Comparative Transcriptome Analysis of Fungal Pathogen Bipolaris maydis to Understand Pathogenicity Behavior on Resistant and Susceptible Non-CMS Maize Genotypes

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Bipolaris maydis is pathogen of maize which causes maydis leaf blight disease. In India major losses occur due to the B. maydis race “O” pathogen, whereas in other parts of the world, major losses are due to the race “T” pathogen. In the present study, we conducted an \textit{in planta} transcriptomics study of the B. maydis race “O” pathogen after infection on non-CMS maize resistant and susceptible genotypes by mRNA sequencing to understand the molecular basis of pathogenicity for better management of the pathogen. Approximately 23.4 GB of mRNA-seq data of B. maydis were obtained from both resistant and susceptible maize backgrounds for fungus. Differentially expressed genes (DEGs) analysis of B. maydis in two different genetic backgrounds suggested that the majority of highly DEGs were associated with mitochondrial, cell wall and chitin synthesis, sugar metabolism, peroxidase activity, mitogen-activated protein kinase (MAPK) activity, and shikimate dehydrogenase. KEGG analysis showed that the biosynthetic pathways for secondary metabolism, antibiotics, and carbon metabolism of fungus were highly enriched, respectively, in susceptible backgrounds during infection. Previous studies in other host pathogen systems suggest that these genes play a vital role in causing disease in their host plants. Our study is probably the first transcriptome study of the B. maydis race “O” pathogen and provides in-depth insight of pathogenicity on the host.

\textbf{Keywords:} Bipolaris maydis race “O”, non-CMS maize, RNA-seq, host–pathogen interaction, differentially expressed genes (DEGs), effectors

\textbf{INTRODUCTION}

Bipolaris maydis (Cochliobolus heterostrophus) is a necrotrophic ascomycete belonging to the order Pleosporales, which causes maydis leaf blight (MLB) or southern corn leaf blight. In India, yield losses occur due to the B. maydis race “O” pathogen in maize, unlike the rest of the world where major losses are due to the race “T” pathogen. Maize (\textit{Zea mays} L.) is the third most widely grown...
Meshram et al. RNA Sequencing of Bipolaris maydis Race "O" April 2022 | Volume 13 | Article 837056

**FIGURE 1** | (A) Scanning electron micrograph showing abundant fungal hyphae in susceptible (CM 119) line after infection with Bipolaris maydis. (B) Symptoms of maydis leaf blight on susceptible CM 119 (score 4) and resistant SC-7 (score 1) and both genotypes under field conditions.

**TABLE 1** | Summary of the Illumina sequence reads obtained from Zea mays plants inoculated with B. maydis pathogen grown on sorghum seeds.

| Sample* | Total clean reads | Data (GB) | Q30% | Paired total | Paired aligned uniquely | Unpaired total | Unpaired aligned uniquely | Overall alignment rate |
|---------|-------------------|-----------|------|--------------|------------------------|---------------|--------------------------|------------------------|
| RI      | 34527322          | 5.24      | 89.8 | 17263661     | 17215458              | 34430916      | 56440                    | 0.45                   |
| RIR     | 29461518          | 4.46      | 90.54| 14730759     | 14687660              | 29375320      | 59682                    | 0.5                    |
| SI      | 42163024          | 6.3       | 91.11| 21081512     | 20621885              | 41243770      | 68621                    | 2.35                   |
| SIR     | 50012112          | 7.5       | 90.23| 25006056     | 23866276              | 47732552      | 171279                   | 4.91                   |

*Ri, resistant inoculated; RIR, resistant inoculated replicate; SI, susceptible inoculated; SIR, susceptible inoculated replicate.

cereal crop after rice and wheat in India (Hussain, 2011). Losses in India due to MLB disease may extend up to 70% (Kumar et al., 2016). Infected maize typically causes tan and elliptical to rectangular lesions (White, 1999) on the leaves and under the surface of foliage which later coalesce and result in an extensive blight appearance. Among the 65 major foliar diseases of maize, MLB is an important disease of maize (Rahul and Singh, 2002). MLB is reported from almost all maize growing regions in the world but more severe in areas where environmental conditions are hot and humid. Three races (C, O, and T) of *B. maydis* have been identified in maize crop so far. Race “O” is more prevalent than “T” in India, whereas worldwide, race “T” is a major concern; race “C” is reported only in China (Mubeen et al., 2017).

*Bipolaris maydis* race “O” is predominant in tropical and sub-tropical areas. It infects a broad range of maize genotypes including CMS and non-CMS maize lines. Studies reported that inoculated susceptible lines with race “O” showed a 50% yield loss (Fisher et al., 1976; Gregory et al., 1978). Typical symptoms of race “O” are small lesions which eventually become diamond-shaped and rectangular as they mature and are restricted to leaf veins (Ali et al., 2011). Race “T” attacks CMS maize which promotes Texas male-sterile cytoplasm (cms-T), this race historically caused an epidemic in the United States in 1970 and 1971. Typical symptoms of race “T” develop on leaves, husks, and ears, and produce small lesions on maize (Ullstrup, 1972). Races “O” and “T” can be identified best with a host differential test, viz., a pathogenicity test of cms-T plants, and also by physiological/morphological characteristics on culture media (Leonard, 1977; Warren et al., 1977).

Combating losses caused by MLB resistance varieties is the best solution. Maize crop is resistant to race “T” with normal cytoplasm therefore management of race “T” can be achieved with elimination of cms-T from cultivars of high agronomic importance (Hyre, 1970; Ullstrup, 1972). In India, a broad range of maize genotypes serve as the major host of race
“O,” which causes huge loss. So far, we only know that the rhm recessive gene of C. heterostrophus confers resistance to race “O” (Zaitlin et al., 1993). Various screening techniques, viz., detached leaf techniques (Lakshmi and Sharma, 1987), tissue culture (Kuehnle and Earle, 1988), and seedling assays (Tajimi et al., 1985) have been investigated for disease resistance. Conventional breeding or recurrent selection is also an effective method to improve resistance against MLB (Shieh and Lu, 1993). On the pathogen side, very few studies have been conducted to understand the race “O” pathogen. In the present study, whole transcriptome analysis was done by mRNA sequencing of the B. maydis race “O” pathogen after infection of resistant and susceptible non-CMS maize inbred lines to understand the molecular basis of pathogenicity leading to better management of the pathogen. The race “O” pathogen used in this study was re-confirmed by Venkatesh et al. (2021). So far transcriptome analysis of fungal pathogen B. sorokiniana on infected wheat (Ye et al., 2019) and B. sorghicola on sorghum (Mizuno et al., 2012; Yazawa et al., 2013) has been completed. Here we present probably the first in planta transcriptome study of the B. maydis race “O” pathogen on non-CMS maize lines. RNA-seq and fold change were calculated by comparing B. maydis infection on susceptible inoculated (SI) versus resistant inoculated (RI) lines.
# Materials and Methods

## Plant Material and Fungal Inoculation

Two extreme genotypes of maize inbred lines differing in their susceptibility to *B. maydis* were used in this study. Line SC-7-2-1-2-6-1 (SC-7) which is registered (INGR 07025) as a highly resistant non-CMS line and CM 119 which is established as a standard susceptible marker against *B. maydis*. The experiment was conducted under greenhouse conditions. The *B. maydis* New Delhi isolate was maintained in pure culture and later mass-multiplied on soaked sorghum seeds. After 30 days, old plants were inoculated with pathogen *B. maydis* according to the method described by Payak and Sharma (1983). Inoculated samples were collected for RNA-seq at 48 h post inoculation (disease phase, Liu et al., 2015). Symptoms started appearing and fungal signs were noticed more clearly on susceptible line CM 119 (Figure 1).

## RNA Extraction, Library Preparation, and Sequencing

Total RNA was extracted from infected CM 119 and SC-7 at 48 h post inoculation and their non-inoculated controls with an RNAeasy plant mini kit (Qiagen) following the manufacturer's instructions. Total RNA of each sample was quantified and qualified by an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, United States), NanoDrop (Thermo Fisher Scientific Inc.), and 1% agarose gel. One microgram of total RNA with an RNA quality score was 30 (Qphred). HISAT2 software was selected according to the characteristics of the reference genome. The reference genome used for this study was Bipolaris_maydis_c5_gca_000338975.CocheC5_3.dna.toplevel.fa. Raw read counts mapped to each gene from the HiSat2-generated alignments were obtained using the feature counts command (Liao et al., 2014) of the subread package (Liao et al., 2013).

### Quality Control and Read Mapping to the Reference Genome

Quality checks for the raw fastq files were conducted through a pipeline consisting of FastQC. The minimum quality score was 30 (Qphred). HISAT2 software was selected according to the characteristics of the reference genome. The reference genome used for this study was Bipolaris_maydis_c5_gca_000338975.CocheC5_3.dna.toplevel.fa. Raw read counts mapped to each gene from the HiSat2-generated alignments were obtained using the feature counts command (Liao et al., 2014) of the subread package (Liao et al., 2013).

### Differential Gene Expression Analysis

For differentially expressed gene (DEG) identification, DESeq2 V1.21.17 with a replicated package was run with parametric fit Padj < 0.05. A false discovery rate (FDR) of 0.05 and a fold change of >2 were set as thresholds for DEG calling, as previously described (Bagnaresi et al., 2012; Li and Lan, 2015) and P-value >0.05 was set. The list of all DEGs is provided (Supplementary Table 1) to allow any further DEG sub-setting based on different FDRs or fold changes.

### GO Enrichment and KEGG Analyses

GO enrichment analyses were conducted with topGO, an R-bioconductor package for enrichment analysis version 2.28.0, and a P-value of 0.001 was used with classic Fisher ordering, ranks=topgoFisher. The Bioconductor package ClusterProfiler version 3.10.0 was used to generate relevant KEGG pathway pictures incorporating color-coded expression values (Padj < 0.05). A pie chart for GO enrichment is provided along with enriched genes (Supplementary Tables 2–4).

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**Supplementary Table 3** Top 20 highly downregulated genes of *B. maydis* differentially expressed in inoculated resistant and susceptible plants.

| Gene ID | Log fold | Annotation | Protein ID |
|---------|----------|------------|------------|
| COCHEDRAFT_1195 | -3.86 | 40S ribosomal protein | EMD9020 |
| COCHEDRAFT_1177804 | -4.23 | 40S ribosomal protein S3 | EMD9815 |
| COCHEDRAFT_1021505 | -4.21 | 60S ribosomal protein | EMD91574 |
| COCHEDRAFT_1225360 | -3.86 | L 21 protein | EMD97420 |
| COCHEDRAFT_1157448 | -4.43 | 40S ribosomal protein S2 | EMD90435 |
| COCHEDRAFT_1173335 | -4.54 | 40S ribosomal protein S1 | EMD91948 |
| COCHEDRAFT_1214849 | -4.81 | 60S ribosomal protein L11, N-terminal domain | EMD91114 |
| COCHEDRAFT_1225361 | -5.09 | 40S ribosomal protein S9 | EMD98741 |
| COCHEDRAFT_1127623 | -3.48 | 40S ribosomal protein S8 | EMD96126 |
| COCHEDRAFT_1224611 | -3.53 | Ribosomal protein | EMD91465 |
| COCHEDRAFT_1145202 | -3.55 | Glyceraldehyde-3-phosphate dehydrogenase | EMD87494 |
| COCHEDRAFT_1019764 | -3.44 | Ribosomal protein S13/S18 | EMD94767 |
| COCHEDRAFT_1164310 | -3.78 | Ribosomal protein L11, N-terminal domain | EMD97422 |
| COCHEDRAFT_1139445 | -4.09 | Promotes the GTP-dependent binding of aminoacyl-tRNA to the A-site of ribosomes during protein biosynthesis (by similarity) | EMD90278 |
| ENSRNAG049949382 | -3.80 | – | EMD84642 |
| ENSRNAG049949379 | -5.40 | – | EMD91248 |
| ENSRNAG049949459 | -4.76 | – | EMD91248 |
| COCHEDRAFT_1122518 | -5.72 | H3 | EMD91248 |
| COCHEDRAFT_1103804 | -5.87 | 3027035.1 | EMD91248 |
**TABLE 4** | Important upregulated genes for pathogen fitness and pathogenesis.

| Gene and gene ID | Log fold change | Description | References |
|------------------|-----------------|-------------|------------|
| **Mitochondrial genes** | | | |
| Mitochondrial carrier protein COCHEDRAFT\_1139719 | 7.7685614 | Transport of ions, nucleotide, amino acid, and cofactors across membrane | Bertrand, 2000; Arcila et al., 2021 |
| Mitochondrial 18 kDa protein COCHEDRAFT\_1024553 | 8.172178 | Mitochondrial fission, morphology, and development of mitochondria, mutation leads to apoptosis | |
| AMP-binding enzyme COCHEDRAFT\_1105220 | 10.68332 | Mitochondrial biogenesis | |
| ATP-dependent serine protease COCHEDRAFT\_1207993 | 8.95483288 | Participates in the regulation of mitochondrial gene expression and in the maintenance of the integrity of the mitochondrial genome | |
| Yml38 Yml34 7.407734745 | 7.407734745 | Mitochondrial 54S ribosomal protein | |
| Mitochondrial protein synthesis | 7.976810257 | Promotes mitochondrial ribosomes in a GTP-dependent manner | |
| **Fungal cell wall and chitin synthesis genes** | | | |
| Chitin synthase III catalytic subunit COCHEDRAFT\_1197445 | 7.451803741 | Responsible for the synthesis of the majority of the chitin found in the cell wall periphery | Banks et al., 2005; Langner and Göhre, 2016; Pusztahelyi, 2018; Garcia-Rubio et al., 2020 |
| Chitin synthase, COCHEDRAFT\_1192892 | 10.12960501 | Chitin synthases (CHSs) are key enzymes in the biosynthesis of chitin, an important structural component of fungal cell walls | |
| Chitin synthase regulation, resistance to Congo red COCHEDRAFT\_1152857 | 3.273618 | Regulates chitin deposition in the fungal cell wall | |
| **Sugar metabolism** | | | |
| Sugar transporter COCHEDRAFT\_1186319 | 11.2559271 | Sugar transporters (STs) that are essential for taking up the mono- and short oligosaccharides, resulting from extracellular enzymatic digestion of lignocellulose, into the fungal cell | Vankuyk et al., 2004; Lv et al., 2020; Monfared et al., 2020 |
| Glucanases COCHEDRAFT\_1118047 | 7.355847304 | Plays a role in cell expansion during growth, in cell–cell fusion during mating, and in spore release during sporulation. This enzyme may be involved in beta-glucan degradation | |
| **Genes related to toxin (polyketide cyclases)** | | | |
| Polyketide cyclases family COCHEDRAFT\_1167946 | 7.332835 | T-toxin is a family of linear polyketides 37–45 carbons in length, of which the major component is 41 carbons | Gaffoor et al., 2005; Schindler and Nowrousian, 2014 |
| Acetoacetate decarboxylase COCHEDRAFT\_1103773 | 8.894696 | Catalyzes the conversion of acetoacetate to acetone and carbon dioxide | |
| LAM1 COCHEDRAFT\_1167244 | 8.228607 | 3-Hydroxyacyl-CoA dehydrogenase, NAD-binding domain | |
| **Other important genes associated with secondary metabolites and signaling** | | | |
| Peroxidase COCHEDRAFT\_1125365 | 7.721354 | Peroxidases are a group of oxidoreductases which mediate electron transfer from hydrogen peroxide (H₂O₂) and organic peroxide to various electron acceptors | Mir et al., 2015 |
| Reactive mitochondrial oxygen species modulator COCHEDRAFT\_1022035 | 8.233186 | Required in fungal differentiation processes that are necessary for virulence | Hansberg et al., 2012; Mir et al., 2015; Martinez-Soto and Ruiz-Herrera, 2017 |
| Mitogen-activated protein kinase COCHEDRAFT\_1207640 | 9.375412784 | Involved in fungal development, sexual reproduction, pathogenicity and/or virulence in many filamentous plant pathogenic fungi | |
| Catalase COCHEDRAFT\_1179052 | 8.872981819 | Catalyzes the reaction of cyanate with bicarbonate to produce ammonia and carbon dioxide | |
| Shikimate dehydrogenase substrate binding domain COCHEDRAFT\_1228346 | 9.422273 | Catalytic domain at N terminus binds to the substrate, 3-dehydroshikimate | |
| Signal-recognition-particle (SRP) COCHEDRAFT\_1187286 | 8.077532 | Signal-recognition-particle assembly has a crucial role in targeting secretory proteins to the rough endoplasmic reticulum membrane | |
**TABLE 5** | List of candidate effector genes identified in *B. maydis*.

| Gene ID                     | Annotation                                           | CDS  | Effector prediction | Description based on UniProt/InterPro and similarity |
|-----------------------------|------------------------------------------------------|------|---------------------|------------------------------------------------------|
| COCHEDRAFT_1093124         | EthD domain Ethyl tert-butyl ether degradation       | 103  | 0.916               | Contributes to conidial pigmentation that provides protection from UV radiation, heat and cold stress |
| COCHEDRAFT_1202737         | Hypothetical protein                                 | 138  | 0.918               | Protein occurs in the cell wall of the fungus and is involved in the host-plane interaction and induces both cell necrosis and phytoalexin synthesis which is one of the first plant defense-related events |
| COCHEDRAFT_1134423         | Hypothetical protein                                 | 130  | 0.999               | Component of the endoplasmic reticulum-associated degradation (ERAD) pathway |
| COCHEDRAFT_1155213         | Hypothetical protein                                 | 162  | 0.997               | Integral component of cell membrane                  |
| COCHEDRAFT_1193149         | Conserved hypothetical protein                       | 165  | 0.691               | Integral component of cell membrane                  |
| COCHEDRAFT_1020438         | Conserved hypothetical protein                       | 86   | 0.539               | NADH dehydrogenase (ubiquinone) 1 alpha subcomplex subunit 1 |
| COCHEDRAFT_1094426         | Conserved hypothetical protein                       | 211  | 0.888               | Thioredoxin-like fold domain-containing protein       |
| COCHEDRAFT_1168141         | Hypothetical protein                                 | 169  | 0.965               | Uncharacterized protein                              |
| COCHEDRAFT_1149474         | Proteasome subunit                                   | 205  | 0.767               | Cleavage of peptide bonds with very broad specificity, endopeptidase |
| COCHEDRAFT_1199047         | ATP synthase E chain                                 | 90   | 0.71                | Mitochondrial membrane ATP synthase                  |
| COCHEDRAFT_1024834         | Cytidine and deoxyxycytidylate deaminase zinc-binding region | 185  | 0.948               | Scavenges exogenous and endogenous cytidine and 2′-deoxyxycytidine for UMP synthesis |
| COCHEDRAFT_11186866        | Antibiotic biosynthesis monooxygenase                | 108  | 0.992               | ABM domain-containing protein                        |
| COCHEDRAFT_1148984         | Hypothetical protein                                 | 72   | 0.966               | Membrane-associated and mitochondrion-associated cellular component |
| COCHEDRAFT_1207896         | Synaptobrevin                                        | 200  | 0.976               | Vesicle-mediated transport                           |
| COCHEDRAFT_1088730         | Dienelactone hydrolase family                        | 250  | 0.555               | DLH domain, hydrolase activity                       |
| COCHEDRAFT_1019519         | Redoxin                                              | 167  | 0.856               | Thiol-specific peroxidase that catalyzes the reduction of hydrogen peroxide and organic hydroperoxides to water and alcohols, respectively. Plays a role in cell protection against oxidative stress by detoxifying peroxides |
| COCHEDRAFT_1221715         | Protein of unknown function (DUF1687)                | 150  | 0.98                | Putative mitochondrial redox protein which could be involved in the reduction of small toxic molecules |
| COCHEDRAFT_1221723         | Ubiquitin-conjugating enzyme                         | 149  | 0.779               | Glycol thioester intermediate, ATP binding, transferase activity |
| COCHEDRAFT_1019537         | Mitochondrial ribosomal protein L51/S25/Ci-B8 domain | 93   | 0.986               | Electron transport, respiratory chain                |
| COCHEDRAFT_1166992         | ATP synthase delta (OSCP) subunit                    | 231  | 0.978               | ATP synthase subunit 5, mitochondrial, proton-transporting ATP synthase activity |
| COCHEDRAFT_1191234         | Cytochrome c domain-containing protein               | 108  | 0.999               | Electron carrier protein. The oxidized form of the cytochrome c heme group can accept an electron from the heme group of the cytochrome c1 subunit of cytochrome reductase. Cytochrome c then transfers this electron to the cytochrome oxidase complex, the final protein carrier in the mitochondrial electron-transport chain (by similarity) |
| COCHEDRAFT_1127833         | Stress-response A/B barrel domain-containing protein | 110  | 0.931               | Stress-response A/B barrel domain-containing protein |
FIGURE 3 | Mean expression versus log fold change plots (MA-plots). Transcriptional changes of *B. maydis* are presented in SC-7 and CM 119 48 h post inoculation. Normalized P-values are plotted versus Log2 fold changes. Genes with an FDR < 0.05 are plotted.

FIGURE 4 | Pie chart of enriched GO terms of *B. maydis* for biological functions differentially expressed in inoculated resistant and susceptible plants.
qRT-PCR for Expression of Selected Genes
The validation of the RNA-seq technique was performed by quantitative RT-PCR through monitoring the expression levels of seven selected transcripts (Figure 2 and Supplementary Table 5) after designing primers for selected genes (Supplementary Table 6). A melt curve is also provided in Supplementary Figure 1.

Statistical Analysis
qRT-PCR data were analyzed by analysis of variance (ANOVA) using the Statistical Package for Social Science (SPSS, IBM, Chicago, IL, United States) version 16.0. The statistical significance was judged at $P < 0.05$.

RESULTS

Disease Development
There were no observable phenotypic differences between the susceptible and resistant maize inbred lines without pathogen inoculation at all-time points. We observed prominent symptoms on susceptible line CM 119. Non-inoculated controls never showed necrotic lesions. Lesions were visible from 72 h after inoculation. In the resistant line, lesions were small and fewer in numbers. There was a noticeable symptom difference between the susceptible and resistant maize inbred lines at 96 h after inoculation (Figure 1).

Sequencing and Mapping
From the sequencing of in planta libraries, approximately 31,994,420 resistant and 46,087,568 susceptible lines reads were generated (Table 1). The genome was mapped with reference genome Bipolaris_maydis_c5_gca_000338975.CocheC5_3.dna.toplevel.fa., and mapping statistics are provided in Supplementary Figure 2.

Differential Gene Expression Analysis
Approximately 23.4 GB of mRNA-seq data of B. maydis were obtained from both resistant and susceptible maize backgrounds. DEG analysis was conducted to detect B. maydis transcriptome changes during the pathogenesis of maize. Out of 10,363 mapped genes, 3313 genes were upregulated and only 100 genes were downregulated, and 6949 genes were commonly expressed in B. maydis SI plants compared to RI plants. A list of genes and their expression is provided in Supplementary Table 1. Log FCs and $P$-value are shown in Figure 3.
FIGURE 6 | Pie chart of enriched GO terms of B. maydis for molecular functions differentially expressed in inoculated resistant and susceptible plants.

TABLE 6 | Top 10 important enriched GO terms of B. maydis for biological functions differentially expressed in inoculated resistant and susceptible plants.

| Gene ID | Annotation                  | Enriched genes | Function |
|---------|-----------------------------|----------------|----------|
| 1       | GO:0000003 Reproduction     | 188            |          |
| 2       | GO:0002181 Cytoplasmic translation | 67         |          |
| 3       | GO:0007275 Multicellular organism development | 41         |          |
| 4       | GO:0000054 Ribosomal subunit export from nucleus | 21         |          |
| 5       | GO:0000096 Sulfur amino acid metabolic process | 21         |          |
| 6       | GO:0000122 Negative regulation of transcription | 22         |          |
| 7       | GO:0000272 Polysaccharide catabolic process | 21         |          |
| 8       | GO:0000375 RNA splicing via transesterification | 33         |          |
| 9       | GO:0008150 Biological process | 730          |          |
| 10      | GO:0000001 Mitochondrion inheritance | 14         |          |

TABLE 7 | Top 10 important enriched GO terms of B. maydis for cellular functions differentially expressed in inoculated resistant and susceptible plants.

| Gene ID | Annotation                     | Enriched genes | Number |
|---------|--------------------------------|----------------|--------|
| 1       | GO:0005575 Cellular component  | 555            |        |
| 2       | GO:0000139 Golgi membrane      | 72             |        |
| 3       | GO:0000322 Storage vacuole     | 58             |        |
| 4       | GO:0000502 Proteasome complex  | 25             |        |
| 5       | GO:0000313 Organellar ribosome | 22             |        |
| 6       | GO:0000151 Ubiquitin ligase complex | 12        |        |
| 7       | GO:0000243 Commitment complex  | 6              |        |
| 8       | GO:0000428 DNA-directed RNA polymerase complex | 10         |        |
TABLE 8 | Top 10 important enriched GO terms of B. maydis for molecular functions differentially expressed in inoculated resistant and susceptible plants.

| Gene ID | Annotation          | Enriched genes                  | Number |
|---------|---------------------|---------------------------------|--------|
| 1       | GO:0003674          | Molecular function              | 626    |
| 2       | GO:0000166          | Nucleotide binding              | 97     |
| 3       | GO:0000030          | Mannosyl transferase activity   | 10     |
| 4       | GO:0001671          | ATPase activator activity       | 3      |
| 5       | GO:0000049          | tRNA binding                    | 4      |
| 6       | GO:0000295          | Succinate dehydrogenase activity| 2      |
| 7       | GO:000104           | Adenine nucleotide transmembrane transport | 2 |
| 8       | GO:0000271          | (R,R)-butanediol dehydrogenase activity | 2 |
| 9       | GO:0000048          | Peptidyltransferase activity    | 2      |
| 10      | GO:0000062          | Fatty-acyl-CoA binding          | 2      |

Transcriptome analysis of the top 40 DEGs suggested 20 upregulated and 20 downregulated genes, the detailed description of genes along with their fold change and function is provided in Tables 2, 3. Further, these DEGs were studied for genes related to pathogenesis and pathogen fitness which revealed important aspects, as shown in Table 4 and Supplementary Table 1. On the other hand, the data were also analyzed in silico to find out putative effectors using the Effector database and 22 effectors were predicted (Table 5).

GO Categories and Enrichment Analysis

GO enrichment suggested that most of the genes under DEGs were associated with pathogen fitness and reproduction. The results are presented in Figures 4–6 and described in Tables 6–8, with additional information available in Supplementary Tables 2–4.

DISCUSSION

Enrichment analysis and the expression patterns of the highly upregulated genes indicated that successful pathogenicity of B. maydis depends on pathogen fitness genes such as mitochondrial genes, cell wall synthesis, toxin-related effector molecules, and on other hand, cell wall degradation of the host, detoxification, and host defense evasion (Figures 7–9). Understanding the pathogen's molecular pathways during the infection process using transcriptome analysis can contribute significantly to identifying new targets for SSR control, novel genes, and pathogenicity-related pathways (Ribeiro et al., 2020; Poretti et al., 2021).

Mitochondrial Genes

Mitochondria have diverse functions to perform in fungal cells. Mitochondria play a major role in fungal metabolism.
and fungicide resistance (Arcila et al., 2021). In the present study, genes associated with mitochondrial functions for B. maydis race "O" were upregulated in the susceptible line (CM 119), which suggests that CM 119 supports the growth of B. maydis, and on the other hand, SC-7 restricts the cellular activity of fungus (Figure 8). Previously studies suggested that fungal mitochondria play a significant role in determining fungal fitness and virulence (Calderone et al., 2015; Medina et al., 2020). A recent study suggests that endoplasmic reticulum (ER) and mitochondrial interactions along with the ER-mitochondria organizing network (ERMIONE) play important roles in adaptive responses in fungi, particularly in response to cell surface-related mechanisms that facilitate fungal invasion, growth, and stress responsive behaviors that support fungal pathogenicity (Koch et al., 2017). An investigation on C. parasitica study suggested the role of mitochondria in hypo virulence (Bertrand, 2000). Another study on the mitochondria genome of phytopathogens Synchytrium endobioticum and Phlebia radiate showed that alteration in the mitochondrial genome majorly affects the ability of fungi to adapt to changing environments (Medina et al., 2020). Overall, fungal mitochondria play a crucial role in determining pathogenicity of the host, and in susceptible backgrounds of the host, these genes are more expressed whereas resistant genotypes have a tendency to suppress the mitochondrial genes and ultimately the pathogen becomes less virulent, which we found in the present study.

**Fungal Cell Wall and Chitin Synthesis Genes**

The cell wall is an important component of fungal cells which mediates fungal cell interactions with its external environment and hyphal development (Castro et al., 2022). Chitin is the main component for cell wall synthesis. The cell wall protects the cell content, provides rigidity, and determines the cellular structure. It also protects the cell from various stresses including osmotic changes which are significant for healthy fungal cells. It also carries some proteins that play a role in recognition, adhesion, and receptor activity. There are several studies which establish the role of the fungal cell wall in pathogen fitness and its association with pathogenesis (Banks et al., 2005; Langner and Göhre, 2016; Pusztahelyi, 2018; Garcia-Rubio et al., 2020). It has also been investigated whether the fungal cell wall plays a crucial role in spore development (Backes et al., 2020) and in antifungal resistance activity (Díaz-Jiménez et al., 2012). In the present study, the high expression of cell wall-associated genes reconfirmed the fact that the fungal cell wall plays an
essential role in disease development in susceptible hosts and also showed that the resistant genotype had the capacity to hinder the expression of fungal cell wall genes during the interaction (Figure 7).

Sugar Metabolism
Sugar transporter genes of filamentous fungi are associated with multiple physiological and biochemical processes, such as the response to various stresses (Vankuyk et al., 2004; Lv et al., 2020; Monfared et al., 2020). They were also found to be linked with many salt tolerance and sophisticated transcriptional processes. In the present transcriptome profile of B. maydis, genes of sugar metabolism (Figure 9) such as sugar transporter and glucanases were upregulated which suggests that these genes play an essential role in pathogen proliferation under susceptible backgrounds of the host.

Gene-Related Polyketides
Polyketides (PKs) play a role in mycelial growth and development of asexual and sexual structures of fungi (Gaffoor et al., 2005; Schindler and Nowrousian, 2014) including shikimate dehydrogenase (Kinghorn, 2000). A study on B. maydis race “T” demonstrated the association of a toxin-related locus with polyketide biosynthesis and high virulence on T-cytoplasm maize (Rose et al., 2002), similarly in the present study, high expression of PKs was investigated which suggests there could be a possible association of toxin “O” with PKs.

Other Important Genes Associated With Secondary Metabolites and Signaling
Recent studies proposed peroxidases, catalases, and reactive oxygen species (ROS) as components of the antioxidant defense system in fungal pathogens and were also associated with conidial production (Zhang et al., 2020). A study on fungal pathogen Magnaporthe oryzae suggested significant and positive correlations among sensitivity to H$_2$O$_2$, peroxidase activity, and fungal pathogenicity (Garre et al., 1998; Hansberg et al., 2012; Mir et al., 2015; Chittem et al., 2020). On the other hand, mitogen-activated protein kinase (MAPK) signaling pathways play an important role in cell cycle control, mating, morphogenesis, response to different stresses, resistance to UV radiation, temperature changes, cell wall assembly and integrity, degradation of cellular organelles, virulence, cell–cell signaling, fungus–plant interaction, and response to damage-associated molecular patterns (DAMPs) (Martínez-Soto and Ruiz-Herrera, 2017). Upregulation of these genes in the B. maydis race “O”
pathogen indicated the interconnected nature in determining the fungal infection strategy in susceptible hosts (Figure 7).

**Candidate Effector Genes Identified in Bipolaris maydis**

At least 22 transcripts showing homology to genes previously reported to be involved in fungal infection were predicted as effector proteins (Table 5 and Figure 9) in the Effector database using the CSIRO tool EffectorP2 (a machine learning method for fungal effector prediction in secretomes) (Saperschneider et al., 2018). The majority of them showed a probability above 60–90%. This fact can provide evidence for the pathogenicity behavior of *B. maydis* race “O” on susceptible lines.

**CONCLUSION**

Based on the observation of present and previous studies in other host pathogen systems, we suggest that the above cited genes play a vital role in causing disease in their host plants. The DEG study of pathogen genes can provide evidence for its sensitive targets, virulence toward hosts, and resistance against chemicals. This is probably the first transcriptome study of the *B. maydis* pathogen during infection in a non-CMS maize genotype, differing in their susceptibility to the pathogen. The findings from this study emphasize the role of mitochondrial-associated genes and pathways. In addition, cell wall synthesis, genes related to synthesis of polyketides, toxins, and putative candidate effector genes were found to be the key compounds underlying the pathogenesis of the *B. maydis* race “O” pathogen.

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**DATA AVAILABILITY STATEMENT**

The datasets presented in this study can be found in online repositories. The names of the repository/publication and accession number(s) can be found below: NCBI BioProject – PRJNA689117.

**AUTHOR CONTRIBUTIONS**

SM, RG, BB, PM, FH, and AK were involved in the conceptualization of the project, study design, critical inputs, and finalization of the manuscript. PM, AK, and BB were involved in wet lab experiments. BB and PM were involved in bioinformatics analyses and data compilation. SM, RG, BB, and PM drafted the manuscript. All authors have read and approved the final manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.837056/full#supplementary-material
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