bond-hydrolysis-driven protein transmembrane transporter activity; | transcript_2789 | quinone binding; oxidoreductase activity, acting on NAD+ or NADP+; |
| 23. transcript_1184    | mannosyl oligosaccharide 1,2-alpha-mannosidase activity; calcium ion binding; | | |
| 24. transcript_1517    | cellulose synthase (UDP-forming) activity; | | |
| 25. transcript_1584    | SNAP receptor activity; | | |
| 26. transcript_3939    | P-P bond-hydrolysis-driven protein transmembrane transporter activity; | | |
| 27. transcript_4002    | N-acetyltransferase activity; | | |
| 28. transcript_4037    | aspartate-type endoprotease activity; | | |
| 29. transcript_6112    | serine-type endoprotease activity; | | |
| 30. transcript_6441    | mannose-1-phosphate guanylyltransferase (CGT) activity; | | |
| 31. transcript_6977    | inositol 1,3,4-trisphosphate 5/6 kinase activity; inositol tetakisphosphate 1-kinase activity; ATP binding; magnesium ion binding; | | |
| 32. transcript_1967    | NADH dehydrogenase (ubiquinone) activity; | | |

Table 3. Representative differential transcripts from RRL and SRL and their putative function as mapped in KEGG-KASS database
| S.No | Transcripts                                                        | Function            | RR | SR |
|------|-------------------------------------------------------------------|---------------------|----|----|
| 1    | Chitin elicitor binding protein (CEBiP)                          | Recognition         | √  | √  |
| 2    | Serine Threonine protein kinase (STPK)                           | Signalling          | √  | √  |
| 3    | Mitogen activated protein kinase kinase1 (MAPKKK1)                | Signalling          | √  | x  |
| 4    | Mitogen activated protein kinase kinase1 (MAPKK1)                 | Signalling          | √  | x  |
| 5    | Disease resistance protein RPM1 (DRP RPM1)                        | Defense             | √  | x  |
| 6    | Disease resistance protein RPS2 (DRP RPS2)                        | Defense             | √  | x  |
| 7    | Disease resistance protein RPS5 (DRP RPS5)                        | Defense             | √  | x  |
| 8    | Protein kinase (PK)                                               | Signalling          | √  | x  |
| 9    | Brassinosteroid signalling kinase (BRSK)                          | Signalling          | √  | √  |
| 10   | 14-3-3 protein (14-3-3 P)                                        | Multifaceted roles  | √  | √  |
| 11   | Phospholipase C (PLC)                                             | Signalling          | √  | √  |
| 12   | Phospholipase D (PLD)                                             | Signalling          | √  | √  |
| 13   | Phosphoinositide 3 kinase (PI3K)                                  | Signalling          | √  | x  |
| 14   | Phosphoinositide 4 kinase (PI4K)                                  | Signalling          | √  | x  |
| 15   | Linoleate 9S lipoxygenase (LOX)                                   | Signalling          | √  | x  |
| 16   | Jasmonic acid amino synthetase (JAAS)                             | Signalling          | √  | x  |
| 17   | Calcium binding protein CML (CBP CML)                             | Signalling          | √  | √  |
| 18   | Calcium binding protein 39 (CBP 39)                               | Signalling          | √  | x  |
| 19   | Calmodulin (CaM)                                                  | Signalling          | √  | √  |
| 20   | Calcium dependent protein kinase (CDPK)                           | Signalling          | √  | √  |
| 21   | Cyclic nucleotide gated channel (CNGC)                            | Redox homeostasis   | √  | x  |
| 22   | Respiratory burst oxidase (RBO)                                   | Oxidative stress    | √  | √  |
| 23   | Catalase (CAT)                                                    | Oxidative stress    | √  | √  |
| 24   | Superoxide dismutase (SOD)                                        | Oxidative stress    | √  | √  |
| 25   | Glutathione peroxidase (GPOX)                                     | Oxidative stress    | √  | √  |
| 26   | L-Ascorbate peroxidase (L-APX)                                    | Oxidative stress    | √  | √  |
| 27   | Peroxidase (POX)                                                  | Oxidative stress    | √  | √  |
|    | Protein Name                                | Function                          | Found in | Missing in |
|----|--------------------------------------------|-----------------------------------|----------|------------|
| 28 | Glutathione S transferase (GST)             | Redox homeostasis                 | √        | √          |
| 29 | Glutathione S reductase (GSR)               | Redox homeostasis                 | √        | √          |
| 30 | Abscisic acid receptor PYR/PYL (ABAR PYR/PYL) | Signalling                       | √        | √          |
| 31 | Protein phosphatise 2C (PP2C)               | Signalling                       | √        | √          |
| 32 | Ethylene receptor (ETR)                    | Recognition & Signalling          | √        | x          |
| 33 | Ethylene insensitive 2 (EIN2)               | Signalling                       | √        | x          |
| 34 | Ethylene insensitive 3 (EIN3)               | Signalling                       | √        | x          |
| 35 | S-adenosyl methionine synthetase (SAMS)    | Secondary metabolism              | √        | √          |
| 36 | S-adenosyl methionine decarboxylase (SAMDC) | Secondary metabolism              | √        | √          |
| 37 | Hexokinase (HK)                            | Glycolysis                        | √        | x          |
| 38 | Phosphoglycerate mutase (PGM)              | Glycolysis                        | √        | x          |
| 39 | Pyruvate dehydrogenase (PDH)               | Glycolysis                        | √        | x          |
| 40 | Succinate dehydratase (SDH)                | Tricarboxylic acid cycle          | √        | x          |
| 41 | Phenylalanine ammonia lyase (PAL)          | Phenyl propanoid biosynthesis     | √        | x          |
| 42 | 4-Coumarate CoA ligase (4-CouCoAL)         | Phenyl propanoid biosynthesis     | √        | x          |
| 43 | Coniferyl aldehyde dehydrogenase (ConADH)  | Phenyl propanoid biosynthesis     | √        | x          |
| 44 | Cinnamyl alcohol dehydrogenase (CinAlcDH)  | Phenyl propanoid biosynthesis     | √        | x          |
| 45 | Trans-cinnamate 4 monooxygenase (TC4MO)    | Phenyl propanoid biosynthesis     | x        | √          |
| 46 | Tyrosine aminotransferase (TyrAT)          | Alkaloid biosynthesis             | √        | √          |
| 47 | Tyrosine decarboxylase (TyrDC)             | Alkaloid biosynthesis             | √        | √          |
| 48 | Phosphomevalonate kinase (PMK)             | Terpenoid biosynthesis            | √        | x          |
| 49 | Diphosphomevalonate decarboxylase (DPMDC)  | Terpenoid biosynthesis            | √        | x          |
| 50 | Farnesyl diphosphate synthase (FDPS)       | Terpenoid biosynthesis            | √        | x          |
| 51 | Phytoene synthase (PhyS)                   | Carotenoid biosynthesis           | √        | x          |
| 52 | Zeaxanthin epoxidase (ZXEOX)               | Carotenoid biosynthesis           | √        | x          |
| 53 | Violaxanthin de-epoxidase (VXDOX)          | Carotenoid biosynthesis           | √        | x          |
| 54 | Chalcone synthase (CS)                     | Flavonoid biosynthesis            | x        | √          |
|   | Description                                                                 | Location/Pathway         | Presence | Absence |
|---|------------------------------------------------------------------------------|--------------------------|---------|---------|
| 55| Photosystem I P 700                                                          | Light harvesting complex | ✓       | ✓       |
| 56| Photosystem II                                                               | Light harvesting complex | ✓       | x       |
| 57| Cytochrome b6                                                                 | Light harvesting complex | ✓       | x       |
| 58| Apocytochrome f                                                              | Light harvesting complex | x       | ✓       |
| 59| F-type H+-transporting ATPase subunit                                        | Ion transport            | ✓       | ✓       |
| 60| Clathrin heavy chain                                                         | Secretory pathway        | ✓       | x       |
| 61| Charged multivesicular body protein                                          | Secretory pathway        | ✓       | ✓       |
| 62| Vacuolar protein sorting associated protein                                  | Secretory pathway        | ✓       | ✓       |
| 63| Ras-related protein                                                          | Secretory pathway        | ✓       | x       |
| 64| Programmed cell death 6 interacting protein (PCD6IP)                         | Secretory pathway        | ✓       | x       |
| 65| E3 ubiquitin protein ligase (E3UbL)                                          | Secretory pathway        | ✓       | x       |
| 66| Vesicle transport protein                                                    | Secretory pathway        | ✓       | x       |
| 67| Signal recognition particle receptor subunit                                 | Protein processing in ER  | ✓       | ✓       |
| 68| Signal peptidase                                                             | Protein processing in ER  | ✓       | ✓       |
| 69| Chitinase                                                                    | Defense                   | ✓       | ✓       |
| 70| Endoglucanase                                                                | Defense                   | ✓       | x       |
| 71| Pathogenesis related 1 (PR-1)                                                 | Defense                   | ✓       | x       |
| 72| Pectin esterase                                                              | Cell wall metabolism      | x       | ✓       |

RR - Resistance response, SR - Susceptible response, ✓ - Presence of the transcripts, x - absence of the transcripts

**Table 4. Transcript homologous to *Glomerella graminicola* from SRL identified through BLASTx homology search**
| S.No | Transcripts                                             | Function                  |
|------|--------------------------------------------------------|---------------------------|
| 1    | Endoribonuclease L-PSP                                  | Translation               |
| 2    | Hexokinase                                             | Glycolysis                |
| 3    | Ribosomal L2 domain containing protein                 | Translation               |
| 4    | G-patch domain containing protein                      | RNA splicing              |
| 5    | 25s ribosomal protein                                  | Translation               |
| 6    | Ribosomal protein s28e                                 | Translation               |
| 7    | Ribonucleotide reductase                               | Translation               |
| 8    | Hypothetical protein GLRG                              | Unknown                   |
| 9    | Ribosomal protein L18ae protein family                 | Translation               |
| 10   | Ras family protein                                     | Intracellular signal transduction |
| 11   | AMP binding enzyme                                     | Intracellular signal transduction |
| 12   | Sulfate permease                                       | Transmembrane protein     |
| 13   | Zinc finger containing protein                         | Proteasome system         |
| 14   | Translation elongation factor EF-1                     | Translation               |
| 15   | Ribosomal L29e family protein                          | Translation               |
| 16   | Ribosomal protein s12                                  | Translation               |
| 17   | Alanine-glyoxylate amino transferase                   | Fungal morphogenesis      |

**Figures**
Figure 1

Phenotypic symptoms of sugarcane cv Co 7805 exhibiting differential response to inoculation with two different C. falcatum pathotypes Cf87012 (1) and Cf94012 (2); arrows indicate point of pathogen inoculation.

Figure 2

Gene ontology distribution in resistant (RRL) and susceptible (SRL) responses in SSH libraries of sugarcane challenged with virulent and less virulent pathotypes of C. falcatum.
**Figure 3**

Functional categorization of transcripts involved in resistant (RR) and susceptible (SR) responses in SSH libraries of sugarcane challenged with virulent and less virulent pathotypes of C. falcum.
Figure 4

Gene expression profiling of transcripts in incompatible (Co 7805 vs Cf 87012) and compatible interactions (Co 7805 vs Cf 94012). C – Mock control, h – hours post C. falcatum inoculation, X- axis – hours post C. falcatum inoculation; Y – axis – RQ values.
Figure 5

Schematic representation of probable hypothetical events occurring in sugarcane variety Co 7805 to two different C. falcatum pathotypes during incompatible (Cf87012) and compatible (Cf94012) interactions. Transcripts in green represents those involved in incompatible interaction. Red- transcripts involved in both compatible and incompatible interactions. Blue- transcripts involved compatible interaction. Lavender-represents transcripts involved in the reproductive stage of C. falcatum pathotype Cf94012 inside the host cells. CEBIP – Chitin elicitor binding protein, LRR-NBS-CC- Leucine rich repeat- Nucleotide binding site, Coiled coil, DRP-Disease resistant protein, RPM1-, RPS2-, RPS5-, STPK- Serine threonine protein kinase, MAPKKK1 – Mitogen activated protein kinase kinase kinase 1, MAPKK1 – Mitogen activated protein kinase kinase 1, BRSK – Brassinosteroid signalling kinase, 14-3-3P – 14-3-3 Protein, JA-
Jasmonic acid, LOX – Lipoxygenase, DAG – Diacylglyceol, PI, PI3K, PI4K, IP3, IP4, CBP– Calcium binding protein, Ca2+– Calcium, CDPK – Calcium dependent protein kinase, CNGC – Cyclic nucleotide gated channel, ROS– Reactive oxygen species, RBO – Respiratory burst oxidase, CAT – Catalase, SOD – Superoxide dismutase, GPOX- Glutathione peroxidase, L-APX – L-Ascorbate peroxidase, POX – Peroxidase, ABA – Abscisic acid, ABAREBP – ABA response element binding protein, PYR/PYL – ABA receptor, PP2C-, SAM – S-adenosyl methionine, SAMDC – SAM decarboxylase, ET- Ethylene, ETR – ET receptor, EIN2 – Ethylene insensitive 2, EIN3 - Ethylene insensitive 3, Glc-1-P – Glucose 1 phosphate, PDH – Pyruvate dehydrogenase, TCA – Tricarboxylic acid cycle, SDH – Succinate dehydrogenase, PEP – phosphoenol pyruvate, E4P – Erythrose 4 phosphate, PAL – Phenylalanine ammonia lyase, 4-CCoAL-, CoADH – Coniferyl aldehyde dehydrogenase, CiADH – Cinnamyl alcohol dehydrogenase, TyrAT-, TyrDC-, PMK-, DPMDC-, FDPS-, PS-, ZXEOX-, VXDOX-, PCD – Programmed cell death-, Ub- Ubiquitin, SCF – Skp1-Cullin- F-box, HR – Hypersensitive response, TC4MO-, CS – Chalcone synthase,

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