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RESEARCH ARTICLE

UNRAVELING STAGE-SPECIFIC IMMUNE RESPONSES IN ZEA MAYS THROUGH TRANSCRIPTOME PROFILING AND MACHINE LEARNING UNDER EXSEROHILUM TURCICUM INFECTION

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ABSTRACT

Maize (*Zea mays*), the "Queen of Cereals," is the world's third most cultivated crop after rice and wheat, with global production exceeding one billion tons annually. However, fungal diseases such as Northern Corn Leaf Blight (NCLB), caused by *Exserohilum turcicum*, significantly reduce maize yield, threatening food security under changing climatic conditions. This study employs transcriptomic, network biology, and machine learning approaches to investigate maize defense responses against fungal infection. Publicly available RNA-Seq data comprising 27 maize leaf samples across three genotypes EtEC81 (fungal effector), GFP (control), and ZmEIP1 (elicitor-induced protein)were analyzed at 10, 14, and 21 days post-infection. Reads were aligned to *Zea mays* B73 genome, followed by differential expression (DEG) analysis, Weighted Gene Co-expression Network Analysis (WGCNA), and classification using Random Forest, XGBoost, Support Vector Machine, and Logistic Regression models. A total of 1,202 significant co-expressed genes and 350 DEGs were identified, enriched in immune signaling, hormone regulation, and metabolic pathways. Machine learning models converged on 4,280 key genes, highlighting pathogen recognition, transcriptional regulation, and cell wall reinforcement. Functional characterization revealed 65 transcription factors (notably bHLH), 145 resistance (R) genes dominated by kinase-class receptors, and 54 carbohydrate-active enzymes (CAZymes), particularly GT1, involved in secondary metabolism and defense responses. Protein–protein interaction analysis uncovered central hub genes, including stress-related kinases and glutathione transferases, underpinning immune suppression and metabolic reprogramming by the pathogen. These findings reveal stage-specific defense activation and recovery mechanisms in maize, offering potential disease-resistance candidates for breeding strategies. Integrating transcriptomics with machine learning provides a robust framework to dissect host–pathogen interactions in crop systems.

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INTRODUCTION

Zea Mays is also known as the Queen of Cereals. It is the 3rd largest produced crop in the world after rice and wheat. It is one of the most important crops in the world, and it has multi-billion dollars in annual revenue(1). Currently, over 170 nations are producing a big 1147.7 million tons from 193.7 million hectares of land, with each hectare yielding about 5.75 tons (2). Earlier it was domesticated in the continents of America and Africa. It belongs to the *Zea* species of *mays* genus in *poaceae* family. Various biotic and abiotic factors are currently affecting maize production. Drought is a serious threat to maize, and climate change is making this stress worse. Other biotic stressors like weeds, insect pests, and bacterial, viral, and fungal illnesses also affect the crop. Despite the widespread application of antifungal treatments, agricultural producers continue to experience annual crop losses ranging from 10% to 23% due to fungal phytopathogens(3). These fungi result in losses that would provide 600 million people with 2000 calories a day for a full year, and if nothing is done, the number of people going hungry will rise due to climate change(4). Its order and class are *Poales* and *Magnoliopsida,* which belong to the phylum *Tracheophyta* and the kingdom is *Plantae*(5) were aerials used mainly for feed and food, while nowadays they have other uses such as medicine, cosmetics, biofuel and etc (6), (7), (8). The leaves of *Zea mays* show an alternate arrangement along the stem, which helps collect the maximum amount of sunlight for the process of photosynthesis.The leaf tissue itself, particularly the lamina and surfaces (both the upper/adaxial and lower/abaxial

surfaces), is the main component of a leaf that is impacted by fungus. Visible signs of fungal infections frequently include lesions, patches, or blights on the leaves (9) .*Exserohilum turcicum* is a fungus that causes the disease of the northern leaf blight of maize and loss of production yields. It's known as *Setosphaeria turcic*of*Pleosporaceae* family. The morphological structure of this fungus has olive-gray color spindle-shaped conidia that measure 5 X 20 cm and have 1-9 septa(10). These fugus affect the *Zea mays* plant and loss the production of yield (11), was initially reported in 1876 Italy under 15-25 °C and 90-100 % level of humidity (12), and reduced surface area of leaves and which affect the photosynthesis as well as the pathogenic race affect the size and shape of lesions (13). It develops very fast in number as well as spread to other portions of plant tissues. These spots develop into elliptic lesions which are running parallel to the lower leaf margin and spreading upward. Northern Corn Leaf Blight (NCLB) can lead to stunted growth and reduced kernel development as the infections affect the whole leaves. In this study, where used transcriptome data of maize leaves infected with *Exserohilum turcicum* were found from the NCBI (National Center for Biotechnology Information), where contain 27 samples of three conditions, including EtEC81 (*Exserohilum turcicum* Effector 81), GFP (Green Fluorescent Protein as a control), and ZmEIP1 (Zea mays Elicitor-Induced Protein 1, a maize resistance-associated protein) and different time periods, like 10 days, 14 days, and 21 days. Futher analysis and identified gene expression patterns in fungal infection through WGCNA, DEGs, and ML(14).This study uses Weighted Gene Co-expression Network Analysis (WGCNA), Differentially Expressed Genes (DEGs) analysis, and Machine Learning (ML) models such as Random Forest (RF), XGBoost, Support Vector Machine (SVM), and Logistic Regressionto unravel maize's transcriptional responses to fungal infection. WGCNA, groups genes into co-expression modules to identify clusters linked to trait. DEG analysis detects genes with significant expression changes between control and infected, highlighting active biological processes. Together, these methods reveal how immune signaling, hormone regulation, and defence systemcoordinate maize's stage-specific defense and recovery mechanisms. Additionally 65 transcriptional regulations, 146 plant immunity (R genes), and 54 carbohydrate-active enzyme (CAZyme) genes were identified with protein-protein interactions. which contributes to the scientific community's disease-resistant genes which help farmers cultivate maize varieties.

MATERIALS AND METHODS

Data Collection: Transcriptome data of fungal-infected leaf of *Zea mays* were obtained from the Bioproject database in NCBI (accession no PRJNA1144095). The dataset contained 27 samples with three replicates of genotypes such as EtEC81(exserohilum turcicum Effector 81), GFP (Green fluorescent protein), and ZmEIP1 (Zea mays Elicitor-Induced Protein 1) at different time periods like 10 days, 14 days, and 21 days.

RNA-Seq data pre-processing and alignment: The quality of all samples were checked by the tool FASTQC, further read sequences were aligned with the reference sequence of *Zea mays* B73 (V5.0) (http://ftp.ebi.ac.uk/ ensemblgenomes/ pub/release-51/plants/fasta/zea_mays) using the tool HISAT2 (15)under Galaxy web tool (https://usegalaxy.org/). The read count of genes were identified by the htseq-count tool. Additionally, we checked batch effect and variation between replicate samples through LaneNormalization-methods (Fig. 1), and datasets were chosen(Dillies et al., 2013).

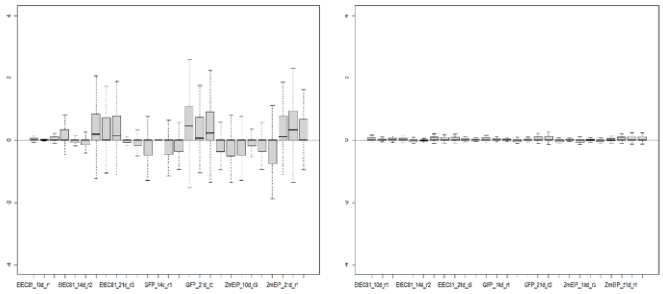

Fig 1: Box plot of un-normalized and normalized count of samples

Weighted Gene Co-expression Network Analysis: Weighted gene co-expression network analysis (WGCNA) was conducted on read count of genes through R package. Gene modules were identified via a dynamic tree-cutting algorithm, employing a minimum module size of 30 and a default cut-off height of 0.99. These modules genes were hierarchically clustered within a dendrogram based on their structural similarities. Pairwise gene correlation across all samples was determined using Pearson's correlation coefficient, which was utilized to construct an adjacency matrix. Subsequently, the topological overlap matrix (TOM) and the corresponding dissimilarity values (1-TOM) were computed to assess network connectivity(17).

Differential Expression Genes Analysis: DEG was identified by R package with RStudio, where GFP 10,14 and 21 day samples were used as control and EtEC81(10 day ,14 day 21) and ZmEIP (10,14and 21 days) were used as treated.further the significant DEGs were identified based on the $|\log_2(\text{Fold Change})| \geq 1$ and FDR < 0.05.

Machine Learning Models: We used Random Forest, XG Boost, SVM and Logistic regression Models of ML.RF uses many decision trees, handles large amount of data and find out key genes linked with certain traits(18) .XG Boost build decision tree by correcting errors each time and find complex gene expression patterns(19).Logistic regression shows how much one genes expression depends on others(20) .SVM helps to separate gene classes based on gene expression data(21). We identified top 10 percent key genes which are highly expressed., further more simplified and found highly significant genes through venn diagram which revealed the number of genes expressed commonly expressed genes in all models. Additionaly, Highly significant 26 genes were identified common between WGCNA, DEGs and ML models.

Resistance gene, CAZyme, and TF identification: Significant genes encoding Transcription Factors (TFs) and resistance (R) genes were identified through BLASTx analysis against the Plant Transcription Factor Database (PlantTFdb) and the Plant Resistance Gene Database (PRGdb) version 4.0. The identification process involved aligning query sequences with known TF and R-gene sequences to determine functional homology and domain conservation(22). Furthermore, R-gene classes and their associated functional domains were systematically identified and classified using DRAGO, a specialized tool within the PRGdb platform. This classification

enabled the differentiation of resistance gene categories based on structural characteristics and evolutionary relationships, facilitating a more comprehensive understanding of their roles in plant immunity(23).

PPI Network Analysis: The interactions between ML models, DEGs and positively or negatively co-expressed genes network visualization through Cytoscape, which allowed for the identification of clusters and strongly hubs genes within the network(24) under various fungal infection. Further circumstances were examined interactions among identified TFs, R-genes, and CAZyme-encoding genes.Click or tap here to enter text.

Functional Annotation: Functional annotation for gene and pathway identification was performed using ShinyGo and UniProt. Gene Ontology (GO) annotations were obtained using the following categories: Biological process (BP), Molecular function (MF), and Cellular component (CC) with a cut-off at FDR=0.05. Shiny Go gives the result in a more graphical way, such as a bar graph, tree, etc., and the UniProt ID mapping tool was used to convert stable ID to string ID downstream process.

RESULTS

The publicly available 27 transcriptome samples of 3 replicates were screened for adaptors, GC content, and per-base N content, and quality checked, further aligned and mapped uniquely with more than 70 %, further processed for read counts downstream analysis.

Co-expression genes cluster identification: The significant co-expressed genes were found by the R package (WGCNA) (25). The scale-free fit index and mean connectivity were calculated, and the power of β = 12 (scale-free R2 = 0.8) was selected (**Fig. 2**).

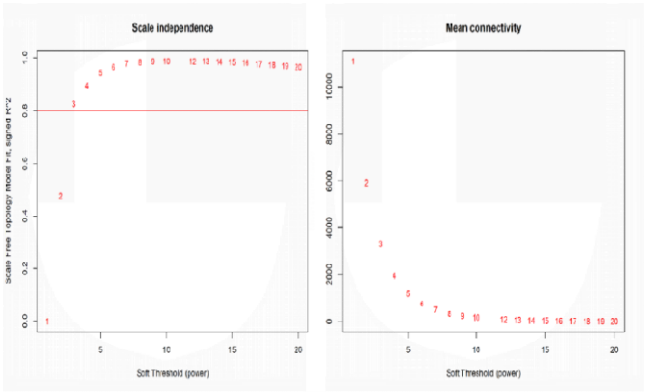

Fig. 2. Scale independence and mean connectivity analysis

Further, based on cluster dendrogram merge modules with a similarity greater than 0.25 and finally 16 modules, including MEsalmon, MEdargery, MEgreenyellow, MEdarkolivegreen, MEgrey60, MEviolet, MEpaleturquoise, MElightgreen, MEred, MElightyellow, MEblack, MEwhite, MEblue, MEmagenta, MEsteelblue, MEgrey (Fig. 3), in which 13 modules have the gene significance |GS| >0.5 and P-value <0.05. Further intramodular analysis based on the GS (Gene Significance) and MM (Module Membership) of genes identified key genes within the 13 modules. A filter of |MM>0.7 & GS> 0.5| was applied for significant gene identifications. The number of significant genes in different modules is as follows 20 in ME white,6 in ME violet, 12 in MEsteelblue, 20 in MEpaleturquoise, 98 in MEmagenta,33 in MElightyellow,133 in MElightgreen,14 in MEgrey60,2 in MEgrey, 67 in MEgreen yellow, 5 in MEdarkolive green, 636 in MEblue and 156 in MEblack.No significant genes were observed in MEsalmon, MEred and MEdarkgrey. Finally, 1202 significant genes were identified in this analysis (Fig. 4; Sup. Table 1).

Sup.

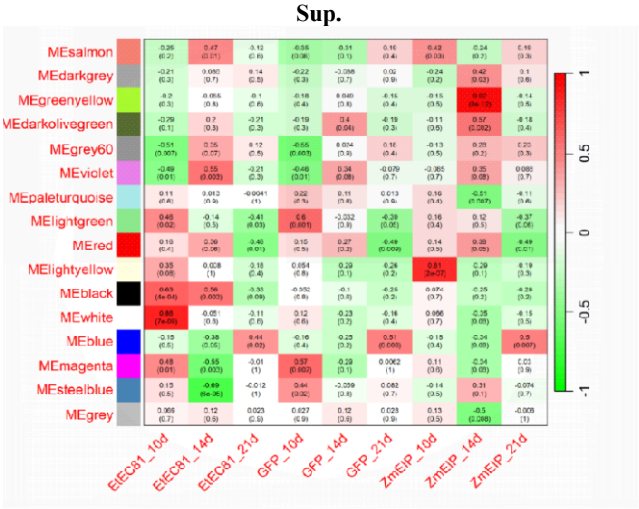

Fig 3: Module–trait relationship with samples

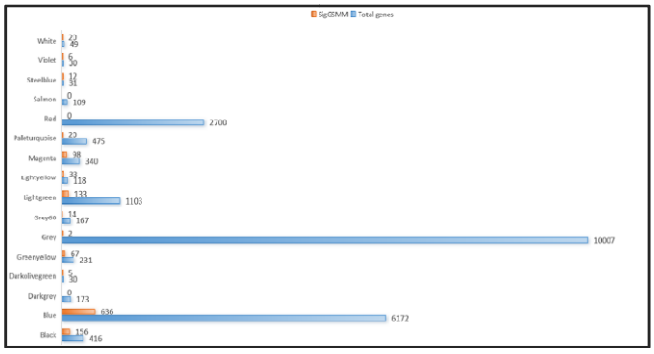

Fig 4. Bar graph showing the number of significant and total genes

Differentially Expressed Genes: Functional annotation of significant DEGs, filtered at log2FC>1 and p<0.05 revealed significant DGE responses under *EtEC81* infection and *ZmEIP* expression. Across all comparisons, the highest number of DEGs was observed under the *ZmEIP* late-stage condition. Here we have found out that 350 genes were significantly differently expressed. Out of which 208 genes were significantly upregulated and 142 genes were significantly downregulated. It has been find out that in ZmEIP 14 day sample , less genes were downregulated.

Significant genes identified by machine learning: We were used multiple machine learning models such as Random Forest (RF), XGBoost, Support Vector Machine (SVM), and Logistic Regression, and found genes 4459, 4281, 4333, and 4458 genes, respectively have been found, in which 4,280 genes that were consistently present all four model algorithms (Fig.5). These genes showed high importance scores in the ML models, indicating that their expression changes are closely associated with maize responses to *Exserohilum turcicum* infection and ZmEIP.

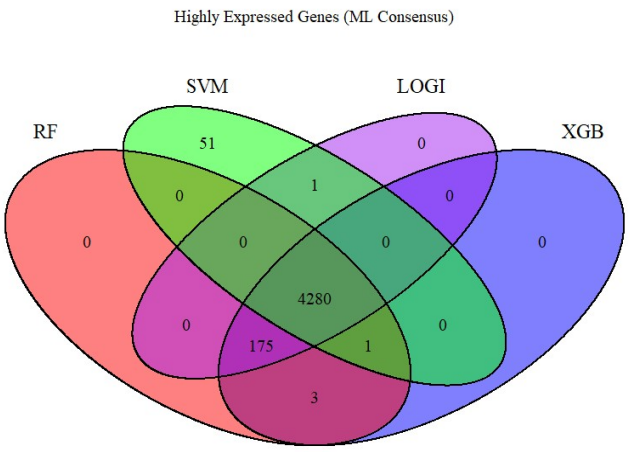

Fig. 5. Venn Diagram generated by M-L model

Role of TF, R genes, CAZyme: In the more significant positive and negative expressed genes used to identify transcription Factors (TF), CAZymes (Carbohydrate Active enzymes), and R (Resistance) genes, which have an inevitable role in the molecular behavior in plant systems. TFs control the regulation of gene transcription and affect their expression levels, and thus, play a pivotal role in plant development under biotic and abiotic stress conditions and regulate secondary metabolism (26). 65 transcription factors genes were found which were belong 25 different families such as HSF, NFYA, FAR1, bHLH, LBD, GRAS, GATA, ZFHD, Trihelix, NAC, WRKY, bZIP, SBP, ARF, B3, ERF, DBB, CO-like, G2-like, MIKC_MADS, TALE, C2H2, C3H, MYB, MYB_related. bHLH TF is the major TF identified in the study, which plays an important role in the range of biological processes in Zea mays, which is identified in the samples GFP 21-day blue, GFP 10-day magenta, and GFP 10-day light green (**Sup. Table 2A**).

A total of 54 genes encoding cell wall-related proteins, specifically carbohydrate-active enzymes (CAZyme) were identified. The CAZymes identified were AA, CE, GH and GT. Out of these GT 1 (Glycosyltransferase 1) is the most significant CAZyme. GT 1 is a large group of proteins that play important roles in secondary metabolite biosynthesis in plants (27). GT 1 is identified in the samples GFP 10-day lightgreen, ZmEIP 21-day blue, EtEC81 14-day steelblue, GS greenyellow MM greenyellow, EtEC81 10-day black. 2 genes (Zm00001eb349810 and Zm00001eb209550) are expressed both the modules, GFP 21-day blue and ZmEIP 21-day blue (Sup. Table 2B).

The 145 R genes were identified, where 10 classes of seven domains contain including LYP, CN, N, KIN, RLK, LECRK, CNL, RLP, TRAN, and CK, while domains like TM, LYSM, NBS, CC, Kinase, LRR, LECM. KIN major class were found out of them with TM (Transmembrane) domains in EtC81-10d samples with different clusters grey60, EtC81-14d magenta. EtEC81_10d and EtEC81_14d remain black clusters. EtEC81_10d found white. EtEC81_14d samples have Violet. GFP_10d found Light_green, GFP_21d blue, GFP-10d magenta, Light_yellow, ZmEIP_10d, ZmEIP_21d blue, ZmEIP-14d grey, ZmEIP-14d contain paletqurqo is cluster (Sup. Table 2C).

Complex network analysis: The genome annotation database was used in UniProt to identify protein IDs from significant genes, in which 525 protein IDs construct a protein-protein interactions network for further analysis (Sup. Table 3).

Network visualization and analysis were performed by Cytoscape, which identified a total of 353 nodes and 2052 edges. This node corresponds to the gene Zm00001eb229930(string id: 4577.P24067), present in the ZmEIP_21d under blue modules. It has a significant role in protein folding in the ER(28). While other C2H2 transcription factors (4577.B4FEJ7) were positively expressed which have to interact with resistant proteins Serine/threonine-protein kinase ATG1t (string id: 4577.C0PMW7) (Fig. 6). Functional annotations: The genes involved in biological processes, molecular functions, cellular components, and pathways were identified using ShinyGo8.0 and UniProt. Functional annotation of 1162 positive regulated genes and 39 negative-regulated genes were filtered using a False discovery rate (FDR)<0.05. The blue module was positively expressed in ZmEIP_21d and GFP_21d, while in other conditions not significant (Fig. 7). Other black modules also have positive expressions in EtEc81_10d and 14d; these positively expressed genes are involved in multiple functions (Fig. 8). White module is highly expressed in EtEC81 10 day sample. It functions include transmembrane transport, polysaccharide catabolic process, lignin catabolic process, defense response, carboxylic acid metabolic process. Lightyellow module is higly expressed in ZmEIP1 10 day sample. Its functions include trehalose biosynthetic process, translation, RNA modification, response to oxidative stress, regulation of DNA-templated transcription, etc. Green yellow is highly expressed kin ZmEIP1 14 days sample It's which is involved in ubiquitin-dependent protein catabolic process, translation, sucrose biosynthetic process, RNA splicing, etc. Future studies on stress adaptation and plant defense systems will be made easier by our findings, which offer important insights into the molecular mechanisms controlling gene regulation and plant responses under fungal infection conditions. In the EtEC81 condition, the early-stage of infection, upregulated genes were predominantly enriched in functions associated with immune system activation and signal transduction, such as defense response, protein kinase activity, and Toll-like receptor signalling pathway. Under the mid-stage, the genes upregulated were having functions like metabolic adaptation functions, including lipid metabolism and amino acid catabolism, while downregulated gene's activated in the following functions such as DNA replication, chromosome organization, and cell cycle progression. In the late-stage, glycolysis/gluconeogenesis, fatty acid metabolism, and other metabolic remodelling pathways were upregulated, with sustained repression of proliferative and biosynthetic functions. The early stage of *zmeip* condition upregulated genes were involved in pathogen recognition, cytokine-mediated signalling, and antigen processing and presentation, while downregulated genes work in nucleosome assembly, DNA replication, and translation. Additionally, the functional annotation of these ML-selected genes (Sup. Table 4) revealed that they upregulate many genes, which are having functions such as multiple defence-related categories, including pathogen recognition, hormone signalling pathways, transcriptional regulation, secondary metabolism, and developmental reprogramming. Furthermore, were found the common genes between all machine learning models, Differential Gene Expression analysis and WGCNA. The most significant 26 genes (Fig. 9) involved in multiple biological process, in which 5 genes insist to stimulus.

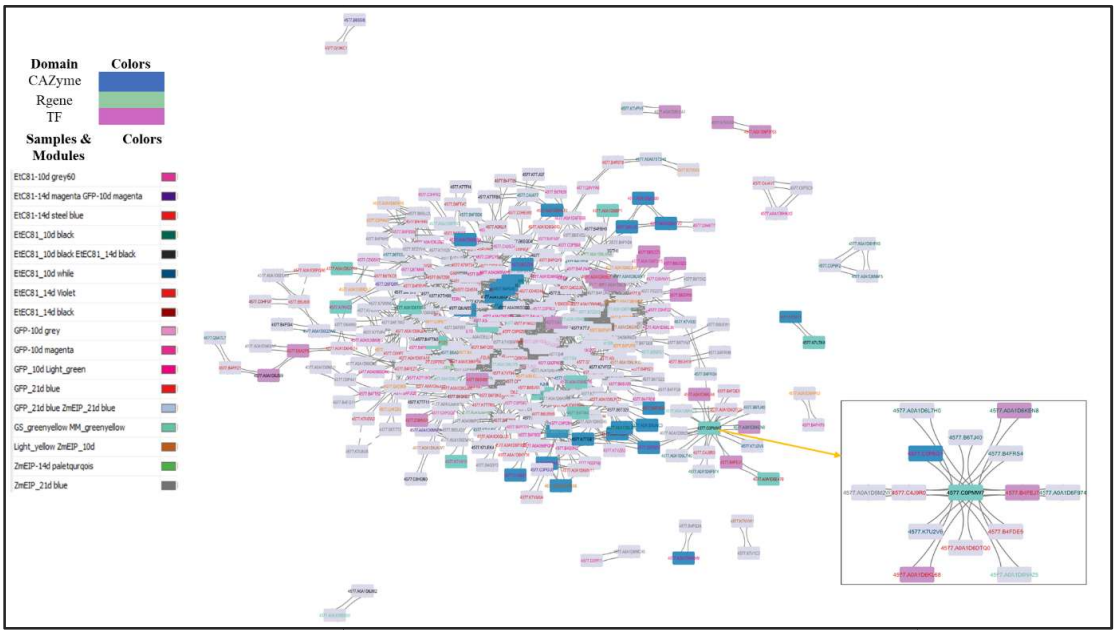

Fig. 6. Protein-protein interaction network; Label colors denote samples and modules and fill colours represent Domains

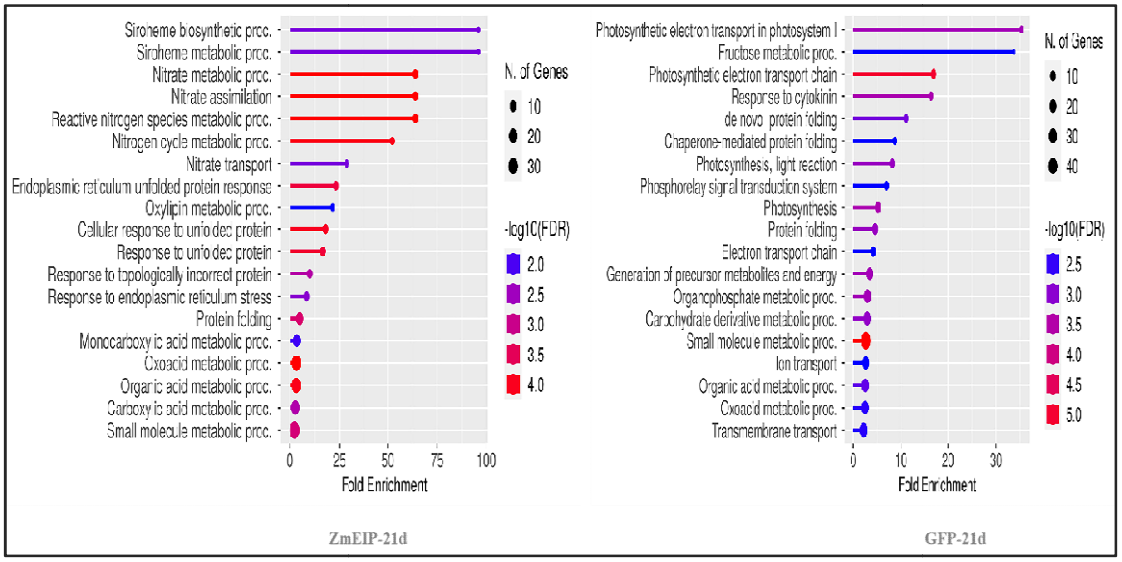

Fig 7. Gene ontology (GO) enrichment analysis of blue module of samples ZmEIP 21 day and GFP 21 day

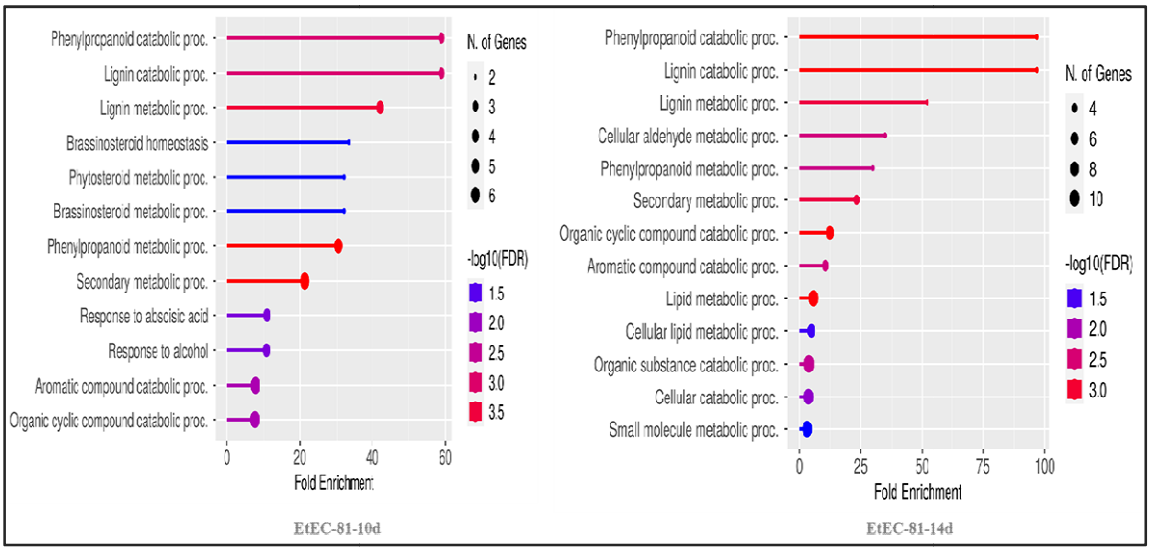

Fig. 8. Gene ontology (GO) enrichment analysis of black module of samples EtEC81-10 day and EtEC81-14 day

DISCUSSION

Fungal infections are the 2nd most common pathogen that affects*Zea mays* after insect attacks. It can cause up to 5% to 42% yield loss. In this study, we focus on the fungus *Exserohilum turcicum*. *Exserohilum turcicum* causes the disease of Northern Corn Leaf Blind (29). The disease is particularly seen in areas with high humidity and moderate temperature of 22-25 C. The fungus *Exserohilum turcicum* secretes the protein EtEC81, and this protein alter cellular pathways. The Zea mays Elicitor-Induced Protein 1 (ZmEIP1) and its interaction with the exserohilum turcicum Effector 81 (EtEC81) could provide insights into maize's resistance mechanisms. GFP (Green fluorescent protein) is typically used as a control to ensure the changes observed are specific to EtEC81. In the current study, we used computational approaches on the publicly available transcriptome data of multiple fungal-affected silk of Zea mays and identified key gene modules associated with maize defense responses through WGCNA. Several biological significant modules that exhibit distinct expression patterns under different fungal infection conditions. A total of 22151 genes from the sample were subjected with significance values GS> 0.5 and MM>0.8 in 16 modules. The total number of significant genes present here is 1202. A notable observation in our study was the differential regulation of the Magenta module, which was upregulated in GFP 10-day samples but significantly downregulated in EtEC81 14-day samples. This suggests that maize initially had immune-related genes (Zm00001eb315910, Zm00001eb209480) but *E. turcicum* manipulates the host transcriptome to suppress these pathways at later stages of infection (30). Transcription factors (TFs) play crucial roles in plant defence by regulating hormone signaling pathways, stress responses, and secondary metabolis. The role of bHLH is particularly in growth, development, stress responses, and defence mechanisms (31). Which were negative co-expression in EtEC81-infected samples indicates that the pathogen may be

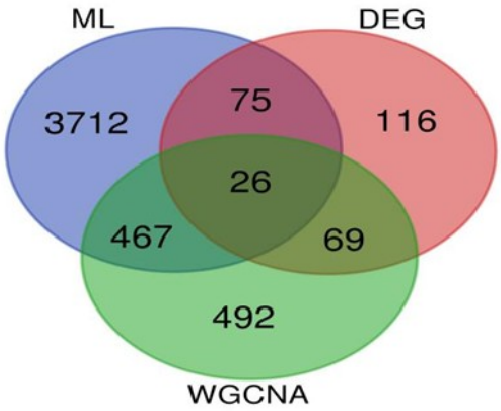

Fig. 9. Core genes identification

targeting these transcription factors to disrupt JA/ET-mediated immune responses, which are known to be crucial for fungal pathogen resistance (30). Several scientists have been finding that pathways controlling the adaptation to stress, such as resistance to mechanical damage, drought, high salt, oxidative stress, low-temperature stressors, heavy metal stress, iron deficiency, and osmotic stress (32). JA/ET(jasmonic acid and ethylene) signaling pathway has been documented in *Pseudomonas syringae*, where JA biosynthesis dampens host defense (33). In this study, the bHLH transcription factor might regulate JA/ET pathways and suppression in EtEC81-treated samples, affecting immune suppression to weaken host resistance.

The plant cell wall is an elaborate extracellular matrix that encloses each cell in a plant and help from biotic and abiotic stress (29). we have many genes that synthesize cell wall-related proteins and polysaccharide recognition, thus promoting efficient catalysis(34). GT1 (Glycosyltransferase 1) enzymes, which were expressed in GFP 10 days (Light Green modules), suggest that Glycosyltransferase 1 plays a key role in maize's defense response. However, the expression of GT1 genes was negative co-expression in EtEC81 14 days (Steel Blue module models), indicating a potential pathogen attack on the host's carbohydrate metabolism. The CAZymes that are significant after GT1 are N-Acetyltransferase and GT2 (Glycosyltransferase 2). GT2 has a role in the defense, signaling, and storage of carbohydrate biosynthesis, etc. It helps in the production of cellulose and hemicellulose (35).

Resistance (R) genes are crucial components of plant innate immunity. The Kinase-Class R (KIN) genes have key roles in pathogen recognition and signaling(36).KIN-class R genes were the dominant class, characterized by the presence of Kinase and Transmembrane (TM)domains. Our analysis identified KIN-class R genes as significant R genes that contribute to maize defense. The effector molecules are recognized by the LRR domain, and this recognition signal is transmitted through the TM domain to the intracellular kinase domain, which further 9 activates a downstream signaling cascade resulting in the activation of plant defense (37). Kinases have an inevitable role in mediating plant immune responses; pathogen effectors have adapted mechanisms to directly target and inhibit these kinase-mediated pathways, effectively undermining the plant's ability to mount a robust defense (38). The KIN-class R genes are negatively co-expressed in EtEC81-infected samples, this suggests a direct suppression of kinase-mediated immunity by the pathogen. The other significant gene after the KIN class is RLK (Receptor-like kinase), and the domains are TM, LRR, and kinase.Transmembrane proteins known as receptor-like kinases (RLKs) are distinguished by the presence of an external receptor domain, which might comprise wall-associated kinase (WAK) domains, lectin (Lec), leucine-rich repeats (LRRs), or lysine motifs (LysM). These RLKs play an important role in controlling plant signaling pathways, especially when it comes to modulating reactions to abiotic stressors including drought, high temperatures, and low temperatures. Furthermore, RLKs are essential for plant immunity because they recognize pathogen-associated molecular patterns (PAMPs) and trigger immunological signaling cascades that improve defenses against pathogen invasion (39).

Protein-protein interactions were identified from significantly positively expressed under ZmEIP_21d present in the under blue modules like Zm00001eb229930 (string id 4577.P24067). Which have been identified a significant role in protein folding in the ER (21). Other C2H2 transcription factors (4577. B4FEJ7) exhibited positive expression and are required to interact with resistance-related proteins such as Serine/threonine-protein kinase ATG1t (STRING ID: 4577.C0PMW7). These proteins regulate multiple target proteins, including Endoglucanase (4577.C0P6G1), which plays a crucial role in the breakdown of plant material into sugars. Additionally, the transcription factor SHOOT

GRAVITROPISM 5 (4577.A0A1D6KL68) also interacts with these resistance (R) genes (Fig. 6). Previous studies in Arabidopsis thaliana have reported the positive involvement of these transcription factors in stomatal responses to darkness (40)

The most significant 26 genes involved in multiple biological process, such as response to stimulus, localization role of peptide-based signaling and nutrient transport in defense responses. Protein kinase domain-containing protein coding genes (Zm00001eb158040) were downregulated in EtEC81 21d,(41) ,have been suggested that these factors cause the misregulation of cellular activities and thus induce immune responses. While Iron-phytosiderophore transporter yellow stripe 1 protein genes (Zm00001eb249020) also showing downregulated in Exserohilum turcicum Effector 81(EtEC81 21 d) which response to iron ion. While 3 genes upregulated in Zm00001eb141080 (Glutathione transferase) in ZmEIP 14 d, Zm00001eb253890 (dihydropyrimidine dehydrogenase (NADP(+))) in EtEC81 14 d, Zm00001eb418060(Glutathione S-transferase 4) in ZmEIP 14 d. Its play significant role in herbicide detoxification(42).

CONCLUSION

Significant modules were identified through Weighted Gene Co-expression Network Analysis (WGCNA). The transcription factor (TF) identification, CAZyme screening, and R gene classification were performed, which revealed critical regulatory pathways that contribute to maize's immune responses. This study provides significant information on *Exserohilum turcicum* effector EtEC81 and molecular mechanisms involved in maize defense and pathogen-induced immune suppression. This finding suggests that *E. turcicum* suppresses maize immunity and manipulates the plant's metabolic pathways. The pathogen disrupts bHLH TF-regulated hormone signaling, alters GT1-mediated glycosylation, and suppresses KIN-class R genes, leading to compromised immune responses in silk of *Zea mays*. DEG analysis revealed stage-specific transcriptional responses under both *EtEC81* infection and *ZmEIP* expression, highlighting shifts from immune activation in early stages to growth restoration in later stages.Machine learning models identified 4,280 key genes involved in pathogen recognition, hormone signalling, transcriptional regulation, and structural defence reinforcement.The integration of DEG and ML analyses confirmed maize prioritizes immune defence in early stages and recovery processes in late stages.

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Author Contributions

RS and AK designed the research. RS and AK performed the analysis and analyzed the results. RS wrote the initial manuscript. AK and ST reviewed and analyzed the draft and finalized the manuscript. All authors contributed to the article and approved the submitted version.

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