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Research article

Comparative transcriptome analysis reveals resistance-related genes and pathways in *Musa acuminata* banana 'Guijiao 9' in response to Fusarium wilt

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ABSTRACT

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*), is one of the most devastating diseases in bananas resulting in significant loss of Cavendish bananas production worldwide. Here we show the agronomic traits and the resistance of 'Guijiao 9′ in the field trials from 2012 to 2017. And then we dissect and compare the transcriptome response from these two cultivars (cv. 'Guijiao 9′ and cv. Williams) in an attempt to understand the molecular basis that contribute to the enhanced *Foc* tropical race 4 (*Foc*-TR4) resistance.

'Guijiao 9′ is a Cavendish cultivar with strong resistance to *Foc*-TR4, which was reflected in a lower disease severity and incidence in glasshouse and field trails, when compared to the susceptible cultivar Williams. Gene expression profiles of 'Guijiao 9′ and Williams were captured by performing RNA-Seq analysis on 16 biological samples collected over a six day period post inoculation with *Foc*-TR4. Transcriptional reprogramming in response to *Foc*-TR4 was detected in both genotypes but the response was more drastic in 'Guijiao 9′ than in Williams. Specific genes involved in plant-pathogen interaction and defense signaling including MAPK, calcium, salicylic acid, jasmonic acid and ethylene pathways were analyzed and compared between 'Guijiao 9′ and Williams. Genes associated with defense-related metabolites synthesis such as NB-LRR proteins, calmodulin-binding protein and phenylpropanoids biosynthesis genes were significantly up-regulated in 'Guijiao 9′ resistant to *Foc*-TR4 infection. Taken together, this study highlights the important roles of plant hormone regulation and defense gene activation in mediating resistance in 'Guijiao 9'.

1. Introduction

Banana, cooking banana and plantain (*Musa* spp.), with an annual global production over 120 million tons, are among the most important fruit crops in the world and serve as the major staple food crop for millions of people in the sub-tropics of Africa and South East Asia (Ghag et al., 2015). The edible varieties are derived from *Musa acuminata* and *Musa balbisiana* hybridisations, carrying genomes A and B, respectively (Ploetz, 2015a). The most significant varieties are the triploids, including the Cavendish (genome AAA) and plantains (genome AAB) (Ploetz, 2015a). These two, out of 50 subgroups, are responsible for 64% of the world banana production. In China, the banana cultivation areas are mainly distributed in Guangdong, Guangxi, Hainan, Fujian, Yunnan and Taiwan province. Guangxi is one of the major banana producing areas and its annual yield accounts for 31% of the total national production.

However, Fusarium wilt of banana caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*) is the most destructive plant disease in banana production (Siamak and Zheng, 2018), It has devastated thousands of hectares of banana crops in the world. *Foc* infects the roots of banana plants, colonizes and occludes the xylem vessels, causing wilt syndrome with necrosis and rotting (Zhang et al., 2018). The spores of pathogen can spread through soil and water, and the chlamydospore can survive in soil for decades (Ploetz, 2015b). Fusarium wilt was firstly reported in 1870s and caused a major epidemic in commercial banana plantations of the cultivar Gros Michel in South and Central America. The Fusarium wilt epidemic was caused by *Foc* race 1 and decimated the large-scale monocultures of Gros Michel. No effective control methods were found

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other than replacing Gros Michel with resistant Cavendish bananas during the 1960s. However, in the early 1990s, a damaging new variant of *Foc*, tropical race 4 (TR4) was detected in South East Asia that is capable of infecting Cavendish bananas and has since spread in the tropics of Asia (Ploetz, 2015b). *Foc*-TR4 devastated Cavendish plantations in Indonesia, China, Malaysia, the Philippines, Australia, and Mozambique. It is capable of spreading out relatively fast from a source. For example, TR4 was detected around the same time in banana growing regions of Jordan, Pakistan and Labanon (Syed et al., 2015; Ordonez et al., 2016). TR4 has caused significant economic losses to the banana exports in countries including Malaysia and Indonesia with each having reported losses of up to 121 and 243 million dollars, respectively (Aquino et al., 2013). In China, *Foc*-TR4 has spread to Guangdong, Fujian, Hainan, Guangxi and Yunnan provinces, where 80% of the banana plantations were affected (Zheng et al., 2018).

Through better understanding of the epidemiology of *Foc*, measures can be put in place to control the disease from spreading, through good practices and early diagnosis methods. However, there is no true ways to remove the pathogen from the infested soil once the plants are infested (Ploetz, 2015b). The use of resistant varieties is the most effective means to manage Fusarium wilt disease (Dita et al., 2018). Through breeding and genetic manipulation, plant host resistance has the potential to offer a solution that is more sustainable, through the deployment of resistant lines in the TR4 affected areas. It is very difficult to develop resistant cultivar by conventional hybridization breeding because of the parthenocarpy phenotype associated with the triploid Cavendish bananas which make them highly sterile. Most of banana varieties used in industry are developed by somaclonal variation. However planting a single variety over a long period of time can often lead to susceptibility due to the fungus being able to overcome host resistance by acquiring new virulence in the monoculture fields.

The cultivar 'Guijiao 9' (Guishenguo, 2,015,008), released by Guangxi Committee of Crops Variety Examination in 2015, was identified as good resistant to *Foc*-TR4 from a resistance screen of natural variant lines grown in the field where banana plants were severely infected by TR4. 'Guijiao 9′ showed good yields in the assessment of agronomic traits and that is correlated with its resistance to *Foc*-TR4 over five years of field trials. Understanding of the host resistance would be very useful towards the development of disease resistance in cultivar improvement.

The rapid and low-costing high-through deep sequencing technology has been successfully used for comparative genomics, expression profiling and molecular mechanism investigation of plants after pathogen infection. The draft sequence of a doubled-haploid *Musa acuminata* is publicly available (D'Hont et al., 2012). The genome of cultivated banana is expected to be more complex due to its polyploidy and heterozygosity. Therefore, the genome of a doubled-haploid *Musa acuminata* was used as a reference for mapping. Transcriptome analysis of bananas infected by *Foc*-TR4 will enable us to understand the molecular mechanism through discovering genes and pathways related to *Foc* resistance. A study is designed to evaluate the resistance of 'Guijiao 9′ and conduct comparative transcriptome analysis by using mRNA-Seq method. Analysis leads to identification of genes that control differences in pathogenesis process of *Foc*-TR4. The long-term objective is to understand the resistance mechanism of bananas to Fusarium wilt disease, and eventually to identify, isolate and utilize the *Foc* resistance-related genes for banana cultivar improvement.

2. Materials and methods

2.1. The breeding of 'Guijiao 9′

In order to obtain Cavendish banana cultivar tolerant to *Foc*-TR4, selection of somaclonal variation on Cavendish banana was performed. Nine healthy banana plants that survived in fields severely infested with *Foc*-TR4 were found in Huangliu town, Hainan, China (18°50′N, 108°79′E) in 2010. The cultivar Williams was grown in the infested field. The nine banana plants were collected as original parental plants to conduct tissue culture of mutant. The mutant plantlets were selected as suitable parents from which clones were multiplied and propagated through tissue culture of suckers of the parents. The somatic mutant plantlets derived from parent were screened for resistance against *Foc*-TR4 in pots and fields. After selection and purification, resistant lines were further assessed in the field for agronomic traits through variety comparative tests and regional trials during 2012–2017. The resistant lines were planted in the commercial banana plantations in different season in Guangxi, Hainan and Guangdong regions. The growth cycle was recorded and the plant height and yield were measured during the mature period. Thirty banana fruits were prepared and the peel were separated from pulp. The banana peel thickness and banana pulp were measured. The data of peel thickness and banana edible percentage were calculated in average. Soluble solids concentration and was evaluated by a refractometer (Model N1, Atago Co., Japan) and the Vitamin C was determined according to Ding et al. (2015) method. The total sugar and soluble titratable acid were detected and quantified using the method of Bernard et al. (2008). The cultivar 'Guijiao 9′ which showed superior *Foc*-TR4 resistance and yield related traits was released by Guangxi Committee of Crops Variety Examination in 2015.

2.2. Fungal culture, plant materials and pathogen infection

The *Foc*-TR4 strain (CNSD1) was originally collected from Wuming banana plantations at Nanning, China in 2012. In 2016, vegetative compatibility group (VCG) testing performed on this strain by Department of Plant Pathology, Stellenbosch University (Stellenbosch, South Africa) confirmed that it is VCG 01213/16. The *Foc*-TR4 isolate was routinely cultured on potato dextrose agar (PDA) plates for 7 days at 28 °C. Spores were harvested from the plates by rubbing the surface mycelium gently with a rubber swab and collecting the spores in distilled water. The concentration of the suspension was adjusted to 1×10^6 spores per ml with a hemocytometer and used for infection. Experiments were conducted using Williams (*Musa acuminate* L. AAA Cavendish cv. Williams) and 'Guijiao 9' (*Musa acuminate* L. AAA Cavendish cv. 'Guijiao 9′), which were obtained from Institute of Biotechnology of Guangxi Academy of Agricultural Sciences. Tissue cultured banana plants (cv. Williams and cv. 'Guijiao 9') were transplanted into pots and incubated at 28 °C with a 16-h light/8-h dark photoperiod and 60% relative humidity (RH). Banana plants grown with five or six leaves and healthy root system were selected for *Foc*-TR4 inoculation. The roots were immersed in *Foc*-TR4 spore suspension for 4 h and replanted into pots. The xylems of the roots of three inoculated plants were collected for each treatment at 0, 2, 4 and 6 days post inoculation (dpi), and each genotype had two biological replicates. The xylems of the roots harvested from the water-inoculated plants at 0 day were served as a control. The harvested tissues were snap frozen in liquid nitrogen and stored at −80 °C for RNA extraction.

2.3. RNA extraction, cDNA library construction and illumina sequencing

High-throughput Illumina sequencing was completed by using the HiSeq™ 2000 platform. Total RNA was extracted from the xylems of banana roots by a modified CTAB method (Li et al., 2012). RNA integrity was confirmed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA; http://www.agilent.com) with a minimum RNA integrated number (RIN) value of seven. Two biological replicates for each treatment were subjected to RNA-Seq library construction. The library for sequencing was constructed using the Illumina's kits following the manufacturer's recommendations. Briefly, poly (A)-enriched mRNA was purified from total RNA using Oligo (dT) magnetic beads and cleaved into small pieces with divalent cations under elevated temperature. First-strand cDNA was synthesized using random hexamer (N6) primers and reverse transcriptase (Invitrogen,

Carlsbad, CA, USA), and followed by second-strand cDNA synthesis using DNA polymerase I (NEB, Ipswich, MA, USA) and RNaseH (Invitrogen, Carlsbad, CA, USA). After end repairing, cDNA were ligated to adapters, purified and enriched by PCR to create the final cDNA library. The cDNA library prepared was sequenced on Illumina HiSeq™ 2000 platform and 100-bp raw PE reads were generated.

2.4. Transcriptome assembly and analysis from RNA-seq

Raw reads were filtered to obtain clean reads, by removing the adaptor sequences, the low-quality sequences, and the reads with unknown base pairs 'N'. All high-quality reads of each sample were aligned to the *Musa* genome sequence (http://banana-genome.cirad.fr version 1) using TopHat 2.0 and Bowtie 2 with default parameters (Trapnell et al., 2009). Cufflinks 1.0.3 (Trapnell et al., 2010) was then used to assemble the transcripts from the TopHat alignment results to determine gene expression values. Two biological replicates of each sample were used for differential gene expression analysis, and Pearson's correlation coefficients between biological replicates for each sample were calculated in R (logiciel). The genes with absolute values of $\log_2$ (fold change) ≥ 1 and adjusted false discovery rate in q-value ≥ 0.99 were identified as differentially expressed genes (DEGs). The gene ontology (GO) functional classification of DEGs (Fisher, P-value < 0.05) was carried out by BLAST2GO 2.5 program (Conesa et al., 2005). Kyoto Encylopedia of Genes and Genomics (KEGG) enrichment analysis (P-value < 0.05) of DEGs was performed using KOBAS2. The gene expression patterns was analyzed with MeV software (www.tm4.org/mev.html) to construct the heat maps.

2.5. Real-time RT-PCR for validation of transcript levels

Gene-specific primers were designed by using the software primer premier 5.0 (Premier Biosoft Interpairs, Palo Alto, CA) and the primer sequences are listed in Table S1. Total RNA was isolated from the xylems of banana roots as mentioned for the transcriptome sequencing. The first-strand cDNA was synthesized from 1.0 μg of total RNA using the PrimeScriptTM RT Master Mix Kit (TaKaRa,Bio Inc., Japan). The rps2 gene of banana was used as the reference gene for normalization (Chen et al., 2011). The qRT-PCR was carried out using the SYBR Premix ExTaq Kit (TaKaRa, Bio Inc., Japan), with a reaction (20 μl) included 20 ng cDNA, 0.3 μM of each primer, and 1 × SYBR Premix ExTaq. All qRT-PCR reactions were performed on the Light Cycler 480 (Roche Diagnostics, Germany) under the following conditions: 95 °C for 30 s, 40 cycles of 95 °C for 10 s, 60 °C for 20 s and 72 °C for 10 s to calculate cycle threshold (Ct) values. Each treatment was examined in three technical replicates and the melt curves were analyzed to ensure the primer specificity. The relative expression of each gene was estimated by using the $2^{-\triangle\triangle Ct}$ method (Livak and Schmittgen, 2001).

2.6. Evaluation of 'Guijiao 9′ resistant to Foc-TR4

In order to ascertain the resistance of the cultivars, Williams and 'Guijiao 9′ was evaluated in glasshouse and field trials by investigating the disease incidence and severity. Disease severity was graded according to the method described by Mak et al. (2004). Banana plants were inoculated with *Foc*-TR4 as described above for the sample preparation of sequencing. The pot trial experiment was repeated three times and each treatment had 30 plants. Validation of resistance in field experiments were carried out over a period spanning 6 years from 2012 to 2017. 'Guijiao 9′ was planted in *Foc*-TR4 infested fields in Hainan, Guangdong and Guangxi province.

2.7. Statistical analysis

The false discovery rate (FDR) was used to determine the threshold of P value in multiple test and analysis. The significance of gene expression difference was defined by FDR ≤ 0.001 and the absolute value of fold change |log2Ratio| ≥ 1 as the threshold (Smyth, 2004). Statistical analysis of the data was conducted using SPSS 25.0 software. Significance in all the comparisons among means was calculated by analysis of variance with Duncan's multiple comparison adjustment.

3. Results

3.1. Agronomic traits of 'Guijiao 9′

The new breeding variety 'Guijiao 9′ was the somatic mutant of Musa AAA Cavendish, which is the first *Foc*-tolerant banana variety bred by Guangxi Academy of Agricultural Sciences. It is suitable for planting in the autumn and the early spring growing seasons in Guangxi, Hainan and Guangdong regions. The whole growth cycle takes 310–350 days to complete when planting is performed in early spring in Guangxi province. The fruit pulp had 22.3% of soluble solid content and 0.43% of soluble titratable acid, with 19.6 mg/100 g of total sugar and 16.38 mg/100 g of Vitamin C (Table 1). The fruit showed an improved storability of 3–5 days at ambient temperature after ripening and before the fruit quality starts to degrade. Pseudostem is green accompanied with brown blotch, can reach 230.0–320.0 cm in height with a stem circumference of 70.0–90.0 cm. The yield of a single plant can total up to 40.0 kg of harvest weight.

3.2. Evaluation of 'Guijiao 9′ resistant to Foc-TR4 in glasshouse and field trials

'Guijiao 9′ was shown to have significantly lower disease incidence and severity after inoculation with *Foc*-TR4, compared to susceptible cultivar Williams in glasshouse (Table 2). The disease incidence of 'Guijiao 9′ was approximately 10% while the incidence of Williams under the same treatment reached up to 95%, and this reflected in the death rate of 'Guijiao 9' (11.7%) which is significantly lower than that of Williams. The resistant response in 'Guijiao 9′ is correlated with phenotypes that show few yellow leaf symptoms and healthy corms (Fig. 1A and B), while the leaves of Williams showed yellowing and necrotic symptoms as well as browning of the corms in cross dissections (Fig. 1C and D) at 30 days after inoculation with *Foc*-TR4. The pseudostems from all of the inoculated plants were scored for vascular discoloration and the infested samples were selected for fungal isolation and PCR-based assays to test existence of *Foc*-TR4 (Dita et al., 2010). All of the 20 samples were tested positive for *Foc*-TR4, which confirmed the accuracy of the diagnostic.

MR and HS abbreviate moderately resistant and highly susceptible phenotypes, respectively. Data are the means of three replicates. The

Table 1
Quality characteristics performance of Guijiao 9.

Banana variety	Banana peel thickness (mm)	Banana edible percentage (%)	Total soluble solid content (%)	Total sugar content (mg/100 g)	Vitamins content (mg/100 g)	Titratable acid content (%)
Guijiao 9	3.64a	69a	22.3a	19.6b	6.38a	0.43a
Williams	3.67a	72a	22.9a	20.3a	6.62a	0.33a

Table 2
Evaluation of banana germplasm for resistance to *Foc*-TR4.

Variety	Number of treated plants	Glasshouse Evaluation						Evaluation results
		Incidence (%)	Death rate (%)	corm symptoms		leaves symptoms		
				Severity grade	Resistance grade	Severity grade	Resistance grade	
Williams	30	95a	78.4a	6.1a	6a	3.9a	6a	HS
Guijiao 9	30	10b	11.7b	2.4b	2b	1.6b	2b	MR

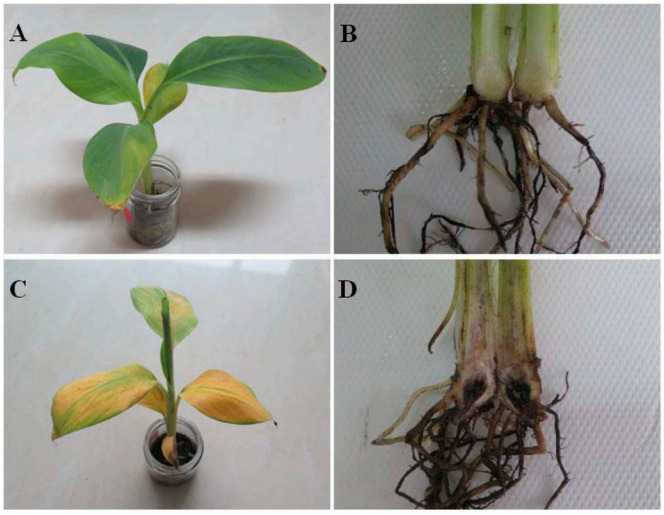

Fig. 1. Characteristic symptoms of Foc-TR4 in susceptible and resistant banana in glasshouse. Plants were inoculated with Foc-TR4 and photographs were taken 1 month after inoculation. A, a clonal plant of 'Guijiao 9'. B, the rhizome of 'Guijiao 9′ cut in half. C, a clonal plant of Williams. D, the rhizome of Williams cut in half.

data in the same column followed by different letters are significantly different at 0.05 levels. Statistical analysis of the data was performed using SPSS 25.0 software.

In order to determine whether 'Guijiao 9′ could confer TR4 resistance in infested regions, we assessed 'Guijiao 9′ for resistance in field trials over a 6 year period. The trial sites were commercial banana plantations in south China where *Foc*-TR4 has destroyed Cavendish banana plants. 'Guijiao 9′ was planted in *Foc*-TR4 infested plantations in Hainan, Guangdong and Guangxi provinces. The controls in the field trials were the *Foc*-TR4-susceptible cultivars Baxi and Williams. The trials were regularly inspected for plants showing leaf yellowing, wilting and/or pseudostem splitting (Fig. 2A, B, C and D). The susceptible Williams showed the typical *Foc*-TR4 symptoms such as leaf yellowing and pseudostem splitting while the resistant 'Guijiao 9′ has no obvious symptoms. The pseudostems were further examined for the presence of the reddish-brown vascular discoloration characteristic of *Foc*-TR4 infection (Fig. 2E, F, G and H). In general, disease developed faster in the control plants Baxi and Williams (Table 3). By the end of the trials, all of the control plants were either infected or dead in Huangliu and Dongying, Hainan. The incidence of *Foc*-TR4 infection of 'Guijiao 9′ plangting in Huangliu increased from 8.1% to 21.4%, while the incidence of Baxi increased from 72.5% to 100% in 2012 and 2013. The results in Dongying showed the similar situation in Huangliu. The incidence of 'Guijiao 9′ plangting in Dongying increased from 4.6% to 11.3%, while the incidence of Baxi increased from 52.7% to 100%. The results of *Foc*-TR4 screen performed in Xiaqiao, Guangdong field trial also indicated that 'Guijiao 9′ was more resistant to *Foc*-TR4 than Williams. In the trial of Ningwu of Guangxi, the disease incidence of 'Guijiao 9′ developed slowly, only increased to 7.23% from 2014 to 2017, while the incidence of Williams increased up to 33.6% in 4 years (Table 3).

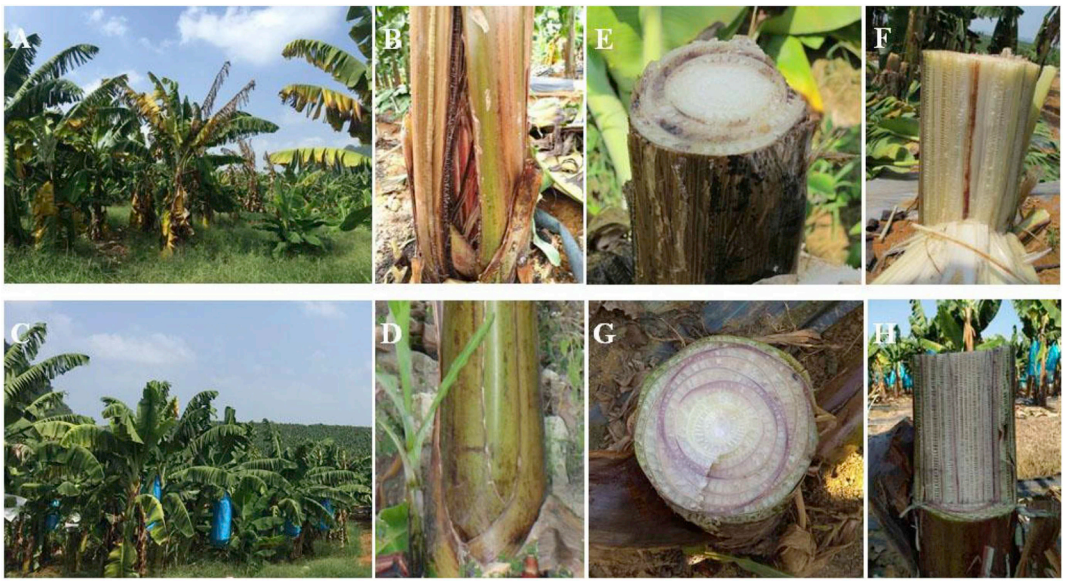

Fig. 2. Characteristic symptoms of *Foc*-TR4 in susceptible and resistant banana in field trials. External symptoms of *Foc*-TR4 in infected Williams **A** and **B** compared with resistant 'Guijiao 9′ **C** and **D**. Reddish-brown internal vascular discoloration of *Foc*-TR4 in infected Williams **E** and **F** compared with resistant 'Guijiao 9′ **G** and **H**. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Disease incidence and yield of 'Guijiao 9′ in different plantations from 2012 to 2017.

Location	Year	Line	Tested area (ha)	Number of treated plants	Yield per plant (kg)	Total yield (kg/ha)	Incidence of *Foc*-TR4 infection (%)
Huangliu, Hainan	2012	Guijiao 9	1.33	900	25.3b	62776.5a	8.10b
		Baxi		900	26.7a	19825.5b	72.50a
	2013	Guijiao 9	1.33	900	26.6a	51747.0a	21.40b
		Baxi		900	0b	0b	100.00a
Dongying, Hainan	2012	Guijiao 9	6.67	5000	27.6b	67663.6a	4.60b
		Baxi		5000	31.2a	39846.0b	52.70a
	2013	Guijiao 9	6.67	5000	28.7a	64915.5a	11.30b
		Baxi		5000	0b	0b	100.00a
Xiaqiao, Guangdong	2013	Guijiao 9	1.33	900	27.7b	65740.5a	12.10b
		Baxi		900	30.7a	38461.5b	53.60a
Ningwu, Guangxi	2014	Guijiao 9	0.87	655	24.8b	46500.0a	0b
		Williams		655	26.8a	46682.3a	7.10
	2015	Guijiao 9	0.87	655	25.3b	45084.6b	0.95b
		Williams		655	27.9a	46036.7a	8.33a
	2016	Guijiao 9	0.87	655	22.8b	44460.0a	5.05b
		Williams		655	26.5a	36296.5b	29.76a
	2017	Guijiao 9	0.87	655	26.2a	52149.0a	7.23b
		Williams		655	26.9a	35746.5b	33.60a

The data in the same column followed by different letters are significantly different at 0.05 levels.Statistical analysis of the data was performed using SPSS 25.0 software.

3.3. Illumina sequencing and mapping of reads to the Musa genome

In order to identify differentially expressed genes (DEGs) putatively involved in the resistance response, RNA-Seq analysis was carried out on total RNA samples from both resistant (cv. 'Guijiao 9′) and susceptible (cv. Williams) banana cultivars infected with *Foc*-TR4 and mock controls. Low-complexity and low quality reads were filtered out to obtain reliable reads. More than 48 million high quality reads were acquired and an average of 80% of the filtered reads mapped to the Musa genome (D'Hont et al., 2012) (mapped reads) for each of the two biological replicates (Table 4). Among the mapped reads, 75% matched to unique locations (unique mapped reads) and 5.8% displayed multiple matches (multiple mapped reads) (Table 4). Two biological replicates for each sample were sequenced and high Pearson's correlation coefficients (R^2) of FPKM (Fragments Per Kilobase of transcript per Million mapped reads) distribution between the two biological replicates were detected (R2 = 0.92–0.96, $p < 0.001$) (Figure S1). This indicated that the sequencing data of different replicates has a good level of repeatability.

3.4. Musa genes differentially expressed at Foc-TR4 different infection stages

To get a better understanding of the mechanism underlying *Foc*-TR4 resistance, comparative transcriptome analysis was performed. According to the method described by Audic (Audic and Claverie, 1997), we identified the differentially expressed genes (DEGs) of 'Guijiao 9′ and Williams at different *Foc*-TR4 infection stages. A total of 9612 DEGs were identified, highlighting the complex transcriptional reprogramming of banana roots in response to *Foc*-TR4 inoculation. The comparison of DEGs between different genotypes were summarized in Table 5.

Overall, a large number of genes were found up- or down-regulated in Williams at all times, whereas the pattern in 'Guijiao 9′ is different. A much smaller number of genes showed altered expression levels in 'Guijiao 9′ at 2 dpi (Table 5). After 2 d of *Foc*-TR4 infection, a total of 841 and 1876 TR4-responsive genes were identified in Williams and 'Guijiao 9′, respectively. Out of the 841 DEGs, 580 TR4-responsive genes were exclusively identified in Williams, whereas 1615 TR4-

Table 4
Mapping results of high quality reads against the Musa genomic sequence.

Sample	Experiment	Total high quality reads	Mapped reads (%)[a]	Multiple mapped reads (%)[a]	Uniquely mapped reads (%)[a]
Williams_0 dpi	1	50,856,000	40,943,320 (80.51)	2,681,628 (5.27)	38,261,692 (75.23)
	2	63,487,090	52,646,206 (80.90)	2,618,630 (4.97)	50,027,576 (75.93)
Williams _2 dpi	1	49,624,898	38,887,134 (78.42)	1,928,097 (4.96)	36,959,037 (73.44)
	2	48,942,902	39,533,395 (80.79)	1,082,970 (2.74)	38,450,425 (78.06)
Williams _4 dpi	1	58,923,602	47,126,727 (80.02)	2,763,527 (5.86)	44,363,200 (74.14)
	2	55,198,676	44,562,449 (80.68)	4,552,900 (10.21)	40,009,549 (70.49)
Williams _6 dpi	1	56,693,766	46,181,547 (81.54)	2,032,626 (4.40)	44,148,921 (77.10)
	2	56,614,438	45,219,423 (79.90)	2,234,364 (4.94)	42,985,059 (74.96)
Guijiao 9_0 dpi	1	75,428,666	62,006,014 (82.21)	7,343,219 (11.84)	54,662,795 (70.36)
	2	55,535,912	44,443,299 (80.04)	2,566,088 (5.77)	41,877,211 (74.23)
Guijiao 9_2 dpi	1	55,409,196	45,035,819 (81.33)	957,669 (2.13)	44,078,150 (79.17)
	2	55,472,936	44,396,265 (80.00)	2,186,182 (4.92)	42,210,083 (75.08)
Guijiao 9_4 dpi	1	50,274,456	40,298,975 (80.24)	2,222,985 (5.52)	38,075,990 (74.68)
	2	56,141,012	45,264,017 (80.57)	1,510,676 (3.34)	43,753,341 (77.26)
Guijiao 9_6 dpi	1	57,305,696	46,627,871 (81.42)	3,254,608 (6.80)	43,373,263 (74.6)
	2	53,147,606	43,181,881 (81.24)	4,013,087 (9.29)	39,168,794 (71.91)
Average		56,191,053	45,397,146 (80.61)	2,746,829 (5.81)	42,650,318 (74.49)

[a] The numbers in parentheses are the percentage of reads over total number of high quality reads.

Table 5
Differentially[a] expressed genes (DEGs) in Guijiao 9 and Williams during *Foc*-TR4 treatment.

		Williams	Unique in Williams	Guijiao 9	Unique in Guijiao 9	Common in both
DEGs at 2 dpi	Total	841	580	1876	1615	261
	Up-regulated	411	238	909	736	173
	Down-regulated	430	382	967	919	48
DEGs at 4 dpi	Total	904	771	590	457	133
	Up-regulated	443	332	386	275	111
	Down-regulated	428	419	204	195	9
DEGs at 6 dpi	Total	1034	379	4310	3655	655
	Up-regulated	320	40	3306	3026	280
	Down-regulated	714	342	1004	632	372

[a] Differentially refers to difference with the T0 point of the time course.

responsive genes were uniquely observed in 'Guijiao 9'. The remaining 261 genes were commonly regulated by *Foc*-TR4 in both Williams and 'Guijiao 9'. Similarly, a total of 904 and 590 TR4-responsive genes were identified in Williams and 'Guijiao 9′ after 4 d of *Foc*-TR4 infection, respectively. Out of the 904 DEGs, 771 TR4-responsive genes were exclusively identified in Williams, whereas 457 TR4-responsive genes were uniquely observed in 'Guijiao 9′ and 133 genes were commonly regulated by *Foc*-TR4 in both Williams and 'Guijiao 9'. After 6 d of *Foc*-TR4 infection, a total of 1034 and 4310 TR4-responsive genes were identified in Williams and 'Guijiao 9′, respectively. Out of the 1034 DEGs, 379 TR4-responsive genes were exclusively identified in Williams, whereas 3655 TR4-responsive genes were uniquely observed in 'Guijiao 9'. The remaining 655 genes were commonly regulated by *Foc*-TR4 in both Williams and 'Guijiao 9' (Table 5). It is noteworthy that among DEGs unique to Williams more are down-than up-regulated at all times, whereas the pattern in Guijiao 9 is different. We pay more attention to the DEGs that are either specifically regulated in 'Guijiao 9′ or those with transcript levels that are significantly different between 'Guijiao 9′ and Williams at a given time point. These two categories of genes are probably involved in the transcriptome signaling of *Foc*-TR4 resistance in 'Guijiao 9'.

3.5. Functional annotation of DEGs

To functionally categorize the DEGs, the analysis of Gene Ontology (GO) was performed using the Blast2go software on the DEGs identified in resistant cv. 'Guijiao 9'. These DEGs in 'Guijiao 9′ were assigned to three principal GO categories separately and the percentage of DEGs belonging to each category: biological process (72.5%), cellular component (42.2%) and molecular function (82.2%). In the biological function category, the DEGs were assessed by Blast2go resulting in 63 enriched GO terms. Amongst these the most relevant GO terms that are consistent with the response to fungal infection were response to stress (GO:0006950), signal transduction (GO:0007165), generation of precursor metabolites and energy (GO:0006091), and secondary metabolic process (GO:0019748) (Fig. 3). Thirty GO terms were grouped into the category of cellular component which are enriched for DEGs involved in the localization and targeting of resistance response and they include intracellular membrane-bounded organelle (GO:0043231), cell wall (GO:0005618) and cytoplasm (GO:0005737) (Fig. 3). Finally the GO category for molecular function identified DEGs that are involved in Ion binding (GO:0043167), transcription factor binding (GO:0008134) and oxidoreductase activity (GO:0016491) (Fig. 3). The 6581 DEGs specific to Guijiao 9 or significantly different in expression level between Williams and Guijiao 9 (threshold ≥ 2) were further analyzed.

3.6. Differentially expressed genes in response to Foc-TR4 infection

In order to facilitate the inspection of the plant and fungus networks and understand their interactions, the biological pathways mapping of the DEGs activated in resistant *cv.* 'Guijiao 9′ or significantly expression

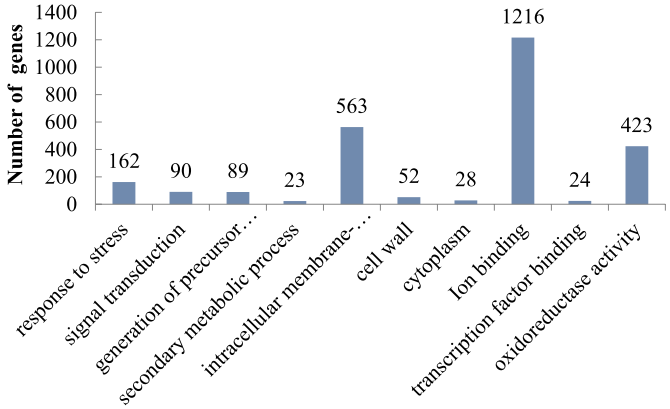

Fig. 3. GO annotation of DEGs response to *Foc*-TR4 found in resistant cv. 'Guijiao 9′.

between Williams and Guijiao 9 were performed using KEGG database (http://www.genome.jp/kegg/). Top 25 KEGG pathways were significantly enriched in 'Guijiao 9' (P-value < 0.05) (Table 6), of which 4 pathways, namely *Plant-pathogen interaction*, *Plant hormone signal transduction*, *Phenylpropanoid biosynthesis* and *Flavonoid biosynthesis* are normally regarded as disease-resistance related events. In addition, our data also suggested that ubiquitination may also participate in the incompatible interactions between 'Guijiao 9′ and *Foc*-TR4. These genes from the 4 categories which related to disease-resistance were mainly analyzed as follows.

3.7. Plant-pathogen interaction

The resistant plant 'Guijiao 9′ exhibited higher up-regulation of genes involved in plant-pathogen interaction compared to the susceptible plant Williams. A well known NBS-LRR gene RPS2 (GSMUA_Achr7G08530_001) accumulated more abundantly in 'Guijiao 9′ than in Williams throughout the whole experiment (Fig. 4A). At the late stages of infection, two disease resistance protein genes (RPM1, GSMUA_Achr9G28020_001, GSMUA_Achr6G29800_001) and one pathogenesis-related transcriptional activator gene (PTI6, GSMUA_Achr7G21980_001) were significantly up-regulation in 'Guijiao 9′ compared to Williams (Fig. 4A). A gene encoding chitin elicitor-binding protein (CEBiP, GSMUA_Achr1G02750_001) was only induced in 'Guijiao 9′ at 6 dpi. Three genes encoding Ca^{2+} -dependent protein kinase (CDPK, GSMUA_Achr1G05040_001, GSMUA_Achr2G17730_001, GSMUA_Achr3G18430_001) involved in hypersensitive response and signal transduction was up-regulated in 'Guijiao 9′ during the time course experiment. PR proteins have been regarded as pivotal components of defense proteins against pathogens attack in plants (Li et al., 2015). Three PR1 genes (GSMUA_Achr2G13210_001, GSMUA_Achr2G13240_001, GSMUA_Achr4G23100_001) were strongly

Table 6
Significantly enriched KEGG pathways of the defense-related DEGs.

pathays	ko term	DEGs tested	pathay ID
Plant hormone signal transduction	30	115	ko04075
Starch and sucrose metabolism	27	79	ko00500
Biosynthesis of amino acids	50	70	ko01230
Carbon metabolism	44	65	ko01200
Phenylpropanoid biosynthesis	14	55	ko00940
Protein processing in endoplasmic reticulum	22	53	ko04141
Plant-pathogen interaction	18	51	ko04626
Amino sugar and nucleotide sugar metabolism	22	44	ko00520
Cysteine and methionine metabolism	25	42	ko00270
Ribosome	34	40	ko03010
Viral carcinogenesis	18	36	ko05203
Glycolysis/Gluconeogenesis	18	32	ko00010
Glutathione metabolism	12	29	ko00480
Cell cycle	19	26	ko04110
Glycine, serine and threonine metabolism	20	25	ko00260
Oxidative phosphorylation	14	24	ko00190
RNA transport	19	23	ko03013
Glycerophospholipid metabolism	18	23	ko00564
Phenylalanine metabolism	11	20	ko00360
Flavonoid biosynthesis	10	16	ko00941
Peroxisome	17	18	ko04146
Carbon fixation in photosynthetic organisms	14	18	ko00710
Ubiquitin mediated proteolysis	11	22	ko04120
MAPK signaling pathway	5	10	ko04010
Calcium signaling pathway	5	10	ko04020

up-regulated in ‘Guijiao 9′ for 35, 41 and 38 fold change at 2 dpi. Another PR1 gene (GSMUA_Achr4G28250_001) was expressed rarely in Williams but expressed much higher at 6 dpi in ‘Guijiao 9'. A gene encoding MEKK1 (GSMUA_Achr2G07690_001) was expressed higher in ‘Guijiao 9′ at 6 dpi (Fig. 4A). A gene encoding heat shock protein 90 kDa beta (HSP90, GSMUA_Achr1G07620_001), downstream components of hypersensitive response, also showed positive expression in the time course experiment. A pathogenesis-related genes transcriptional activator gene PTI5 (GSMUA_Achr3G15550_001) was abundantly expressed at 2 dpi in ‘Guijiao 9' (Fig. 4A). However, two WRKY transcription factor 22 genes (GSMUA_Achr10G01150_001, GSMUA_Achr10G14710_001) were down-regulated in ‘Guijiao 9′ in the infection process.

3.8. Signal transduction

Plant hormones such as auxins, abscisic acid (ABA), ethylene (ET), salicylic acid (SA) and Jasmonates (JA) are known to play roles in mediating plant defense response against biotic stress. In this study, five SA-signaling genes were identified to be associated with ‘Guijiao 9′ against *Foc*-TR4. Among them, a gene encoding regulatory protein NPR1 (GSMUA_Achr6G00950_001) and a gene encoding transcription factor TGA (GSMUA_Achr8G05800_001) were up-regulated to high levels only at 6 dpi (Fig. 4B). In ET-signaling pathway, two genes encoding ethylene receptor (ETR, GSMUA_Achr11G02140_001, GSMUA_Achr8G14350_001) and two genes encoding EIN3-binding F-box protein (EBF1_2, GSMUA_Achr4G30680_001, GSMUA_Achr9G28510_001) had high expression levels at 2 dpi in ‘Guijiao 9' (Fig. 4B). A gene encoding ethylene-responsive transcription factor 1 (ERF1, GSMUA_Achr5G19610_001) was induced higher at the early stage of infection, while another ERF1 gene (GSMUA_Achr4G05520_001) was more abundant at the later stage (Fig. 4B). For JA signaling, a jasmonate-ZIM-domain (JAZ) gene (GSMUA_AchrUn_randomG27570_001) was induced higher in ‘Guijiao 9′ infected by *Foc*-TR4, but not expressed in Williams. A gene encoding jasmonic acid-amino synthetase (JAR1, GSMUA_Achr4G08460_001) and a gene encoding transcription factor MYC2 (GSMUA_Achr4G16980_001) involved in JA signaling were significantly up-regulated in ‘Guijiao 9′ compared to Williams at the early stage of infection (Fig. 4B). Of all ABA-signaling related genes, two genes encoding protein phosphatase 2C (PP2C, GSMUA_Achr10G14430_001, GSMUA_Achr11G21980_001) were up-regulated rapidly to a high level in ‘Guijiao 9′ at 4 dpi and 6 dpi in response to the *Foc*-TR4 infection. Two ABA responsive element binding factor genes (ABF, GSMUA_Achr6G15970_001, GSMUA_Achr6G30550_001) and one serine/threonine-protein kinase gene (SnRK2, GSMUA_Achr11G11970_001) were found up-regulated dramatically in ‘Guijiao 9′ at the late stage of infection. A gene belong to abscisic acid receptor PYR/PYL family (GSMUA_Achr10G27940_001) was found up-regulated in ‘Guijiao 9′, and down-regulated in Williams at the time course experiment (Fig. 4B). Other genes associated with auxin also responded to pathogen challenge. Of the auxin related genes, two encoding auxin influx carrier (LAX, GSMUA_Achr11G24200_001, GSMUA_Achr6G25630_001) and three encoding auxin-responsive protein (IAAs, GSMUA_Achr3G16300_001, GSMUA_Achr4G21030_001, GSMUA_Achr9G02960_001) were up-regulated in ‘Guijiao 9′ during the infection process (Fig. 4B).

In addition, MAPK and Calcium signals were also identified. A MAPK-signaling gene, mitogen-activated protein kinase kinase 3 (MEKK3, GSMUA_Achr9G19130_001) was up-regulated in ‘Guijiao 9′ and down-regulated inWilliams. Two calcium signaling genes (CaM, GSMUA_Achr7G01390_001, GSMUA_AchrUn_randomG12510_001) presented more repression in ‘Guijiao 9′, indicating their negative regulation in the Fusarium wilt disease resistance of ‘Guijiao 9'.

3.9. Secondary metabolism

Phenylpropanoid biosynthesis and flavonoid biosynthesis, belonging to secondary metabolism, have been proved to be involved in plant defense response through reinforcement of plant cell walls and phytoalexins synthesis. At 2 dpi, two beta-glucosidase genes (bglB, GSMUA_Achr1G26250_001, GSMUA_Achr3G29530_001), a 4-coumarate–CoA ligase gene (4CL, GSMUA_Achr2G18040_001) and a cinnamyl-alcohol dehydrogenase gene (CAD, GSMUA_Achr4G26450_001) accumulated more abundantly in ‘Guijiao 9′ than in Williams (Fig. 4C), indicating cell wall responses that reinforce plant's defense at the cellular level. Peroxidases (POD) are PR-9 gene expressions are induced in plant tissues upon pathogen infection and this may lead to the production of reactive oxygen and nitrogen species which prevent cellular diffusion of pathogens into the host plants. In this study, more than a dozen of POD related genes are identified in the phenylpropanoid biosynthesis pathway. The transcriptional changes may suggest an activated defense mechanism in response to *Foc*-TR4 infection. Among them, Four genes encoding Peroxidases (POD, GSMUA_Achr10G01840_001, GSMUA_Achr5G29600_001, GSMUA_Achr2G15480_001, GSMUA_Achr2G21710_001) were dramatically expressed in ‘Guijiao 9′ throughout the entire period of the experiment (Fig. 4C). In the flavonoid biosynthesis pathway, a gene encoding leucoanthocyanidin dioxygenase (LDOX, GSMUA_Achr5G04080_001) was expressed much higher in ‘Guijiao 9′ than in Williams at 2 dpi (Fig. 4C). A gene encoding chalcone synthase (CHS, GSMUA_Achr10G12260_001) was induced exclusively in ‘Guijiao 9′ and another CHS gene (GSMUA_Achr6G10910_001) accumulated abundantly in the resistant cultivar at the whole infection process. Two genes encoding caffeoyl-CoA O-methyltransferase (CCoAMs, GSMUA_Achr8G15650_001, GSMUA_Achr9G25230_001) were up-regulated in ‘Guijiao 9′ at 6 dpi (Fig. 4C).

3.10. Ubiquitin mediated proteolysis

Ubiquitination is known to play an important role in plant defense. In response to *Foc*-TR4 infection, ‘Guijiao 9′ induces the expression of a gene encoding RING finger and CHY zinc finger domain-containing protein 1 (RCHY1, GSMUA_Achr8G12910_001) and two genes encoding ubiquitin-conjugating enzyme E2 H (UBE2H, GSMUA_Achr1G01320_001 and GSMUA_Achr5G13210_001) at 2 dpi (Fig. 4D). At the later stage of

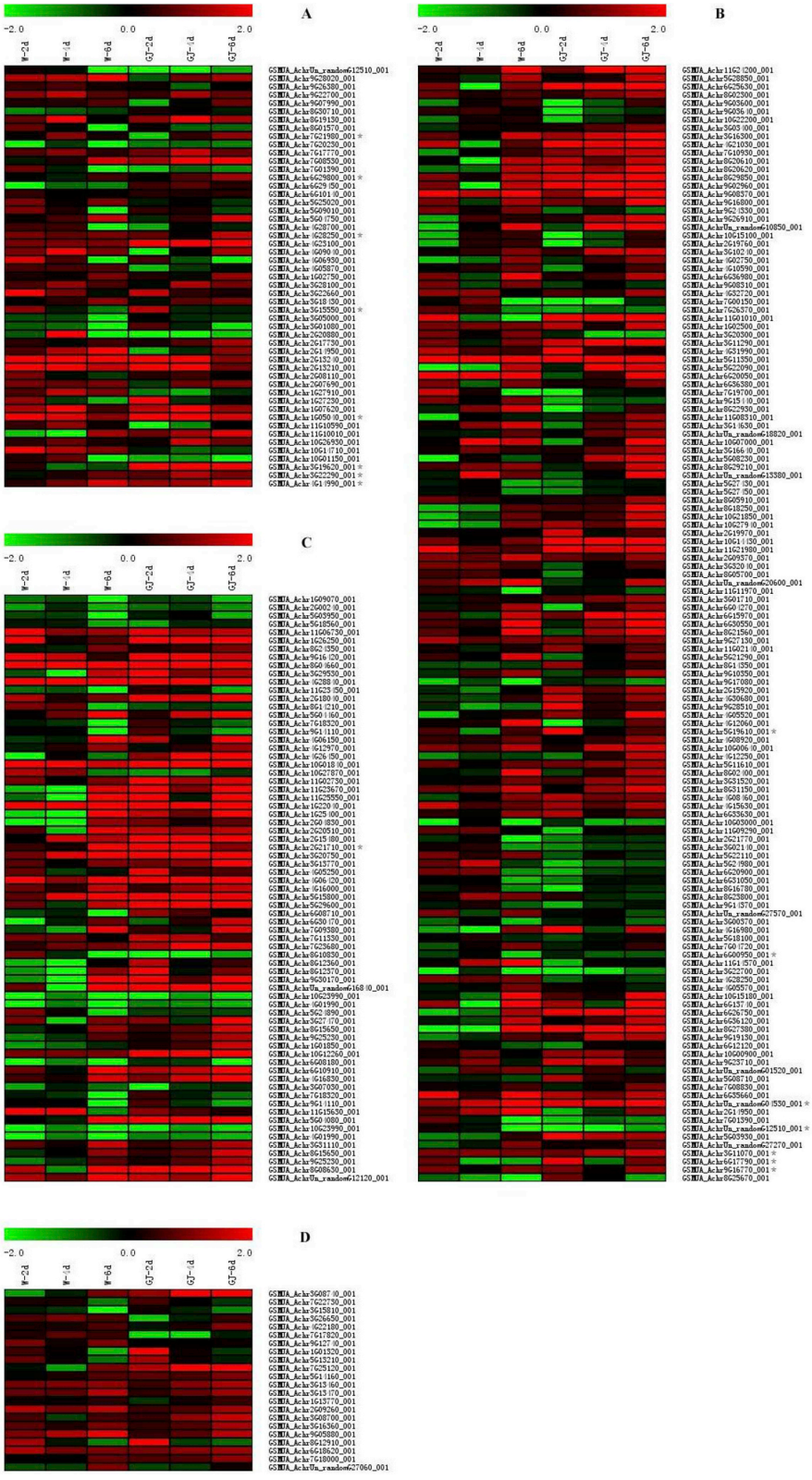

Fig. 4. Expression patterns of *Foc*-TR4 resistance-related genes. The color bars represent the values of log2-fold change at the same time point, ranging from green (−2) to red (2). A: plant-pathogen interaction; B: signal transduction; C: secondary metabolism; D: ubiquitin mediated proteolysis. The asterisk means the gene has been validated by qRT-PCR in the following chapter. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

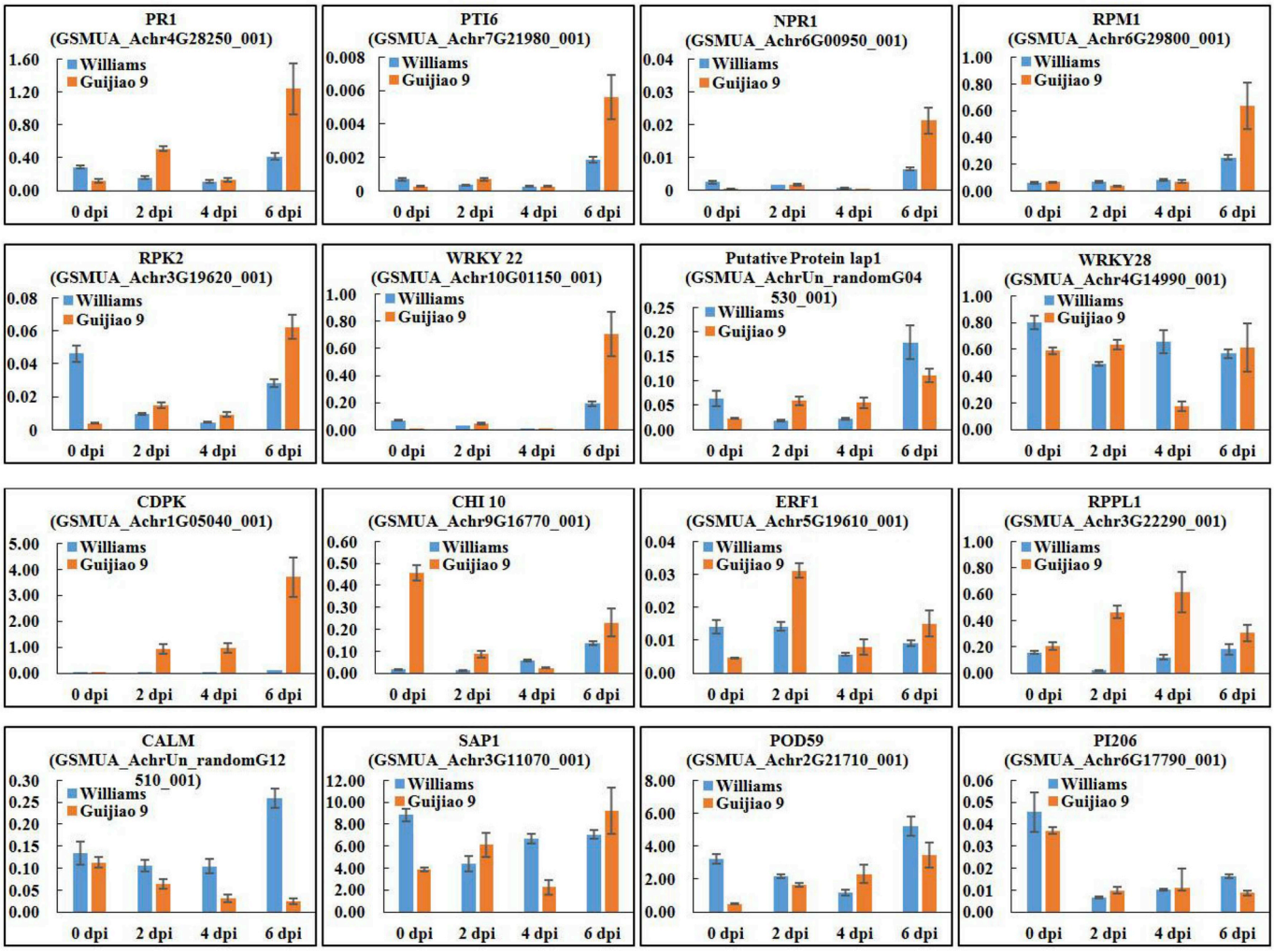

Fig. 5. Expression profiles of the 16 DGEs in 'Guijiao 9′ and Williams from 0, 2, 4 and 6 day inoculations with *Foc*-TR4. The qRT-PCR of each sample was performed three times and each treatment was amplified in triplicate. The red column indicates the resistant 'Guijiao 9′, and the blue column indicates the susceptible var. Williams. Dpi indicates days after inoculation. These DEGs were marked with an asterisk in Fig. 4. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

infection, one S-phase kinase-associated protein 2 gene (SKP2, GSMUA_Achr6G18620_001), one ubiquitin-conjugating enzyme E2 C gene (UBE2C, GSMUA_Achr3G08740_001), one ubiquitin-conjugating enzyme E2 O gene (UBE2O, GSMUA_Achr7G25120_001) and three E3 ubiquitin-protein ligase SIAH1 genes (SIAH1, GSMUA_Achr3G08700_001, GSMUA_Achr3G16360_001 and GSMUA_Achr9G05880_001) were more expressed in 'Guijiao 9' (Fig. 4D). In contrast, in the incompatible interaction, a gene encoding ubiquitin-conjugating enzyme E2 D (UBE2D, GSMUA_Achr7G17820_001) was less expressed at 2 dpi and 4 dpi.

3.11. Validation of DEGs by qRT-PCR

In order to confirm the results of the Illumina sequencing, eighteen DEGs were selected based on their expression patterns in 'Guijiao 9′ and Williams at different time points for quantitative RT-PCR (qRT-PCR) by using the same RNA extracts as for RNA-seq experiments. Of the 16 genes analyzed, six genes (PR1, GSMUA_Achr4G28250_001; NPR1, GSMUA_Achr6G00950_001; RPM1,GSMUA_Achr6G29800_001; PTI6, GSMUA_Achr7G21980_001; RPK2, GSMUA_Achr3G19620_001; WRKY22, GSMUA_Achr10G01150_001) were significantly up-regulated and one gene (Putative Protein lap1, GSMUA_AchrUn_randomG04530_001) was down-regulated in 'Guijiao 9′ compared to Williams at 6 dpi (Fig. 5). These results are consistent with the findings from the RNA-Seq results. CDPK (GSMUA_Achr1G05040_001), CHI10 (chitinase 10, GSMUA_Achr9G16770_001) and RPPL1 (Putative disease resistance RPP13-like protein 1, GSMUA_Achr3G22290_001) were expressed abundantly in 'Guijiao 9′ throughout the whole experiment and a similar trend in their expression profiles were characterized by the RNA-Seq technique (Fig. 5). ERF1 (GSMUA_Achr5G19610_001) was expressed at a higher level in 'Guijiao 9′ than Williams at the early stage of infection but its expression level appears to be down-regulated at 4 dpi. Two genes (CaM, GSMUA_AchrUn_randomG12510_001; PI206, GSMUA_Achr6G17790_001) were also down regulated in 'Guijiao 9′, while other three genes (POD59, GSMUA_Achr2G21710_001; WRKY 28, GSMUA_Achr4G14990_001; SAP1 Zinc finger A20 and AN1 domain-containing stress-associated protein 1, GSMUA_Achr3G11070_001) presented the similar expression profile in the two varieties (Fig. 5). As expected, these data confirmed the reliability of the transcriptome analysis by RNA-Seq.

4. Discussion

RNA-Seq, based on deep sequencing, is a reliable and cost-effective approach for huge sequence data collection and analysis. Transcriptome analyses can help uncover mechanisms that control resistance to

Fusarium wilt disease in banana. Previous studies have reported the gene expression profiling for the host-pathogen interaction (Li et al., 2013), and comparison of transcriptomes between resistant and susceptible genotypes (Li et al., 2012; Bai et al., 2013).

'Guijiao 9′ is a somatic Cavendish type mutant that has shown to produce improved yields under *Foc*-TR4 infested fields. The resistance and agronomic traits of 'Guijiao 9′ have been investigated and well characterized for multiple years (Wei et al., 2016). The resistance and agronomic traits of 'Guijiao 9′ had already been investigated and well characterized for five years before the RNA-seq experiment. The genome of banana (*Musa acuminata*) was firstly sequenced in 2012 (D'Hont et al., 2012), and the sequencing reads we obtained are aligned primarily by mapping onto the sequenced reference genome. Plants have evolved complex signaling and defense pathways in response to pathogen attacks. Identifying key components of transcriptome response to *Foc*-TR4 may facilitate the discovery and annotation of important genes in the plant's defense response. Here, we performed a comprehensive transcriptome profiling against *Foc*-TR4 on the resistant and susceptible cultivars 'Guijiao 9′ and Williams respectively.

4.1. Gene expression changes in response to Foc-TR4 infection

We identified differentially expressed genes (DEGs) of 2, 4 and 6 dpi for each cultivar compared with the 0 dpi. A total of 6776 and 2779 DEGs in resistant 'Guijiao 9′ and susceptible Williams banana, respectively, were identified. qRT-PCR results validated the 16 candidate DEGs identified from the RNA-Seq study. The expression levels of candidate genes correlated positively with the number of reads mapped to these genes. During the onset of *Foc*-TR4 infection, the resistant 'Guijiao 9′ samples showed a much higher number of DEGs than the susceptible Williams, which may indicate that the different banana cultivars may activate different genes or gene networks which control the underlying response to *Foc*-TR4 infection. These findings are similar to what has been previously described for the interaction between banana and *Foc*-TR4, where the resistant cultivar showed much more DEGs than the susceptible one (Bai et al., 2013).

4.2. Plant-pathogen interaction

Plants have established a series of defense mechanisms against pathogens during their co-evolution. The innate immunity in plant is triggered via the response of pattern recognition receptors (PRRs) to pathogen-associated molecular patterns (PAMPs), thereby providing the first layer of defense mechanism for pathogens (PTI) (Bernoux et al., 2011). Chitin, a major component of fungal cell walls, is one of the most common PAMPs in fungi (Chen and Ronald, 2011). The chitin elicitor-binding protein (CEBiP) and the chitin elicitor receptor kinase (CERK1) have been identified as critical components of the plant signaling pathway that recognizes chitin oligosaccharides (Chen and Ronald, 2011). In this study, CEBiP was only induced in 'Guijiao 9′ at 6 dpi, which is consistent with the reports that the expression of CEBiP were up-regulated in resistant banana cultivar (Li et al., 2012; Bai et al., 2013). The results suggested that TR4 infection trigger an immune response in resistant 'Guijiao 9'. The time course of CEBiP up-regulation in 'Yueyoukang 1′ and 'Nongke No 1' (Li et al., 2012; Bai et al., 2013) was a little earlier than in 'Guijiao 9′, which may be caused by the difference of cultivar. The genes related to CERK1 showed no significant changes in their expression levels in the resistant cultivar, which may indicate that they did not play a role in incompatible interaction between 'Guijiao 9′ and *Foc*-TR4. The second layer of immunity relies on the recognition of pathogen virulence molecular effectors by plant-specific resistance proteins (R proteins) in direct or indirect ways, which leads to ETI (Jones and Dangl, 2006). ETI is often initiated by a subset of R genes. The largest class of R genes encodes a NB-LRR class of proteins (Dangl and Jones, 2001). RPM1 and RPS2 genes, belonging to NB-LRR genes, were accumulated abundantly in 'Guijiao 9′ after *Foc*-TR4 inoculation compared to Williams. RPM1 conferred resistance to *Pseudomonas syringae* expressing either avrRpm1 or avrB effector (Boyes et al., 1998). The accumulation of RPM1 in 'Guijiao 9′ indicated that it may be required to mediate incompatible interactions in banana, which is in agreement with the previous work (Li et al., 2012; Bai et al., 2013). The up-regulation of RPS2 in 'Guijiao 9′ at the all stages of infection may suggest that it plays an important role in mediating or maintaining host resistance against the pathogen, while the previous reports did not find the accumulation of RPS2 in other resistant banana cultivars (Li et al., 2012; Bai et al., 2013).

Calcium, which is regarded as an important secondary messenger, plays an essential role in the production of hypersensitive reactions in the plant response to biotic stress. The genes related to CNGC and Rboh (Respiratory burst oxidase homolog) in 'Guijiao 9′ were not expressed differentially after TR4 infection, suggesting there was no HR-like reaction in inoculated Guijiao 9. The calcium (Ca^{2+}) related proteins include CaM, CaM-binding protein, CDPK, and Ca^{2+}-CaM-regulated protein phosphatase all of which are involved in calcium mediated signaling in plants (Lecourieux et al., 2006). Recent evidence suggests that Ca^{2+} signaling via CDPKs, CBL/CBL-interacting protein kinases and Ca^{2+} signaling may play a part in the melon-*Monosporascus cannonballus* interaction and melon-*Fusarium oxysporum* f. sp. *melonis* Snyd. & Hans race 1.2 interaction (Sebastiani et al., 2017). In this research, we observed the expression of CDPK gene was up-regulated in 'Guijiao 9′ in response to *Foc*-TR4 infection, while CaM and CML genes had lower transcript abundance, indicating Ca^{2+} signaling may be engaged in a complicated manner in banana-TR4 interaction. The PR1 gene is often used as a molecular marker of disease resistance. Researches have shown that the expression level of PR1 increased with pathogen infection (Hamamouch et al., 2011). Here, we found PR1 genes accumulated dramatically in 'Guijiao 9′ at 2 dpi (Fig. 4), suggesting that PR1 genes may be involved in the incompatible interaction at the early stage of infection.

4.3. Transduction of defense signals

Multiple phytohormones, including Jasmonic acid (JA), salicylic acid (SA), and ethylene (ET), are involved in plant defense processes. JA plays regulatory roles in plant development and responses to fungal infection. Plant resistance to necrotrophic pathogens, such as *Fusarium oxysporum* and *Fusarium fujikuroi*, is activated by JA signaling (Matic et al., 2016). We observed that JAR1, COI1, and MYC2, were up-regulated in 'Guijiao 9′ and down-regulated in Williams, indicating that JA pathway was activated in the disease resistance response to *Foc*-TR4. This is in line with previous reports showing that the JA pathway mediates plant resistance to pathogens (Li et al., 2012; Bai et al., 2013). SA is a critical factor in local and systemic acquired resistance responses. The SA response pathway is usually thought to be effective against biotrophic pathogens. NPR1 is an essential regulator of plant systemic acquired resistance (SAR), which confers immunity to abroad-spectrum of pathogens. SAR induction results in accumulation of the signal molecule SA, which induces defense gene expression via activation of NPR1 (Mou et al., 2003). NPR1 has been shown interacting with the TGA subclass of basic leucine-zipper (bZIP) transcription factors. These TGA factors can bind to the as-1 element present in the PR1 gene promoter, which is required for SA-responsiveness of the gene (Mou et al., 2003). In this present study, SA-signaling associated genes encoding NPR1 and TGA did not show significant difference between the two cultivars, suggesting that SA may or may not be directly involved in the defense response towards *Foc*-TR4. Future work may involve the determination of SA levels in the roots of these cultivars. In Arabidopsis, PR1 and SA levels can be quantified to measure the pattern of SAR (Kiefer and Slusarenko, 2003). In our study, PR1 gene expression was strongly induced in 'Guiijao 9′ but not in Williams suggesting that PR1 may play a role in the plant-pathogen interactions, but future experiments will need to address whether the response mediated through

PR1 is part of the SA-signaling pathway. Pathogen attack triggers complex signaling cascades regulated by these signaling molecules, resulting in the expression of defense-related genes such as those encoding PR proteins. In ET-signaling pathway, genes including ETR, EBF1 and ERF1 played as positive regulators in 'Guijiao 9′ response to *Foc*-TR4 infection, which could have activated ET signaling in the disease resistance.

4.4. Expression of defense related products

Secondary metabolism has been proved to play an important role in plant disease resistance. The pathway of phenylpropanoid, flavonoids and stilbenoid biosynthesis participate in the formation of secondary resistance metabolites, such as phytoalexin, lignin and phenolic compounds (Zhang et al., 2017). Here, eight DEGs were identified to participate in phenylalanine metabolic pathways. Among them, two bglBs (GSMUA_Achr1G26250_001, GSMUA_Achr3G29530_001), 4CL (GSMUA_Achr2G18040_001) and CAD (GSMUA_Achr4G26450_001) showed increased transcript levels in *Foc*-TR4 treated roots of the resistant 'Guijiao 9'. These genes are involved in the synthesis of lignin polymers which could lead to the reinforcement of the cell wall during the activation of the resistance response. In comparison, up-regulation of transcripts were not detected in all four genes in Williams. A series of peroxidase (POD) genes (GSMUA_Achr10G01840_001, GSMUA_Achr5G29600_001, GSMUA_Achr2G15480_001, GSMUA_Achr2G21710_001) were also found to be induced after *Foc*-TR4 infection. Studies have shown that POD participates in the polymerization of monolignols into lignin and confers resistance to a wide range of plant pathogens (Marjamaa et al., 2009). Two CHSs genes (GSMUA_Achr10G12260_001, GSMUA_Achr6G10910_001), and a LDOX gene (GSMUA_Achr5G04080_001) involved in flavonoids biosynthesis accumulated in the roots of 'Guijiao 9′ during banana-TR4 incompatible interaction at all the time points. CcoAM (GSMUA_Achr8G15650_001) displayed an increased level of transcripts in the resistant cultivar 'Guijiao 9′ at 6 dpi. Taken together, our results suggest that the biosynthesis of secondary metabolites may be important components of the plant defense in response to *Foc*-TR4 infection.

4.5. Regulatory role of ubiquitination in disease resistance

Ubiquitination is proposed to play a critical role in plant defense response against pathogen challenge (Li et al., 2015; Sebastiani et al., 2017). There are three major stages during the tagging of a protein to ubiquitination and consequently there are three major enzyme classes, including E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzyme and E3 ubiquitin ligase (Craig et al., 2009). Here, two genes encoding E2 enzyme were significantly up-regulated at the early stage of infection in the resistant cultivar, suggesting a positive regulation of this enzyme in *Foc*-TR4 resistance. Studies have indicated a prominent role for E3 ubiquitin ligases during plant defence (Li et al., 2015). We found that four genes encoding E3 ubiquitin ligases were positively correlated with *Foc*-TR4 resistance. This is the first ever study in which E3 ubiquitin is shown to participate in *Foc* resistance. A gene (GSMUA_Achr6G18620_001) encoding SKP2 subunit of E3 ligase SCF complex that is a crucial component of R-mediated resistance (Craig et al., 2009), exhibited up-regulation at 6 dpi in the incompatibility, indicating SCF complex may also exert positive effect in *Foc* resistance. This is not consistent with the previous reports. Overall, ubiquitination driven by E2 conjugating enzymes and E3 ligases could have played important roles in R-mediated *Foc* resistance in 'Guijiao 9'.

5. Conclusion

In this study, we performed root xylem transcriptome analysis of resistant 'Guijiao 9′ and the susceptible Williams banana using RNA-Seq and compared expression profile differences between the two cultivars infected by *Foc*-TR4 for 2, 4 and 6 dpi. The differences in terms of DEGs between the resistant cultivar 'Guijiao 9′ and the susceptible cultivar Williams provide a comprehensive overview of the transcriptome of two banana cultivars with contrasting disease responses. The resistant 'Guijiao 9′ activated multiple resistance pathways, and DEGs were involved in the plant-pathogen interaction, signal transduction, second metabolism and other processes, suggesting that pathogen response is regulated by multi-gene networks. Among the DEGs, some defense-related genes showed different expression patterns between 'Guijiao 9′ and other resistant cultivars ('Yueyoukang 1′ and 'Nongke No 1′) challenged with *Foc*-TR4, suggesting that 'Guijiao 9′ had a different resistance mechanism. The resistance to *Foc*-TR4 was mainly signaled by JA/ET, which act in tandem to induce local and systemic expression of defense genes. It will provide insights into the host-pathogen interactions and uncovering the resistant mechanism of banana.

Author's contributions

J S, J Z, S W and J L proposed, organized and planned the experiments. J S, L P, J Z, H F and C L carried out and performed the experiments, J S wrote the manuscript draft, S Z polished the draft, all authors commented and contributed to the preparation of the final manuscript.

Conflicts of interest

The authors declare that the submitted work was not carried out in the presence of any personal, professional or financial relationships that could potentially be constructed as a conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.05.022.

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