Transcriptomic analysis of bakanae disease resistant and susceptible rice genotypes in response to infection by *Fusarium fujikuroi*

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Abstract

Background *Fusarium fujikuroi* causing bakanae is one of the most significant pathogen of rice and much responsible for yield losses thereby emerging as a major risk to food security.

Methods In the present study transcriptomic analysis was conducted between two contrasting resistant (C101A51) and susceptible (Rasi) genotypes of rice with the combinations of C101A51 control (CC) vs C101A51 inoculated (CI); Rasi control (RC) vs Rasi inoculated (RI) and C101A51 inoculated (CI) vs Rasi inoculated (RI).

Results In CC vs CI commonly expressed genes were 12,764. Out of them 567 (4%) were significantly upregulated and 1399 (9%) genes were down regulated. For the RC vs RI 14, 333 (79%) genes were commonly expressed. For CI vs RI 13,662 (72%) genes commonly expressed. Cysteine proteinase inhibitor 10, disease resistance protein TAO1-like, oleosin 16 kDa-like, pathogenesis-related protein (PR1), (PR4), BTB/POZ and MATH domain-containing protein 5-like, alpha-amylase isozyme 3D-like (LOC4345814), were upregulated in resistant genotype C101A51. Whereas, GDSL esterase/lipase At5g33370, serine glyoxylate aminotransferase, CASP-like protein 2C1, WAT1-related protein At4g08290, Cytoplasmic linker associated proteins, xyloglucan endotransglucosylase/hydrolase protein and β-D xylosidase 7 were upregulated in susceptible genotype Rasi. Gene ontology analysis showed functions related to defence response (GO:0006952), regulation of plant hypersensitive type response (GO:0010363), Potassium ion transmembrane activity (GO:0015079), chloroplast (GO:0009507), response to wounding (GO:0009611), xylan biosynthetic process (GO:0045492) were upregulated in resistant genotype C101A51 under inoculated conditions.

Conclusions Real time PCR based validation of the selected DEGs showed that the qRT-PCR was consistent with the RNA-Seq results. This is the first transcriptomic study against bakanae disease of rice in Indian genotypes and will be helpful for the development of bakanae management strategies.

Introduction

Rice is an important cereal crop with an overall production of 735 million metric tons [1]. Basmati varieties are of high significance due to its rich aroma, flavor and other unique properties [2]. India produces a large share with an overwhelming 90% yield contribution worldwide and is the largest exporter of basmati returning approximately 30000 crores of revenue in year 2019 [3]. However, the yield of rice is affected by various biotic and abiotic stresses in the environment.

Bakanae disease of rice caused by *Fusarium fujikuroi* is emerging as the most devastating disease causing yield losses in almost all rice producing states of India [4–5]. It is responsible for upto 70% yield losses thereby emerging as a major risk to food security [6–8]. The disease symptoms include abnormal elongation of seedlings, etiolation, root and crown rot and stunted growth with few tillers which bears empty panicles if survive upto later stages [4, 9]. However, the symptoms vary according to the fungal strain and the rice variety [10–12].

Although the effect of bakanae on rice crop has been comprehensively studied however; molecular basis for acquisition of resistance in plants is still a debatable issue. A total of 28 QTLs for bakanae resistance were mapped in different chromosomes of rice, no causal genes have been cloned and functionally characterized. Hence, the transcriptome mediated study of defense genes involved in various host pathogen interaction and tolerance mechanisms is crucial to unravel the possible pathways for disease resistance and susceptibility. It's

been widely reported that RNA sequencing is used in illustrating the resistance mechanism in rice cultivars against various pathogens [13–15]. For instance, the upregulation of defence genes imparting blast resistance to rice cultivars were identified [13]. Matic et al [16] also described transcriptome analysis between contrasting genotypes (Selenio and Dorella) of rice against *Fusarium fujikuroi*. A number of defense genes such as PR1, germin-like proteins, glycoside hydrolases, MAP kinases, and WRKY transcriptional factors were up-regulated in the resistant cultivar. Further, the whole genome sequencing studies on *Fusarium fujikuroi* and interaction with susceptible and resistant cultivars of rice suggested an up and down regulation of some enzymes in resistant cultivars [17]. However, knowledge about resistant and susceptibility of rice genotypes against bakanae pathogen *Fusarium fujikuroi* is still limited. Therefore, the present study evaluates whole transcriptome analysis in bakanae resistant and susceptible rice cultivars post inoculation with *Fusarium fujikuroi*. The expression of some important gene is validated by qRT PCR.

Material And Methods

Seed material and source

Seeds of two contrasting cultivars of rice (*Oryza sativa*) Rasi (highly susceptible) and C101A51 (resistant) [17–18] were procured from Division of Genetics, Indian Agricultural Research Institute, New Delhi.

Inoculation And Plant Growth Conditions

The virulent *Fusarium fujikuroi* isolate “F250” (GenBank Accession no. KM50526; MBPO00000000) which was isolated from symptomatic bakanae infected rice plant in Hisar, Haryana (India) was used for the study [4, 17]. Spore suspension was prepared with sterile distilled water using 15 days old culture of *F. fujikuroi* and filtered through two layers of sterile muslin cloth and brought to a final concentration of 10^6 spores ml^{-1} to be used for inoculation. The seeds of resistant and susceptible genotypes were first surface sterilized with 1% (v/v) sodium hypochloride followed by washing with sterilized distilled water and kept in *F. fujikuroi* “F250” inoculum suspension for 18 h at room temperature (25°C) and control seeds were soaked in sterile distill water. The seeds were then sown in earthen pots containing sterilized sand: soil (3:1) mixture and grown under day/night temperatures of 30–35°C/18°C and a relative humidity of 80/90%. The pots were divided into two groups: one group was used as control (RC, Rasi control; CC, C101A51, control) and to the other bakanae inoculation (RI, Rasi infected; CI, C101A51 infected) was done. Each treatment had a randomized block design with three replications, hence, overall 18 plants for each cultivars (9 control and 9 inoculated; 3 blocks per sampling time). The plant samples were harvested after 7, 10 and 30 days of inoculation and stored immediately using liquid nitrogen at -80°C for transcriptome analysis [16, 17].

Total Rna Extraction

Total RNA extraction

Total RNA was isolated from 8 samples using trizol (TRI reagent, Molecular research centre, Ohio, USA) following the manufacturer’s instruction. Briefly, a 100 mg seedling of rice was homogenized using trizol and incubated for 5 min at room temperature. Then a 200 µl of chloroform was added to it and incubated for 10 min at room temperature after shaking vigorously. The samples were centrifuged (Eppendorf AG, Heidelberg, Germany) at

12000 g for 15 min and the upper aqueous phase was separated. A 500 µl of isopropanol was added to it and incubated at room temperature for 5 min. The RNA was pelleted by centrifuging at 10000 rpm for 10 min and purified by washing twice with 75% (v/v) alcohol. The quality and quantity of the isolated RNAs were checked on denatured RNA agarose gel and NanoDrop (Thermo Fisher Scientific, Wilmington, USA) reading, respectively.

Transcriptome Analysis

The RNA Seq paired end sequencing libraries were prepared from the isolated total RNA using illumina TruSeq stranded mRNA sample preparation kit (Illumina, San Diego, California U.S.A.). For this, mRNA was enriched from the total RNA using poly-T attached magnetic beads, followed by enzymatic fragmentation, 1st strand cDNA conversion. The 1st strand cDNA was then synthesized to second strand using second strand mix and Act-D mix to facilitate RNA dependent synthesis. The ds cDNA samples were then purified using Ampure XP beads (New England Biolabs, Ipswich, Massachusetts, USA) followed by A-tailing, adapter ligation and then enriched by limited no of PCR cycles.

Quantity and quality check (QC) of library on Agilent 4200 Tape Station

The PCR enriched libraries were analyzed in 4200 Tape Station system (Agilent Technologies) using High sensitivity D1000 Screen tape as per manufacturer instructions.

Cluster Generation And Sequencing

After obtaining the Qubit concentration for the libraries and the mean peak size from Agilent Tape Station profile, the PE illumina library was loaded onto NextSeq 500 for cluster generation and sequencing. Paired-End sequencing allows the template fragments to be sequenced in both the forward and reverse directions on NextSeq 500. The samples were bound to complementary adapter oligos on paired-end flow cell. The adapters were designed to allow selective cleavage of the forward strands after re-synthesis of the reverse strand during sequencing. The copied reverse strand was then use to sequence from the opposite end of the fragment.

Gene expression analysis by real time reverse transcriptase PCR (qRT-PCR)

The qRT-PCR was done to validate the significant contribution of the candidate genes in imparting host resistance in rice cultivars against bakanae pathogen, *Fusarium fujikuroi*. All the qRT-PCR experiments were conducted in three replicates. For gene expression analysis, first RNA was extracted as stated above. Then, a 3 µg of total RNA was used for cDNA synthesis according to manufacturer's protocol. To this nuclease free water is added to make up the volume to 9 µl followed by addition of 2 µl random hexamer and spun down at 1 000 rpm and incubated at 65°C for 10 min followed by incubation at room temperature for 2 min. Different reagents were added in an indicated order as follows: 1 µl of an RNase inhibitor, 1 µl of 100 mM dithiotreitol, 4 µl of a 5× assay buffer, 2 µl of a 10 mM dNTP mix, 1 µl of M-MuLV reverse transcriptase and 1 µl of nuclease free water; mixed gently and spun slowly at 1000 rpm for 15 s. The tubes were incubated at 37°C for 60 min. The reaction was terminated by heating at 70°C for 15 min.

The PCR reaction mix was prepared using the primer pairs specific to rice (Supplementary), and GAPDH was used as internal control [19]. The reaction comprises a 2 µl of 1:10 diluted cDNA mixed with a SYBR Green PCR master

mix (Thermoscientific, Thermo Fisher Scientific Baltics, Vihius, Lithuania), 5 pmol of a forward primer and 5 pmol of a reverse primer in a final volume of 20 µl. Template controls were analyzed for all genes. The PCR was performed using a miniOpticon real time PCR system (Biorad, Hercules, California, USA) with the following conditions: an initial activation step at 94°C for 4 min, denaturation at 94°C for 15 s, annealing at 58°C for 15 s, and extension at 70°C for 20 s. Melt curve analysis of the PCR product was carried out at 72°C for 1 min and ramped from 75 to 95°C with a rise by 1°C every 5 s. The specificity of the reaction was confirmed by a melt curve and gel electrophoresis. Relative gene expressions were calculated in terms of fold changes using the ΔCt method. The results are expressed as arithmetic means and standard deviations of three replicates.

Analysis Of Data

The data obtained for differential expression of genes was analyzed by bioinformatics tools. The highly up regulated and down regulated genes in CC vs CI, RC vs RI and CI vs RI were selected. Then the sequences of the genes were searched using gene ID and subjected to BLAST for similarity search to assign functions. The expression of genes contributing in defense pathways involved in various metabolic processes was validated using real time PCR.

For Hierarchial cluster analysis a heat map was constructed using the log-transformed and normalized value of genes based on Euclidean distance as well as based on average linkage method. The heatmap shows level of gene abundance. Cluster analysis was performed for the top 100 differentially expressed genes in CI and RI. For the qPCR analysis 3 biological replicates were taken with 3 technical replicates of each and gene expression was calculated using in terms of fold changes using the ΔCt method.

Pathway Analysis

The functional annotations of genes were carried out against the curated KEGG GENES database using KAAS (KEGG Automatic Annotation Server). (http://www.genome.jp/kegg/ko.html) KAAS was used for functional annotation of commonly expressed genes against KEGG GENES database. Subsequently, each gene is provided with KO (KEGG Orthology) assignment and its association with KEGG metabolic pathway. The KEGG Orthology database of *O. sativa* was used as the reference for pathway mapping. The result contains KO (KEGG Orthology) assignments and automatically generated KEGG pathways using KAAS the BBH (bidirectional best hit) method against *O. sativa* Ref-seq database.

Results

Gene profile and differential expression study

Gene profile study of CC, CI, RC and RI samples were acquired by Illumina NextSeq500 platform. Overall a total of 16,831,988 (2 x 150 bp), 15,407,625 (2 x 150 bp), 15,742,730 (2 x 150 bp) and 14,563,773 (2 x 150 bp) high quality reads were retained for CC, CI, RC and RI sample respectively. Further, differential gene expression analysis was carried out between the samples. Transcriptomic analysis was conducted between CC vs CI (Supplementary table 1), CI vs RI (Supplementary table 2) and RC vs RI (Supplementary table 3). In CC vs CI differentially expressed genes in both the conditions were 12,764 (79%), 429 (3%) genes were observed

exclusively in CC, whereas 239 (1%) genes were identified exclusively in CI. Out of them 567 (4%) were significantly upregulated and 1399 (9%) genes were downregulated. For the RC vs RI 14, 333 (79%) genes were expressed in both inoculated plants and control plants. 346 (2%) genes were exclusive in RC whereas 359 (2%) genes were exclusive in RI. Out of them 1575 (9%) genes were significantly upregulated and 892 (5%) genes were significantly down regulated. When we compared CI vs RI 13,662 (72%) genes were identified to be commonly expressed. Further, 280 (1%) genes were exclusive in CI and 532 (3%) genes were exclusive in CI. A total of 2438 (13%) genes were observed to be significantly upregulated whereas 750 (4%) genes were observed to be down regulated (Table 1, Fig. 1 and Fig. 2).

Table 1
Statistics of differential expression analysis of CC and CI, CI vs RI and RC vs RI

Sample name	No. of genes Expressed in Both	Exclusive in CC	Exclusive in CI	Up Regulated	Down Regulated	Up Regulated FDR Value	Down Regulated FDR Value
CC Vs CI	12,764	429	239	567	1,399	183	549
		Exclusive in CI	**Exclusive in RI**				
CI Vs RI	13,662	280	532	2,438	750	913	333
		Exclusive in RC	**Exclusive in RI**				
RC Vs RI	14,333	346	359	1,575	892	327	268

The genes showing significant expression were considered for establishing relation with the rice cultivars' response to pathogen. In the resistant cultivar, C101A51 the expression of defense related genes such as lipoxygenase, transcription factor WRKY, UDP glucosyltransferase were mostly upregulated whereas, a number of chloroplastic genes (sedoheptulose-1, 7 bisphosphotase, fructose-1,6 bisphosphatase) were downregulated. However, in the susceptible cultivar Rasi expression of the genes mixed expression were observed for defense genes where genes like, disease resistant RPP 13, sialyl transferase, glutamate receptor, sulfotransferase, RGA2, protein kinase, beta glucanase, etc.) were upregulated whereas TAO1-like, cysteine proteinase inhibitor were down regulated (Fig. 3; Supplementary table 2A). Whereas, genes related to glycin rich wall structural protein 2-like (LOC4348767, BGIOSGA030193), sulfite exporter Tau/SalE family protein 3(loc4345475), gdsl esterase/lipase At5g3337U (LOC4348826), glyoxylate aminotransferase (LOC4345962), CASP-like protein 2c1 (LOC4341716), WAT1- related protein At4g08290 (LOC4329749), ribulose biphosphate carboxylase small chain, chloroplastic like (LOC4352021), kinesin like protein kin12a (loc4335552) were more expressed in RI (Fig. 3; (Supplementary table 2A).

Gene Ontology Study

Gene Ontology analysis revealed GO representation in CC and CI sample. A total of 295, 160, 389, 141, and 11 significant GO terms obtained in commonly expressed genes, upregulated, downregulated, exclusive expressed genes in CC and CI sample respectively based on Singular Enrichment Analysis (SEA) and Hypergeometric test with Hochberg FDR was performed using agriGO (Supplementary table 4). Gene Ontology analysis revealed GO representation in CI and RI sample. A total of 267, 499, 177, 11 and 144 significant GO terms obtained in commonly expressed genes, upregulated, downregulated, exclusively expressed genes in CI and RI sample respectively based on Singular Enrichment Analysis (SEA) and Hypergeometric test with Hochberg FDR was performed using agriGO (Supplementary table 5). Gene Ontology enrichment analysis revealed GO representation in RC and RI sample. A total of 249, 466, 250, 16, and 14 significant GO terms obtained in commonly expressed genes, upregulated, downregulated, exclusively (Supplementary table 6). Some of the GO IDs commonly present in all the treatments related to host pathogen interactions were GO:0004601 (Peroxidase), GO:0009507 (Chloroplast), GO:0006952 (Defense response) GO:0015979 (Photosynthesis), GO:0003700 (Transcription factor), GO:0009611 (Response to wounding). Maximum no. of these ids were observed in the treatments CI vs. RI followed by RC vs RI and CC vs. CI (Table 2). Gene ontology analysis showed functions related to defence response (GO:0006952), regulation of plant hypersensitive type response (GO:0010363), Potassium ion transmembrane activity (GO:0015079), chloroplast (GO:0009507), response to wounding (GO:0009611), xylan biosynthetic process (GO:0045492) were upregulated in resistant genotype C101A51 under inoculated conditions. GO IDs GO:0009733 (Response to auxins), GO:0015079 (Potassium ion transmembrane activity), GO:0006506 (GPI anchor biosynthetic process), were observed only under inoculated conditions. GO IDs GO:0045492 (Xylan biosynthetic process) and GO:0008146 (Sulfotransferase activity) was observed more in number) in susceptible genotype Rasi (17, 6 respectively) compared to resistant genotype C101A51 (5, 3 respectively).

Table 2
GO enrichment analysis of the genes responsible in host pathogen interaction

GO: ID	Function	CI VS RI		RC vs RI		CC vs CI	
		Up-regulated	Down-regulated	Up-regulated	Down-regulated	Up-regulated	Down-regulated
GO:0004601	Peroxidase	17	19	12	9	10	5
GO:0006952	Defense response	54	12	40	18	9	18
GO:0006950	Response to stress	3	9	3	6	3	6
GO:0008061	Defense to fungus	2	2	3	0	3	0
GO:0010363	Regulation of plant hypersensitive type response	12	0	0	11	1	1
GO:0003700	Transcription factor	38	36	26	21	23	21
GO:0010279	Indole-3-acetamide synthetase activity	0	2	0	0	2	0
GO:0015079	Potassium ion transmembrane activity	4	0	5	0	2	0
GO:0009733	Response to auxins	5	2	3		3	
GO:0009507	Chloroplast	143	19	63	123	11	124
GO:0008146	Sulfotransferase activity	1		3	3	0	3
GO:0009611	Response to wounding	15	3	13	7	2	7
GO:0006506	GPI anchor biosynthetic process	3	0	3	0	3	0
GO:0008299	Isoflavonoid biosynthetic process	2	1	0	2	1	2
GO:0045492	Xylan biosynthetic process	10	1	8	9	2	3
GO:0010223	Secondary shoot formation	0	1	0	0	0	0

GO: ID	Function	CI VS RI		RC vs RI		CC vs CI	
GO:0009826	Undimensional cell growth	2	5	1	1	4	1
GO:0015979	Photosynthesis	35	0	7	35	0	35
GO:0019748	Secondary metabolic process	3	0	0	4	0	4

Pathway Analysis

Commonly expressed genes were classified into 22 functional Pathway categories (Fig. 4).

CC vs CI

Genes related to extracellular region part structural molecule, cellular component biogenesis, cellular component organization; establishment of localization were exclusively expressed in CC whereas genes related to membrane enclosed lumen, enzyme regulator, immune system process, multi organism process were exclusively regulated in CI (Supplementary table 7).

CI vs RI

Genes related nutrient reservoir immune system processes were exclusively expressed in CI. Further, genes related to envelope, enzyme regulator, structural molecule, anatomical structural formation, cellular component biogenesis, cellular component organization, growth, reproduction, reproductive process were exclusively expressed in RI (Supplementary table 8).

RC vs. RI

Genes with function of membrane enclosed lumen, electron carrier, molecular transducers, biological adhesion, death were exclusively up regulated while genes related to nutrient reservoir translation regulator were exclusively down regulated in RI compared to RC (Supplementary table 9).

Commonly up regulated

Overall no. of hits related to aging and cell growth and death observed were more in susceptible genotype compared to resistant one.

Validation Of Differentially Expressed Genes Through Real Time Pcr

Expression of genes like CTP synthase were observed more at 7 days of inoculation in resistant genotype, whereas its expression decreased after 10 days and significantly increased expression of CTP synthase was observed at 10 days after inoculation. Genes like disease resistance protein TAO1-like, putative disease resistance protein RGA3, and putative NBS-LRR disease resistance protein, miniribonuclease 3 were expressed

more at early days after inoculation and expression of these genes reduced after 10 days of inoculation. Expression of β-glucanase was more at 10 days of after inoculation in resistant genotype. Further, expression of WRKY transcription factor 33, was observed more at 7 and 10 days of inoculation in resistant genotype. Expression pattern of genes related to glucose and ribitol dehydrogenase and TBC domain family member 8B was increased along with the days of inoculation in both the genotypes and it was expressed maximum at 30 days after inoculation in genotype Rasi (Fig. 5).

Discussion

Bakanae is a major disease of rice causing risk to food security at a large scale. Amongst all the varieties of rice, the yield losses in basmati are of high economic importance. Hence, the present study focuses on elucidation of some possible pathways and gene expression analysis through whole transcriptome analysis in contrasting cultivars of Indian basmati rice (*Oryza sativa* L.).

The data presented clearly demonstrates some defense mechanisms exhibited by the resistant cultivar, C101A51 in response to infection with *Fusarium fujikuroi*. A large no. of peroxidase genes were observed in susceptible genotype Rasi compared to resistant genotype C101A51. This may be due to low spread of pathogen in resistant genotype as peroxidases are required to prevent the cellular diffusion of pathogen. Similary, no. of genes related to chloroplast were observed more in Rasi compared to C101A51. A decrease in the expression of chloroplastic genes was observed which may be considered as a resistance strategy of the rice by limiting the source of available carbon for pathogen growth and diverting the pathway towards defense responses [20]. Heat stress related transcripts (BIP4 like protein, transcription factor A-2a like, BHLH148) were upregulated in resistant genotype compared to susceptible genotype. Heat stress transcription factors are also reported against the fungal disease resistance in different plant species [21–22]. Similarly, upregulation of heat stress related transcription factors was also reported in bakanae resistant genotype Selenio [16].

ABA receptor PYL9 like protein helps in stomatal closure and reduced transpiration against environmental stresses. Gene ID BGIOSGA033746 coding antimicrobial peptides also expressed more in C101A51. Antimicrobial peptides or host defense peptides are produced as a first line of defense by plants as an antibiotic to protect against potential pathogenic microbes. Antimicrobial peptides were observed to be effective against *Verticillium* and *Fusarium* wilts of cotton [23]. Kaurene synthase like genes are responsible for biosynthesis of various diterpenoids including phytoalexins. NAC gene diterpenoids including phytoalexins. NAC gene families have been suggested to play important roles in the regulation of the transcriptional reprogramming associated with plant stress responses. These transcriptional factors proved to be involved in resistance against *Magnaporthe grisea* [24] and *Phytophthora infestans* [25]. *Fusarium graminarium* infections [26]. Most of the chloroplast related proteins like were down regulated in resistant genotype compared to susceptible one. Down regulation of genes related to photosynthesis were strongly associated with disease may be due to failure in electron transport and conserved energy for photosynthesis were significantly observed in CI.

Cysteine proteinase inhibitor 10 (LOC4335551), disease resistance protein TAO1-like (LOC112939055), oleosin 16 kDa-like (LOC4336570), pathogenesis-related protein (PR1), pathogenesis-related protein (PR4), BTB/POZ and MATH domain-containing protein 5-like (LOC112936838), alpha-amylase isozyme 3D-like (LOC4345814), were upregulated in resistant genotype C101A51. The decrease in their expression may be considered as a source of their entry. CTP is a specific cysteine proteinases inhibitor which has role in the defense against pests and

pathogens and endogenous processes regulation [27]. Expression of TAO like putative disease resistance protein RGA3 was more in resistant genotype C101A51. TAO was observed to be responsible for expression of pathogenesis realted protein 1 (PR1) gene in *Pseudomonas syringe* rice interaction [28] which also suggestive of its role against *F. fujikuroi* in rice more expression of oleosin 16 kDa like protein in resistant genotype suggested then more oil bodies are present in seed of resistant rice genotype C101A51 which may be resistant to the pathogen entry in rice plant. The family of pathogenesis related protein 4 (PR4) are generally possess a broad range of antifungal activity and involved in plant defense responses and observed effective against *Phytophthora sojae* soybean interaction [29].

Lipooxygenase are enzymes which catalyze oxygenation of polyunsaturated fatty acids with one or several pentadiene units to hydroperoxy fatty acids which possess a range of biological functions such as diverse signal molecules, oxidants and modifiers of membrane structures [30–31]. Lipooxygenase are identified as an enzyme of early response in host pathogen interaction [31]. For instance lipooxygenase expression is induced in rice cultivars upon infection by blast fungi, *Magnaporthe grisea*. A significantly higher expression of lipooxygenase is observed in resistant cultivars of *Cajanus cajan* against the fungi, *Fusarium udum*. The study observed the role of LOX metabolites as antifungal agents in imparting resistance in *Cajanus cajan* [32].

α amylase activity was observed to be increased in inoculated plants compared to control. In rice α amylase isozymes are critical for the formation of the storage starch granules during seed maturation and motivate the stored starch to nourish the developing seedlings during seed germination which directly affects the plant growth and yield. α amylase activity generally depends on gibberellic acid content. Increased gibberellic acid content in *F. fujikuroi* inoculated plant may have increased the activity of this enzyme.

GDSL esterase/lipase At5g33370 (LOC4348826), serine-glyoxylate aminotransferase (LOC4345962), CASP-like protein 2C1 (LOC4341716), WAT1-related protein At4g08290 (LOC4348283), Cytoplasmic linker associated proteins (CLASPs) are found to be upregulated in susceptible genotype Rasi. GDSL esterase/lipases characterized by conserved GDSL motif, are a subfamily of lipolytic enzymes and GDSL lipases are known to be involved in lipid metabolism and negatively modulate rice immune responses against *Magnaporthe oryzae* and *Xanthomonas oryzae* pv. *oryzae* [33]. Serine glyoxylate aminotransferase (SGAI) converts glyoxylate and serine to glycine and hydroxypyruvate during phosphorespiration. Elevated SGAI activity causes changes in metabolism and interferes with photosynthetic CO_2 uptake and biomass accumulation in *Arabidopsis* [34]. Similar mechanism may be responsible for the reduced biomass in bakanae infected plants. Casparian membrane domain proteins (CASP) like protein 2C1 are required for casparian strip formation in plants. It regulates membrane cell wall junctions and localized cell wall depositions. WAT1 exhibits enhanced resistance to vascular plant pathogens including *Ralstonia solanacearum*. It is related to cell wall modifications decrease in cell elongation, changes in mechanical properties and severe reduction in stem fibre secondary wall thickness [35]. Cell wall degrading proteins like xyloglucan endotransglucosylase/hydrolase protein and β-D xylosidase 7 were upregulated in susceptible genotype Rasi which may be due to degradation of cell wall by *F. fujikuroi*. Bashyal et al. [17] also reported more expression of cell wall degrading enzymes in susceptible genotype Rasi.

Cytoplasmic linker associated proteins (CLASPs) are microtubule associated proteins involved in regulation of dynamics of microtubules that play an important role in plant growth and development. Zhu et al, [36] indicated that the suppression of CLASP protein related to the shorter internodes and semi dwarfism in plants. Significantly positive expression of CLASP transcript in rice genotype Rasi may be responsible for the elongated plant

phenotype. BTB/POZ and MATH domain proteins are upregulated in resistant genotype C101A51. Generally, BTB domain participates in plant responses against biotic and abiotic stresses. He et al, [37] reported down regulation of BTB gene under *Phytophthora capsici* infection. Upregulation of this gene in resistant genotype may have role in defence against *F. fujikuroi.*

Results of the selected DEGs showed that the qRT-PCR was consistent with the RNA-Seq results showing similar expression pattern of up- and down-regulated genes by using both, RNA-Seq and qRT-PCR, analyses.

Conclusions

The results showed that general expression of glucanases, peroxidases in both, resistant and susceptible, genotypes could represent the basic defence mechanism of rice against *F. fujikuroi*. What was found strikingly different in resistant genotype is the modulation of WRKY transcriptional factors, Cysteine proteinase inhibitor 10 (LOC4335551), disease resistance protein TAO1-like (LOC112939055), oleosin 16 kDa-like (LOC4336570), pathogenesis-related protein (PR1), pathogenesis-related protein (PR4), BTB/POZ and MATH domain-containing protein 5-like (LOC112936838), alpha-amylase isozyme 3D-like (LOC4345814). Further, GDSL esterase/lipase, serine-glyoxylate aminotransferase, CASP-like protein 2C1, WAT1-related protein, Cytoplasmic linker associated proteins (CLASPs) may be determinant of susceptibility of rice plants against *F. fujikuroi.* RNAseq data of resistant and susceptible genotype was submitted to SRA with the accession no. accession PRJNA562996.

Abbreviations

qRT-PCR
Real time reverse transcriptase PCR

Declarations

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Author Contributions

BM Bashyal, Rashmi Aggarwal and S Gopalakrishnan designed the experiments. Kirti Rawat, Pooja Parmar and BM Bashyal performed the experiments. BM Bashyal, Pooja Parmar and Sangeeta Gupta wrote the manuscript with input from Rashmi Aggarwal and S Gopalakrishnan. Ashish Kumar Gupta, Ravish Choudhary, Sezai Ercisli and Antonija Kovacevic proofreaded the manuscript. All authors reviewed the manuscript.

Data availability: The data that support the findings of this study are available from the first author [Bishnu Maya Bashyal], upon reasonable request.

Conflict of interest: The authors declare that they have no conflict of interest.

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Figures

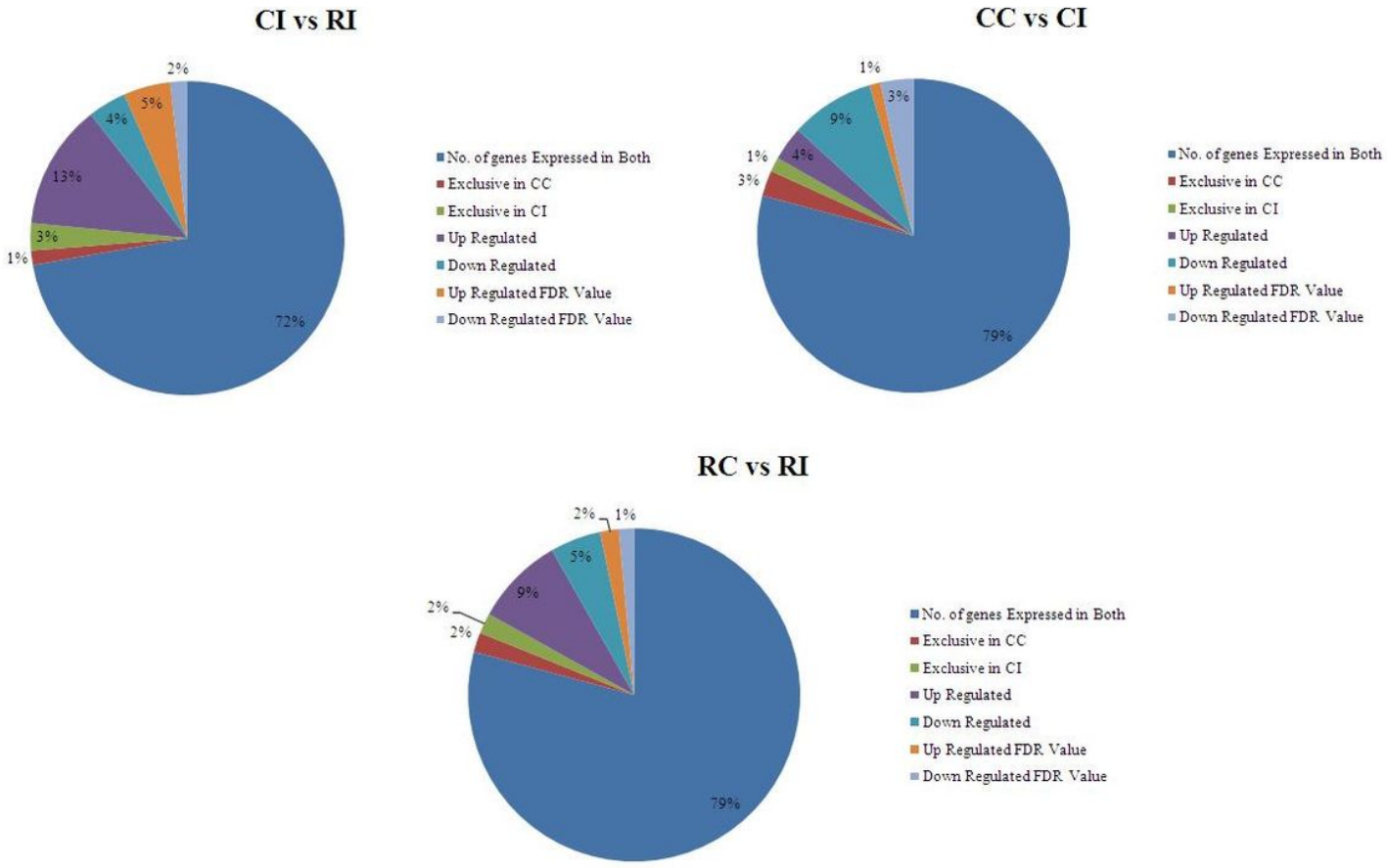

Figure 1

Distribution of genes expressed in (a) CC vs CI, (b) CI vs RI , (c) RC vs RI

Figure 2

Scatter plot of differentially expressed genes between (A) CC vs CI, (B) CI vs RI , (C) RC vs RI

Figure 3

Heatmap of differentially expressed genes between CI vs RI. Levels of expression are represented as log2 ratio of gene abundance between CC – CI, CI – RI and RC - RI samples. (In this, each horizontal line refers to a gene. The color represents the logarithmic intensity of the expressed genes. Relatively high expression values are shown in red.)

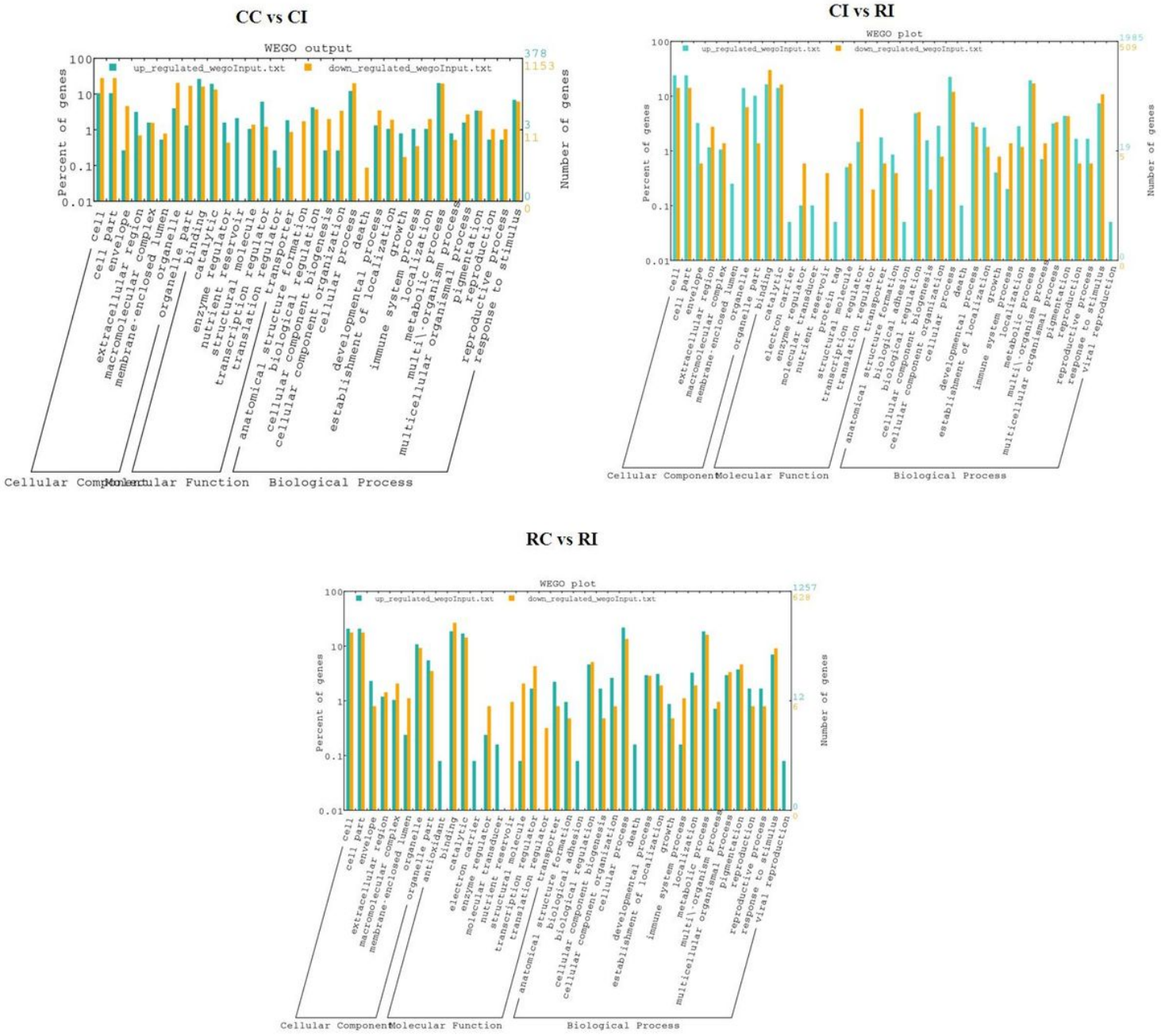

Figure 4

WEGO Plot for differentially expressed genes in (a) CC vs CI, (b) CI vs RI , (c) RC vs RI

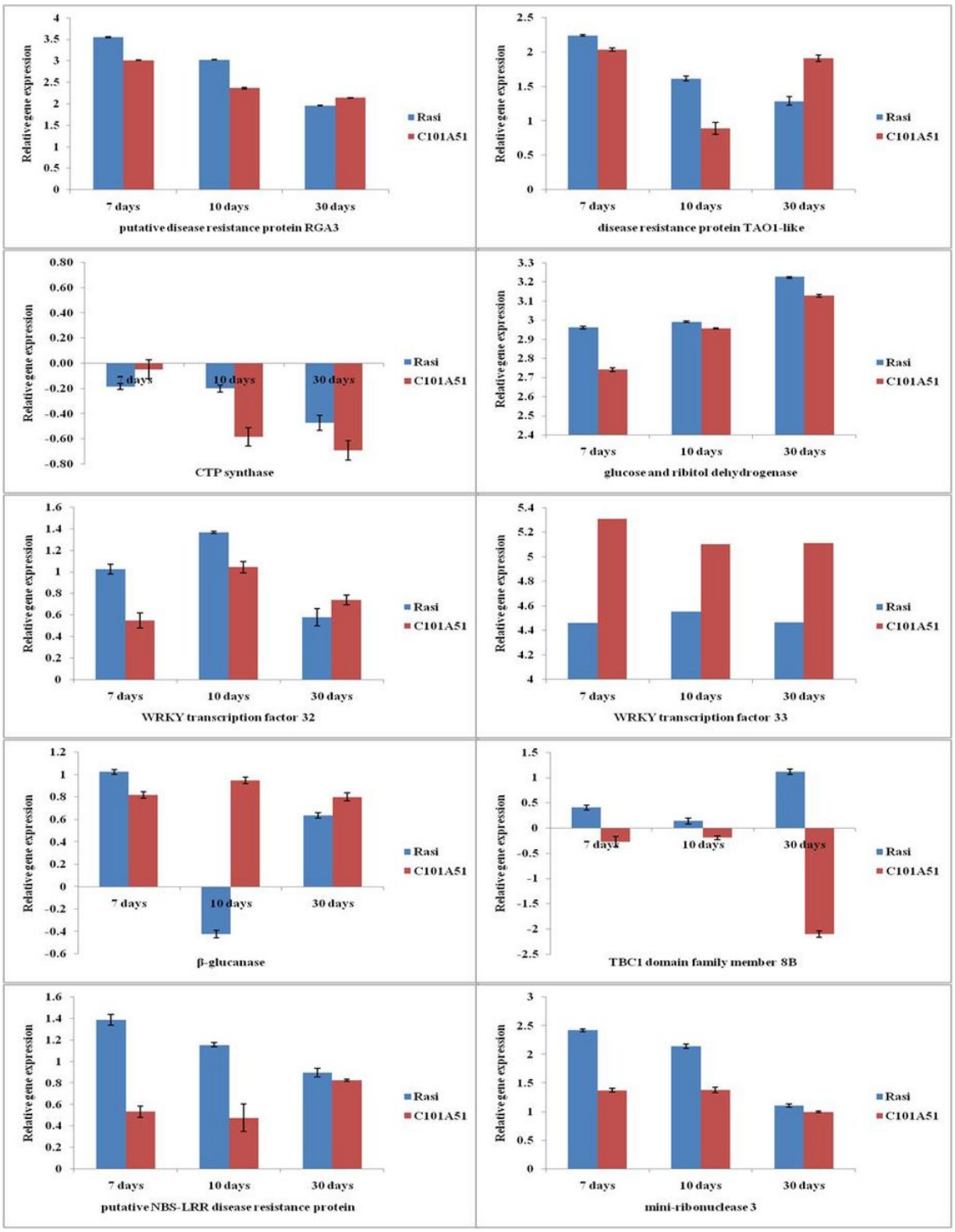

Figure 5

qPCR based analysis of *F. fujikuroi* transcriptome genes at different time points (7, 10, 30 d) post inoculation in resistant rice genotype, C101A51 and susceptible genotype,"Rasi". Mean values ± standard deviation of triplicate data from three biological replicates are shown.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable01CCvsCIdeg.xls
- SUPPLEMENTARYTABLE2A.doc
- SupplementaryTable02CIvsRIdeg.xls
- Supplementarytable03RCvsRIdeg.xls
- SupplementaryTable04CCvsCIGO.xls
- SupplementaryTable05CIvsRIGO.xls
- SupplementaryTable06RCvsRIGO.xls
- SupplementaryTable07CCCIpathway.xls
- SupplementaryTable08CIRIpathway.xls
- SupplementaryTable09RCRIpathway.xls