Transcriptome analysis of sesame-*Macrophomina phaseolina* interactions revealing the distinct genetic components for early defense responses

Nidhi Radadiya[1,3] · Naman Mangukia[2,5] · Virali Antala[1,3] · Hiral Desai[1] ·
Hemangini Chaudhari[1] · T. L. Dholaria[3] · Denish Dholaria[3] · Rukam Singh Tomar[1] ·
B. A. Golakiya[1] · Mahesh Kumar Mahatma[4]

Abstract Sesame (*Sesamum indicum* L.) is an oilseed crop
challenged by many biotic stresses. Charcoal rot caused by
Macrophomina phaseolina (MP) is one of the most dev-
astating diseases of sesame. Till date, molecular mecha-
nisms of resistance to charcoal rot in sesame is not yet
reported. In this study, two sesame variety GT-10 (resis-
tant) and RT-373 (susceptible) were identified with con-
trasting disease incidence when infected with MP. To get
the molecular insight, root samples were collected at 0, 24,
48- and 72-h post inoculation (hpi) with the pathogen and
generated RNAseq data was analyzed. A total of 1153 and
1226 differentially expressed genes (DEGS) were identi-
fied in GT-10 and RT-373, respectively. During the inoc-
ulation with MP, resistant genotype showed high number
DEGs at early time point of 24 hpi and when compared to
late expression in susceptible genotype at 48 hpi. Distinct
clusters were represented for each time period represented
by cytochrome P450 83B1-like, single anchor, hypothetical
protein C4D60, kirola like and heat shock proteins in the
resistant genotype contributing for resistance. Analysis of
differentially expressed genes, catalogued the genes
involved in synthesis of pathogenesis-related (PR) proteins,
MYB, WRKY, leucine zipper protein, bHLH, bZIP and
NAC transcription factors, ABC transporters (B, C and G
subfamily), glutathione metabolism, secondary metabo-
lites, fatty acid biosynthesis and phytohormones like auxin,
abscisic acid, ethylene and gibberellic acid. Additionally,
in the resistant response we have found three unique GO
terms including ATP binding, ribonucleotide binding and
nucleic acid binding in molecular function category. The
molecular clues generated through this work will provide
an important resource of genes contributing for disease
resistance and could prioritize genes for functional vali-
dation in the important oil crop.

Introduction

Sesame (*Sesamum indicum* L.) is an important and oldest
oilseed crop which is grown widely in tropical and sub-
tropical regions for its highly nutritive oil (Pathak et al.,
2014). Besides being a food item, sesame has become an
important ingredient for several industries like cosmetic,
chemical and pharmaceutical industries (Hahm and Kuei
2014). The crop is infected by various fungal diseases like
Alternaria leaf spot, *Fusarium* stem rot, powdery mildew
and charcoal rot or dry root rot resulting in low produc-
tivity. Among all, charcoal rot is caused by the soil born
necrotrophic fungus *Macrophomina phaseolina* (Tassi)
Goid is most devastating disease of sesame (Thiyagu et al.
2007). This fungus has wide host range, capable of
infecting plants from more than 500 families and causes
pre- and post-emergence plant mortality. India is the lar-
gest producer and exporter of sesame seeds around the

✉ Mahesh Kumar Mahatma
maheshmahatma@gmail.com

[1] Department of Biotechnology, Junagadh Agricultural
University, Junagadh, Gujarat, India

[2] Department of Bioinformatics, Gujarat University,
Ahmedabad, Gujarat, India

[3] Solar Agrotech Pvt. Ltd. Bhaichand Mehta Industrial Estate,
Rajkot, Gujarat, India

[4] Department of Biochemistry, ICAR-Directorate of
Groundnut Research, Junagadh, Gujarat, India

[5] Bioinnovations, Mumbai, India

world with a production of 0.689 million tonns in 1.42 million hectors of area with productivity 485.21 kg/ha (FAOSTAT 2019) along with the reported losses 5%-100% reported earlier (Kolte 1985). The phenotypic symptoms of the disease include sudden wilting, stunting of plant, incomplete pod filling, early maturation and charcoal type coloration in the colonized plant tissue, (Kaur et al. 2012). Although all the developing stages of crops are affected by disease yet they are most vulnerable to the disease at post-flowering stages. An infection is established when the micro-sclerotia from soil comes in contact with the root of host plant, leading to its germination and penetrataion into the root tissue followed by the intra cellular invasion on vascular tissues. This leads to blockage in the vascular tissues leading to the plant death (Collins 1991). Upon germination the multicellular microsclerotia takes about 24 h and starts root infection by the root exudates (Bowen and Schapaugh 1989). These fungi alter the level of host phytohormones thereby disrupting the defense signaling (Prins et al. 2000) or manipulating the host cellular machinery which suppresses defenses mechanism and allowing disease progression (Laluk and Mengiste 2010).

On the plant side, as a second line of defense, effector-triggered immunity (ETI) occurs, when the MP avirulence (Avr) protein recognizes sesame resistance (R) protein, which prevents the fungal growth through hypersensitive response (Jamir et al. 2004; He et al.2006; Nomura et al. 2006). The second line of response is a strong and specific immune response towards those isolates of pathogen that produce the recognized effectors. Furthermore, invasion of pathogen may also activate salisylic acid (SA) and jasmonic acid (JA)/ ethylene mediated signaling pathways for defense mechanism, which is commonly known as systemic acquired resistance (Whipps 2001).

Sesame genome sequencing (Wang et al. 2014), has opened up the door of genome research and molecular sesame breeding. Transcriptome analysis is an effective way to obtain information regarding the state of a cell or tissue under a specified condition, including development or differentiation, responses to the environmental stress or during the diseases (Saliba et al. 2014). RNA-sequencing data can be utilized to illustrate differential expression which facilitates to better understanding of the comprehensive host–pathogen interaction (Ward et al. 2012). This data can also be utilized for molecular marker mining. Dutta et al. (2020) identified 26,880 simple sequence repeats, 90,181 single nucleotide polymorphisms and 25,063 insertion deletion by using transriptome data of non-infected and MP infected wild, cultivated and inter-specific hybrid sesame genotypes. Yan et al. (2021) reported resistance mechanism of sesame against MP by comparative transcriptome analysis between disease resistant and susceptible sesame genotype. However, they

carried out the experiments on seedlings at three pairs of true leaves which were cultured in artificial climate box. Thus aim of present study was to understand the molecular components and delineate the distinct response which occur in two contrasting (resistance-R and susceptible-S) genotypes at post flowering stage i.e. 60 days after sowing (at this stage disease is mostly prominent) in field condition. RNAseq data of the present indicates novel elements for the involvement of auxin and abscisic acid related genes, ethylene responsive transcription factors, MYB, WRKY, bHLH, bZIP and NAC transcription factors, genes related to phenyl propanoid pathway and energy metabolism in sesame against MP.

Materials and methods

Isolation and multiplication of fungus

Charcoal rot infected plants were collected from the sesame growing fields at Krishigadh, oil research station farm of Junagadh Agricultural University, Junagadh and sesame fields of nearby villages of Junagadh district. Infected stem was cut into 5 mm pieces and then surface sterilized by immersion for 30 s in 1% sodium hypochlorite followed by rinsing several times with sterile distilled water to eliminate excess sodium hypochlorite. These pieces were finally blotted dry between two autoclaved paper towels, plated on to PDA plates and incubated at 30 °C in the dark. Developed fungal growth was transferred onto new PDA plates and maintained on PDA plates at room temperature until used (Akhtar et al. 2011). Isolated fungus was verified by morphological identification. The pathogen *M. phaseolina* was multiplied on sorghum grains to produce microsclerotia. Polypropylene bags were filled up with about 200 g of grains. Open end of these bags was packed with 4 cm piece of PVC pipe and nonabsorbent cotton. Then it was autoclaved at 121 °C and 15 psi for 20 min. for sterilization. Then after inoculated with 2–3 discs of size 5 mm diameter from 6 days old culture of pathogen, put in BOD incubator after seven days of incubation.

Screening of sesame genotypes against charcoal rot

Twelve genotypes of sesame were procured from Agricultural Research Station, Junagadh Agricultural University, Amreli (Fig. S2). In vitro and in vivo screening test was carried out suggested by Thiyagu et al. (2007) with slight modifications. Seeds of each genotypes were sown in pots containing sick soil (inoculated with MP) in three replication. In each pot 10–12 plants were maintained. At post flowering stage, observations were recorded daily.

Disease scoring of each variety was based on the degree of necrosis of roots, stems and other plant part. The genotypes were graded as resistant, moderately resistant, moderately susceptible, susceptible or highly susceptible based on their infection percentage using the scale given by Dinakaran and Naina Mohammed (2001).

Scanning electron microscopy (SEM)

After screening experiment, most resistant (GT-10) and susceptible (RT-373) genotype was selected for the SEM study. Sampling from both the genotypes was done at the interval of 24 h (from 0 to 144 hpi) after inoculation of pathogen. SEM was performed as described by Ajmal et al. 2016 with slight modification. SEM was performed on a ZEISS EVO 18 scanning electron microscope by ZEISS Research Microscopy Solutions.

Plant materials and treatments

Five biological replications of GT-10 (resistant) and RT-373 (susceptible) were sown in pots, containing sterile soil during summer 2018 at Junagadh Agricultural University, Junagadh, India. Pots were kept at field condition and 15–20 plants were maintained in each pot. Each pot was inoculated with suspension culture of microsclerotia (6×10^3 sclerotia per ml) at post flowering stage (60 days after sowing) of sesame plants. On the basis of SEM results plant roots were sampled at 0 hpi (control treatment) and again at 24, 48 and 72 hpi for RNA sequencing. The whole plant was uprooted from the pot and soaked in distilled water to remove soil. Then the whole root was wrapped in tin foil and immediately snap-frozen in liquid nitrogen and stored at $-80\ °C$. Five biological replications were collected from individual genotype at each time point and pooled for RNA extraction. Three independent RNA extraction was carried out for each genotype. Samples were referred to as R0, R1, R2, R3 and S0, S1, S2, S3 for 0, 24, 48 and 72 hpi for resistant and susceptible, respectively.

RNA sequencing and data processing

Total RNA was extracted using modified protocol with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and RNeasy® Plant mini kit (Qiagen,Valencia, CA). The RNA integrity number (RIN) quality and concentration of RNA were assessed by capillary electrophoresis (Qiaxcel system, Qiagen) and using Qubit® 2.0 Fluorometer (Thermo Scientific, USA). Purification of mRNA from total RNA (> 7.0 RIN) was carried out using oligo-dT-attached magnetic beads (Dynabeads® mRNA DIRECT™ Micro Kit) and cDNA libraries were prepared using standard

procedure of Ion total RNA seq—kit v2 (Thermo Fisher Scientific). Sequencing was conducted with two biological replications (8 samples in each replication) in an Ion S5 Machine (S5TM, Life Technologies). The raw data quality of all 16 samples was checked using FASTQC (version 0.11.7, Babraham Bioinformatics) software. Reads with quality value < Q20 was filtered out including the minimum length threshold set by 36 bases using Trimmomatic (version 0.38, Bolger et al. 2014). The rerun of FASTQC software on filtered HQ (High Quality) data to check the quality.

Data analysis

The 7 available genome sequences of *Macrophomina phaseolina*, bio project number: PRJNA78845, PRJNA291859, PRJNA291857, PRJNA291861, PRJNA291860, PRJNA291855, PRJNA271369 were downloaded from NCBI in FASTA format and concatenated in a single file using shell script. The HQ data was mapped on the *Macrophomina phaseolina* genome using Bowtie1 (version1.2.2, Johns Hopkins University) to avoid possible contamination of the fungus in *Sesamum indicum* data. The unmapped reads were extracted. These unmapped clean reads were processed for further downstream analysis. The reference genome and gff of *Sesamum indicum* were downloaded from NCBI (Wang et al. 2014). The fasta file of gene sequences were fetched out from the genome file using coordinate information from gff file through bedtools (version 2.27.1). Clear reads were mapped on sesame genes using BWA (version 0.17.7). sam2bam, sort and idxstats utility of Samtools (version 1.9) were used to extract read counts from bam files. Deseq (version 1.22.1, Anders and Huber 2010) was run to find out the differential gene expression, where dispersion estimation was applied with 2 biological replicates for each sample and used 'nbinomTest' to find the difference between the base means of control and treatment conditions. The P-value was adjusted using Benjamini and Hochberg's approach to control the false discovery rate. Genes with P-value < 0.05 were considered as significantly differential expressed (Benjamini and Yekutieli 2001). Venn diagrams were created by convenient and powerful online tools (Heberle et al. 2015). Heat maps were generated by an open source web tool ClustVis (Metsalu and Vilo 2015).

Functional annotation, gene ontology analysis and pathway assignments

Functional annotation of significantly expressed genes of each group was carried out by using Standalone Blastx

against Nr Database. Gene Ontology (GO) enrichment analysis was performed using Blast2go software to identify over-represented (significantly enriched) GO terms in the differentially expressed genes. Pathway assignments were carried out according to KEGG (Kyoto Encyclopedia of Genes and Genomes database) mapping using custom Blast2GO (Conesa et al 2005). The dataset is available from the NCBI Short Read Archive (SRA) under accession number PRJNA656950.

Validation of gene expression by qRT-PCR

The total RNA used in gene expression analysis by qRT-PCR (ABI-7500 Fast Real-Time PCR System) was same as transcriptome RNA study. The cDNA was synthesized by using QuantiTect Reverse Transcription Kit (Quagen) which uses the random primer scheme for initiating cDNA synthesis. The Primers were designed from the contig of randomly selected significant genes by using BatchPrimer3 (Table S1), a web primer design You et al. 2008). The PCR efficiency was checked for each primer pairs which shows constant amplification efficiency. Quantitative real-time-PCR following the standard protocol for SYBR green assay and 18S rRNA primer as an endogenous control (Wei et al. 2013). Relative RNA quantities were determined with delta-delta ($\Delta\Delta$) Ct and expressed as Fold increase = $2^{-\Delta\Delta Ct}$ (Rao et al. 2013).

Results

Screening of the sesame genotypes against charcoal rot

The pathogenicity of MP was significantly different on 12 sesame genotypes. During the screening experiment the observed lifecycle of the MP is depicted in Fig S1. The tested genotypes showed varying degree of disease incidence ranging between 4.667 to 95.214% in vivo and 7.03 to 96.66% in vitro screening method (Fig. 1). Among the twelve genotypes evaluated against charcoal rot, two genotypes viz., GT-10 and Rama were resistant to root rot disease recording the mean incidence of 4.67 and 6.68 per cent respectively during in vivo condition and 3.63 and 7.03 per cent respectively during in vitro condition. RT-373 was identified as a highly susceptible genotype as it shown the highest per cent disease incidence in both the screening methods. For clarity, we categorized the two genotypes as resistant, two as susceptible and eight as highly susceptible (scale given by Dinakaran and Naina Mohammed 2001). To provide the molecular basis of the resistance against this disease, a comparative transcriptome study was done on GT-10 (resistant) and RT-373

(susceptible) were selected on the basis of disease incidence (Fig. 2). Infection was confirmed by histopathological study as well as Koch's postulates were also confirmed.

Scanning electron microscopy (SEM) of fungal invasion on the root

The germination of microsclerotia was observed on the roots of susceptible variety (RT-373) within 24 hpi. The hyphae grew over the root surface, with secondary branching arising from the principal hyphae. At 24 hpi, SEM revealed that hyphal branches tended to wrap around root hairs (Fig. 3D). However, hyphal growth was not observed on the root surface of the resistant variety, GT-10 (Fig. 3A, B, C). Compact hyphal growth was seen on the root surface of the susceptible (RT-373) genotype which further invaded through the vascular bundle resulted to clog the vascular tissue (Fig. 3E). While the root section of non-inoculated control plants and resistant variety showed clear lumens (Fig. 3B, C). Attachment of microsclerotia on the surface of roots is essential for the successful establishment of the pathogenesis of MP. The formation of microscelrotia was observed in vascular tissues of RT-373 at 144 hpi at when plant tissues were completely dried. The symptoms of wilting started at 120 hpi in susceptible genotype, but the wilting symptoms were not observed on the resistant. By 144 hpi (6 days) significantly higher disease incidence was noticed in the susceptible variety than that of resistant variety. On the basis of SEM observation sampling time was decided at 0, 24, 48 and 72 hpi for the current study, where sampling at 0 hpi served as control.

Transcriptome profiles of resistant and susceptible sesame genotypes

The transcriptomes of the individual inoculated samples in genotype GT-10 and genotype RT-373 from 0, 24, 48 and 72 h were sequenced using IonTorent S5 genome sequencing platform. A total of 65.77 million raw reads and 9.62 Gb original data were generated in first replication whereas in second biological replication total of 61.25 million raw read and 8.56 Gb data were generated. After the removal of low-quality sequences, 42.90 and 33.01 million reads were left in replication one and two, respectively. A total of 38.51 and 37.40 million reads were obtained in GT-10 and RT-373, respectively. After filtering and trimming, the clean reads were mapped from 1.15 to 6.93 million on the *Sesamum indicum* L. genome database and the mapping percentage was 61.80% to 93.25% (Table 1). Subsequently, these reads were processed for further transcriptome analysis.
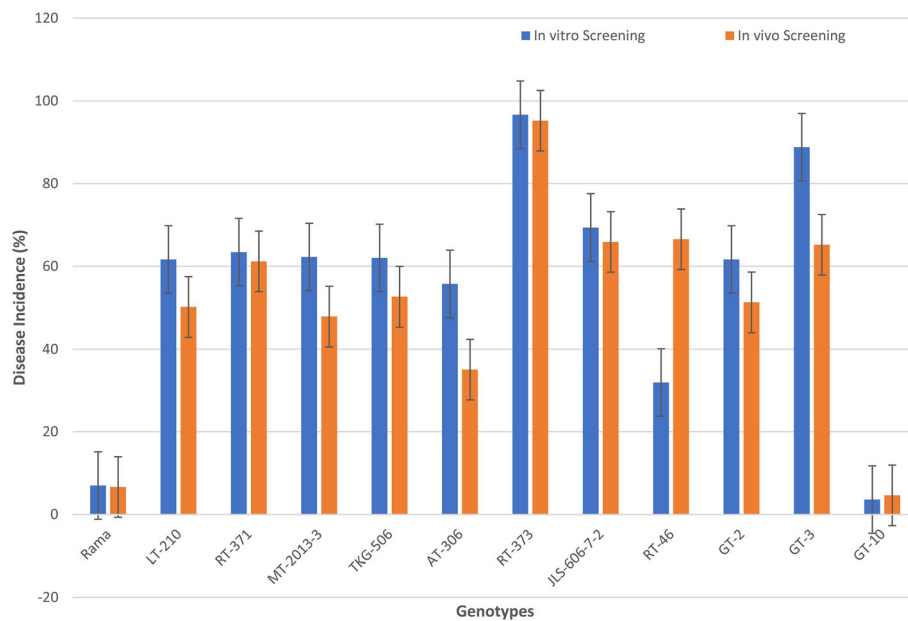
Fig. 1 Per cent disease incidence of 12 sesame genotypes against *M. phaseolina*. For in vivo screening, seeds of each genotypes were sown in sick soil (inoculated with MP sclerotia) in three replication. In each pot 10–12 plants were maintained. Observation were recorded daily at post flowering stage and the incidence was noticed after flowering. The percent disease incidence was recorded at maturity stage. For in vitro screening the seeds were grown in sterile soil in three replication and after 20 days of sowing 8–10 seedlings/replication were transferred in paper towel. Roots of each seedlings were dipped in the inoculum with up and down movement for 10–15 s and then again placed side by side on a paper towel. The infected seedlings died gradually within 10 days and the percentage of disease incidence was calculated

Identification of specific genes in resistant and susceptible genotype during infection process

To detect differentially expressed genes (DEGs) involved in MP infection response, we compared genes expression among data sets of four treatments in both genotypes. The basal response in the resistant genotype GT-10 under uninfected condition lies within the cluster of DEGs under infected conditions (Fig. 4). In resistant genotypes, some important DEGs were observed which encodes for genes such as two-component response regulator-like PRR95, vinorine synthase-like, cytochrome P450 93A3-like, 4-coumarate–CoA ligase-like 7, protein NIM1-INTERACTING 2-like and Auxilin-like protein and related proteins containing DnaJ domain. As expected, DEGs in uninfected and under infected conditions are placed in different cluster of PCA plot in susceptible genotype RT-373. In GT-10, a total of 1153 differentially expressed candidate genes were found (Fig. 5, 6). Of which, 409 genes were up regulated at maximum fivefold and 744 genes were down regulated at maximum sevenfold compared to those in the control (0 hpi). Among all these DEGs genes began to express at 24 hpi time point and also shows the highest number of expressed genes. Meanwhile, in RT-373 a total of 1226 genes were expressed, of which 729 genes were upregulated at max eightfold while 497 genes were downregulated max at ninefold compare to control. Contrary to GT-10, the greater number of DEGs were found at 48 h of inoculation. Further comparison indicated that the number of DEGs in resistant genotype was higher than that of susceptible one. In GT-10 the number of upregulated genes was found higher at 24 h post inoculation stage (PIS) and in RT-373 the number of upregulated genes was found highest at 48 h followed by 72 h PIS. We observed 35 DEGs in GT-10 and 31 DEGs in RT-373 which are commonly expressed throughout the infection (Fig. 7). The expression pattern of those DEGs at all inoculation stages in both the genotypes was depicted by the heat map (Fig. 8). In GT-10 at 24 hpi the brown cluster shows regulation of cytochrome P450 93A3 (NC_026148.1_632770-634,655), senescence associated protein (NW_011628374.1_216-1301, NW_011628380.1_914-7377), chlorophyll a-b binding protein 7 (NC_026152.1_12683503-12,685,133), protein TAR1 (NC_026151.1_30096-32,590) and ATPase subunit 1 (NW_011628115.1_47797-57,233) which were down regulated with progress in inoculation as shown in blue cluster at 72 hpi (Fig. 7A). Simultaneously we also found some DEGs which were up regulated with increase with time of post inoculation. In RT-373, UDP-glycosyltransferase 74E2 (NC_026147.1_19059977-19,062,602), sodium transporter HKT-1 like (NC_026149.1_1815509-1,821,048), beta-carotene isomerase D7 (NC_026150.1_1998483-2,001,584), hexose carrier protein (NC_026148.1_11124385-11,129,071), putative membrane protein (NC_026154.1_14692865-

Fig. 2 Disease phenotypes of sesame genotypes against *M.phaseolina*. i) disease phenotypes such as wilting and drying in susceptible genotype RT-373. ii) arrows show the dense mycelial growth due to germination of microsclerotia surrounding the root surface of RT-373. iii) healthy plants of resistant genotype GT-10 shows no disease phenotypes. iv) healthy root of GT-10. The pictures of plants and roots were captured at 6 days post inoculationand 24 h post inoculation, respectively
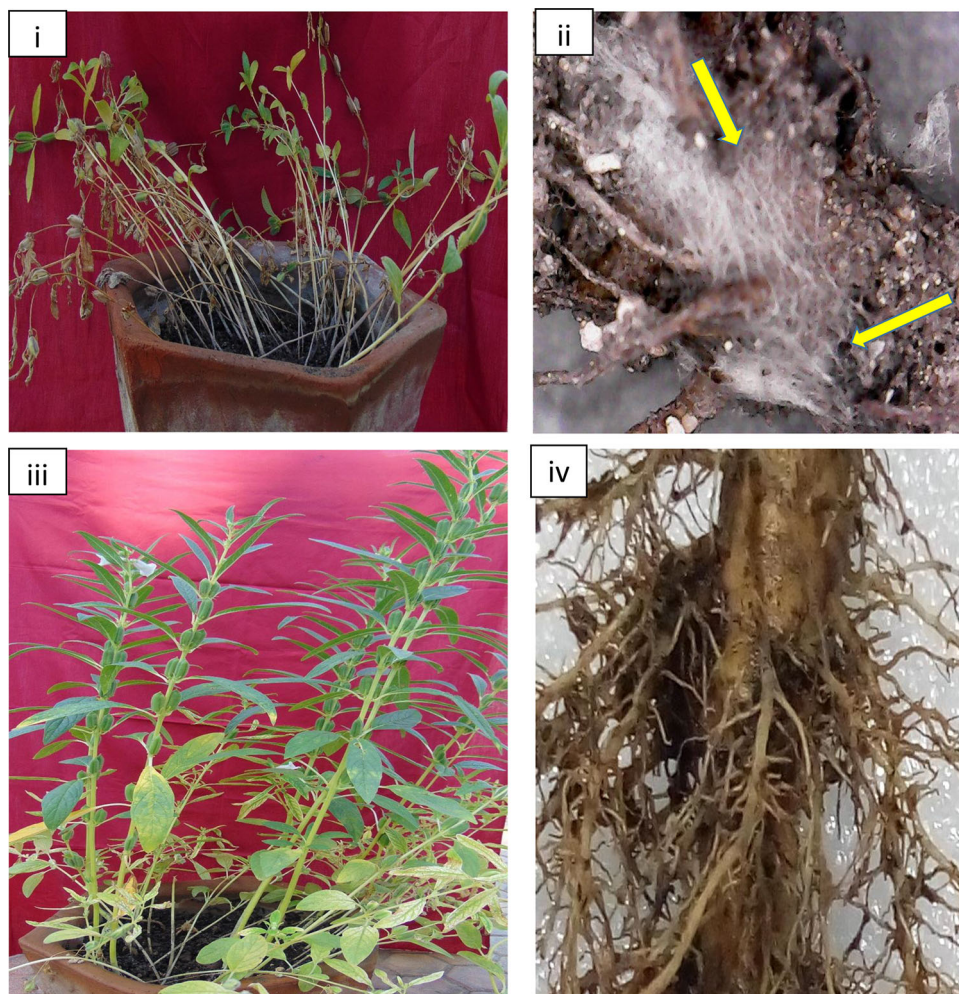
14,693,916), nicotianamine synthase-like (NC_026154.1_ 13105000-13,106,317) and transcription factor bHLH123 (NC_026156.1_287774-290,419) were up regulated as shown in brown cluster at 24 hpi and the expression was decreased (blue clusters) with disease progression (Fig. 8B). The DEGS shown in other cluster were down regulated at 24 hpi and expressed positively at 48 hpi.. The description of genes present in different clusters were given in supplementary Table 11.

DEGs involved in biosynthesis of secondary metabolites

Biosynthesis and activation of secondary metabolites (SMs) are triggered with the recognition of MAMPs by pattern recognition receptors, similar to other defense responses. In this study we observed 7 and 19 DEGs in GT-10 and in RT-373, respectively which are annotated as catalytic enzymes in phenylpropanoid biosynthesis pathway (Table S2). These includes up regulation of beta-

glucosidase BoGH3B-like, catalase and glucan endo-1,3-beta-glucosidase 4, cyanogenic beta-glucosidase, glucan endo-1,3-beta-glucosidase 12 and glucan endo-1,3-beta-glucosidase 3-like were down regulated in GT-10. Expression of cytochrome P450 CYP73A100-like, glucan endo-1,3-beta-glucosidase, acidic, peroxiredoxin-2F, caffeic acid O-methyltransferase, cinnamoyl-CoA reductase were observed at 24 h in GT-10 while in RT-373 these genes were up regulated at 48 h. Expression of glucan endo-1,3-beta-glucosidase, acidic-like gene was found up regulated by 1.99 fold at 24 hpi and 6.23 fold at 48 hpi. Lignin-forming anionic peroxidase was up regulated by 3.44 fold at 48 hpi and 3.78 fold at 72 hpi. Peroxidase (POD, 7 genes) was significantly expressed at 48 and 72 hpi. Among them POD 4 and POD 51 were up regulated by 3.05 and 2.68-fold, respectively at 48 hpi. For indole alkaloid biosynthesis pathway 4 gene were found up regulated in GT-10 and 7 genes were up regulated in RT-373. In GT-10, 6 genes and 15 genes in RT-373 were found which are involved in terpenoid biosynthesis pathway.
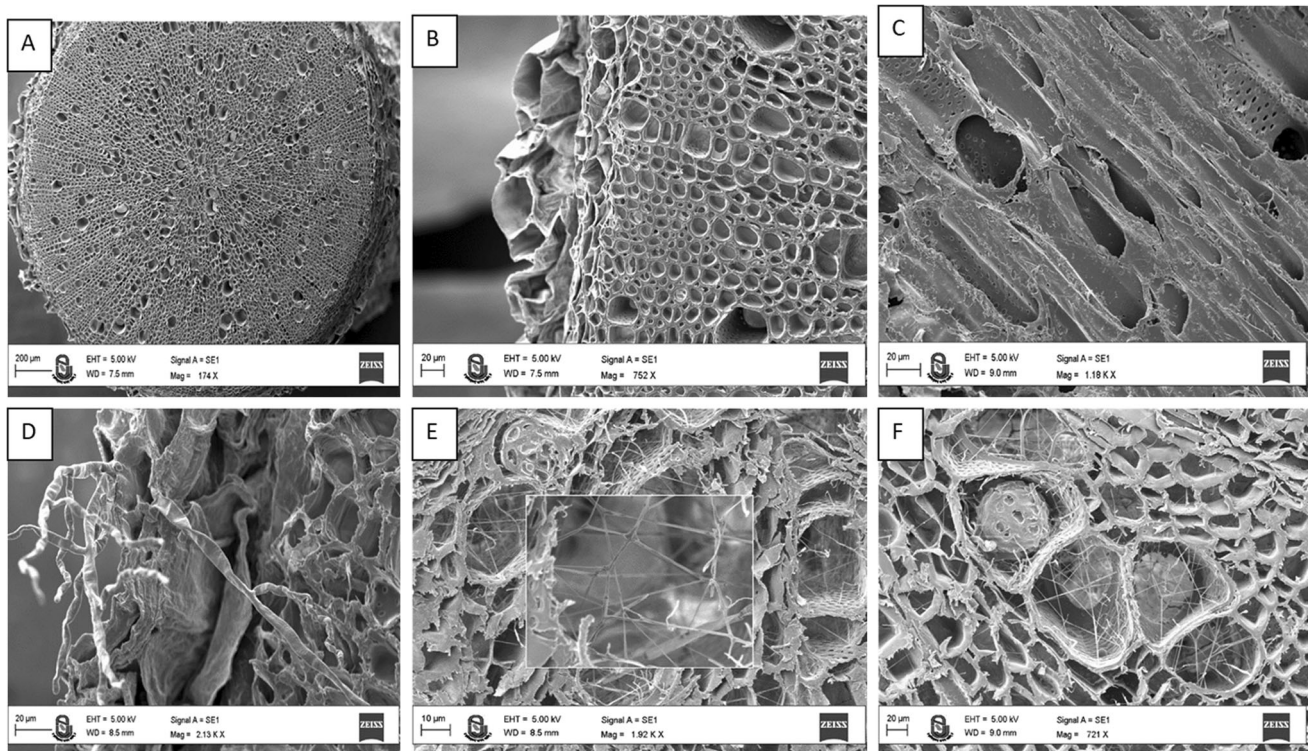
Fig. 3 Scanning electron micrograph of *M. phaseolina* infected root samples of resistant genotype GT-10 (**A-C**) and susceptible genotype RT-373 (**D-F**). Note: Root transverse and longitude section of GT-10 shows no mycelial growth in epidermal cells, cortex and vascular tissues. However in RT-373 penetration by hyphae in the root epidermal cells was observed at 24hpi (**D**). Intra cellular mycelial network as a result of disease progression at 72hpi (**E**) Microsclerotia formation in vascular tissues of dried root after 6 days post inoculation (**F**). Microscopy of resistant genotype was also done at the same time as of susceptible genotype

Transcription factors and phytohormones signaling represent major sigalling components of the DEGs

Transcription factors (TFs) play an important role in plant growth and development, leaf senescence, signaling and plant resistance to pathogens. We analyzed the TFs encoding DEGs in each library that resulted in identification of a total of 67 TFs genes in GT-10 and 74 TFs were identified in RT-373. Among all transcription factors, expression of MYB family TFs were observed in highest number followed by WRKY, leucine zipper protein, bHLH, bZIP and NAC in both the genotypes however, significant expression observed in the resistance genotype (Table S3). In our study, we observed a total of 69 significantly expressed genes, having major role in phytohormone (auxin, zeatin, gibberellin, ethylene, ABA, SA and JA) biosynthesis, signaling and transcriptional regulation (Table S4). In GT-10, total 7 DEGs were involved in auxin-activated signaling and response to auxin, in which 6 were (3 up regulated and 3 down regulated) differentially expressed at 24 hpi whereas one gene was up regulated at 48 hpi. One positively regulated gene was found at 24 hpi which was involved in cytokinin biosynthesis and 3 genes for gibberellin regulated protein were down regulated. For ethylene, total of 9 DEGs were found in which 3 were up regulated and 4 were down regulated at 48 hpi. Additionally, one was down regulated at 24 hpi and 72 hpi whereas, 2 DEGs (one up and one down regulated) involved in salicylic biosynthesis process were expressed at 24 hpi. There were also 4 DEGs found positively regulated for abscisic acid-activating signaling pathway. In RT-373 a total of 45 DEGs were significantly expressed with 9 (4 up regulated and 5 down regulated) genes were found to be in response to auxin, one was expressed positively for zeatin biosynthesis, 5 (2 up regulated and 3 down regulated) were significantly expressed for gibberellin regulated protein, 8 positively regulated DEGs, were found to ethylene responsive transcription factor, one was up regulated for jasmonic acid biosynthesis process and 21 up regulated DEGs were involved in abscisic acid-activated signaling pathway.

DEGs involved in PR proteins

Plants produce PR proteins as an innate immune response under biotic stresses. We observed 3 (2 up regulated and 1 down regulated) significantly expressed genes in GT-10 which shows chitinase activity at 24 hpi. In RT-373 total of

Table 1 Summary of RNA seq data

Sample	Raw reads	Clean reads	Total mapped	Mapped reads (%)
R1_replicate1	8,099,087	5,135,580	4,052,990	73.22%
R1_replicate 2	5,893,222	3,307,843	2,340,576	67.36%
R2_replicate 1	5,423,599	3,591,212	2,485,729	64.57%
R2_replicate 2	9,600,248	4,789,806	4,265,052	82.09%
R3_ replicate 1	8,981,542	5,829,801	4,922,374	76.41%
R3_ replicate 2	7,791,506	4,313,323	3,417,797	73.57%
R4_ replicate 1	9,056,581	6,453,004	6,937,463	93.25%
R4_ replicate 2	9,434,474	5,099,098	3,684,713	67.30%
S1_ replicate 1	5,229,444	3,195,078	2,775,380	78.50%
S1_ replicate 2	11,003,834	5,518,360	5,118,524	86.32%
S2_ replicate 1	9,864,804	6,376,696	4,718,059	68.65%
S2_ replicate 2	3,707,716	1,772,541	1,154,950	61.80%
S3_ replicate 1	9,281,546	6,064,442	4,679,996	72.07%
S3_ replicate 2	8,780,807	5,200,812	5,245,138	91.76%
S4_ replicate 1	9,840,479	6,264,003	4,200,946	62.75%
S4_ replicate 2	5,044,675	3,017,488	2,771,125	85.45%

Fig. 4 Principal component analysis (PCA) for the differentially expressed genes. PCA-cluster of genes across different time interval 0 hpi as control, 24 hpi, 48 hpi and 72 hpi in susceptible genotype RT-373 and resistant genotype GT-10
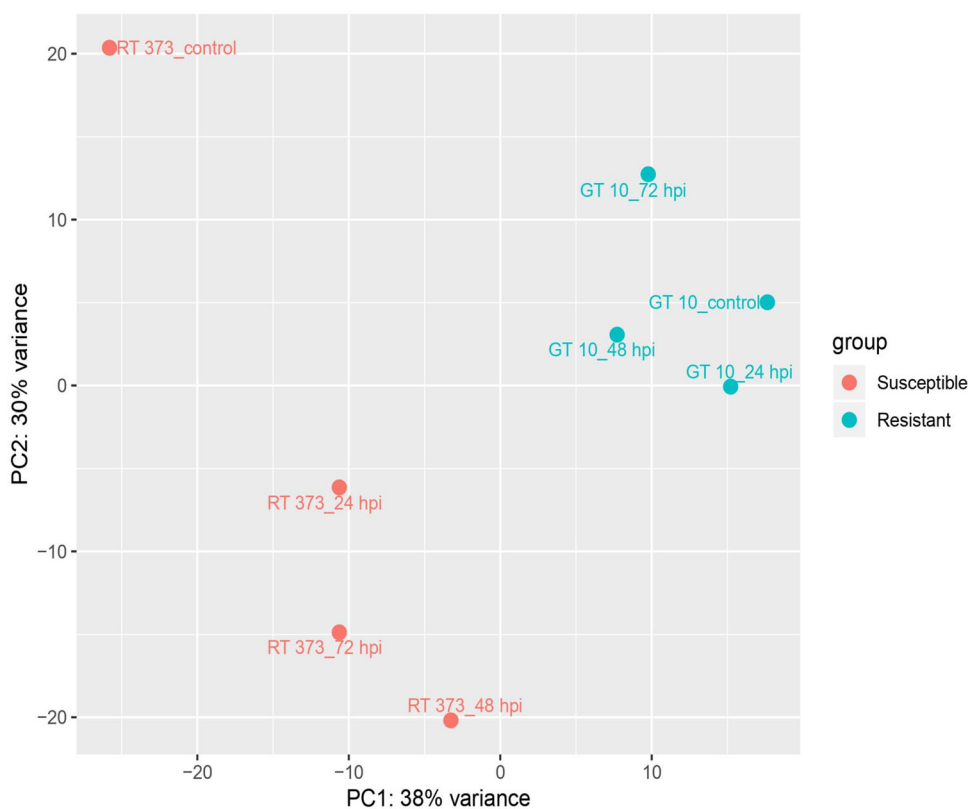
16 up regulated genes were found for PR proteins in which 15 were expressed at 48 hpi and one was expressed at 72 hpi. These genes include mainly PR protein STH-2 and chitinase (Table S5), having the role in carbohydrate metabolic process, abscisic acid- activated signaling pathways, chitinase activity and defense response to biotic stimulus.

DEGs involved in energy metabolism, glutathione metabolism, transportation and defense response

Energy is critical during the implementation of defense responses due to the expression of hundreds of genes from multiple defense pathways. So, plants try to compensate by downregulation the genes involved in energy metabolism
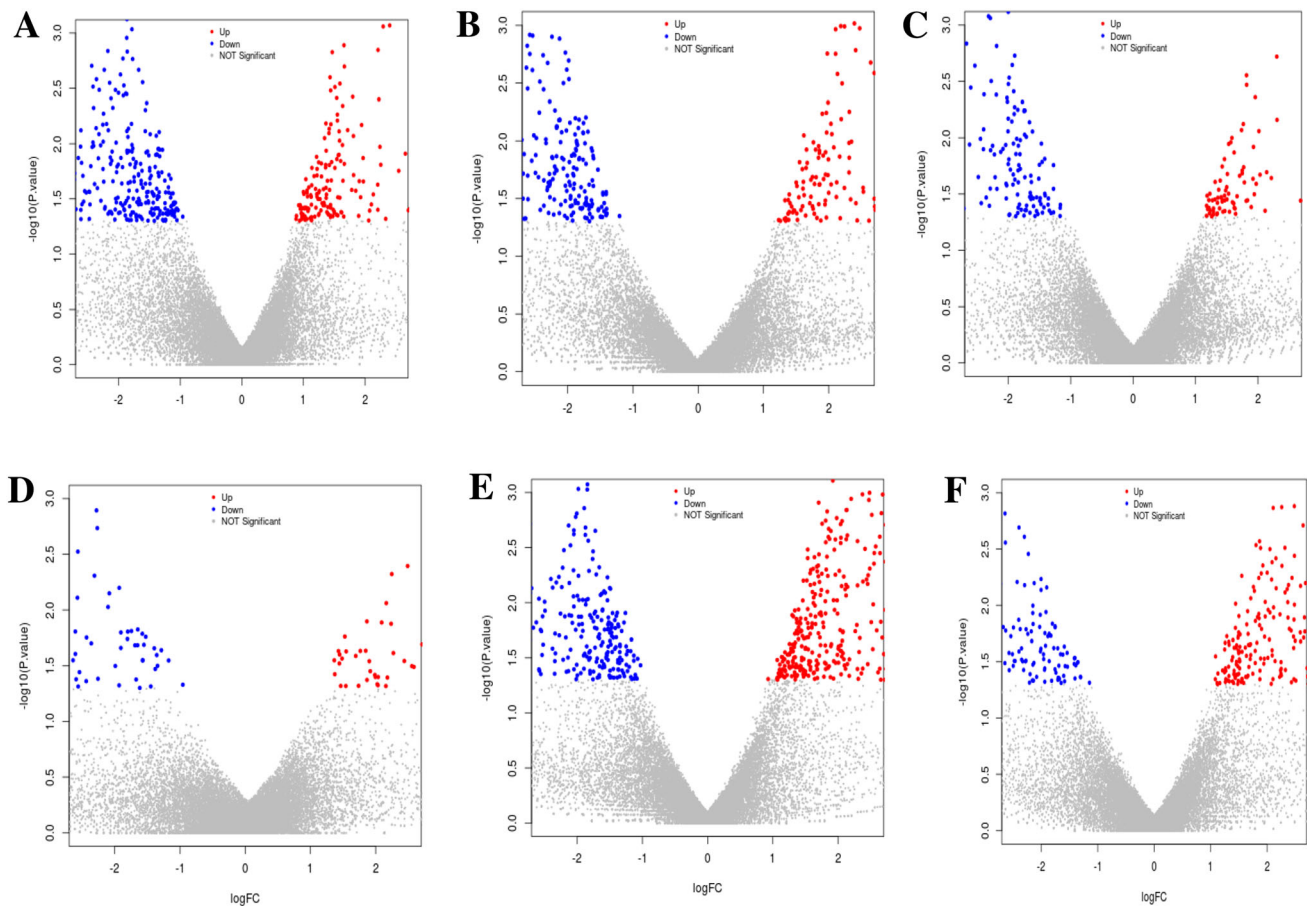
Fig. 5 Volcano plot distribution of differentially expressed genes at control versus all post inoculation stages in GT-10 and RT-373. The X-axis represents fold change at logarithm to the base 2 between control and treated samples. The Y-axis represents P-value at negative logarithm to the base 10. The red and blue color dots represent up and down regulated significant differentially expressed genes respectively having p-value < 0.05. The dots in grey color represent nonsignificant differentially expressed genes. **A** displaying DEGs in R0vsR1 **B** DEGs in R0vsR2 **C** DEGs in R0vsR3 **D** DEGs in S0vsS1 **E** DEGs in S0vsS2 **F** DEGs in S0vsS3. R and S represent 'Resistant GT-10' and 'Susceptible-RT-373' respectively; 0, 1, 2 and 3 represents 0, 24, 48 and 72 hpi

and support cellular energy requirements for plant defense response (Rojas et al. 2014). We found total 17 genes including glucan endo 1,3-beta-glucosidase, endoglucanase, alpha amylase and sucrose synthase were significantly expressed in GT-10 at 24 hpi (Table S6). In RT-373 total of 13 DEGs were found significantly expressed involved in energy metabolism. Here, we found 4 DEGs in GT-10 and 9 DEGs in RT-373 responsible for glutathione metabolism and transferase activity (Table S7). The ATP binding cassette (ABC) superfamily is a large and diverse group of proteins, transport compounds across cellular membrane and involved in diverse range of biological processes (Bhati et al. 2015). We found that 8 genes encoding for ABC transporter family in GT-10 and 9 genes in RT-373 (Table S8). In our study we found 16 genes involved in fatty acid metabolism in which 5 genes are involved in cutin, suberine and wax biosynthesis process in RT-373 (Table S9). In GT-10 down regulated 2 genes were found in which one was involved in fatty acid metabolism and one was involved in wax biosynthesis.

Comparative analysis of DEGs in resistant genotype with reference to susceptible susceptible

To analyze the genes involved in the resistance response, DEGs were also compared at each infection stage between the resistant and susceptible genotypes. At 24 hpi a total of 7 genes were differentially expressed in both the genotypes (Table S10). The DEGs involved in photoperiodism regulation, amino acid export, UDP-glucosyltransferase activity and proteasome-mediated ubiquitin-dependent protein catabolic process. With the progression of disease, greater number of DEGs were (54 DEGs) observed at 48 hpi between the two genotypes. These genes are involved in DNA-binding transcription factor activity, transcription regulation, endopeptidase inhibitor activity, oxidoreductase activity, protein serine/threonine kinase activity,
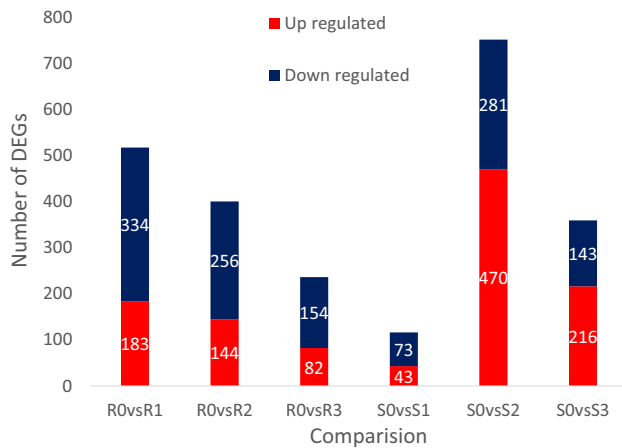
Up regulated
Down regulated
Number of DEGs
800
700
600
500
400
300
200
100
0
334
183
256
144
154
82
73
43
281
470
143
216
R0vsR1
R0vsR2
R0vsR3
S0vsS1
S0vsS2
S0vsS3
Comparision

Fig. 6 Total number of up regulated and down regulated DEGs in each comparison of resistant (GT-10) and susceptible (RT-373). X-axis and Y-axis shows the number of DGEs and comparisons, respectively. Red bar shows up regulation and blue bar shows down regulation of DGEs. Deseq (version 1.22.1) was run to find out the differential gene expression and the P-value was adjusted using Benjamini and Hochberg's approach to control the false discovery rate. Genes with *P*-value < 0.05 were considered as significantly differential expressed. R and S represent Resistant and Susceptible respectively; 0, 1, 2 and 3 represents 0, 24, 48 and 72 hpi

glutathione transferase activity and structural constituent of cell wall. DEGs were significantly decreased at 72 hp with a total number of 12 DEGs. These genes involved in fructose-bisphosphate aldolase activity, fructose 1,6-bisphosphate metabolic process, glycolytic process, ligase activity and sequence-specific DNA binding. This analysis specifically provides insight for the molecular components contributing for the resistant genotype with respect to the susceptible genotype.

GO based analysis of DEGs

The gene ontology (GO) analysis was done by Blast2GO online tool. GO was categorized in three different categories i.e. 1) biological process, 2) cellular compartment and 3) molecular function. DEGs from different time points in GT-10 were found to be linked mainly to the biological processes. These DEGs specifically were grouped in to 17 different major GO terms including "macromolecular metabolic process" (197), "organonitrogen compound metabolic process" (142) and "protein metabolic process" (111), within cellular process, different 6 GO terms including "integral component of membrane" (207), "intracellular membrane bound organelle" (193) and "cytoplasm" (121) whereas within molecular function 8 major GO terms like "anion binding" (125), "nucleoside phosphate binding" (111) and "nucleotide binding" (111) were significantly enriched in these comparisons (Fig. 9A). For the DEGs from different time points in RT-373, within the biological process, cellular macromolecular metabolic process" (160), "organic substance biosynthetic process" (126) and "cellular biosynthetic process" (116) were significantly enriched among 18 major GO terms, within cellular component "integral component of membrane" (238), "intracellular membrane bound organelle" (145)

R0R1
R0R2
322
130
205
35
30
30
141
R0R3
S0S1
S0S2
47
33
541
31
5
146
177
S0S3
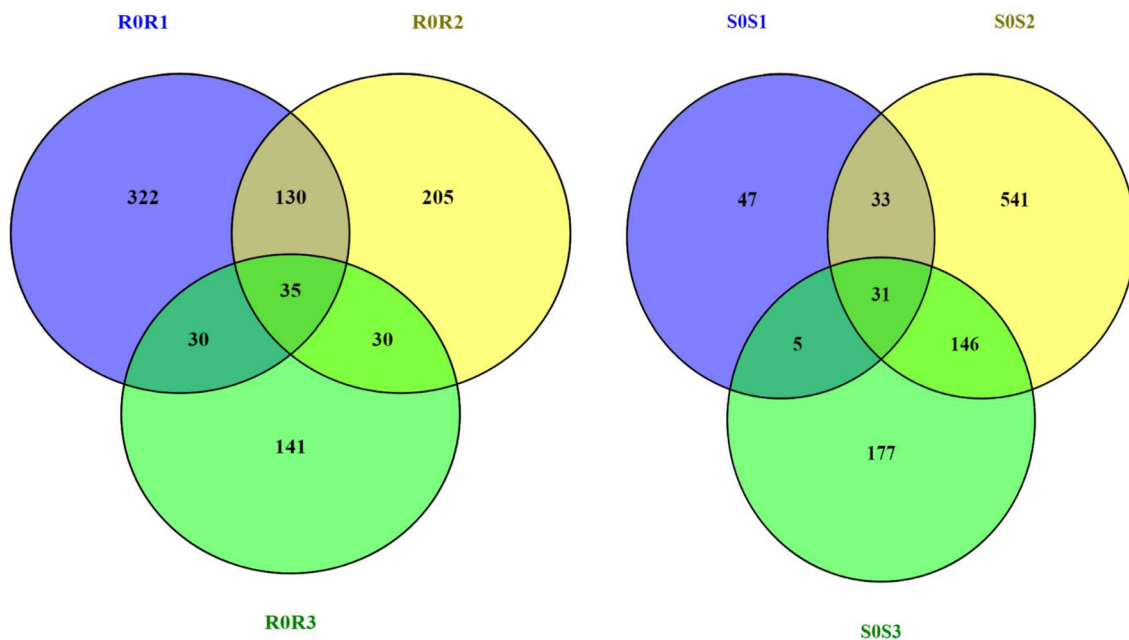
Fig. 7 Venn diagrams of differentially expressed genes (DEGs) modulated by *M. phaseolina*. Venn diagrams represent DEGs in GT-10 and RT-373 challenged by *M. phaseolina* at 0, 24, 48 and 72 h post inoculation. R and S represent 'Resistant GT-10' and 'Susceptible-RT-373' respectively; 0, 1, 2 and 3 represents 0, 24, 48 and 72 hpi

Fig. 8 Hierarchical clustering of DEGs which expressed commonly in all comparisons in both resistant (**A**) and susceptible (**B**) genotypes. Up-regulation and down-regulation were represented by brown and blue shading, respectively. White shading indicated non-modulation.

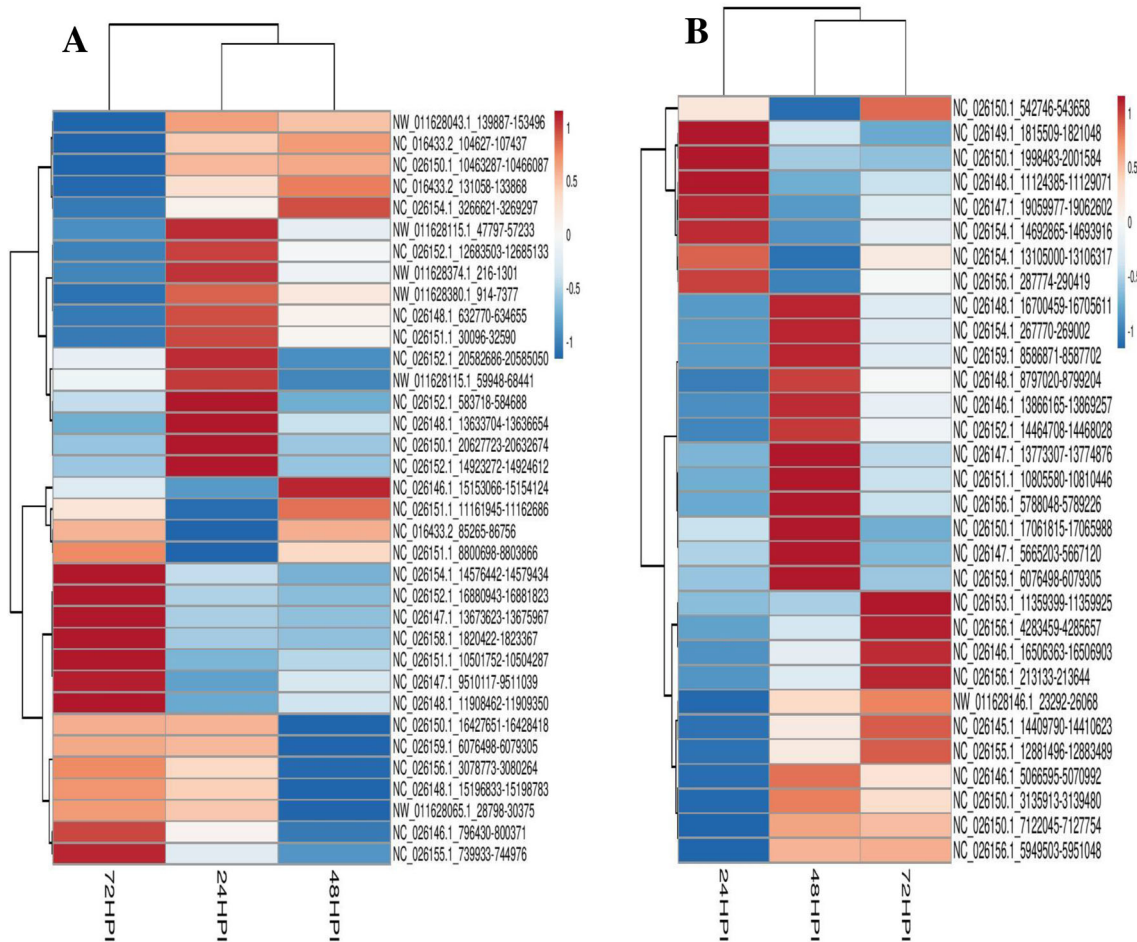Each column represents a comparison and each row means a gene. The details of genes presented by each raw is given in supplementary table 11

and "membrane bound organelle" (145) and in molecular function, "cation binding" (157), "anion binding" (114) and "nucleoside phosphate" (104) binding were significantly enriched among 4 and 5 major GO terms respectively (Fig. 9B).

KEGG pathway enrichment analysis for DEGs identified

Changes in metabolic pathways under different post inoculation stages were determined using Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of DEGs. At 24 hpi total 63 pathways were enriched in GT-10 and at 48 hpi total 89 pathways were enriched in RT-373. The top 14 enriched pathways of GT-10 and the top 23 pathways of RT-373 were enlisted in table S11. The pathways mainly include starch and sugar metabolism, fatty acid biosynthesis, secondary metabolism (zeatin biosynthesis, flavonoid biosynthesis, caffine metabolism

and indole alkaloid biosynthesis), phenylpropanoid biosynthesis and drug metabolism-cytochrome P450. However, the number of enzymes and the gene sequences involved in enzyme expression were different in both genotypes.

Validation of DEGs by qRT-PCR

Quantitative RT-PCR of 5 randomly selected DEGs was performed with gene specific primers to validate the gene expression data from RNA-seq (Table S1). Expression timing patterns of the investigated genes were analyzed in sesame root samples at four time-points (0, 24, 48, and 72 hpi). The expression of glycerol-3-phosphate acyltransferase 5 and bZIP TFs 53-like were down regulated at all four time-points in both the genotypes. The expression of another randomly selected gene, proline-rich receptor-like protein kinase PERK2 was down regulated in RT-373 at all four time-points, while it was up regulated in GT-10 at 24
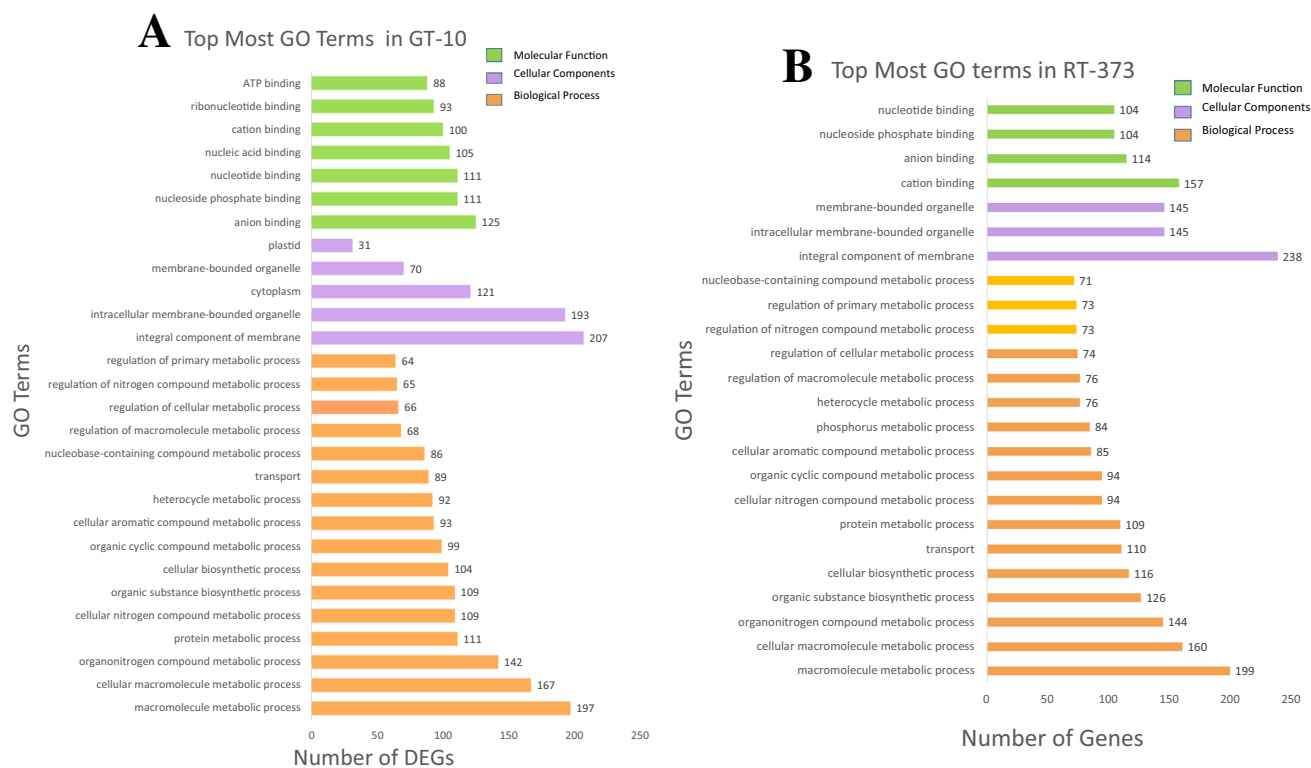
Fig. 9 Classification of DEGs based on gene ontology in the resistant interaction (**A**) and susceptible interaction (**B**). The number of DEGs categorized under biological process in sesame roots infected by

M.phaseolina were presented as orange bars. Genes belonging to molecular function and cellular components were represented as green and purple bars, respectively

and 48 h after inoculation (Fig. 10). Our analysis for the expression data of the randomly selected DEGs were found during the qRT-PCR were similar with those obtained by the RNA-Seq analysis. These results implied that the RNA-seq data showed the real expression patterns of the sesame genes in the compatible interaction.

Discussion

Transcriptome analysis

Transcript sequencing of MP resistant and susceptible genotypes of sesame at four different post inoculation stages revealed total 16 cDNA libraries (with 2 replication) and single pair end data were generated by using Iontorent S5 platform. With a combination of functional genomics and various bioinformatics tools, the differentially expressed gene were obtained in this study.

The roots inoculated with MP showed impact in cell structure in resistant genotype GT-10, whereas in susceptible genotype (RT-373), the epidermal cells and vascular tissues of infected roots were clogged (Fig. 3). In the roots of susceptible genotype hyphal penetration was seen at 24 hpi which became denser with the increase of time.

Chowdhury et al. (2014) reported maximum fungal hyphae growth in susceptible variety of sesame cv. VRI-1 at 48 hpi. They also observed greater number of melanized microsclerotia on the root surface of susceptible variety than the resistant at 86 hpi. This study supported our SEM results and sampling time after fungal inoculation. It implied that the development of MP hyphae was inhibited by GT-10 and therefore characterized as a resistant genotype.

A total of 1153 and1226 candidate genes in resistant genotypes, GT-10 and susceptible genotype RT-373, respectively, were identified (Fig. 6). During the inoculation with MP, resistant genotype showed higher number DEGs at 24 hpi while at 48 hpi in case of susceptible genotype. These results indicate the early response in resistant genotypes, however a greater number of DEGs in susceptible than those in resistant indicates there may be some changes of normal metabolic system in susceptible genotype after inoculation with MP. Similarly, in sesame varieties inoculated with *Fusarium oxysporum* showed greater number of DEGs in susceptible than the resistance (Wei et al. 2016). Many genes expressed in a similar way in both resistant and susceptible genotypes during the plant pathogen interaction, bur early response in resistant genotype indicates the success of defense. The most significant
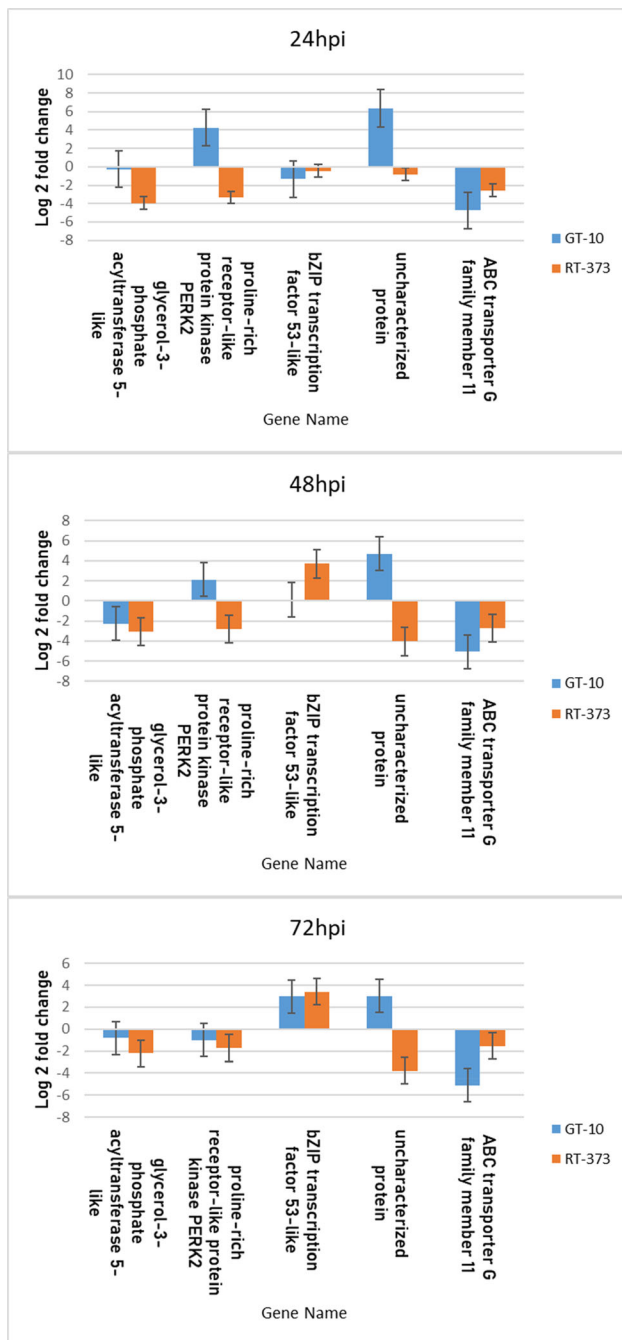
Fig. 10 Validation of RNA-seq data by qRT-PCR. Five differentially expressed genes (DEGs) were selected for validation, and they showed a similar tendency with RNA-Seq. The y-axis showed the fold changes values indicating up-regulation and negative values indicating down-regulation

DEGs involved in defense strategy against MP are depicted using Circos plot (Fig. 11) which are also discussed below.

Role of transcription factors

Transcription factors (TFs) are key components of plant innate immune system and play major role in basal defense. TFs lead to activation of PR proteins, HR response which accompanied by the induction of SAR. In defense related TFs family bZIP, AP2/ERF, NAC, MYC, MYB and WRKY gene families are included (Amorim et al. 2017). Among them WRKY and AP2/ERF families are reported to activate plant defense against necrotrophic fungi (Chi et al. 2013; Berrocal-Lobo et al. 2002). In this study we also observed up regulation of WRKY genes NC_026150.1_22905954-22,909,832, NW_011628049.1_212875-216,013, NC_026155.1_15298480-15,300,438 and NC_026148.1_4812653-4,818,181 in resistant genotype. Among these four genes NW_011 628,049.1_212875-216,013 was annotated as WRKY13. Up regulation of WRKY 13 gene may be responsible for suppression of JA pathway and activation of salicylic acid biosynthesis in GT-10 in our study. In rice, OsWRKY13 participate in fungal blast disease resistance by activating the salicylic acid biosynthesis pathway and the suppression of jasmonic acid pathway (Qiu et al. 2007). In cotton, *GbWRKY1* is a negative regulator of jasmonate-mediated defense response and plant resistance to the pathogen *Botrytis cinerea* and *Verticillium dahliae* (Li et al. 2014). MYB TFs were also observed to be involved against plant biotic stresses. In *A. thaliana* over-expression of *BjMYB1* significantly increase the resistance against *B. cinerea* (Gao et al. 2016). *TaRIM* positively regulated the expression of five defense genes (Defensin, PR10, PR17c, nsLTP1, and chitinase1) through binding to R2R3-MYB in wheat against *Rhizoctonia cerealis* (Shan et al. 2016). In our study we identified total 16 MYB TFs like *MYB52* (NC_026152.1_13989999-13,993,211), *MYB8* (NW_011628051.1_225578-227,066), *MYB44* (NC_026147.1_22229592-22,230,640), *MYB59* (NC_026153.1_4907511-4,909,058), *MYB86* (NW_011628063.1_87455-89,511) and *MYB3R*-1 (NC_026145.1_1549173-1,558,593) which might suggest similar defense mechanism in sesame.

TF bZIP domain containing transcription families are the largest TF family in plants regulating the genes in response to pathogen defense, flower development, seed maturation and abiotic stresses (Alves et al. 2013). Kaminaka et al. (2006) found that AtbZIP10 as appositive mediator of the uncontrolled cell death observed in *Arabidopasis* mutants, act antagonistically in both pathogen-induced HR and basal defense response. However, in susceptible wheat bZIP showed significantly down regulation during interaction with *Fusarium graminearum* (Erayman et al. 2015). These results suggest bZIP TFs may serve both a positive and negative regulator of plant defense. We also identified bZIP 53, bZIP 11, bZIP 43, homeobox-leucine
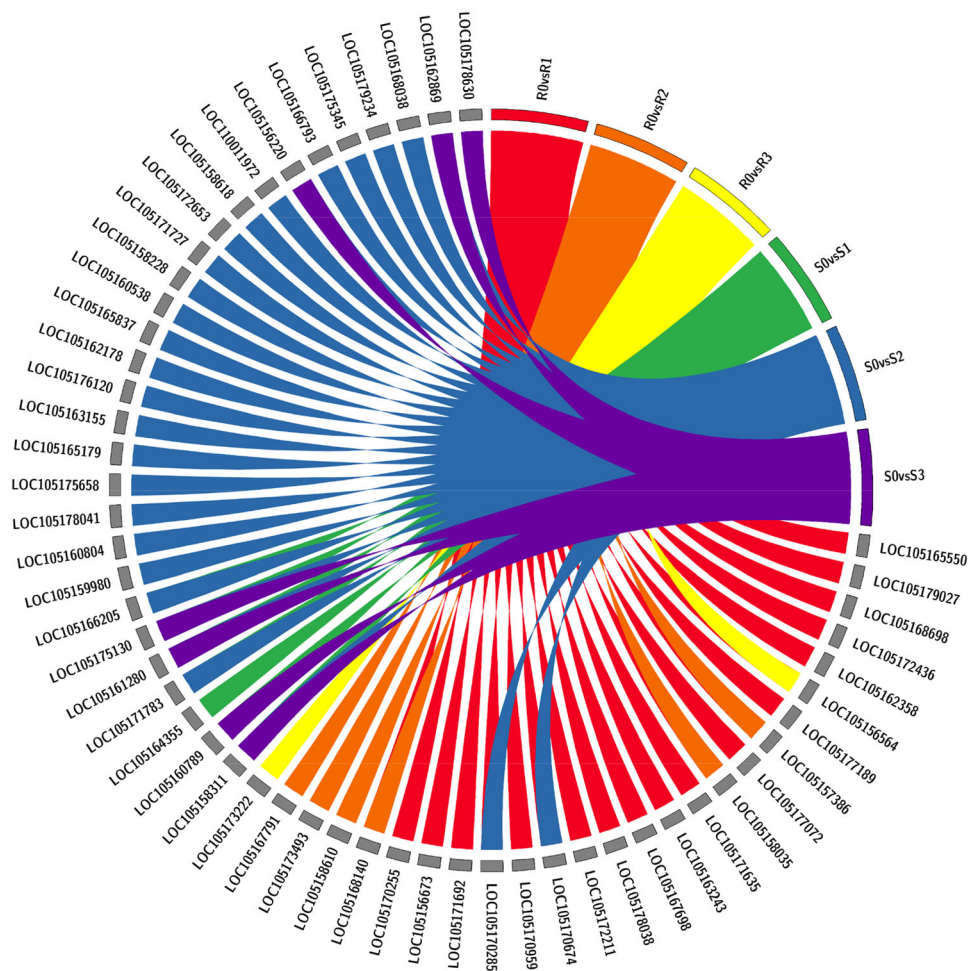
zipper protein HAT 22, HAT 5 and ANTHOCYANIN-LESS 2 in resistant and bZIP 63, bZIP 53, homeobox-leucine zipper protein HAT 4, ATHB 7 and ATHB 52 in susceptible genotype. In *Arabidopsis* bZIP 11 is involved in activation of auxin-induced transcription by recruiting histone acetylation machinery (Weiste and Droge-Laser 2014). In resistant genotype up regulation of bZIP 11 (NC_026147.1_23354798-23,356,010) indicates its probable involvement in response to auxin against MP.

NAC transcription factors were observed to play a crucial role in plant immunity via hypersensitive response and ETI against number of pathogens (Yuan et al. 2019). In wheat, TF TaNAC8 (Xia et al. 2010), in maize, ZmNAC41 and ZmNAC100 (Voitsik et al. 2013), in rice, OsNAC122 and OsNAC131, in tomato, two homologous NACTFs, JA2 and JA2L (Sun et al. 2013) were found positively regulated during various pathogen infection where as in wheat, TaNAC30 (Wang et al. 2018) and TaNAC2 (Zhang et al. 2018) were negatively regulated. In our study no significant expression of NAC TFs was found in case of resistant genotype but in susceptible genotype total six (NC_026148.1_4093122-4,094,547, NC_026159.1_

6137846-6,139,616, NC_026146.1_5041837-5,043,462, NC_026158.1_4755147-4,756,720, NC_026158.1_4737996-4,739,437 and NC_026145.1_13414852-13,416,482) NAC TFs were significantly expressed. We also identified up regulation of bHLH TFs in resistant genotype but in susceptible genotype this transcription factor showed negative expression. This result suggests positive role of bHLH in defense response. Our result is consistent with the Cheng et al. (2018) who reported a bHLH transcription factor, *GmPIB1* enhance resistance to a causal organism for *Phytophthora* root rot in soybean.

ABC transporters form a large protein family in plants, involved in detoxification, response to pathogen, phytate accumulation in seed, surface lipid deposition and transport of the phytohormones like auxin and ABA. Participation of ABC transporters in secondary metabolite transport (Yazaki 2006) and plasma membrane remodeling which potentially affects the membrane bound proteome (Deppe et al. 2018). We have also found total eight and nine ABC transporters in resistant and susceptible genotypes respectively, which may involve in secondary metabolites

transport and membrane integrity during the pathogen interaction in sesame.

GSTs are ubiquitous and multifunctional enzymes encoded by a large gene family, which induced during various biotic and abiotic stresses. We observed an early induction of GST gene in resistant genotype at 24 hpi while in susceptible genotype higher expression of GST observed at 48 hpi. A microarray analysis showed significant upregulation of GST1 gene in *A. thaliana* infected with necrotrophic fungus *Alternaria brassicicola* at initial infection site (Schenk et al. 2000). In another study *TaGST5* was induced at early (3–6 h after inoculation) stage in wheat resistant to seedling blight caused by *Fusarium asiaticum* (Li et al. 2010). These results suggest the early induction of GST in plants during necrotrophic fungal invasion. Moreover, we have found down regulation of genes which are involved in energy metabolism in resistant genotype where as in susceptible genotype these genes showed significant up regulation. Our findings are supported by Wei et al. (2016) who also found a greater number of upregulated genes related to energy metabolism like carbohydrate, lipid and amino acid metabolism in susceptible sesame variety against *F. oxysporum*.

Fatty acids and their derivatives are reported in local defense and in systemic acquired resistance as well. During SAR, localized infection by a primary pathogen results in the induction of broad-spectrum resistance against secondary infections in the systemic tissues of the plant (Lim et al. 2017). Our results show the positive regulation of SAR genes (NIM1-INTERACTING 2 and lipase-like PAD4) in resistant genotype. In *Arabidopsis* NIM1-INTERACTING 2 (*NIMIN2*) is reported as the immediate early SA induced gene (Hermann et al. 2013). We also noticed up regulation *NIMIN2* (NC_026147.1_11264369-11,265,004) at 24 hpi which was slightly decreased at 48 hpi in GT-10. Interestingly, we couldn't find any genes involved in SAR in susceptible genotype. But in susceptible we found total of 11 significantly expressed genes which are involved in fatty acid biosynthesis and metabolic process. Besides the structural integrity to cell lipids, fatty acids play a vital role in signaling also. Fatty acids also serve as precursor of the jasmonic acid which is involved in the signaling pathways. We also observed up regulation of allene oxide cyclase (AOC) which involved in jasmonic acid biosynthesis process in susceptible genotype. Riemann et al. (2013) found two mutants of rice defective in the gene encoding *OsAOC* were susceptible to blast pathogen *Magnaporthe oryzae* than the wild type as hyphal growth was enhanced in the mutants. In addition, other hormones like auxin, gibberellin, cytokinin, ABA, ethylene and salicylic acid are also well documented in plant defense during biotic stress. In *Arabidopasis* mutants, axr1, axr2 and axr3, disruption of auxin signaling leads to resistance

enhancement to *F. oxysporum* (Kidd et al. 2011). Nonetheless, it was also found positively regulated in axr2-1 and axr1-1 *Arabidopsis* mutants which are more susceptible than the wild type to the necrotrophic fungi *Plectosphaerella cucumerina* and *B. cinera* (Llorente et al. 2008). Similarly, in our study auxin-responsive protein IAA4, SAUR32 and auxin induced protein 22D shown down regulation and auxin-responsive protein SAUR7, auxin efflux carrier component 7, auxin repressed 12.5 kDa protein and auxin response factor 19 shown up regulation in GT-10. Contrary, in RT-373 we observed up regulation of auxin induced 22D protein and the highest up regulation (eightfold) of 2-oxoglutarate-dependent dioxygenase (DAO) was also recorded. Gibberellic acids are the terpenoid hormone compound involved in germination, flowering and cell division but in plant pathogen interaction its role is not well known. Zumaquero et al. (2019) reported positive regulation of two GA biosynthesis pathway genes, GA4 disaturase family protein and Gibberellin 20-oxidase in avocado susceptible to root rot, however in another report no any correlation was found between GA and *Fusarium* pathogenicity (Manka 1980). In present study positive regulation of GA in GT-10 while both positive and negative regulation in RT-373 observed.

PR proteins

PR proteins comprise the large protein family and classified in to different 17 classes. These proteins are mainly synthesized and induced during incompatible host–pathogen interaction. Several DEGs encoding PR proteins were identified and found to be upregulated at 48 hpi in RT-373 but in GT-10 a smaller number of DEGs were identified and regulated in both positive and negative manner. We identified major allergen pru which was significantly up regulated in both the genotypes and involved in abscisic acid mediated signaling. Major allergen pru is a novel gene encoding PR10 protein and also reported in soybean, which could be involved in resistance against *Phytopthera sojae* (Fan et al. 2015). Constabel et al. (1992) reported pathogenesis related STH-2 gene show unaltered susceptibility to *P. infestans* in transgenic potato. There was no increase in resistance after overexpression of PR STH-2 gene in potato. Similarly, we did not observe any PR STH-2 (NC_026147.1_16917097-16,919,408) genes in GT-10 but in RT-373 we found positively regulated three PR STH-2 genes which might alter resistance. Another PR protein chitinase was also up regulated in both the genotypes. The chitinase of plants are generally endochitinase and can be detected at the early stage of growth. Early induction (24 hpi) of chitinase in resistant genotype (GT-10) restricts the growth and development of MP. Plant chitinase triggers two major plant defense responses first is degradation of

the fungal cell wall which in turn inhibits hyphal growth and second, is elicitor mediated plant immune responses by cleaved chitin fragments (Shibuya and Minami, 2001; Radadiya et al 2020).

Role of secondary metabolites biosynthesis genes

Plants are rich source of low molecular secondary metabolites (SM) such as terpenoids, tannins, saponins, flavonoids and alkaloids which are crucial for survival of plants against biotic and abiotic stresses (Ribera and Zuniga 2012). Various constitutive and pathogen inducible SMs with antimicrobial activity have been reported in different plants (Ahuja et al. 2012; Jadhav et al. 2013). Lignin is the most abundant biopolymer and produced by the phenylpropanoid pathway. Cinnamoyl CoA reductase is involved in the first step of lignin biosynthesis pathway as it converts the cinnmolyl-coA esterase into monolignols (Ghosh et al. 2015; Kawasaki et al. 2006). Lignins are the major component of the plant secondary cell walls and play a role in defense against pathogen (Bagniewska-Zadworna et al. 2014). In our experiment we found positive expression of cinnamoyl-CoA reductase 1 (NC_026153.1_2108859-2,111,045) in both the genotypes which shows the initiation of lignin biosynthesis pathway. Goujon et al. (2003) found up to 50% reduction in lignin contents with down regulation of AtCCR1 gene in Arabidopsis. Moreover, in susceptible genotype lignin forming anionic peroxidase (NC_026146.1_15027834-15,029,531) also found positively regulated at 48 hpi.

Terpenoids are the important secondary metabolites in plants synthesized by two independent pathways, mevalonic acid and non mevalonic acid or 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway (Chen et al. 2011). Howell et al. (2000) demonstrated the induction of terpenoid biosynthesis in cotton roots during interaction with Rhizoctonia solani, however the roles and nature of terpenes produced by the plant roots below the ground is not very well documented (Huang and Osbourn 2019). In present study we found up regulation of genes involved in mono-, di-, sesqui-, tri-terpenoid, ubiquinone and other terpenoid quinone biosynthesis pathways in susceptible genotype which might indicate the crucial function of terpenoids in plant defense and below ground communication between plants and soil-dwelling microorganisms.

Flavonoids are synthesized by phenylpropanoid pathway and protects plants against insects and pathogens which are regulated by MYB, bHLH and WD40-type transcription factors (Falcone Ferreyra et al. 2012). We also observed upregulation of genes involved in flavonoids biosynthesis genes including naringenin 3-dioxygenase, dihydrokaempferol 4-reductase and trans-cinnamate 4-monooxygenase. Our results are supported by Camargo-

Ramirez et al. (2018) who stated that kampherol, naringenin and p-coumaric acid showed antifungal activity against the necrotrophic fungus *Plectosphaerella cucumerina* in *Arabidopsis*.

Conclusions

Transcriptome analysis of resistant and susceptible sesame genotypes during MP infection unravels molecular mechanisms of defense response against charcoal rot of sesame. The key difference between resistant and susceptible plants is the timely recognition of pathogen invasion along with early and rapid activation of host defense mechanisms. Resistant genotype had the highest number of expressed genes at 24 hpi while the susceptible genotype showed highest number of expressed genes at 48 hpi. In addition, flavonoids, flavones, carotenoids and caffeine biosynthesis via phenylpropanoid pathway and salicylic acid biosynthesis at 24 hpi may contribute to the sesame root rot resistance. Significantly higher expression of MYB, WRKY, leucine zipper protein, bHLH, bZIP and NAC transcription factors were observed in resistant genotype. Moreover, resistant genotype also showed higher expression chitinase gene at 24 hpi, which may restrict the pathogen entry. Thus genes/transcription factors imparting disease resistance could serve as markers for resistant sesame breeding by marker assisted selection.

Declarations

Conflicts of interest The authors declare that they do not have any conflict of interest.

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