Comparative transcriptome analysis and weighted gene co-expression network analysis to identify core in Musa acuminata sub-varieties infected by Fusarium oxysporum f.sp. cubense TR4

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Abstract

Bananas a high-nutrient fruit crop that ranks fourth in terms of gross value production after rice, wheat, and maize. Fusarium wilt of banana, caused by fungal pathogens *Fusarium* oxysporum *f. sp. cubense*tropical race 4 (Foc TR4), is considered as the most destructive disease in the world and results in extensive damage of the crop leading up to complete productivity loss. These cavendish cultivars are vulnerable to *Fusarium oxysporum* f. sp. cubense tropical race 4. (Foc TR4). Benzothiadiazole (BTH) is a plant resistance inducer that can enhance the defensive mechanism of plants against this pathogen. However, the mechanism behind the defensive response is still unknown. In this study, bioinformatics tools were used to publicly available transcriptome data of multiple (Baragan, Brazilian, and Williams) cultivars of cavendish banana under *Fusarium oxysporum* on Benzathiadiasole (BTH) of Barzillian cultivar, which was further used for comparative transcriptome analysis. The widespread use of plant resistance inducers (PRIs) such as Benzathiadiasole (BTH) is a new strategy to stimulate the defence response in banana plants to protect against pathogen infection. The differential expression genes (DEGs) analyses with time series of cultivars were done, of which approximately 56.3 % (19835) genes were found to be significantly differentially expressed. The DEGs have been compared with genes found in modules of weighted gene co-expression network followed by the analysis of relevant (trait) cultivars to identify core genes, and it was found that 1110 genes were down regulated, and 671 were upregulated genes in Berangan cultivars 4 days Foc4 fungal infected samples, whereas in Brazilian cultivars one day Foc4 infections with BTH treated, 171 genes were upregulated and 125 genes were downregulated. Further, in pathway analysis, 20 pathways were identified under significant enrichment FDR, many proteins were also involved in different pathways, including sphingolipid metabolism, peroxisome, ubiquitin-mediated proteolysis and endocytosis in the roots of banana.

Introduction

The banana (*Musa acuminata*), commonly known as the 'Apple of paradise', is the oldest fruit known to mankind. It belongs to the Musaceae family and is considered one of the most valuable primary agricultural commodities. It is consumed raw, fried, or brewed. According to FAO (https://www.fao.org/markets-and-trade/commodities/bananas/en/), banana is one of the most produced, traded, and consumed fruit worldwide and is cultivated in over 150 countries. It is believed that this fruit originated in Southern Asia and was the first cultivated crop (Ploetz 2015). Between 2000 and 2019, global fruit production increased by 54% from 311 million tones to 883 million tones. Five (plantains, watermelons, apples, oranges, grapes) fruit species accounted for 57 percent of the total output in 2019; among them, bananas and plantains hold the highest global share (18 percent). Since 2000, the global share of bananas and plantains has slightly increased compared to watermelons (11 percent), apple (10 percent), orange, and grapes (9 percent). (FAO. 2021. *World Food and Agriculture – Statistical Yearbook 2021.* Rome https://doi.org/10.4060/cb4477en. Among the 1000 varieties of banana, the cavendish cultivar contributes half of the global production and trades that belong to the edible varieties of banana derived from the hybridization of diploid species, *Musa acumianata* and *Musa balbisiana* carrying genomes A and B, respectively, but is triploidic containing the genome AAA for cavendish (Sun et al. 2019)while for plantains has the copy number of the genome AAB(Simmonds and Shepherd 1955).

The banana has quite a better digestion than most other fruits and is very nutritious. Furthermore, it has a great source of dietary fiber, vitamin C, vitamin B6, potassium (K), manganese (Mn). It has a high calorie count with the little amount of fat(Alzate Acevedo et al. 2021). It is also rich in several bioactive antioxidant compounds like phenolics, flavonoids, dopamine etc. (Mohapatra et al. 2010), and adding banana to a regular diet can help to lower the risk of various chronic degenerative diseases. The antioxidant compounds effectively protect the body against various oxidative stresses, and the flavonoids and saponins have an anti-diarrheal activity.

This study used cultivars belonging to the Cavendish subgroup with AAA genome like Brazilian, Berangan, and William. Brazilian is a dwarf banana, known as Santa Catarina silver, belongs to Brazil and is sweet and plumpy in nature. Barangan or Pisang Berangan is a medium-sized banana that belongs to West Malaysia with a balanced sweet and sour flavour. Williams' is a cultivar of the giant cavendish type in the cavendish subgroup(https://www.promusa.org/)

Fusarium wilt of banana, a severe and fatal fungal disease caused by soilborne *Fusarium oxysporum* f. sp. cubense that threatens 15% of worldwide banana production and nearly half of the world's total banana cultivated area(Thangavelu et al. 2021). *Fusarium oxysporum* f. sp. cubense has been divided into four physiological races, namely race 1, race 2, race 3, and race 4, that infect banana. *F. oxysporum* f. sp. cubense R4 again has two biotypes that are *F. oxysporum* f. sp. cubense tropical race 4 (TR4) and *F. oxysporum* f. sp. cubense subtropical race 4 (STR4). Each race is specific to its host organism. Race 1 infected banana cultivars like "Gros Michel" (Musa sp. AAA genome), "Pome," "silk," and "Pisang Awak" (Musa sp. AAB group) and was responsible for the 20th -century epidemic (Zhan et al. 2022). Race 2 infects the cultivar "Bluggoe" and its closely related cultivars. Race 3 does not contaminate any of the *Musa* species buton the contrary, race 4 has a broad host range, infecting almost all cultivars, including "Dwarf Cavendish" (Musa sp. AAA genome), as well as the hosts of race 1 and race 2 (Guo et al. 2014). Hence, race 4 is considered as the most prevalent and devastating biotype of *Fusarium oxysporum f. sp. cubense* in tropical area that causes Panama (fusarium wilt) disease in bananas, and its occurrence is affecting up to 50% yield in Bihar and Uttar Pradesh states of India (Thangavelu et al. 2021)

However, the identification of novel cultivars through resistant breeding is one method to overcome this, but this effort is frequently hampered by triploid bananas that limited seed production. Another option is to use nontoxic plant resistance inducers (PRI), which can help plants to fight pathogens by stimulating their defence mechanisms. Exogenous compounds such as - Beta aminobutyric acid (BABA), hexanoic acid, and benzothiadiazole (BTH) all have been found to be effective against the pathogen in plant species.(Li et al. 2012; Niu et al. 2018; Kaushal et al. 2021). Although comparative studies between *Fusarium oxysporum* f. sp. cubense subtropical race 4 affected and normal cavendish banana group cultivars

have revealed the critical roles of pathogenesis related proteins, signalling, cell wall lignification, hypersensitive response in host resistance against FocTR4, These kind of comparison seemed to provide a scope to determine the resistant mechanism. Accordingly, the role of BTH (Benzothiadiazole) in enhancing banana plant defence response to FocTR4 infection was reported(Cheng et al. 2018) has revealed that BTH too selectively affects the biological processes associated with plant defence. They also observed several genes that were up- and down-regulated in the roots and leaves. However, no comparative study of a cultivar with BTH induced resistance and a susceptible cultivar of FocTR4 has been published, which could help us understand the genes responsible for enhancing the growth of BTH-treated plants in pathogen-induced stressed conditions and it is still unclear how BTH levels affect banana protection against Foc 4, thus to understand the mechanism behind infection process and effect of BTH treatment was carried out. Studies were undertaken to compare the three different cultivars (Brazilian, Berangan, and Williams) under the normal conditions (control) and further a comparison at the separate organism level under the influence of the fungal infections to examine the differential expression gene pattern existing within and between the variety.

Materials And Methods

Dataset Collection

Multiple dataset samples were retrieved from the National Centre for Biotechnology Information – Sequence Read Archive (NCBI SRA), a public domain database. BioProject accession numbers PRJNA417328(Cheng et al. 2018), PRJNA287860(Munusamy and Zaidi 2021) (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA287860), PRJNA322439 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA322439), PRJNA319058(Gamez et al. 2019) were selected for Brazil, Berangan, and William cultivars respectively for *Fusarium oxysporum* f. sp. cubense tropical race 4 (Foc TR4) infections with benzothiadiazole (BTH) treated on time variances **(Supplementary Table S1A**), Paired-end RNA-Sequence raw read of these cultivars were also collected from banana plant root samples.

Data Pre-processing And Alignment

The quality of all four datasets were computed using the FASTQC tool (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/), and the raw read sequences of the datasets were mapped to the latest reference sequence *Musa acuminata* (v2)using the HISAT2 tool (Kim et al. 2015). The read count of each gene mapped to the reference genome was calculated using the FeatureCount tool (Bates et al. 2014). The different datasets have different sampling time intervals ranging from 0, 24, 48, 72, and 96 hrs and were obtained based on Principal component analysis (PCA) (Fig. 1).

Identification Of Common Genes In Different Combinations Of Degs

Differential expression analysis was performed pairwise using BerFoc0d vs BerFoc4d, BraBTH0d vs BraBTH1d, BraBTH0d vs BraBTH3d, WilNor0d vs WilNor2d, WilNor0d vs WilNor4d on the experimental design (**Supplementary Table S1B**). DESeq2 of Bioconductor R package **(Love et al., 2014**) was used to perform differential expression calculation and significantly differentially expressed genes (DEGs) were identified by applying the cutoff value of log2 fold change of $\geq |1.5|$ with padjcutoff < 0.05.

Weight Gene Co-expression Network Analysis

Weight gene co-expression network was constructed to calculate the relationship between genes by calculating the similarity matrix among the genes. To obtain a scale free network, the type of the network and soft-threshold power 12 have been chosen. Then, Topological Overlap Matrix (TOM) was constructed followed by the module identification by hierarchical clustering and dendrograms using the matrix values. The dynamic tree cut identify the modules, and module eigengenes were identified further to summarise the expression patterns of the module's gene across the sample. The subsequent analyses were narrowed down for certain clusters for the downstream analysis.

Identified Core Genes In Statistically Significant Positive And Negative Co-expressed Genes

Correlation between module eigengenes and gene expression of genes were analyzed among the significantly correlated modules of interest associated with root samples of banana. The heatmap was used to represent the correlation values. The module membership (MM) is the association between each module eigengene and its gene expression as gene significance (GS), defined as the correlation between each trait and its gene expression (Tian et al. 2021). The MM and GS were determined to associate genes with MM > 0.8 closely, and GS > 0.5 were identified as key genes in a module(Farhadian et al. 2021) which correlated with the most significant DEGs(Kumar et al. 2022).

Functional Annotation

The biological information of significant genes were identified by DEGs and WGCNA. Gene Ontology (GO) annotations were obtained using the categories biological process (BP), Molecular function (MF), and Cellular component (CC) with a cutoff at FDR = 0.05. Then, a hierarchical tree summarizing the correlation among the 40 most significantly enriched pathways was also generated using ShinyGO v 0.75(Ge et al. 2020), a tool with

statistical-based analysis and visualization results with R package, that build using Ensembl annotation database and numerous other sources databases.

Protein-protein Interaction Network Analysis

The retrieval of the interacting Genes/Proteins from the STRING (Szklarczyk et al. 2019)database was used to examine the differentially expressed genes conversion into proteins. The core analysis function was used to interpret the identified proteins in the context of biological functions and pathways in the inventive pathway analysis of the identified proteins. Then, the significant modules and hub genes of pathways were identified by using Cytoscape (Doncheva et al. 2018) software and shinyGO tool (Ge et al. 2020) and furthermore, the MapMan programme was used to generate a graphical representation of the core protein genes involved in biotic stress response pathways (Thimm et al. 2004). The input command of the core protein genes were given in the MapMan package to design a particular biological process using the banana annotation.

Results

To understand the mechanism behind BTH-treated and untreated samples, a comparative study of the transcriptome data of *Musa acuminata* sub-cultivars was carried out. The WGCNA and DEG analysis identified genes that were involved in the BTH-treated in *Fusarium oxysporum* f. sp. cubense tropical race 4 (Foc 4) fungal-infected samples. The different datasets have different sampling time intervals ranging from 0, 24, 48, 72, and 96 hrs. They were obtained based on the Principal component analysis (PCA) (Fig. 1) and were used to visualize variation between the expression analysis of the samples, and it was clearly found to be distinguished between the samples.

Differential Expression Genes In Multiple Cultivars

Three different cavendish banana cultivars were used to analyze the differential expression genes in the different time periods. Approximately 56.3% of genes (19835 of 35275 genes) were found to be significantly differentially expressed in this analysis (**Supplementary Table 1**). In the comparative DEGs analysis, 28.04% were expressed in Berangan cultivars 4 days Foc4 fungal infected samples, 15.9% genes were voiced in Brazilian cultivars one day Foc4 infections with BTH treated, 1.1% genes were expressed in Brazilian cultivars three day Foc4 infections with BTH treated, 4.2% genes describe in Williams cultivars two days, and 7.2% genes were expressed Williams cultivars four days (Table 1).

Table 1
Differential expression genes analysis of multiple samples of banana

Samples	Down Regulated	Up Regulated	Total DEG
BerFoc4d	4353	5535	9888
BraBTH1d	1595	3980	5575
BraBTH3d	64	331	395
WilNor2d	656	813	1469
WilNor04d	1215	1293	2508

BerFo4d: Berangan cultivar *Fusarium oxysporum* f. sp. cubense tropical race 4 (Foc 4) four days treated; BraBTH1d: Brazilian cultivar *Fusarium oxysporum* f. sp. cubense tropical race 4 (Foc 4) one day treated with BTH(benzothiadiazole) ; BraBTH3d: Brazilian cultivar *Fusarium oxysporum* f. sp. cubense tropical race 4 (Foc 4) three day treated with BTH(benzothiadiazole); WilNor2d: Two days old normal sample of Williams cultivar; WilNor4d: Two days old normal sample of Williams cultivar.

Furthermore, a detailed comparative analysis of the total (13989) DEGs genes between the three cultivars (Brazillian, Berangan, and William) was done. In total, 0.09% (12) genes were expressed in all three cultivars with different time periods. In the 1.3% (168) genes, connections with four conditions were found, 5.6% (774) genes were intersectionally expressed in three conditions, 26.8% (3749) genes were expressed under two conditions, and 66. 5% (5969, 2244, 36, 794, and 246 genes were expressed in Berangan cultivars Foc4 four days infection, Brazilian cultivars Foc4 two days infections with BTH treated, Brazilian cultivars Foc4 three days infections with BTH treated, Williams cultivars four days, Williams cultivars two days respectively) (Fig. 2) genes were expressed in individual conditions.

Intersectional Co-expression Genes Analysis

The total read counts matrix consists of significant DEGs 13989 genes across 16 samples. The weighted gene co-expression network analysis resulted in seven modules of which 1774 genes were positively co-expressed in four traits and five (blue, green, greenyellow, lightgreen, turquoise) modules where as 539 genes were negatively co-expressed in two traits and two (green and lightgreen) modules **(**Fig. 3; **Supplementary Table 2)**. The correlations of genes and modules of positive and negative coexpressed genes were filtered based on the gene Significance (GS) >|0.5| and Module membership (MM) >|0.8|, and the up- and downregulated genes were compared to the genes in modules with relevant specific traits(Yang et al. 2020;Kumar et al. 2022). We were able to infer that the up- and downregulated core genes in modules blue, green, greenyellow, lightgreen, and turquoise

with upregulated and green and lightgreen were highly stable, 1110 genes were down regulated and 671 were upregulated genes in BerFoc4d while in BraBTH1d, 171 genes were upregulated and 125 genes were downregulated expressed (Table 2**)** in the root (**Supplementary Table 3**) samples of banana.

Table 2 Core genes identified between DEGs and co-expressed genes.

Samples	BerFoc0d	BerFoc0d	BerFoc0d	BerFoc4d	BerFoc4d	BraBTH1d	BraBTH0d	BraBTH0d
Modules	GreenYellow	Lightgreen	Green	Lightgreen	Blue	Blue	Green	Turquoise
Genes	955	73	449	91	292	171	53	230
DEGs	BerFoc0vs4d	BerFoc0vs4d	BerFoc0vs4d	BerFoc0vs4d	BerFoc0vs4d	BraBTH0vs1d	BraBTH0vs1d	BraBTH0vs1d
Core Genes	946	73	411	91	260	171	31	94

Red-levelled positive co-expression; Green-levelled negative co-expression; Red-levelled up-regulated; Green-levelled down-regulated; based on the DEGs; Core Genes- Common between WGCNA and DEGs; BerFo4d: Berangan cultivar *Fusarium oxysporum* f. sp. cubense tropical race 4 (Foc TR4) four days treated; BraBTH1d: Brazilian cultivar *Fusarium oxysporum* f. sp. cubense tropical race 4 (Foc TR4) one day treated with BTH(benzothiadiazole); BraBTH3d: Brazilian cultivar *Fusarium oxysporum* f. sp. cubense tropical race 4 (Foc TR4) three day treated with BTH(benzothiadiazole); WilNor2d: Two days old normal sample of Williams cultivar; WilNor4d: Two days old normal sample of Williams cultivar.

Functional Annotations Of Degs

Functional annotations of a total of 19835 DEGs were performed and overall, 418 high-level GO categories were found, in which 25.2% genes involved in biological processes, 6.3% genes present in a cellular component, 26.9% genes were existing in molecular functions, and 3.5% found in the pathways (Table 3; **Supplementary Table 4**). In BerFoc04d samples the protein phosphorylation, phosphorus metabolic process, photosynthesis, cell wall organizations, and regulations process genes were highly influenced (**Fig. 4A**). The BraBTH01d samples microtubule-based process/ movement, cell wall organizations/ biogenesis hydrogen peroxide catabolic/metabolic process affected the genes. (**Fig. 4B**)of protein folding, response to stress, stimulus, xyloglucan metabolic process, response to hydrogen peroxide, and cell wall polysaccharide metabolic process genes touch in BraBTH03d (**Fig. 4C**). In the WilNor02d sample, many genes were involved in biological processes, including the regulation of the biosynthetic process, regulation of RNA biosynthetic process, photosynthesis, response to red or far red light, response to abiotic stress, RNA biosynthetic process, regulation of RNA biosynthetic process, etc. (**Fig. 4D**). William cultivars were maintained in controlled conditions of temperature and photoperiod and were sampled according to the defined times (Gamez et al. 2019). Williams cultivars had many genes that participated in the different biological processes such as photomorphogenesis, photosynthesis, light harvesting, response to osmotic stress, tryptophan catabolic process, response to heat, benzene-containing compound metabolic process, etc. (**Fig. 4E**).

Table 3
Gene set enrichment analysis of DEGs

Sample	Significant DEGs genes	Genes	High-level GO category	Genes	High-level GO category	Genes	High-level GO category	Genes	High-level GO category
BerFoc04d	9888	2550	40	727	35	4123	40	394	3
BraBTH1d	5575	1555	40	421	36	217	40	195	5
BraBTH3d	395	75	31	14	4	136	26	10	1
WilNoro2d	1469	366	40	8	2	388	10	80	3
WilNoro4d	2508	433	34	69	15	458	12	6	1

BerFo4d: Berangan cultivar *Fusarium oxysporum* f. sp. cubense tropical race 4 (Foc TR4) four days treated; BraBTH1d: Brazilian cultivar *Fusarium oxysporum* f. sp. cubense tropical race 4 (Foc TR4) one day treated with BTH (benzothiadiazole); BraBTH3d: Brazilian cultivar *Fusarium oxysporum* f. sp. cubense tropical race 4 (Foc TR4) three day treated with BTH (benzothiadiazole); WilNor2d: Two days old normal sample of Williams cultivar; WilNor4d: Two days old normal sample of Williams cultivar.

Ppi Interactions And Pathways Analysis Of Core Genes

Candidate hub gene identification was performed by considering both module membership (MM) and Genes significant (GS) and DEG. The 2077 core genes were found **(**Table 2**)** in which 351 string ids were found from 1110 genes that were down regulated and 158 string ids existing in 671 up regulated genes in BerFoc0vs4d conditions, while 34 string ids observed from 171 genes were upregulated and 30 string ids present in 125 genes were down-regulated in BraBTH01d conditions. 13 proteins commonly were upregulated in BerFoc04d and BraBTH01d while 2 proteins varied condition wise in BerFoc04d were up regulated but in BraBTH01d was downregulated (Fig. 5), The 21, 28 genes were uniquely up-regulatedly expressed in BraBTH01dU and were down regulated in BraBTH01d, although 142 protein were upregulated in BerFoc04d and 351 proteins were downregulated in

BerFoc04dD (**Supplementary Table 5I)**. Further, in pathway analysis, 20 pathways were identified under significant enrichment FDR, and it was found that many proteins were involved in different pathways including sphingolipid metabolism, peroxisome, ubiquitin mediated proteolysis, endocytosis, biosynthesis of secondary metabolites, plant hormone signal transduction etc. (Fig. 6; **Supplementary Table 5P**). Further, input was given of core proteins to MapMan package, and 26 bins (term) name were identified that contain functional information of the core genes. After that, we identified 26 genes that were classified into the following seven groups: ABA, JA, Beta glucanase, Redox state, ERF, secondary metabolites, and abiotic stress **(**Fig. 6**)**. Most of the core protein genes in the cell wall organization were under redox state, multi-process regulations in ERF transcriptions factor. The core protein genes with known functions, e.g., TFs, redox state, JA, secondary metabolites, and hormone signaling are shown in **(**Fig. 6**)**. and the detailed data is provided in (**Supplementary Table 6**). Briefly, most of the core protein genes were related to the redox state, ERF, etc. four-day treated sample of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (Foc TR4) infection. Eight out of ten redox state genes were identified as upregulated; moreover, in ERF, four genes were upregulated and two were downregulated genes but in *Fusarium oxysporum* f. sp. cubense tropical race 4 (Foc TR4) Brazilian cultivar one day treated with BTH (benzothiadiazole) one gene was downregulated. The expression levels of four core protein genes were representing the secondary metabolites, of which two were expressed in ABA, JA, and one in beta-glucanase (**Supplementary Table 6**).

Discussion

Panama disease or Fusarium wilt disease, is a destructive soilborne disease caused by the fungus *Fusarium oxysporum f. sp. cubense* (Foc)(Thimm et al. 2004). It attacks various crops like pepper, potato, tomato, eggplant, etc. Fusarium wilt of banana is a typical vascular wilt disease in which the fungus infects the plant through roots of susceptible and resistant banana cultivars. In response to infection, xylem lumina produces tyloses, gums, and gels(Simmonds and Shepherd 1955). This defensive mechanism is more rapidly shown in resistant cultivars than in susceptible cultivars and also prevents the systemic infection of the pseudo stem in resistant cultivars, but the pathogen colonizes susceptible cultivars ahead of these host responses. Infected xylem vessels turns reddish-brown(Thimm et al. 2004) and eventually get clogged which hinders the translocation of water and nutrients to the upper part of the plant. Banana belongs to the Domain Eukaryota Kingdom Plantae Phylum Spermatophyta Subphylum Angiospermae Class Monocotyledonae Order Zingiberales Family *Musaceae* Genus Musa Species *Musa acuminata* Cultivar group AAA and has three Cultivar varieties namely Berangan (Munusamy and Zaidi 2021), Brazilian (Munusamy and Zaidi 2021) and Williams (Stover 1988). During the infection, older leaves begin to wither, die, and split at the base. Yellowing of the leaf lamina is typical, owing to the pathogen's production of phytotoxins, while non-yellowing development of wilt symptoms is also documented. Eventually, the infection leads to younger leaves, and ultimately, the plant dies off.(Ploetz 2015)

The mechanism of Foc pathogenesis in bananas is still a mystery. In order to create a long-term solution to this problem, a detailed study of the elements that cause pathogenicity in bananas is required. However, a few non-pathogenic Foc strains can colonize banana roots but do not cause wilt disease(Ghag et al. 2015). Identification of unique pathogenicity factors in Foc pathogenic strains could contribute to a better knowledge of how this pathogen causes disease in the root of cavendish bananas, as well as a supply of pathogenic isolates that can be identified quickly. In addition, the mechanism underlying host defence in plant in resistance genes varies during fungus *Fusarium oxysporum f. sp. cubunes* TR4 infection (Munusamy and Zaidi 2021), and benzothiazole (BTH) influenced banana plant resistance during Foc 4 infection have also been proved(Cheng et al. 2018). However, there is a lack of research to multiple subvariety (Baragan, Brazilian, and Williams) cavendish banana cultivars under *Fusarium oxysporum f. sp. cubense*tropical race 4(Foc TR4) infections as well as with BTH treated. Therefore, the pathways and identification of genes responsible to defence in these cultivars need to be understood. This current study used publicly available transcriptome data (**Supplementary Table S1A)** and bioinformatics tools (materials and method) for comparative analysis for intersections and core genes identifications in the root of sub-cultivars of the cavendish banana. We identified approximately 56.3% genes that were significantly expressed in the analysis (**Supplementary Table 1**). Further, duplicate genes were removed for modules WGCNA analysis and identified core genes in specific traits were compared with significant DEGs genes (Kumar et al. 2022). Our comparative study has revealed that Bergan cultivars of cavendish banana genes have more influence in four days of Foc4 infection compared to others cultivars under Foc4 with BTH treated and non-infections, non-treated Williams cultivar. Very interesting results were visualized that BTH treatment has an effect on the banana of Foc 4, but after two days, BTH treatment influenced loss(Cheng et al. 2018) was found that BTH sprayed banana plant defence mechanism genes were more positively active in Foc4 than unsprayed plants. In many plants like cucumber, tobacco, wheat, etc. it was proved that BTH treatment has provided resistance against multiple pathogens namely, *C. lagenarium* (Bovie et al. 2004), *Tobacco mosaic virus* (TMV)(Friedrich et al. 1996), Powdery mildew(Friedrich et al. 1996), infections, respectively. If the plant is more resistance towards the Foc4 infection, then genes would be more influenced, just like Brangan cultivars but here not more expression of genes was found (Table 1). In Williams cultivars, we found that genes were linearly influenced by days(Gamez et al. 2019) and co-others have also found the same linear influence with *Pseudomonas fluorescens* Ps006bacterial infections but with *Bacillus amyloliquefaciens* Bs006infection genes were having reverse learner expressions genes. Biological process GO term (**Supplementary Table 4**)like photosynthesis, regulations of biosynthesis process, and response to radiations were found more in two days old plants compared to four days old plants (**Fig. 4D-E**).

Co-expression analysis was constructed of significant DEGs 13989 genes across 16 samples. Hence, five modules were positively expressed in four traits, while two were negatively expressed in two traits. Further, the module genes were linked to the most significant DEGs shared by relevant specific traits(Kumar et al. 2022). All modules were regarded as core genes (Table 2, **Supplementary Table 2–3**), 573 string proteins were found from 2077 cores genes, and further, Protein-protein interaction analysis of many protein genes were having intersectionally relations (Fig. 5), and pathways analysis were also identified under significant enrichment FDR with the help of ShinyGO v0.75(Ge et al. 2020) tool, in which 79 genes were involved in top 20 pathways (**Supplementary Table 4)**, In our study we found that lpha-Linolenic acid metabolism pathway was affected by FocTR4 fungus in Bergan cultivars, and Brazillian and Tianbaojiao' banana cultivars were affected (Wang et al. 2012; Cheng et al. 2021) too. The branched-chain amino acid transaminases (BCATs) plays an important role in the metabolism of the branched-chain amino acids leucine, isoleucine, and valine(Kochevenko et al.

2012). Certain enzymes catalyze the final step of synthesis and the first step of degradation of these amino acids(Wyllie and Fellman 2000; Hu et al. 2021). These enzymes were involved in the biosynthesis of the amino acid pathways but had a negative influence on fungal infections in the banana root. Another protein, glyceraldehyde-3-phosphate dehydrogenases (GAPDH) was also found in many plants' metabolic pathways of glycolysis that generates intermediate products for the primary metabolites such as amino acids (AAs) and fatty acids (FAs)(Schneider et al. 2018; Zeng et al. 2018; Wei et al. 2022).The second largest protein involved in the biosynthesis of secondary metabolites pathways, three proteins were positively expressed out of 31 proteins, and similar results were found by (Zhang et al. 2020; Deng et al. 2022) in the same fungal affected Brazilian and Aifen No. 1 cultivars. The highest genes involved in metabolic pathways, the pectinesterase (PE) protein, have an important role in cell wall modification, cell adhesion, and stem elongation(Micheli 2001; Phan et al. 2007), MapMan (Thimm et al. 2004)based analysis confirmed that the redox state of the pectinesterase protein gene was found upregulated in Bergan cultivars under FocTR4 infections others cultivar Brazillian had no significant (Fig. 6, **Supplementary Table 6**) biotic stress, (Hall et al. 1993; Siddiqui et al. 2020) found that in different cultivars and tissue genes expression values were changed. The peroxisome pathway plays an important role in the biosynthesis of primary bile acid because a peroxisomal β-oxidation step is required for the formation of the mature C24-bile acid from C27-bile acid intermediates(Ferdinandusse and Houten 2006; Poirier et al. 2006), but in our study peroxisomal genes were found to be down-regulated in Bergan root. Plant hormone signal transduction pathways have hormone Jasmonate acid (JA) genes which were involved in the development and drought stress response in tomatoes (Candar-Cakir et al. 2016) in the condition of the FocTR4 infection that also has a negative influence in Bergan banana root but in Brazilian banana roots a positive influence was observed(Supplementary Table 4)and also inother TIFY proteins and ABA, JA phytochrome. (Fig. 6), In *Arabidopsis thaliana* genes were upregulated by drought in an ABA-dependent manner(Li et al. 2008). Ubiquitin mediated proteolysis pathway was found to play a major role in temperature stress responses, that the molecular mechanism in high temperature triggers and stay green ripping banana in association with E3 ubiquitin-ligase, these genes were found to be upregulated in Pre-climacteric banana(Wei et al. 2020), but in fungal FocTR4 fungal infection genes were genes were down regulated in Brangan cultivar of the banana.

Conclusion

The existing transcriptome information was combined and re-examined on NCBI and a critical clue to biotic factors impacting the banana plant was revealed. Based on a comparison investigation employing statistical estimations such as the DEG, the expression level of genes and the behavior of similar coding proteins were shown to be extremely dissimilar during fungal infections and BTH treatment in multiple cavendish banana cultivars. The 12 DEGs were found in all cultivars under fungal infection as well as with treatment. The up-and downregulated genes were compared to the genes in the modules of WGCNA with relevant specific traits, and it was identified that core genes in modules blue, green, greenyellow, lightgreen, and turquoise were with upregulated and green and lightgreen were highly stable, 1110 were down regulated genes and 671 genes were upregulated in BerFoc4d, while in BraBTH1d 171 genes were upregulated and 125 genes were down relatedly expressed. In the current study, core genes were involved more significantly under FocTr4 infection in the Berangan cultivar followed by the Brazilian cultivar. The present study supports that many pathways have been a negative influence in cavendish bananas under FocTr4 infections And it has also been found that gene influence has also increased over time in Williams cultivars. The current study also supports the elevated immune stimulus and resistance genes, photosynthesis, cell wall construction, pectin metabolism, and auxin response in the roots of the cavendish banana.

Declarations

Author contributions

PTVL, AK, AAS, and APR, designed the research; AK performed research; AK and AJ, AS analysed the results; PTVL, AK, AC, AAS, APR wrote the initial manuscript; PTVL, AS, and AC, visualization, investigation; PTVL and AA majorly reviewed, analyzed the draft and finalized the manuscript. All authors approved current version of the manuscript.

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Conflict of interest: None.

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Figures

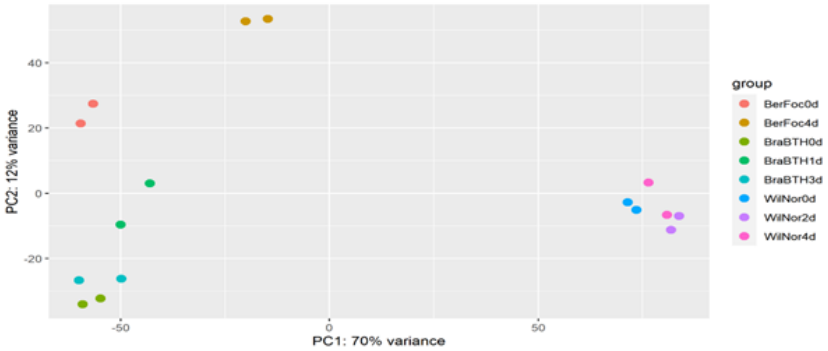

Figure 1

PCA plot, BerFoc0d: control sample of Berangan; BerFo4d: *Fusarium oxysporum* f. sp. cubense tropical race 4 (Foc TR4) four days treated; BraBTH0d: control sample Brazilian cultivar; BraBTH1d: *Fusarium oxysporum* f. sp. cubense tropical race 4 (Foc TR4) one day treated; BraBTH3d: *Fusarium oxysporum* f. sp. cubense tropical race 4 (Foc TR4) three day treated; WilNor0d: control sample of Williams cultivar; WilNor2d: Two days old normal sample of williams cultivar; WilNor4d: Two days old normal sample of williams cultivar.

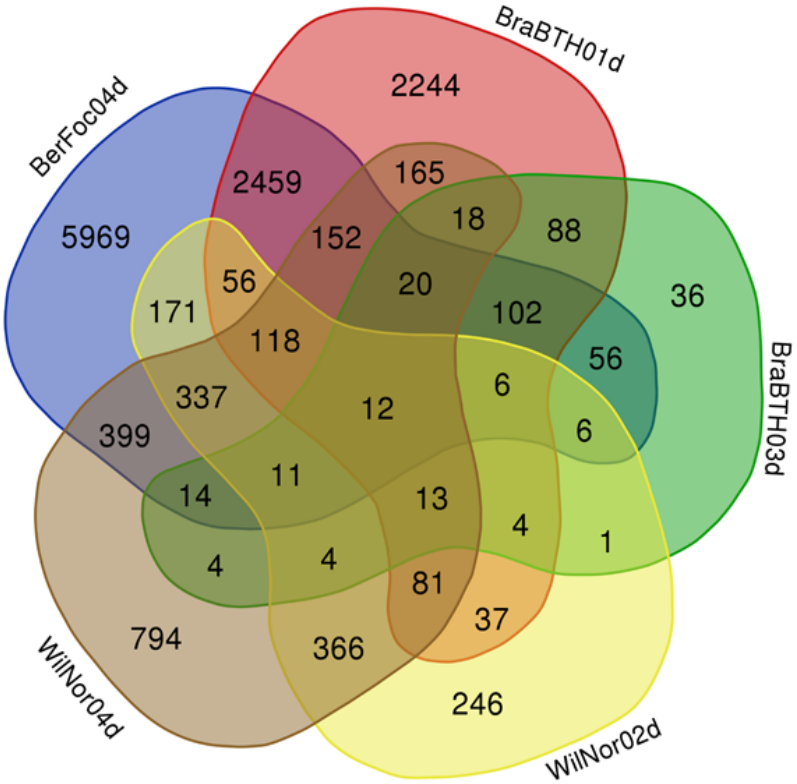

Figure 2

DEG Venn diagram for common genes. The sum of the numbers in the Venn diagram represents the total number of differentially expressed genes. The amount of DEGs is shown by the overlap. BerFoc04d: Berangan cultivar *Fusarium oxysporum* f. sp. cubense tropical race 4 (Foc TR4) four days treated; BraBTH1d: Brazilian cultivar *Fusarium oxysporum* f. sp. cubense tropical race 4 (Foc TR4) one day treated with BTH (benzothiadiazole); BraBTH3d: Brazilian cultivar *Fusarium oxysporum* f. sp. cubense tropical race 4 (Foc TR4) three day treated with BTH(benzothiadiazole); WilNor2d: Two days old normal sample of Williams cultivar; WilNor4d: Two days old normal sample of Williams cultivar.

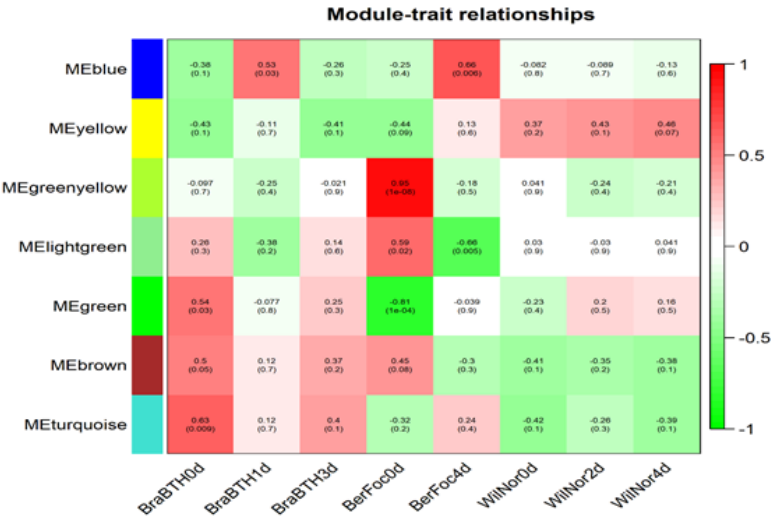

Figure 3

Correlation coefficients between modules and samples. Red to green colour depicted levels of positive and negative regulation of the genes, respectively

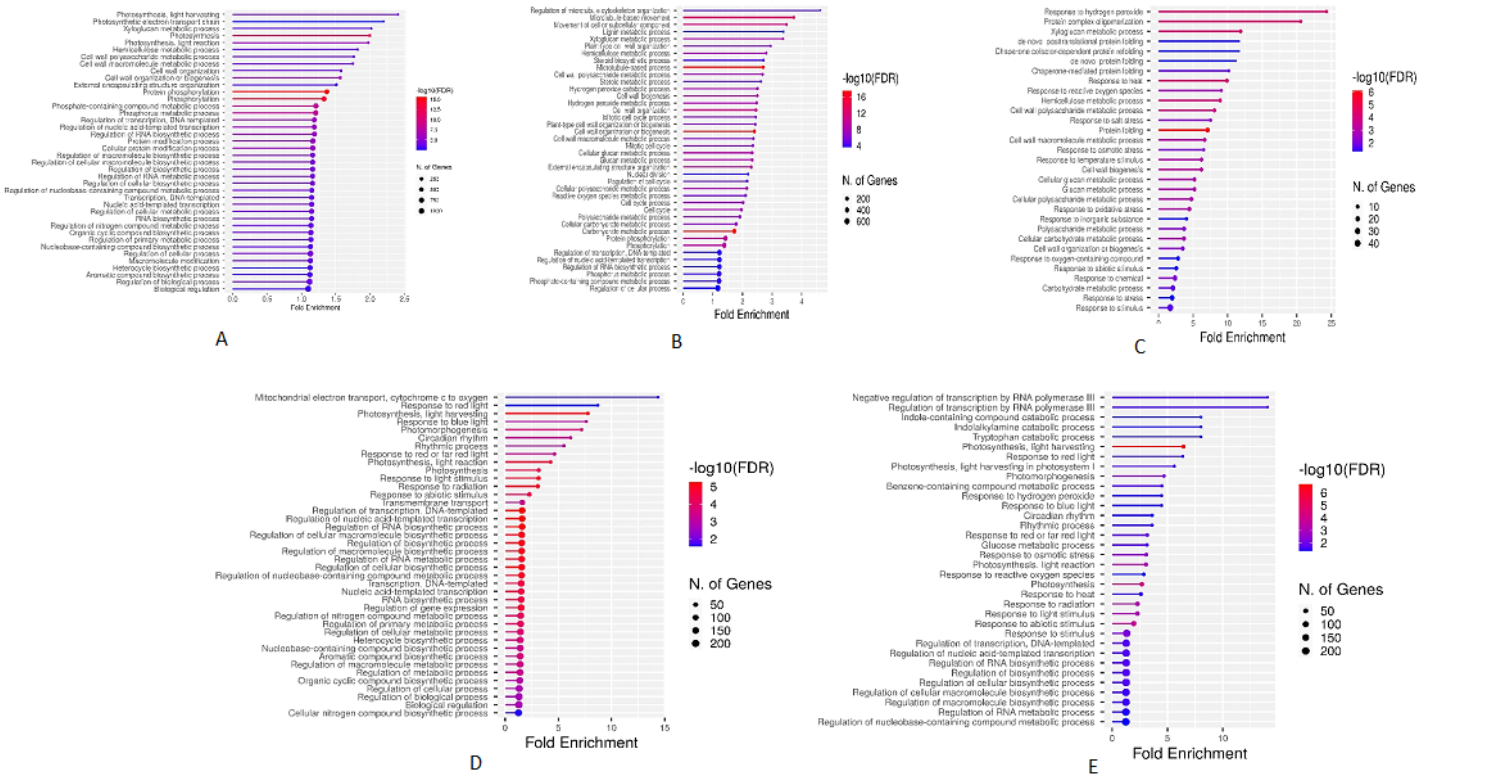

Figure 4

The top 40 biological processes of DEGs. **A** BerFoc04d, **B** BraBTH01d, **C**BraBTH03d, **D** WilNor02d, and **E** WilNor04d were analyzed for functional enrichment using ShinyGO. Fold enrichment is the ratio of the percentage of genes in a list that belongs to a pathway to the comparable percentage in the background.

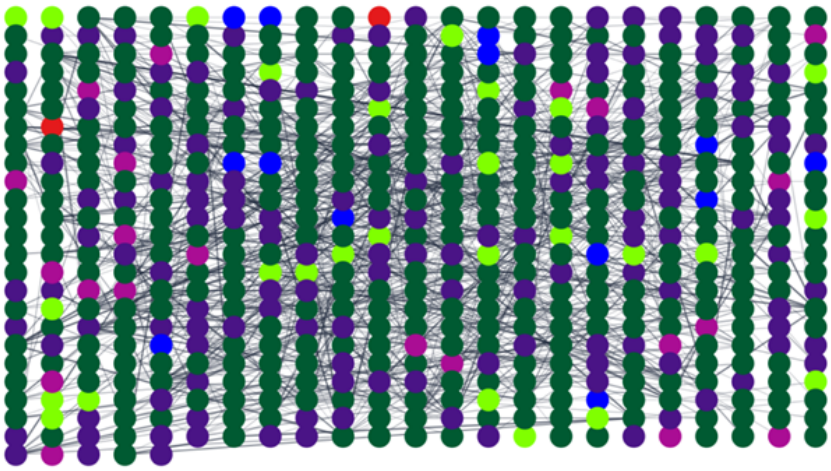

Figure 5

PPI interactions of core genes

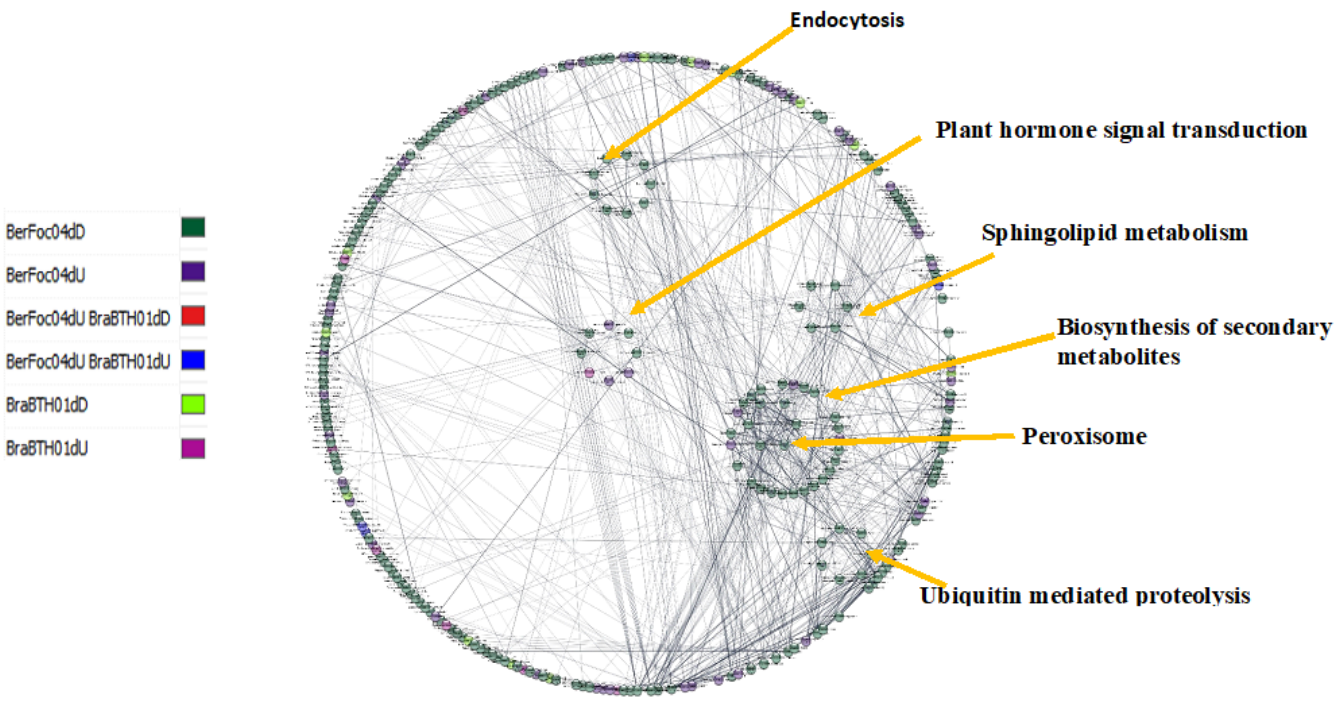

Figure 6

Pathway analysis of core genes. BerFoc04d: Berangan cultivar *Fusarium oxysporum* f. sp. cubense tropical race 4 (Foc TR4) four days treated; BraBTH1d: Brazilian cultivar *Fusarium oxysporum*f. sp. cubense tropical race 4 (Foc TR4) one day treated with BTH (benzothiadiazole); D-downregulated; U-upregulated.

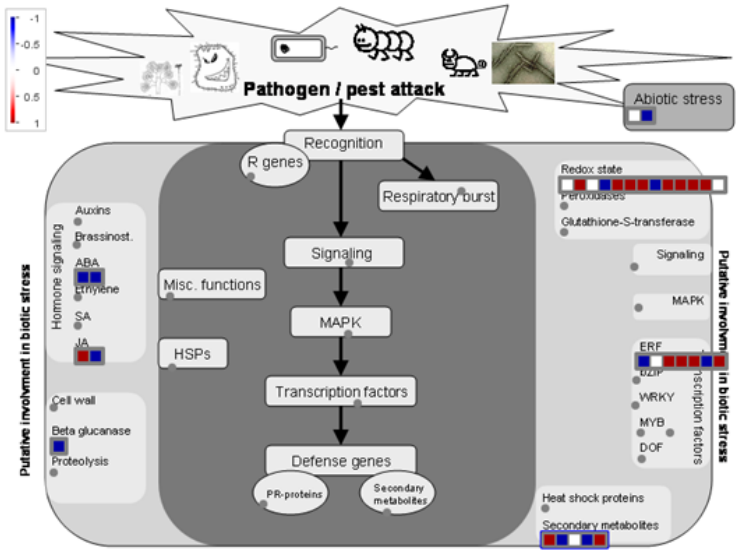

Figure 7

MapMan is a programme that visualizes the major protein genes involved in host-pathogen interactions in Fusarium oxysporum f. sp. cubense tropical race 4 (Foc TR4) treated sample vs. control, the protein gene implicated in the biotic stress pathway is shown by a colour signal, with red representing upregulation and green suggesting downregulation.

Supplementary Files

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

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- SupplementaryTable3.xlsx
- SupplementaryTable4.xlsx
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