



Universidade de Brasília

Instituto de Ciências Biológicas

Departamento de Fitopatologia

Programa de Pós-Graduação em Fitopatologia

**Mecanismos de defesa em *Musa* spp. à Murcha de Fusarium:
análises histológicas e transcritômicas da *interação Musa*
acuminata subsp. *burmannica* acesso 'Calcutta 4' à *Fusarium*
oxysporum f. sp. *cubense* raça subtropical 4**

ÉRICA DE CASTRO COSTA

BRASÍLIA - DISTRITO FEDERAL

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Tese apresentada à
Universidade de Brasília como
requisito para a obtenção do
título de Doutor em
Fitopatologia pelo Programa de
Pós-Graduação em
Fitopatologia.

Orientador

Prof. Dr. Robert Neil Gerard Miller

BRASÍLIA - DISTRITO FEDERAL

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"Nada na vida deve ser temido, apenas compreendido. Agora é hora de compreender mais, para que possamos temer menos."
— Marie Curie

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RESUMO

A banana (*Musa* spp.) é considerada uma das frutas mais consumidas no mundo e componente básico da alimentação de milhões de pessoas. É considerada como fonte importante de carboidratos, vitaminas e minerais. As bananas comerciais são oriundas do cruzamento entre duas espécies selvagens, *Musa acuminata* (A) e *Musa balbisiana* (B). As cultivares que possuem características uniformes são classificadas dentro de subgrupos. Muitas dessas cultivares são estéreis, com o desenvolvimento dos frutos ocorrendo por partenocarpia. Devido à consequente variabilidade genética limitada, as cultivares comerciais tendem a ser suscetíveis ao ataque de inúmeras pragas e doenças, responsáveis pelas maiores perdas na produtividade das lavouras e na qualidade dos frutos. A Murcha de Fusarium é uma doença clássica de murcha vascular causada pelo fungo *Fusarium oxysporum* f. sp. *cubense* (Foc). As perdas podem atingir 100% da produção nas áreas afetadas, uma vez que a persistência de clamidósporos em solos infestados inviabiliza a produção continuada de banana. Foc é subdividida em quatro raças, baseada na patogenicidade a diferentes cultivares de bananas. A raça 1 é patogênica às cultivares Gros Michel (AAA), Maçã e Prata (AAB), a raça 2 é patogênica às cultivares Bluggoe (ABB), e a raça 4 patogênica a todos os subgrupos e à Cavendish (AAA). A raça 4 é subdividida em raça tropical 4 (TR4) e subtropical 4 (STR4), por STR4 ser patogênica a cultivares 'Cavendish' apenas em regiões subtropicais e com menor agressividade que TR4. A medida de controle mais eficiente é através da resistência genética, sendo a genômica uma ferramenta importante de introgressão de genes de cultivares selvagens e resistentes à patógenos, em cultivares comerciais. Com isso, o objetivo desse trabalho foi identificar e validar genes alvo envolvidos na resistência de *Musa* durante a interação com o patógeno Foc STR4, via análise de RNAseq. Para isso, dois genótipos contrastantes foram utilizados para investigação da interação com o isolado de Foc STR4 isolado CNPMF 218A: 'Calcutta 4' (genótipo selvagem resistente) e 'Prata-Anã' (genótipo comercial suscetível). A inoculação foi realizada utilizando arroz inoculado na concentração de 10^6 UFC/g. O RNA total foi extraído de amostras de raízes coletadas aos 1, 2 e 4 dias após a inoculação (DAI) para as análises de RNAseq. Análises histológicas foram realizadas para investigar respostas de defesa da planta, como a deposição de calose e produção de compostos fenólicos nos mesmos tempos de inoculação, bem como acompanhar o processo de infecção do isolado de STR4 em ambos os genótipos acrescidos dos dias 8 e 15 DAI. O estudo da histologia mostrou resposta de defesa pós formadas apenas em 'Calcutta 4' com a formação de calose e compostos fenólicos aos 1 e 2 DAI. Somente foi observada evidência de colonização de Foc STR4 em 'Prata-anã', aos 8 e 15 DAI. Um total de 1416 genes diferencialmente expressos foram observados durante a interação de 'Calcutta 4' e Foc STR4, com 270, 872 e 81 DEGs aos 1, 2 e 4 DAI, respectivamente. Destes, 752 DEGs foram regulados positivamente aos 2 DAI, indicando uma rápida ativação de genes de defesa contra o ataque de Foc STR4. Através das análises funcionais utilizadas, GO, KEGG, KOG, Interpro e Mapman, foram identificados diversos DEGs envolvidos nas respostas imediatas de defesa de 'Calcutta 4' à Foc STR4, como a ativação de PRRs (receptores de reconhecimento de padrões), PRs (proteínas relacionadas à patogênese), quitinases, fitormônios como etileno (ET), ácido jasmônico (JA) e ácido abscísico (ABA), genes de resistência (R) como NLRs (*nucleotide-binding site leucine-rich repeat*), fatores de transcrição (TFs), espécies reativas de oxigênio (ROS), SAR (resistência sistêmica adquirida), fitoalexinas e calose. Esses genes são responsáveis por participarem em diferentes vias funcionais como a resposta a estímulos, interação planta-patógeno, metabolismo secundário,

vias de fitormônios e estresse biótico. A ação conjunta das vias sinalizadas são fundamentais para o processo de resistência do genótipo selvagem à *Foc* STR4. A descoberta desses genes ajudará no processo de seleção de genes candidatos de resistência à *Foc* no uso de ferramentas de melhoramento genético, como a introgressão de genes de resistência de genótipos selvagens em cultivares comerciais, como 'Prata-anã', e nas demais técnicas de edição gênica como CRISPR. Essa estratégia é promissora para o desenvolvimento de variedades de bananas resistentes à Murcha de Fusarium causadas por *Foc* STR4 e TR4, onde a implementação de organismos geneticamente modificados (OGMs) com foco em genes associados a interações planta-patógeno e estresse biótico podem fortalecer o cultivo de banana e reduzir o impacto de epidemias de Murcha de Fusarium na produção nacional.

Palavras-chave: banana, RNAseq, Murcha de Fusarium, estresse biótico, resistência genética, genômica

ABSTRACT

Banana (*Musa* spp.) is one of the world's most important consumed fruits and a basic component of the diet of millions of people, where it is considered an important source of carbohydrates, vitamins and minerals. Commercial bananas originate from crossings of two wild species, namely *Musa acuminata* (A) and *Musa balbisiana* (B). Cultivars with uniform characteristics are divided into subgroups. Many such cultivars are sterile, with fruit development via parthenocarpy. Given the resultant limited genetic variability, commercial cultivars tend to be susceptible to attack by numerous pests and diseases, which are responsible for considerable losses in terms of yield and fruit quality. Fusarium wilt is a classic vascular wilt disease caused by the fungal pathogen *Fusarium oxysporum* f. sp. *cubense* (*Foc*). Losses can reach 100% of production in affected areas, with persistence of fungal chlamydospores in infested soil making land inappropriate for continued banana production. *Foc* is subdivided into four races, based on pathogenicity to different banana cultivars. Race 1 is pathogenic to Gros Michel (AAA), Silk and Pome (AAB) cultivars, race 2 is pathogenic to Bluggoe (ABB) cultivars, and race 4 is pathogenic to all subgroups and Cavendish (AAA). Race 4 is subdivided into tropical race 4 (TR4) and subtropical race 4 (STR4), with STR4 pathogenic to 'Cavendish' cultivars only in subtropical regions and with less aggressiveness than TR4. The most efficient control measure is through genetic resistance, with genomics being one of the important tools for introgression of genes from wild and pathogen-resistant cultivars into commercial cultivars. As such, the aim of this study was to identify and validate target genes involved in the resistance response in *Musa* during the interaction with the pathogen *Foc* STR4, via RNA-seq analysis. For this, two contrasting materials were employed for investigation of the interaction with *Foc* STR4 isolate CNPMF 218A: 'Calcutta 4' (a resistant wild genotype) (AA) and 'Prata-Anã' (a susceptible commercial genotype) (AAB). Inoculation of root tissues was performed using rice inoculated with *Foc* at a concentration of 10^6 CFU/g. Total RNA was extracted from root samples collected at 1, 2 and 4 days after inoculation (DAI) for RNA-seq analyses. Histological analyses were performed to investigate plant defence responses of callose deposition and production of phenolic compounds, as well as to accompany the infection process of the STR4 isolate, with assessments conducted at 1, 2, 4, 8 and 15 DAI. Histological studies revealed post-infection defence responses occurring exclusively in 'Calcutta 4', which included callose deposition and phenolic compound production at 1 and 2 DAI. Colonization of *Foc* STR4 was observed only in 'Prata-anã', at 8 and 15 DAI. Based on RNA-seq analysis, a total of 1,416 differentially expressed genes (DEGs) were identified during the interaction between 'Calcutta 4' and *Foc* STR4, with 270, 872, and 81 DEGs detected at 1, 2, and 4 DAI, respectively. Among these, 752 DEGs were upregulated at 2 DAI, indicating a rapid activation of defence-related genes in response to *Foc* STR4. Functional analyses using GO, KEGG, KOG, InterPro, and MapMan identified several DEGs involved in the rapid defence responses to *Foc* STR4. These included the activation of PRRs (pattern recognition receptors), PRs (pathogenesis-related proteins), chitinases, phytohormones such as ethylene, jasmonic acid, and abscisic acid, resistance genes belonging to the NLR (nucleotide-binding site leucine-rich repeat) family, transcription factors (TFs), ROS (reactive oxygen species), SAR (systemic acquired resistance), phytoalexins, and callose. These genes participate in various functional pathways, such as responses to stimuli, plant-pathogen interactions, secondary metabolism, phytohormone pathways, and biotic stress responses. The coordinated action of these signalling pathways is essential for the resistant response to *Foc* STR4 in this wild genotype. This

characterization of defense responses will facilitate the selection of candidate genes to *Foc* for application in genetic improvement, such as through introgression of resistance genes from wild genotypes into commercial genotypes such as 'Prata-anã', as well as through gene-editing techniques like CRISPR. This strategy offers promise for the development of banana varieties resistant to Fusarium wilt caused by *Foc* STR4 and TR4, where the implementation of genetically modified organisms (GMOs) focusing on genes associated with plant-pathogen interactions and biotic stress response has the potential to enhance banana crop resilience and mitigate the impact of Fusarium Wilt epidemics on national production.

Keywords: banana, RNAseq, Fusarium wilt, biotic stress, genetic resistance, genomics.

INTRODUÇÃO GERAL

A banana (*Musa* spp.) é uma espécie monocotiledônea originária do sudeste asiático e oeste do Pacífico (Perrier et al. 2011). É considerada uma das frutas mais consumidas no mundo e componente básico da alimentação de milhões de pessoas. É considerada como fonte de carboidratos, vitaminas A, B1, B2 B3, B6, C e minerais, como ferro, potássio, fósforo e cálcio (Chandler 1995). Estima-se que a produção mundial de bananas é de 125 milhões de toneladas, onde os maiores produtores mundiais se concentram na Ásia, América Latina e África.

O Brasil ocupa o 4º lugar no ranking de países produtores de bananas, com área plantada de aproximadamente 456 mil hectares e produção de cerca de 6,8 milhões de toneladas de banana (IBGE, 2024). Dessas, apenas 107 mil toneladas foram exportadas, demonstrando a grande demanda do mercado interno (FAO 2022). Apesar da produtividade elevada, o rendimento de produção de banana do Brasil varia aproximadamente de 8 a 27 t/ha, com rendimento médio de 14,9 kg.ha⁻¹ (IBGE 2024) demonstrando que ainda existem desafios para a bananicultura nacional que impedem uma expansão do mercado internacional do produto. As principais cultivares plantadas no Brasil são Prata, Pacovan e Prata anã pertencentes ao subgrupo 'Prata', responsáveis por aproximadamente 60% da área cultivada em todo o país (Borges et al. 2006). O subgrupo 'Cavendish' (AAA) compreende mais 40% de produção mundial de bananas comerciais (IBGE 2024).

As doenças causadas por fitopatógenos são as principais causas de perdas no rendimento e qualidade dos frutos, pois podem atingir a planta desde o plantio até o período de pós-colheita e armazenamento. A pressão exercida pelo estresse biótico causado pelos fitopatógenos, que acometem na redução de produtividade da cultura, e a baixa diversidade genética de bananas comerciais são considerados os fatores limitantes para a bananicultura mundial (Ghag et al. 2015).

A Murcha de *Fusarium* é uma doença clássica de murcha vascular causada pelo fungo *Fusarium oxysporum* f. sp. *cubense* (*Foc*). O gênero *Fusarium* (Snyder e Hansen 1940) possui espécies responsáveis pela maioria das doenças vasculares em diferentes plantas de interesse econômico em todo o mundo. O gênero produz três tipos de esporos: os macroconídios, microconídios e os clamidósporos, que são estruturas de sobrevivência (Leslie e Summerell 2006). A divisão de *F. oxysporum* em

“*formae speciales*” se deu pela capacidade de isolados de infectarem espécies distintas de plantas.

Fusarium oxysporum f. sp. *cubense* W.C. Snyder & H.N. Hansen é subdividida em quatro raças, baseada na patogenicidade a diferentes cultivares de bananas. A raça 1 é patogênica às cultivares Gros Michel (AAA), Maçã e Prata (AAB), a raça 2 é patogênica à cultivar Bluggoe (ABB), também conhecida como Ensete (Ploetz 2006a). A raça 3 não infecta bananas, apenas plantas do gênero *Heliconia* spp. (Su et al. 1986). A raça 4 é subdividida em raça tropical 4 (TR4) e subtropical 4 (STR4), onde STR4 é patogênica a cultivares ‘Cavendish’ apenas em regiões subtropicais (Buddenhagen 2009). Já TR4 compreende isolados altamente virulentos independente das condições climáticas, causando doença em cultivares ‘Cavendish’ em regiões tropicais e subtropicais como no Sudeste da Ásia e Austrália, bem como na América do Sul. Os estudos de identidade e diversidade em *Foc* baseiam-se ainda em análises moleculares, teste de compatibilidade vegetativa (VCG) e análise filogenética (Maryani et al. 2019).

O primeiro registro de Murcha de *Fusarium* em bananeira ‘Sugar’ (subgrupo Maçã AAB) foi em 1874 na Austrália. Durante esse período uma epidemia causada por *Foc* raça 1 dizimou plantações inteiras da cultivar suscetível ‘Gros Michel’ (grupo AAA) na América Central, doença que foi denominada no início de Mal do Panamá (Zheng et al. 2018). No Brasil o primeiro relato da doença foi em 1930 em São Paulo, em plantas da cultivar ‘Maçã’ (Cordeiro et al. 2016a). Simmonds (1966) descreveu como a doença mais destrutiva de bananas, pois não havia métodos de controle efetivos contra a doença. Mais tarde, a cultivar ‘Cavendish’ foi criada como alternativa ao controle genético, por apresentar resistência a *Foc* raça 1 (Buddenhagen 1990). Após a observação de sintomas de Murcha de *Fusarium* em cultivares ‘Cavendish’ em Taiwan em 1967, uma nova raça de *Foc* foi identificada como raça 4 (Su et al. 1986). No final dos anos 90 e início dos anos 2000, TR4 havia difundido para cinco países asiáticos e Austrália, e no início da década de 2010 para o Oriente Médio e África (Jamil et al. 2020). Na América Latina a doença vem se espalhando desde 2019 e já se encontra presente na Colômbia, no Peru e na Venezuela (Vézina 2022b).

No Brasil, a raça TR4 é considerada uma praga quarentenária ausente que apresenta risco iminente de entrada no país, monitorada pelos órgãos de fiscalização governamentais (MAPA). Entretanto, isolados de *Foc* classificados com a VCG 0120,

pertencente à STR4 (raça 4, assim como TR4), são amplamente difundidos e estão presentes em todos os Estados produtores de banana, afetando cultivares do subgrupo 'Cavendish', 'Prata' e 'Maçã' (Batista et al. 2022).

Por ser uma doença sistêmica de entrada via penetração do sistema radicular e posterior colonização dos tecidos vasculares, é comum observar sintomas como a murcha da planta, amarelecimento em folhas mais velhas, queda de folhas sob o pseudocaule em forma de saia, presença de margens pálidas e irregulares em folhas novas, enrugamento e distorção foliar e morte da planta, impedindo a produção de frutos (Dita et al. 2018). Apesar de ser uma doença monocíclica, múltiplas infecções ocorrem em uma plantação de bananas infestadas por *Foc*, principalmente pelo retorno do patógeno ao solo com a morte do hospedeiro infestado, implicando em sua dispersão para plantas vizinhas, e pela ligação da planta-mãe com as plantas-filhas pelo rizoma (Ploetz 2015a).

O manejo mais eficiente para o controle da doença é a exclusão, pelo impedimento da entrada do patógeno em áreas produtoras de banana. Em solos infestados, tendo em vista a persistência do patógeno no solo, o único manejo realmente efetivo é através da resistência genética, presente em diversas cultivares de banana selvagens e híbridas (Buddenhagen 2009; Li et al. 2015; Ploetz 2015a). Demais ferramentas de manejo envolvendo o controle químico, físico e biológico, têm se mostrado ineficiente para o controle da Murcha de *Fusarium* em campos infestados (Siamak e Zeng 2018; Rocha et al. 2021, Guo et al. 2019).

O desenvolvimento dos programas de melhoramento, desde a década de 20, juntamente à inovação em transformação genética, vem possibilitando o surgimento de novos genótipos de banana geneticamente modificados resistentes a doenças, sem comprometer as características comerciais desejáveis. Esses avanços estão diretamente alinhados com as técnicas de sequenciamento mais robusta realizados pelos sequenciadores de nova geração (NGS), que através do sequenciamento massal e rápido, tem como ferramenta o uso de análises transcritômica e genômica de diversos organismos (Reuter et al. 2015). Por meio dessa tecnologia foi possível realizar o sequenciamento do genoma completo de *Musa acuminata* spp. *malaccensis* 'DH-Pahang' e *M. acuminata* subsp. *burmannica* acesso 'Calcutta 4', e de genomas

parciais de *M. acuminata* 'Banksii', *M. acuminata* 'Maia Ao', *M. balbisiana* 'DH-PKW' e *M. balbisiana* 'PKW' (Droc et al. 2013).

A análise da modulação da expressão gênica durante a interação do patógeno e hospedeiro, pode ser realizado utilizando-se a técnica de sequenciamento de Illumina RNAseq, que, junto com o mapeamento dos *reads* contra o genoma de referência *M. acuminata* spp. *malaccensis* 'DH-Pahang', possibilita uma análise global do transcrito e expressão gênica diferencial da planta quando desafiado com o patógeno. Através dessa técnica, diversos genes relacionados a resistência de cultivares de *M. acuminata* e *M. balbisiana* a estresses bióticos e abióticos já foram anotados (Chen et al. 2019; Li et al. 2015b; Xu et al. 2021). Entretanto, as respostas moleculares podem variar para cada patossistema, inclusive de forma intraespecífica, sendo importante identificar as alterações transcricionais de cada espécie sob estresse biótico alvo, e esclarecer quais elementos moleculares são específicos de cada etapa da imunidade vegetal (Kang et al. 2012).

Apesar de vários genes associados a resistência, como os associados a PRRs, PRs, NLRs, ativadores de vias de fitormônios, entre outros, descobertos em 'Calcutta 4' durante as interações com os patógenos *Pseudocercospora musae* (Pinheiro et al. 2022), *Mycosphaerella fijiensis*, *Radopholus similis* (Pillay et al. 2012), e *F. oxysporum* f. sp. *cubense* raça 1 (Kotari et al. 2018b; Swarupa et al. 2013), ainda há limitação na investigação dos genes e vias que controlam os mecanismos moleculares envolvidos na interação desse genótipo com *Foc* STR4, sobretudo no Brasil onde a raça é a mais predominante nos campos produtores de bananas. Com isso, o objetivo desse trabalho é identificar e validar genes alvo envolvidos na resistência de *M. acuminata* subsp. *burmannica* acesso 'Calcutta 4' durante a interação com o patógeno *F. oxysporum* f. sp. *cubense* raça STR4, causador da Murcha de Fusarium.

Capítulo I

Revisão sobre a importância mundial e nacional da cultura da banana, impactos causados pelas raças do patógeno causador da Murcha de Fusarium, e busca de genes de resistência de espécies selvagens de *Musa* voltadas ao uso no melhoramento genético da cultura

1.1 A cultura da Banana (*Musa* spp.)

A banana (*Musa* spp.) é uma espécie monocotiledônea pertencente à classe Magnolopsida, ordem Zingiberales, família Musaceae e gênero *Musa* L. originária do sudeste asiático e oeste do Pacífico (Perrier et al. 2011). Sua distribuição natural compreende as regiões de clima tropical como do Sul da China ao Norte da Austrália, Himalaias, Filipinas e África. É uma planta herbácea que possui um pequeno caule denominado rizoma, de onde originam-se as folhas a partir do meristema apical. Da parte central do rizoma são formadas também as raízes, inicialmente fasciculadas, cordiformes, brancas e tenras, e, à medida que envelhecem, se tornam amarelas, endurecidas e suberosas, distribuindo-se por toda a superfície do rizoma. As radículas que são formadas nas porções externas das raízes são as responsáveis pela absorção de água e nutrientes. A união das bainhas foliares justapostas compõem o pseudocaule de dimensões variáveis entre 1,2 e 8 m de altura e diâmetro de 10 e 50 cm, a depender da cultivar, e em sua terminação é formada a copa de folhas com limbo foliar longo e largo, e nervura central proeminente (Dantas et al. 2016).

Acredita-se que a domesticação tenha se iniciado de um pequeno grupo de espécies selvagens de bananeira, onde as principais são *Musa acuminata* Colla e *Musa balbisiana* Colla, que possuem genomas A e B, respectivamente, de acordo com a classificação desenvolvido por Norman Simonds e Kenneth Shepherd (1955) (Dantas et al. 2016). Dos cruzamentos surgiram os grupos diploides AA, BB e AB, triploides AAA, AAB e ABB, e pelo cruzamento destes, os tetraploides AAAA, AAAB, AABB e ABBB, divididos em subgrupos que compõem o agrupamento de cultivares comerciais, como nos subgrupos ‘Gros Michel’ (AAA), ‘Cavendish’ (AAA), ‘Prata’ (AAB), ‘Maçã’ (AAB), ‘Goldfinger’ (AAAB) entre outros. *M. acuminata* é encontrada na maioria das cultivares comerciais atuais, principalmente por sua predisposição genética para a partenocarpia, pois a presença de sementes e menos polpa é característica indesejável em bananas para consumo (Dantas et al. 2016).

Apesar de as condições ideais de cultivo ocorrerem entre os trópicos 15°N e 15°S de latitude, atualmente o cultivo de bananas comerciais ocorre em aproximadamente 120 países com climas tropicais e subtropicais (Donato et al. 2016).

A banana é considerada uma das frutas mais consumidas no mundo e componente básico da alimentação de milhões de pessoas. É considerada como fonte de carboidratos, vitaminas A, B1, B2 B3, B6, C e minerais, como ferro, potássio, fósforo e cálcio (Chandler 1995). Os maiores produtores de banana se concentram na Ásia, América Latina e África. Estima-se que a produção mundial de bananas é de 125 milhões de toneladas plantadas em aproximadamente 5 milhões de hectares em 2021, se tornando a fruta mais produzida mundialmente de acordo com a *Food and Agriculture Organization of the United Nations* (FAO) (FAO 2022). A exportação mundial de banana atingiu 19,3 milhões de toneladas em 2023, destes, 14,5 milhões de toneladas foram enviados pelas regiões América Latina e Caribe (LAC). Os maiores importadores de banana são a União Europeia e Estados Unidos, que somam 49,7% das importações de bananas (FAO 2024).

No Brasil, que ocupa o 4º lugar no ranking de países produtores de bananas, a área plantada de aproximadamente 453 mil hectares atingiu produção de cerca de 6,8 milhões de toneladas de banana (IBGE 2024). Dessas apenas 107 mil toneladas foram exportadas, demonstrando a grande demanda do mercado interno (FAO 2022). Os estados de São Paulo, Bahia, Minas Gerais, Santa Catarina e Pernambuco foram responsáveis por cerca de 57% da produção nacional em 2023, conforme ilustrado na Figura 1. As principais cultivares plantadas no Brasil são Prata, Pacovan e Prata-anã pertencentes ao subgrupo 'Prata', responsáveis por aproximadamente 60% da área cultivada em todo o país. A cultivar Maçã (AAB) é considerada a preferida entre os consumidores brasileiros, entretanto por ser altamente suscetível à Murcha de Fusarium, teve sua predominância dos mercados substituída pelas cultivares do subgrupo 'Prata'. As cultivares Nanica, Nanicão e Grand Naine pertencentes ao subgrupo 'Cavendish' (AAA) são plantadas em menor escala e utilizadas majoritariamente para a exportação (Borges et al. 2006). O subgrupo 'Cavendish' (AAA) compreende mais 40% de produção mundial de bananas comerciais (IBGE 2024).

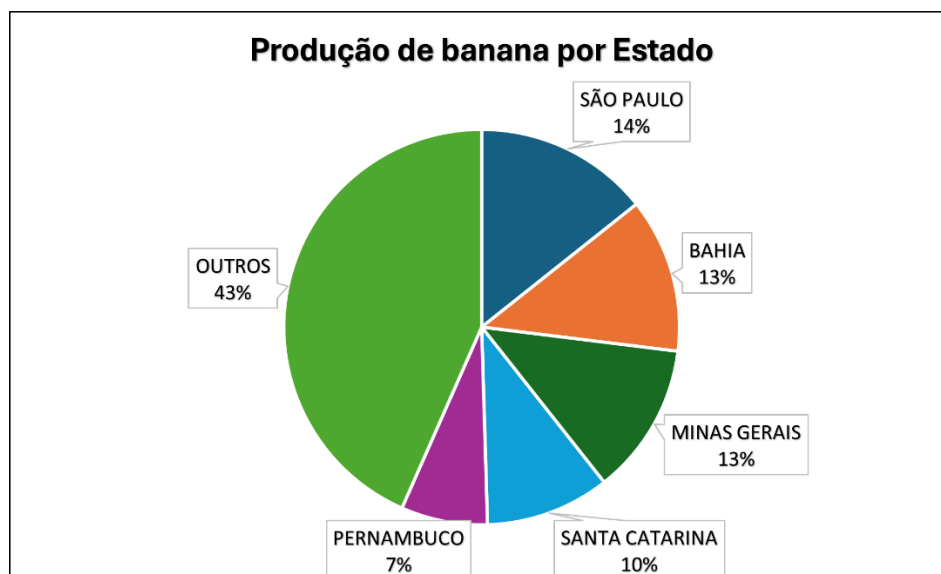


Figura 1. Principais Estados produtores de banana no Brasil em 2023, de acordo com balanço de Produção de Banana do IBGE (2024).

Apesar da produtividade elevada, o rendimento de produção de banana do Brasil varia aproximadamente de 8 a 27 t/ha com rendimento médio de 14,9 kg/ha (IBGE 2024), demonstrando que ainda existem desafios para a bananicultura nacional que impedem uma expansão do mercado internacional do produto. Fatores relacionados às etapas de produção e pós-colheita são fundamentais para garantir além da produtividade, a qualidade final dos frutos (Silva e Cordeiro 2000).

As doenças causadas por fitopatógenos são as principais causas de perdas no rendimento e qualidade dos frutos, pois podem atingir a planta desde o plantio até o período de pós-colheita e armazenamento. Diferentes espécies de vírus, bactérias, fungos e nematoides são capazes de infectar e causar danos à cultura da bananeira (Cordeiro et al. 2016a). Entre as doenças da banana tem-se a bacteriose denominada moko ou murcha bacteriana causado por *Ralstonia solanacearum* raça 2. As viroses causadas por *Cucumber Mosaic Virus* (CMV), *Banana Streak Virus* (BSV), *Banana bunchy top virus* (BBTV), *Banana bract mosaic virus* (BBrMV), *Banana mild mosaic virus* (BanMMV) e *Banana virus X* (BVX) (Tripathi et al. 2016). As causadas pelos nematoides *Radopholus similis*, *Meloidogyne* spp., *Pratylenchus coffea* e *Helicotylenchus multicintus*.

De acordo com Agrios (2004) mais de 10 mil espécies fúngicas são capazes de causar doenças em plantas. São considerados como a classe de patógenos mais importante da cultura da banana, responsáveis por causar doenças em qualquer

estágio da cultura e em frutos após a colheita. As doenças fúngicas Sigatoka-amarela (*Pseudocercospora musae* (Zimm.) Deighton), Sigatoka Negra (*P. fijiensis* (Morelet) Deighton) e a Murcha de Fusarium, causado por *Fusarium oxysporum* f. sp. *cubense* (E. F. Smith) Synder & H.N. Hansen, têm destaque principal pelo potencial destrutivo que causam à cultura, podendo dizimar bananais inteiros (Dita et al. 2018). Entre as doenças de pós-colheita, a antracnose causada por *Colletotrichum musae* (Berk. & M.A. Curtis) é considerada a mais frequente (Cordeiro et al. 2016a; Su et al. 2011).

A pressão exercida pelo estresse biótico causado pelos fitopatógenos, que acometem na redução de produtividade da cultura, e a baixa diversidade genética de bananas comerciais são considerados os fatores limitantes para a bananicultura mundial (Ghag et al. 2015).

1.2 *Fusarium oxysporum* f. sp. *cubense* (Foc)

O patógeno causador da Murcha de Fusarium pertence ao filo Ascomycota, subfilo Pezizomycotina, classe Sordariomycetes, ordem Hypocreales família Nectriaceae e gênero *Fusarium* (Index Fungorum - www.indexfungorum.org). O gênero *Fusarium* (Snyder and Hansen 1940) possui espécies responsáveis pela maioria das doenças vasculares em diferentes plantas de interesse econômico em todo o mundo.

A espécie *F. oxysporum* compreende organismos morfologicamente semelhantes, sem fase sexual conhecida, podendo ser saprofíticos e fitopatógenos polípagos e cosmopolitas. Produzem três tipos de esporos: o macroconídio produzido nas monofiálides em conidióforos ramificados, possui formato falcado, de tamanho pequeno a grande, com geralmente 3 septos, e na maioria dos isolados a célula apical curta e ligeiramente curvada (Figura 2A-B). Os microconídios não possuem septos, de formato oval, elíptico ou reniforme e são formados abundantemente nas “false heads” em pequenas monofiálides (Figura 2C-F) (Leslie & Summerell, 2006). E os clamidósporos que são hifas modificadas em formato globular, e que possuem papel importante na sobrevivência do patógeno por longos períodos e em condições extremas sem a presença do hospedeiro (Figura 2G) (Ploetz 2006a).

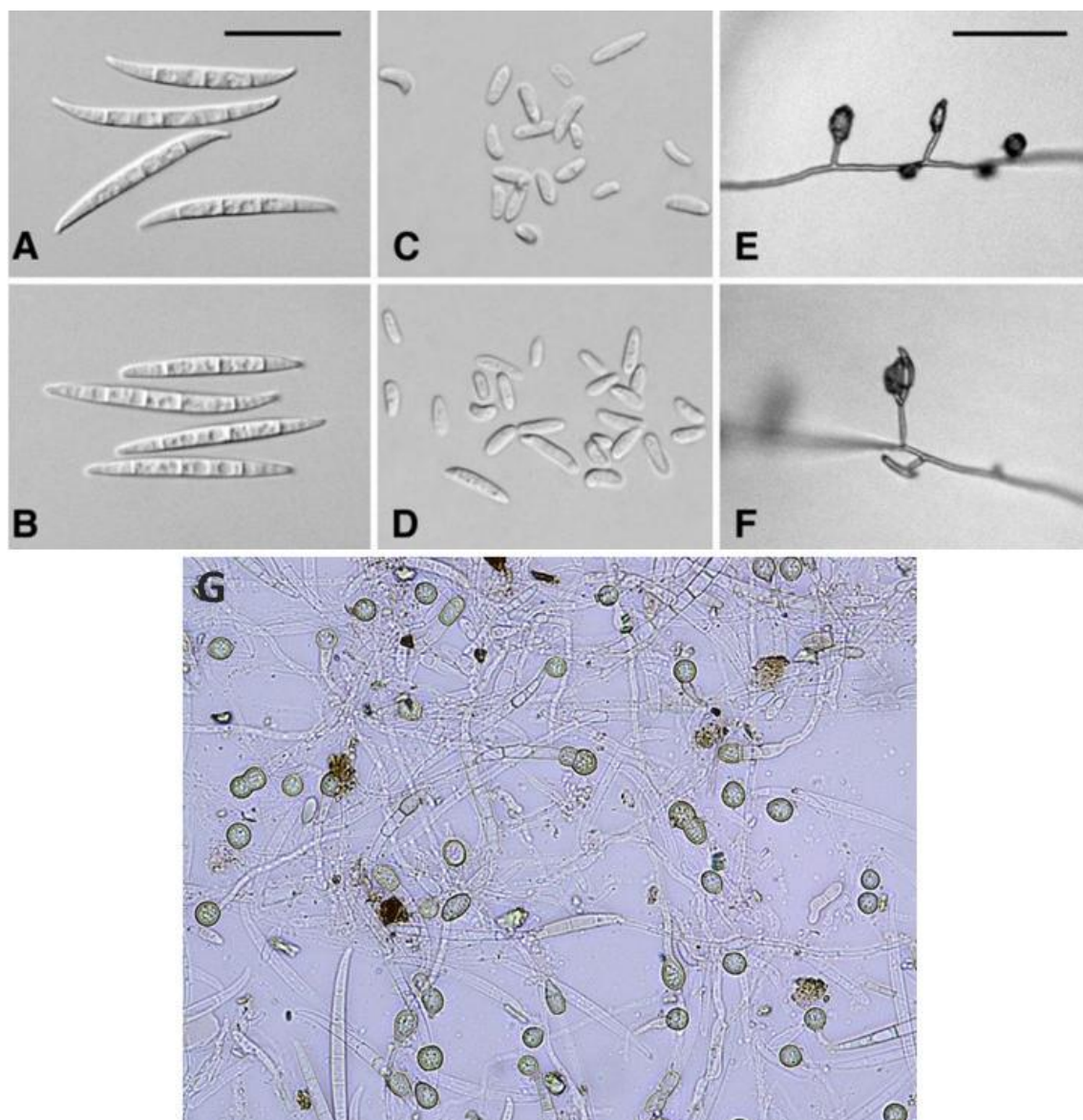


Figura 2. Morfologia de *Fusarium oxysporum*. **A-B.** Macroconídeos; **C-D.** Microconídeos; **E-F.** Microconídeos presos ao conidióforo em meio CLA; **G.** Produção de clamidósporos em cultura de *Fusarium oxysporum* f. sp. *cubense*. Escala: A-D = 25µm; E-F = 50 µm. Adaptado de: Leslie & Summerell 2006; Pegg et al. 2019.

A divisão de *F. oxysporum* em “*formae speciales*” se deu pela capacidade de isolados de infectarem espécies distintas de plantas. Além disso, foi observado também que alguns isolados dentro de “*formae speciales*” conseguiam causar doenças apenas em certas cultivares, e então foram reclassificadas em raças (Edel-Hermann and Lecomte 2019; Leslie and Summerell 2006). Atualmente, 125 classificações de “*formae speciales*” de *F. oxysporum* estão descritas no Index Fungorum (<https://www.indexfungorum.org/>).

Outra classificação comumente utilizada em *F. oxysporum* é através do teste de compatibilidade vegetativa (VCG), dada pela compatibilidade de hifas de isolados distintos formarem heterocarions estáveis (Correll 1991; Leslie and Summerell 2006).

Desde 1910, o agente causal da murcha de Fusarium em bananeira é associado ao nome *cubense*, devido a amostras identificadas serem oriundas de Cuba (Smith 1910). Em 1940 foi descrita como *F. oxysporum* f. sp. *cubense* W.C. Snyder & H.N. Hansen. *Foc* pertence ao complexo de espécies de *F. oxysporum* (FOSC) (Maryani et al. 2019) e é subdividida em quatro raças, baseada na patogenicidade a diferentes cultivares de bananas.

A raça 1 é patogênica às cultivares Gros Michel (AAA), Maçã e Prata (AAB), a raça 2 é patogênica às cultivares Bluggoe (ABB), também conhecidas como Ensete (Ploetz 2006a); e a raça 4 que é patogênica à todas as cultivares suscetíveis às raças 1 e 2, como também à Cavendish (AAA). A raça 3 não infecta bananas, apenas plantas do gênero *Heliconia* spp. (Su et al. 1986). A raça 4 é subdividida em raça tropical 4 (TR4) e subtropical 4 (STR4), onde STR4 é patogênica à cultivares ‘Cavendish’ apenas em regiões subtropicais, como nas Ilhas Canárias (Espanha), África do Sul, Brasil e Austrália (Buddenhagen 2009). Já TR4 compreende isolados altamente virulentos independente das condições climáticas, causando doença em cultivares ‘Cavendish’ em regiões tropicais e subtropicais como no Sudeste da Ásia e Austrália, bem como na América do Sul.

Vinte e quatro VCGs diferentes são conhecidas em isolados de *Foc* (Ghag et al. 2015), onde a maioria está presente no continente Asiático. As VCGs 0123, 0124, 0125, 0126, 0128, 01210, 01212, 01214, 01217, 01218, 01219, 01220, 01221, entre outras, pertencem a *Foc* R1 e R2 (Moore et al. 1993; Ploetz 2006b). *Foc*-TR4 possui uma única VCG: 01213/16, enquanto *Foc*-STR4 possui cinco VCGs: 0120, 0121, 0122, 0129 e 01211 (Buddenhagen, 2009; Dita et al. 2010). Entretanto, a classificação de isolados por VCGs pode indicar a inespecificidade da técnica, como indicada na classificação da VCG 0124 que ocorrem em isolados de raça 1 e raça 2 e a formação de mais de uma VCG por raça (Ghag et al. 2015), ou mesmo pela incapacidade de alguns isolados serem classificados em VCGs conhecidas, por serem incompatíveis (Moore et al. 1993). Um total de 8 VCGs já foram encontradas no Brasil, sendo elas 0120, 0122, 0124, 0125, 0128, 01215, 01220, e 01222, que, de acordo com estudo

de diversidade realizado por Batista et al. (2022), a mais predominante é a VCG 0120 de STR4.

Os estudos de diversidade em *Foc* baseiam-se ainda em análises moleculares como DNA polimórfico amplificado ao acaso (RAPD - *random amplified polymorphic DNA markers*) (Bentley et al. 1995), análise de polimorfismo no comprimento de fragmentos de restrição (RFLP - *restriction fragment length polymorphisms*) (Koenig et al. 1997), análise de polimorfismos de comprimento de fragmento amplificado (AFLP - *amplified fragment length polymorphism*) (Groenewald et al. 2006) e análises de sequência de DNA (O'Donnell et al. 1998). Através da análise filogenética utilizando os marcadores *translation elongation fator 1-alpha* (*tef1*) e *mitochondrial subunit rDNA* (*mtssu*), quatro clados de um complexo de espécies de *Fusarium oxysporum* (FOSC) foram identificados, com isolados de *Foc* agrupando em um único clado (O'Donnell et al. 2004).

Utilizando os marcadores das regiões *RNA polymerase II large subunit* (RPB1), *RNA polymerase II subunit* (RPB2) e *elongation factor 1-alpha gene* (TEF), Maryani et al. (2019) separou isolados de *Foc* da Indonésia, centro de origem do patógeno, em nove linhagens distintas, propondo nova classificação das raças de *Foc* em novas espécies. Assim, todos os isolados de *Foc* Raça 4 do estudo se agruparam na linhagem 1, denominado de *Fusarium odoratissimum*, sem a distinção de isolados de TR4 e STR4. Entretanto, ao replicar as análises utilizadas nesse estudo, Bedoya et al. (2021) reportaram discrepância nos dados, que por não serem robustos, não suportam os mesmos clados monofiléticos descritos por Maryani et al. (2019), demonstrando que foi precipitado propor novas espécies dentro de *Foc*.

1.3 Murcha de Fusarium ou Mal do Panamá

Apesar da probabilidade de ter originado no sudeste da Ásia o primeiro registro da doença foi em 1874 em Brisbane, Austrália, na cultivar de banana 'Sugar' (AAB, subgrupo 'Maçã') por Bancroft (1876) (Pegg et al. 2019). Em 1890 foi registrada no Panamá e Costa Rica, Suriname em 1906, Cuba em 1908, Trinidad em 1909, Jamaica em 1911, Honduras em 1916 e Guatemala em 1919, indicando sua primeira distribuição continental (Vézina 2022a). Durante esse período, uma epidemia causada por *Foc* raça 1 dizimou plantações inteiras da cultivar suscetível 'Gros Michel' (grupo AAA), produzida em larga escala na América Central, que pelo impacto

causado foi considerada como doença de importância mundial (Zheng et al. 2018). No Brasil o primeiro relato da Murcha de Fusarium foi em 1930 em Piracicaba, São Paulo, em plantas da cultivar ‘Maçã’ (grupo AAA), que rapidamente se espalhou para todas as regiões do país, dizimando bananais inteiros dessa cultivar (Cordeiro et al. 2016a).

Por ter sido descrita por Simmonds (1966) como a doença mais destrutiva por todo impacto causado em plantações de ‘Gros Michel’, e sem nenhum método de controle efetivo, pesquisas no melhoramento genético de resistência de genótipos de *Musa* ao patógeno foram impulsionadas, resultando na criação de cultivares ‘Cavendish’, resistente à raça 1 (Buddenhagen 1990).

Em 1967 foi observado sintomas de Murcha de Fusarium em cultivares ‘Cavendish’ em Taiwan. Em 1976, havia cerca de meio milhão de plantas infectadas em uma área de 1200 ha. Após a realização de testes de patogenicidade, em 1977 os isolados coletados nessas áreas foram classificados como *Foc* TR4 (Su et al. 1986). Após se estabelecer em novas plantações de ‘Cavendish’ na Indonésia e Malásia em meados dos 1990 (Pin. 1996), o patógeno ficou popularmente conhecido como raça tropical 4 (TR4). No final dos anos 90, em 1996 TR4 foi relatado na China, em 1997 na Austrália, em 2013 em Omã, Jordânia e Moçambique, em 2015 no Paquistão e Líbano, em 2018 no Vietnã, Laos, Mianmar e Israel (Jamil et al. 2020). O primeiro relato de TR4 na América Latina foi em 2019 na Colômbia (Garcia Bastidas et al. 2019), seguido pelo diagnóstico em 2021 em Piura, no Peru (Acuña et al. 2022) e em 2023 na Venezuela (Mejías Herrera et al. 2023). Na Figura 3 é ilustrada distribuição da Murcha de Fusarium ocasionada pelas R1, R2 e TR4.

A distribuição da raça STR4 não é bem definida nos países, dada a limitação do aparecimento da doença em ‘Cavendish’ nos países tropicais, e quando encontrado em outras cultivares suscetíveis é classificado como raça 1, dificultando a correta identificação e monitoramento da raça quando comparado a TR4 (Munhoz et al. 2024). Entretanto, a presença de *Foc* STR4 em plantações de bananas já foi relatada em diversas regiões da Ásia, Austrália, e Américas Central e do Sul (Munhoz et al. 2024; Olivares et al. 2021; Rocha et al. 2018). No Brasil, a raça TR4 é considerada uma praga quarentenária ausente que apresenta risco iminente de entrada no país, monitorada pelos órgãos de fiscalização governamentais (MAPA).

Entretanto, isolados de *Foc* classificados com a VCG 0120, pertencente à STR4 (raça 4, assim como TR4), são amplamente difundidos e estão presentes em todos os Estados produtores de banana, afetando cultivares do subgrupo ‘Cavendish’, ‘Prata’ e ‘Maçã’ (Batista et al. 2022).

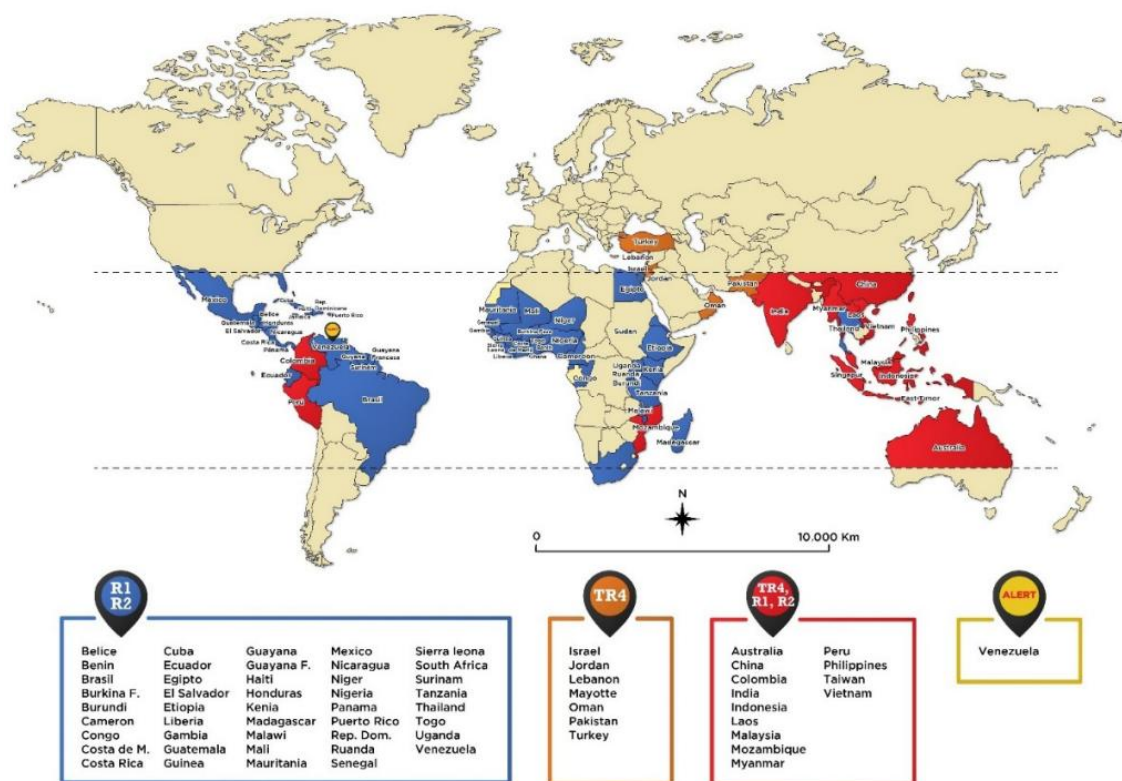


Figura 3. Distribuição geográfica das raças de *Fusarium oxysporum* f. sp. *cubense* (*Foc*), causador da Murcha de Fusarium (Olivares et al., 2021). Em estado de alerta desde 2019, em 2023 foi confirmada a presença da raça TR4 na Venezuela.

A Murcha de Fusarium é uma doença clássica de murcha vascular causada pelo fungo *Fusarium oxysporum* f. sp. *cubense*, classificado como patógeno hemibiotrófico. A infecção se inicia em uma relação biotrófica com o hospedeiro, através da penetração direta do micélio na epiderme de células radiculares até atingir o tecido vascular. O fungo então se espalha para o rizoma causando primeiramente uma descoloração vascular, evoluindo para vermelho escuro a marrom escuro, pela necrose do tecido, transitando para uma relação necrotrófica com o hospedeiro (Dita et al. 2018) (Figura 4B-C). Com a colonização dos demais tecidos do hospedeiro, como o pseudocaule, os vasos do xilema são obstruídos com acúmulo de células

fúngicas resultando no aparecimento dos sintomas externos (Dita et al. 2018; Pegg et al. 2019).

Nesse estágio é comum observar a murcha da planta, a divisão do pseudocaule, e, nas folhas, é possível observar o amarelecimento em folhas mais velhas, queda de folhas sob o pseudocaule em forma de saia, que podem resultar em queda e seca, presença de margens pálidas e irregulares em folhas novas, enrugamento e distorção foliar (Figura 4A) (Cordeiro et al. 2016a). O período de incubação da doença, que compreende o início da infecção até o aparecimento de sintomas de Murcha de Fusarium, pode variar de dois a seis meses, variando de acordo com a quantidade do inóculo inicial, o padrão de resistência e idade do hospedeiro, as condições ambientais e a agressividade do patógeno (Rishbeth 1957).



Figura 4. Sintomas típicos ocasionados pela Murcha de Fusarium em diferentes cultivares de bananas. **A.** Folhas amareladas e murchas, pendidas ao longo do caule, **B.** Descoloração, manchas avermelhadas e necrose no tecido vascular do pseudocaule, **C.** Presença de descoloração, manchas avermelhadas e necrose no

tecido vascular no rizoma e raiz, **D.** Descoloração vascular em talo de cacho infectado, **E.** Invasão do córtex de raiz de banana por micélio de *Foc* realizado por microscopia eletrônica de varredura. Adaptado de: MusaRama (2023) e Pegg et al. (2019).

A entrada do patógeno na área ocorre principalmente com o plantio de mudas infectadas, ou por meio de irrigação com água contaminada com *Foc* (Ghag et al. 2015; Pegg et al. 2019; Warman e Aitken 2018). *Foc* pode sobreviver de forma saprofítica sobre os tecidos do bananas mortas e ou em hospedeiros alternativos e produzir estruturas de sobrevivência, denominadas clamidósporos, até que haja condições favoráveis para sua germinação, como a presença de um novo hospedeiro (Stover 1962; Leslie e Summerell 2006) (Figura 5A). Pela estimulação de exsudatos de raízes de plantas ou pelo contato direto com raízes, os clamidósporos germinam e infectam diretamente ou por ferimentos, o tecido radicular (Figura 5B).

Em um hospedeiro suscetível, o processo de colonização se inicia pela invasão e produção de micélio e esporos nos tecidos do hospedeiro, se espalhando pelas demais raízes secundárias e terciárias (Figura 5C). Apesar da presença de diversos compostos da planta que formam um complexo de polissacarídeos, a maioria pode ser digerida por um complexo de enzimas secretadas pelos patógenos hemibiotróficos, como as PMEs – pectina methylesterases, que degradam a pectina (Caffall and Mohnen 2009; Cantu et al. 2008). Assim, em plantas suscetíveis, o patógeno atinge o rizoma, pseudocaule e caule do hospedeiro através do sistema vascular, resultando em uma infecção sistêmica e entupimento dos feixes vasculares, impedindo a translocação de água e nutrientes, ocasionando os primeiros sintomas externos, como murcha e amarelecimento foliar (Figura 5D), evoluindo para murcha intensa e morte da planta (Figura 5E-F).

Por ser ligada diretamente ao sistema vascular do rizoma da planta mãe, as plantas filhas também são infectadas com o patógeno, e os sintomas iniciais recomeçam, reiniciando o ciclo da doença (Dita et al. 2018; Pegg et al. 2019; Ploetz 2015b).

Apesar de ser uma doença monocíclica, múltiplas infecções ocorrem em uma plantação de bananas infestadas por *Foc*, principalmente pelo retorno do patógeno ao solo com a morte do hospedeiro infestado, implicando em sua dispersão para

plantas vizinhas, e pela ligação da planta-mãe com as plantas-filhas pelo rizoma(Ploetz 2015a).



Figura 5. Ciclo de vida de *Fusarium oxysporum* f. sp. *cubense* em *Musa*. **A.** Os esporos (micro e macroconídios) e clamidósporos permanecem no solo ou em hospedeiros alternativos, como ervas daninhas, **B.** Clamidósporos germinam estimulados por exsudatos radiculares e os tubos germinativos penetram nas raízes da bananeira, **C.** *Foc* cresce através do córtex para a epiderme e o micélio invade o sistema vascular, **D.** Conídios e clamidósporos são constantemente produzidos nos tecidos vasculares. Os conídios são rapidamente distribuídos pela planta via sistema de transpiração. O micélio e a goma bloqueiam os tecidos vasculares e os primeiros sintomas de amarelecimento são observados nas folhas mais velhas, **E.** *Foc* coloniza e destrói mais tecidos vasculares provocando murcha intensa, **F.** A planta infectada morre e a planta seguidora (filha), que foi contaminado pela planta-mãe por meio de conexão vascular, apresenta sintomas iniciais. A planta-mãe eventualmente cai e o ciclo da doença recomeça (Dita et al. 2018).

A idade e genética do hospedeiro também influenciam o desenvolvimento da Murcha de *Fusarium* (VanderMolen et al. 1987). As plantas mais velhas são mais resistentes que as mais jovens, e em cultivares resistentes a infecção é interrompida pela rápida produção de gomas, géis e tiloses no xilema, impedindo que a doença avance para o pseudocaule (Ploetz 2015b).

Alguns hospedeiros alternativos foram relatados para diferentes raças de *Foc*. *Foc* R1 já foi isolado de *Paspalum fasciculatum*, *Panicum purpurascens*, *Ixophorus unisetus* e *Commelina diffusa*. STR4 foram isolados de *Paspalum* sp. e *Amaranthus* sp. e, TR4 de *Cyperus iria*, *C. rotundus*, *Fimbristylis koidzumiana*, *Gnaphalium purpureum*, *Chloris inflata*, *E. heterophylla*, *Cyanthillium cinereum*, *Tridax procumbens*, *Echinochloa colona*, *P. conjugatum*, *E. hirta*, *Ageratum conyzoides*, *Momordica charantia*, *Ipomoea triloba*, *Asystasia gangetica* e *Amaranthus* (Garcia-Bastidas 2022).

À medida que a expansão de TR4 é facilitada pela monocultura de clones de ‘Cavendish’ que ocupam cerca de 40% de área plantada em todo o mundo (Ploetz 2015b), associada a falta de medidas quarentenárias e de controle químico eficiente, o mercado de bananas enfrenta o desafio de prevenir uma nova epidemia de Murcha de Fusarium causada por TR4, através da pesquisa em controle genético.

1.4 Importância e Impactos da Murcha de Fusarium

Bananas e plátanos (*Musa* sp.) fazem parte da subsistência de milhões de pessoas. A banana ocupa o oitavo lugar entre as culturas alimentares mais importantes do mundo e a quarta mais importante entre os países menos desenvolvidos do mundo (Ploetz et al. 2015). É produzida principalmente por pequenos agricultores e cooperativas, distribuídos na Ásia, África, América Central e do Sul, possuindo alto consumo interno e exportação de bananas para o mundo todo, como por exemplo a Europa (Jamil et al. 2020).

A Murcha de Fusarium causada por *Foc* raça 1 no Caribe e América Central findou o comércio de bananas ‘Gros Michel’. A abertura de 40 mil ha de área possibilitou que a produção se mantivesse, entretanto, a área não infestada e acessível se tornou rara em meados de 1950 (Soluri 2002). Em um período de 50 anos, mais de 40 mil ha de bananas na América Central e do Sul foram dizimados por *Foc* raça 1 (Stover 1962). A baixa disponibilidade de terras e o baixo abastecimento do mercado de bananas contribuiu para o aumento no custo de produção e no comércio do fruto.

A mudança no plantio da cultivar resistente ‘Cavendish’ foi motivada pelo comércio do Equador na década de 50. Até então, na África não se tinham preocupações relacionadas as perdas ocasionadas por *Foc* raça 1, visto que a

maioria das cultivares comerciais plantadas na região apresentavam resistência a essa raça (Vézina 2022a).

No surgimento de *Foc* TR4 no Taiwan, país que dominava a exportação de bananas na época, 50 mil ha de 'Cavendish' foram ameaçados. 40 anos após o primeiro relato do patógeno, apenas 6 mil ha de plantações de 'Cavendish' ainda estavam ativas. Os prejuízos relacionados a esse patógeno no Taiwan são estimados em mais de US\$ 250 milhões (Rodriguez and Haddad 2018). Relatos de perdas totais em plantações recém estabelecidas de 'Cavendish' por *Foc* TR4 foram feitas a partir dos anos 1990 na Malásia, Indonésia e Austrália e em 2006 na China (Garcia-Bastidas 2022).

Esses fatores impactaram principalmente os pequenos produtores de banana, que não dispunham de novas terras disponíveis e não podiam arcar com preços de produção e importação de mudas de 'Cavendish'. Nas Filipinas, onde a incidência de *Foc* TR4 nas propriedades monitoradas aumentou de 700 casos em 2005 para 15.000 casos em 2007 (Molina et al. 2008), por exemplo, o governo orientou que os bananicultores afetados por TR4 passassem a cultivar óleo de palma em 2014 (Vézina 2022b, 2022a).

Embora a Murcha de *Fusarium* tenha sido identificada há mais de um século, a falta de soluções eficazes de controle e o aumento do comércio internacional de bananas e outros produtos agrícolas têm contribuído para a disseminação da doença. Estima-se que a Murcha de *Fusarium* causada pelo *Foc* TR4 causou perdas e custos de cerca de US\$ 14,1 milhões na Malásia, US\$ 121 milhões na Indonésia e US\$ 253,3 milhões no Taiwan (Garcia-Bastidas 2022). Somente na Austrália, uma estimativa mostra que a dispersão atual de TR4 no país pode gerar perdas anuais de mais de AUD 138 milhões (Cook et al. 2015). A estimativa mundial é US\$ 400 milhões em perdas de produção a cada ano (Ploetz 2015a).

1.5 Controle

Diante da baixa precisão dos conhecimentos acerca dos fatores climáticos e edáficos que podem influenciar na epidemiologia da Murcha de *Fusarium*, da ampla distribuição da doença nos países produtores de banana e no impacto socioeconômico gerado pelas epidemias ocasionados pelas raças do patógeno ao

longo dos anos, o manejo da doença e da cultura tem se tornado alvo constante de estudos.

Dada as limitações de manejo, a exclusão do patógeno é a medida de controle mais recomendada em todas as regiões produtoras de bananas que não possuem a presença *Foc*, e que adotam medidas quarentenárias, de monitoramento constante e programas de contingência efetivos de materiais vegetais externos (Ploetz 2015b). Organizações internacionais responsáveis pelo programa de contingenciamento contra *Foc* TR4 (VCG 01213), como *International Regional Organization for Agricultural Health* (OIRSA) e a *Food and Agriculture Organization of the United Nations* (FAO) adotaram um método de detecção via PCR por meio da combinação de primers específicos *Foc*TR4-F/*Foc*TR4-R, desenvolvida através da região IGS proposta por Dita et al. (2010). Uma das limitações dessa técnica de manejo se dá pela comercialização de mudas não registradas, que não passam pelo monitoramento dos programas de quarentena, e pelo longo período para aparecimento de sintomas de Murcha de Fusarium após infecção, onde algumas mudas infectadas podem passar despercebidas (Ploetz 2015a). Essa medida também só é eficaz e restrita às regiões que ainda são livres do patógeno. Após a entrada do patógeno na área a doença se torna de difícil controle, uma vez que o patógeno pode sobreviver no solo por vários anos sob condições desfavoráveis devido a produção de clamidósporos (Stover 1962). As medidas de controle nessas condições incluem o uso de variedades resistentes, a rotação de culturas e a prática de higiene adequada, que envolve a eliminação de resíduos vegetais infectados e a desinfecção de equipamentos e ferramentas (Ghag et al. 2015; Pegg et al. 2019; Ploetz 2015a).

A utilização de produtos químicos como o uso de fumigantes para o controle de patógeno de solo, como *Foc*, são desencorajadas, dado a baixa eficácia e danos causados ao meio ambiente (Siamak e Zeng 2018; Rocha et al. 2021). A eficácia de grupos diferentes de fungicidas, como benzimidazóis, inibidores de demetilação, fosfonatos e estrobilurinas, foram testadas *in vitro* e em casa de vegetação, entretanto sem aplicações em campo. A eficiência da aplicação de carbendazim 2% por injeção no rizoma da cultivar 'Rasthali', demonstrou eficiência em apenas um ciclo da cultura, que é em torno de 14 a 18 meses, entretanto o mesmo resultado não foi observado em estudos na África do Sul. Por ser um patógeno de solo, testes utilizando a fumigação do solo com Bromometano reduziram a incidência da doença por alguns

meses, entretanto as áreas foram reinvadidas pelo patógeno, impossibilitando cultivos posteriores de banana 'Williams' (Ploetz, 2015b e todas as referências citadas nele). Demais estudos que envolvem o manejo cultural e físico também possuem eficácia não comprovada em estudos de campo, visto que a maioria são realizados apenas em condições controladas (Ploetz 2015a).

Diversos estudos envolvendo o uso de agentes de controle biológico foram desenvolvidos para o patossistema *Musa* e Murcha de *Fusarium*, com uso de micro-organismos antagonistas ou produtores de toxinas antimicrobianas, como *Trichoderma* spp., *Pseudomonas* spp., *Streptomyces* sp. e *Bacillus* spp. (Bubici et al. 2019). Por outro lado, Guo et al. (2013) mostra grande limitação no uso desses agentes de controle biológico para o controle da murcha de *Fusarium* em campo, baseado em diversos fatores que podem influenciar na eficiência da técnica, como o custo de produção destes micro-organismos em larga escala, os fatores edafoclimáticos que influenciam na eficácia de antagonistas, as competições entre micróbios por sítios ecológicos, nutrientes e energia, a capacidade desses organismos antagonistas serem endofíticos, pois *Foc* penetra na planta hospedeira, entre outros fatores ambientais, genéticos e fisiológicos.

Dessa forma, ainda é defendido que, em solos infestados com *Foc*, o único manejo realmente efetivo é através da resistência genética, presente em diversas cultivares de banana selvagens e híbridas (Buddenhagen 2009; Li et al. 2015; Ploetz 2015a), pois as cultivares suscetíveis apenas podem ser cultivadas em áreas não infestadas, para garantir sua produção. As cultivares resistentes devem suprir além da resistência às diferentes raças de *Foc* (R1 e, sobretudo, a STR4 e TR4), fator que deve ser considerado devido à alta variabilidade do patógeno, também os padrões de mercado e exportação, como sabor, tamanho e qualidade do fruto (Ploetz et al. 2015; Xu et al. 2011). Sobretudo, a criação de variedades resistentes pode aumentar a produtividade da cultura, reduzir danos ao homem e ao meio ambiente e diminuir os gastos com uso de defensivos agrícolas, diminuindo, portanto, o custo de produção e aumento a rentabilidade do produtor (Amorim et al. 2016).

1.6 Mecanismos de defesa de plantas ao estresse biótico

Plantas são atacadas por uma diversidade de patógenos, entre eles fungos, bactérias, mollicutes, vírus e nematoides, possuindo a resistência e imunidade como

regra para a maioria destes micro-organismos. Ainda assim, apesar de alguns patógenos conseguirem causar danos, muitas espécies de plantas são capazes de sobreviver, e até mesmo garantir o crescimento e produtividade (Agrios 2004). Plantas conseguem se defender utilizando uma combinação de estratégias em modelos de interação compatível, quando o patógeno é virulento e o hospedeiro é suscetível, e incompatível, onde o hospedeiro é resistente e o patógeno é avirulento. A teoria de gene-a-gene de Harold Flor (1956) explica a imunidade vegetal demonstrando que, para cada gene de resistência de uma planta (R), há um gene correspondente de avirulência do patógeno (Avr). Desse modo, a interação incompatível é dada quando o hospedeiro reconhece os elicitores do patógeno através dos receptores presentes na membrana plasmática, ativando uma cascata de respostas de defesa e impedindo o processo de infecção.

Alguns patógenos, que de forma coevolutiva com o hospedeiro, adaptaram estratégias para confundir o sistema de defesa vegetal a fim de favorecer o processo de infecção e colonização do hospedeiro, resultando em interações compatíveis.

1.7 Sistema de imunidade vegetal inata – PTI e ETI

As plantas possuem numerosos receptores imunológicos intracelulares e de superfície celular, a fim de reconhecer uma variedade de sinais associados as infecções por patógenos (Jones e Dangl 2006; Wang et al. 2020). Os padrões moleculares associados aos patógenos - PAMPs (*Pathogen-Associated Molecular Patterns*), os padrões moleculares associados aos microrganismos não patogênicos - MAMPs (*Microbial-Associated Molecular Patterns*) e os padrões moleculares associados aos danos - DAMPS (*Damage-Associated Molecular Patterns*) são moléculas conservadas pertencentes a classes específicas de micro-organismos, reconhecidos pelos receptores padrões de reconhecimento - PRRs (*Pattern Recognition Receptors*) presentes na transmembrana de plantas hospedeiras. O reconhecimento de PAMPs/MAMPs por PRRs ativa uma cascata de sinalização induzindo a expressão de genes de defesa da planta hospedeira e impedindo a colonização do patógeno. Esse processo é conhecido como imunidade disparada por PAMPs ou PTI (*PAMP-triggered immunity*), ou resistência horizontal, basal ou de não-hospedeira, resultante da primeira linha de defesa das plantas contra potenciais patógenos (Jones e Dangl 2006; Monaghan e Zipfel 2012; Ngou et al. 2022; Yuan et al. 2021; Zipfel 2014).

Os principais exemplos de PAMPs / MAMPs são a flagelina, o fator de alongação Tu, os lipopolissacarídeos (LPS), os peptideoglicanos (PGN), e os fatores de *quorum sensing* de origem bacteriana; e o β -glucano, o ergosterol e a quitina, de origem fúngica (Boller and Felix 2009; Nurnberger et al. 2004; Zipfel 2014). Os receptores PRR mais conhecidos no reconhecimento de bactérias fitopatogênicas são o FLS2 e EFR do tipo LRR-RLK, e os responsáveis pelo reconhecimento de quitina fúngica são os receptores Lys-MRLKI CERKI e LysM-RLP CEBiP (Boller e He 2009).

Os receptores PRRs presentes na superfície da membrana celular vegetal possuem tipos estruturais: *receptor-like kinases* (RLKs) que possuem domínio quinase intracelular na região C-terminal, e *receptor-like proteins* (RLPs) que não possuem este domínio, mas uma cauda intracelular curta que formam um complexo com a proteína SOBIR1, com a função de reconhecimento do ligante (Yuan et al. 2021). Os PRRs são classificados ainda em subfamílias, sendo eles: domínio de repetições ricas em leucina (LRR – *Leucine-Rich Repeat*); domínio de lisina (LysM – *Lysin Motif*) e domínio semelhante ao do fator de crescimento epidérmico (EGF-like - *Epidermal Growth Factor-like*) ou domínio de lectina (Tang et al. 2017; Zipfel 2014). Após a associação com os ligantes, os RLKs e RLP-SOBIR1 recrutam co-receptores para formar um complexo receptor capaz de fosforilar quinases citoplasmáticas, formando quinases citoplasmáticas semelhantes a receptores (*receptor-like cytoplasmic kinases* - RLCKs), que ativam proteínas no substrato resultantes em alterações fisiológicas (Figura 7A) (Liang e Zhou 2018).

As respostas mais comuns de defesa disparada por PTI em plantas envolve a ativação de uma cascata de sinalização por MAP quinases (MAPKs - *Mitogen-Activated Protein Kinases*) e a ativação de fatores de transcrição do tipo WRKY (Boller e Felix 2009; Rushton et al. 2010; Zipfel e Robatzek 2010), e a produção de espécies reativas de oxigênio (ROS – *Reactive oxygen species*) (Figura 7) que atuam como moléculas sinalizadoras ou impeditivas ao crescimento de micro-organismos, a deposição de calose e lignina que atuam como barreiras físicas na fortificação da parede celular, o acúmulo de quitinases e glucanases que são proteínas relacionadas à patogênese (PR – *Pathogenesis-Related*) e degradam a parede celular de fungos e oomicetos e síntese de compostos secundários antimicrobianos, como as fitoalexinas.

Coevolutivamente, os patógenos adquiriram a capacidade de suprimir as respostas de PTI, atravessando a primeira linha de defesa das plantas, através da secreção e liberação de efetores no citoplasma do hospedeiro, resultando na suscetibilidade disparada por efetores (ETS – *effector-triggered susceptibility*). Nesta etapa, os efetores dos patógenos são reconhecidos por proteínas polimórficas específicas codificadas por genes *R* denominadas de receptores intracelulares com ligação de nucleotídeos e domínios ricos em leucina (NLR), que recebem nomes baseados nos seus domínios característicos de ligação de ATPase (NB- *nucleotide binding*), repetição rica em leucina (LRR – *leucine rich repeat*), e um domínio TIR (*Toll interleukin-1 receptor*) ou CC (*coiled coil*), que podem formar proteínas do tipo TIR-NB-LRR ou CC-NB-LRR, e resultam na imunidade disparada por efetores (ETI – *effector-triggered immunity*), também conhecida como resistência vertical, gene-a-gene ou raça-específica (Dodds e Rathjen 2010; Jones e Dangl 2006).

Os genes *R*, que codificam as proteínas NLR responsáveis pelo reconhecimento dos efetores Avr, possuem ao menos cinco classes reconhecidas dentro dos inúmeros genes de plantas já caracterizados. A exemplo de genes *R* conferindo resistência aos fungos tem-se o gene *I2* que confere resistência em *Solanum lycopersicum* à raça 2 de *F. oxysporum* f. sp. *lycopersici* (Wei et al. 2014); e para as bactérias tem-se os genes *RPS2* e *RPM1* que conferem resistência em *Arabidopsis* à *Pseudomonas syringae* (Reuber e Ausube 1996).

Patógenos podem ainda suprimir genes *R* liberados na primeira resposta de ETI com a produção de efetores e desencadear uma nova cascata de produção de proteínas de reconhecimento por genes *R* da hospedeira resultando em uma nova ETI, representando a quarta etapa da evolução do sistema imune vegetal em um modelo de zigue-zague proposto por Jones e Dangl (2006) (Figura 6).

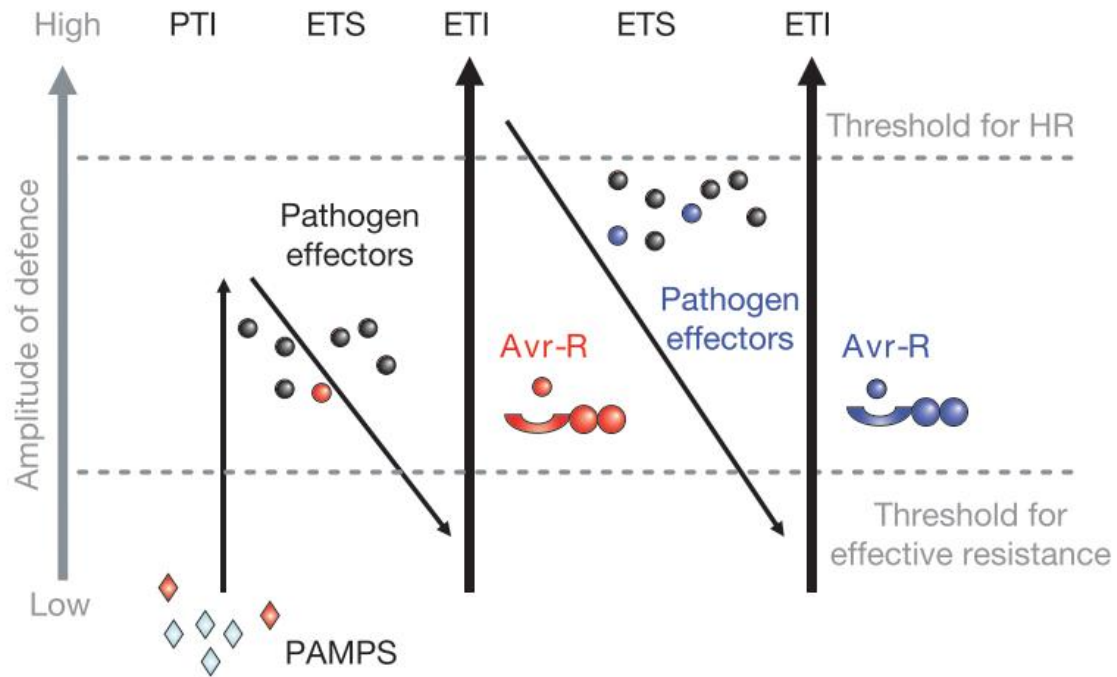


Figura 6. Modelo “zigue-zague” proposto por Jones e Dangl (2006), demonstrando a evolução do sistema imune vegetal em quatro etapas. Na primeira fase as plantas reconhecem os PAMPs/MAMPs/DAMPs de micro-organismos pelo reconhecimento direto e indireto na superfície celular por receptores PRR, resultando na imunidade desencadeada por PAMPs (PTI). Na segunda fase, os patógenos secretam efetores para suprimir as respostas de PTI, resultando em uma resposta de suscetibilidade em plantas desencadeada por efetores (ETS). Na terceira fase, proteínas NB-LRR específicas produzidas por genes R reconhecem os respectivos genes Avr dos efetores produzidos pelos patógenos, resultando na resposta de imunidade desencadeada por efetores (ETI). Na quarta fase, para garantir o sucesso da infecção, novos efetores são produzidos pelos patógenos a fim de suprimir a ETI. Em plantas resistentes, novas proteínas NB-LRR podem reconhecer um efetor recém adquirido e desencadear novamente a ETI.

Diferentemente de PTI, durante o ETI apenas proteínas e moléculas relacionadas a patogenicidade são reconhecidos de forma direta ou indireta por genes *R* da planta que reconhecem genes *Avr* do patógeno, resultando na resistência; e a ausência do gene *R* resultará em suscetibilidade do hospedeiro. As respostas de ETI também são responsáveis pela alteração de hormônios vegetais como ácido jasmônico (JA) e salicílico (SA); acúmulo de proteínas relacionadas a patogênese (PR); e conferir resposta de defesa à diversos ataques subsequentes de patógenos, através da resistência sistêmica adquirida (SAR) (Agrios 2004; Jones e Dangl 2006).

Entretanto, como as linhas de defesa funcionam como uma cadeia de sinalização e respostas, ETI pode acelerar e amplificar as respostas de PTI, resultando na resistência do hospedeiro como também na resposta de

hipersensibilidade (HR) no sítio de infecção (Ngou et al. 2022a). Vários estudos mostraram que componentes são regulados por PTI e ETI, como as MAPKs, ROS, e produção de fito hormônios. A resistência e as respostas da ETI dependem dos componentes da via da PTI, potencializando a PTI, trabalhando de maneira colaborativa para garantir uma imunidade efetiva (Figura 7) (Yuan et al. 2021).

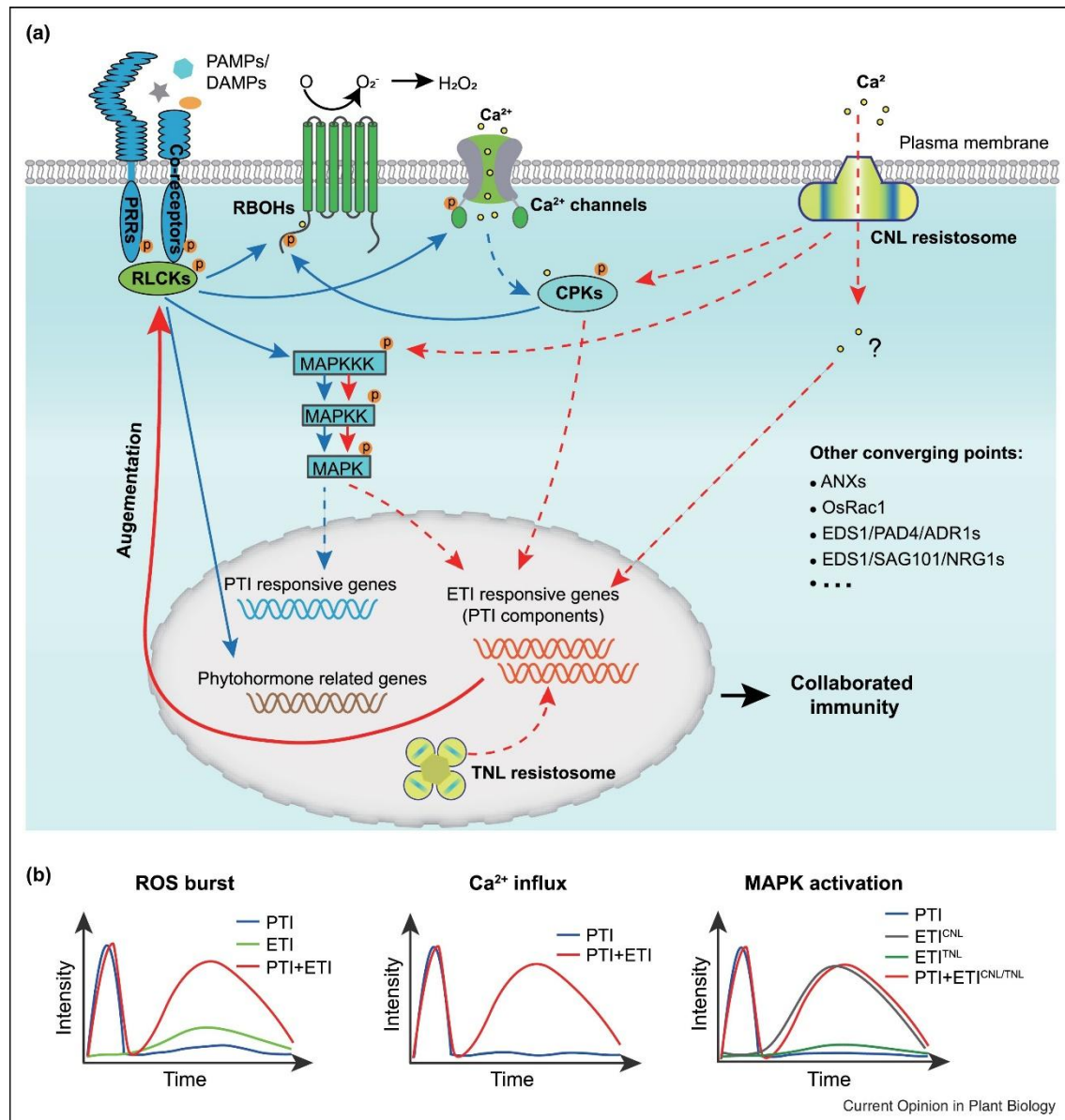


Figura 7. Modelo de imunidade de plantas disparada pela sinalização conjunta de PTI e ETI. **A.** A sinalização de PRR e NLR co-regula as respostas imunes. Após o reconhecimento de PAMPs/DAMPs, os PRRs localizados na superfície celular recrutam co-receptores para formar complexos receptores e ativar RLCKs, que subsequentemente fosforilam componentes (por exemplo, RBOHD, CNGCs/OSCA1.3, MAPKKKs, WRKYs) para desencadear explosão de ROS, influxo de cálcio, ativação de fatores de transcrição MAPK, produção de fito hormônios e reprogramação transcricional. Os NLRs formam resistossomos após a ativação, que eventualmente levam a respostas imunes sobrepostas, mas com dinâmicas

diferentes, demonstradas em (b), e amplitudes geralmente mais fortes em comparação com PTI. As setas sólidas indicam efeitos diretos e as setas tracejadas indicam efeitos indiretos. Pontos de interrogação indicam mecanismos desconhecidos. **B.** Dinâmica e amplitude das respostas imunes em diferentes condições (ou seja, PTI sozinho, ETI sozinho ou PTI + ETI). O eixo x indica o tempo após o tratamento de elicitores ou inoculação de patógenos para ativar diferentes sinalizações. 'PTI' refere-se a respostas após aplicação de PAMP sozinho (PTI) e 'ETI' refere-se a respostas após expressão condicional de efetores indutores de ETI em plantas transgênicas. 'PTI + ETI' refere-se a respostas após aplicação/expressão de efetores indutores de PAMP e ETI ou inoculação de patógenos avirulentos em plantas. Adaptado de Yuan et al. (2021).

1.8 Composição Genômica e Melhoramento de *Musa*

As espécies de *Musa* foram primeiramente classificadas dentro de três subgêneros: *Physocaulis*, *Eumusa* e *Rhodoclamys* (Sagot 1887, Baker 1893). Em uma nova classificação por Cheesman (1947), *Physocaulis* foi considerado como novo gênero denominado *Ensete*, e os dois subgêneros reclassificados em novas seções denominadas *Callimusa* e *Australimusa*. As bananas comestíveis classificadas primeiramente por Cheesman (1947) pertencentes ao subgênero *Eumusa* e *Rhodoclamys* foram concentradas no gênero *Musa* (Sachter-Smith 2023).

Os genomas conhecidos de bananeira AA, BB, AB, AAB, AAAA, AAAB, AABB e ABBB, compreendem organismos diploides, triploides e tetraploides, com 22, 33 e 44 cromossomos, respectivamente, oriundos de cruzamentos entre *Musa acuminata* e *Musa balbisiana*, e por partenocarpia, que ocorre em *M. acuminata* (Sachter-Smith 2023; Silva et al. 2016). A classificação dos grupos genômicos em subgrupos designa o agrupamento de determinadas cultivares que se originaram de um único clone por meio de mutação. Os subgrupos, portanto, podem apresentar uma diversidade de cultivares que apresentam características uniformes, como os subgrupos Prata, Maçã, Terra e Cavendish, existentes no Brasil, com baixa variabilidade genética (Silva et al. 2016). Outros fatores de grande importância para a seleção de cultivares para a produção é a preferência dos consumidores internos e externos, a produtividade, a tolerância aos estresses bióticos e abióticos e o porte da planta, o que reduz o potencial comercial de diversas cultivares encontradas no Brasil (Silva et al. 2013).

A maior variabilidade genética de bananeira é encontrada nas diversas subespécies selvagens de *Musa acuminata* e suas variedades, como *M. acuminata* subsp. *banksii*, *M. acuminata* subsp. *burmannica*, *M. acuminata* subsp. *malaccensis*, *M. acuminata* var. *zebrina*, *M. acuminata* var. *chinensis*, entre outras (Sachter-Smith

2023). *M. acuminata* subsp. *burmannica* acesso 'Calcutta 4' é oriunda do centro de origem de *Musa* que coevoluiu com diversos agentes patogênicos e que, consequentemente, adquiriu um arsenal de mecanismos de defesa através dos genes de resistência a estresses bióticos e abióticos, sendo amplamente utilizada no desenvolvimento de cultivares de bananas comerciais com maior resistência a doenças (Miller et al. 2010b; Sachter-Smith 2023).

O melhoramento convencional de bananeiras é baseado no cruzamento entre dois parentais contrastantes e complementares, que podem produzir sementes, sendo em maior quantidade entre diploides, seguido por triploides e tetraploides (Amorim et al. 2016). No início da década de 1930 o cruzamento de uma cultivar triploide (AAA 'Gros Michel') com um diploide (AA selvagem) resultou no primeiro tetraploide. Esse modelo de hibridação permite o melhoramento de algumas cultivares diploides e triploides de banana, mundialmente utilizado nos programas de melhoramento (Silva et al. 2013).

Como a maioria das cultivares plantadas são triploides, como 'Cavendish', e originárias da hibridização intra e interespecífica, muitas apresentam esterilidade e sua reprodução se dá apenas por partenocarpia, o que diminui consideravelmente a diversidade genéticas das cultivares comerciais (Heslop-Harrison e Schwarzacher 2007). As progênies obtidas entre genótipos triploides e tetraploides ainda apresentam tamanho reduzido e possuem número variado de ploidias, inclusive aneuploides, dificultando o melhoramento genético convencional (Silva et al. 2013). Anormalidades são frequentes entre cruzamentos de diploides, triploides ou tetraploides, como a ausência de embrião e endosperma nas sementes, e inclusive na meiose, o que dificulta a previsão de segregação observada nas progênies (Amorim et al. 2016).

Desde a década de 20 os programas de melhoramento de *Musa* visam a criação de genótipos resistentes às pragas e doenças. O desenvolvimento desses programas, juntamente à transformação genética, vem possibilitando o surgimento de novos genótipos de banana geneticamente modificados resistentes a doenças, sem comprometer as características comerciais desejáveis. Juntamente com outros genótipos selvagens, 'Calcutta 4' tem sido objeto de buscas de genes de resistência para uso em programas de melhoramento genético, por ser diploide, fértil e resistente,

e por possuir dois genes homozigotos e dominantes associados à resistência ao estresse biótico. Entretanto, como muitos genótipos são estéreis e realizam a partenocarpia, o que resulta no desenvolvimento de frutos sem sementes ou com sementes não viáveis, o melhoramento convencional através dos cruzamentos é limitado às cultivares comerciais de banana. A reprodução realizada majoritariamente pela propagação assexuada de mudas, também auxilia na redução da variabilidade genética (Amorim et al. 2016; Silva et al. 2013; Simmonds 1953).

1.9 Genômica de *Musa*

Diante das limitações, novas técnicas buscam aprimorar o melhoramento genético de bananas, com o objetivo de selecionar a maior quantidade de características desejáveis em um mesmo genótipo. Com o auxílio da biologia molecular e o conhecimento das informações genéticas disponíveis, é possível ampliar técnicas moleculares direcionadas que envolvem a identificação de marcadores moleculares, e a expressão diferencial de genes em *Musa*, por exemplo, que podem ser utilizados na engenharia genética, como a mutação, e desenvolvimento de transgênicos (Amorim et al. 2016; Amorim et al. 2012; David et al. 2020; Soares et al. 2021).

O desenvolvimento de técnicas de sequenciamento mais robusta realizados pelos sequenciadores de nova geração (*Next Generation Sequencing* - NGS), como Illumina/Solexa, resultaram num sequenciamento massal e rápido amplamente utilizado em pesquisas de transcrito e genômica (Reuter et al. 2015). Devido a tecnologia NGS, quatro versões completas do genoma de *Musa acuminata* spp. *malaccensis* ‘DH-Pahang’ já foram sequenciadas e anotadas (Belser et al. 2021; D’Hont et al. 2012; Martin et al. 2016). A partir de ‘DH-Pahang’ outras espécies de *Musa* como *M. acuminata* ‘Banksii’, *M. acuminata* ‘Calcutta 4’, *M. acuminata* ‘Maia Oa’ (Rouard et al. 2018), *M. balbisiana* ‘DH-PKW’ (Wang et al. 2019) e *M. balbisiana* ‘PKW’ (Davey et al. 2013) tiveram seu genoma parcialmente anotado.

O estudo de resistência genética de genótipos à *Foc* tem sido alvo de buscas constantes no manejo da Murcha de Fusarium. A caracterização dos loci envolvidos na resistência às raças TR4 e STR4 em genótipos selvagens e resistentes foi observada por Chen et al. (2023) por meio da detecção de loci de características quantitativas - QTLs (*quantitative trait loci*), oriundos de *Musa acuminata* spp.

malaccensis. Demais subespécies diploides silvestres de *M. acuminata* também possuem resistência a *Foc* conhecida, como *M. acuminata* subsp. *burmannica*, *M. acuminata* ssp. *microcarpa* e *M. acuminata* ssp. *siamea* (Chen et al. 2019; Li et al. 2015b; Xu et al. 2021).

1.10 Transcritômica da interação *Musa* e *Foc*

Genes *R* que codificam proteínas NLRs foram encontrados em plantas de bananas selvagens que conferem resistência a patógenos distintos, inclusive para a *Foc* raça 4, como a *Musa acuminata* ‘Pahang’ e *M. acuminata* subsp. *burmmanicoides* ‘Calcutta 4’ (sin. *M. acuminata* subsp. *burmmanica* acesso ‘Calcutta 4’) (Chen et al. 2019; Miller et al. 2008; Zhang et al. 2018).

Desde o sequenciamento do genoma de ‘DH-Pahang’, uma espécie selvagem altamente resistente a *Foc* TR4, diversos estudos de transcritômica da interação *Musa* e fitopatógenos pôde ser realizada. Em ‘DH-Pahang’ 36.769 genes foram identificados, sendo um terço de cópias múltiplas de genes (parálogos) anotados, com cerca de 128 NLR *loci* detectados como NLR gene ou pseudogene (Belser et al. 2021). Por isso, o genoma de referência é utilizado no mapeamento de transcritos, observados nas interações de diversos genótipos e patógenos. Em ‘Calcutta 4’, 114 NLRs potencialmente envolvidos em respostas de defesa a estresses bióticos e abióticos, já foram identificados (Araújo 2020).

Os sequenciadores de nova geração permitiram ainda o desenvolvimento de técnicas capazes de avaliar os níveis de expressão gênica, como o RNA-Seq, que permite que o conjunto de transcritos de uma célula, compostos do RNA mensageiro (mRNA), RNAs não codificadores (ncRNA) e microRNAs (miRNA) sejam analisados e quantificados (Wang et al. 2009). Através da análise de transcritômica é possível compreender os processos de modulação gênica relacionada aos estresses submetidos às plantas, seja pela interação com um patógeno ou por estresses abióticos, que permite a identificação de genes de interesse relacionados a resistência a esses fatores (Staiger et al. 2013; Wang et al. 2009; Zhou & Zhang 2020).

No patossistema *Musa* sp. e *F. oxysporum* f. sp. *cubense* os estudos conduzidos focaram em genótipos resistentes a *Foc* R1 e TR4, como ‘Pahang’ (Zhang et al. 2019), ‘Calcutta’ (Kotari et al. 2018b), ‘Nongke N° 1’ (Li et al. 2012), ‘Yueyoukang

1' (Bai et al. 2013), 'Guijiao 9' (Sun et al., 2019) e 'Tianbaojiao' (Cheng et al. 2019). 'Prata' e 'Maçã' (Rebouças et al. 2018) são os genótipos mais utilizados nas interações suscetíveis a *Foc* R1; e cultivares de 'Cavendish' (Zhang et al. 2019) nas interações suscetíveis a *Foc* TR4. A idade das plantas inoculadas na maioria dos estudos tem variado entre 2 semanas a 6 meses, e a de coleta de amostras, considerando o tempo após a infecção, entre 3 horas a 28 dias após a inoculação (Rocha et al. 2021).

Comumente, o RNA total da planta é extraído de raízes, entretanto, por ser um patógeno vascular, pode ser extraído de outras partes da planta, como realizado por Li et al. (2013), de uma mistura de raízes, pseudocaule, folhas, frutos e flores. Vale considerar que, em cultivares resistentes, o patógeno pode penetrar as células radiculares, mas a colonização é interrompida e a doença não evolui para outras partes da planta (Li et al. 2017).

Dessas análises de transcritômica, as vias mais comumente ativadas foram relacionadas a biossíntese de fenilpropanoides, biossíntese de açúcares, modificações da parede celular, biossíntese de flavonoides e transdução de sinais de fitormônios (Rocha et al. 2021).

A ativação de PTI através do reconhecimento de PAMPs por proteínas PRR foi descrita nas interações com *Foc* TR4 envolvendo a cultivar 'Brazil' do grupo 'Cavendish' (suscetível) e os genótipos resistentes de *M. acuminata* 'Nongke Nº 1', 'Yueyoukang 1' e 'Pahang' (Bai et al. 2013; Li et al. 2013; Li et al. 2017; Zhang et al. 2019). Além dessas, Niu et al. (2018) caracterizou, durante a interação de Cavendish 'Yueyoukang1' resistente e Cavendish 'Baxijiao' suscetível a *Foc* TR4, a atividade de quitinases, que age na degradação de fungos, pectina esterase e produção de xiloglucano, responsável pela produção de hemicelulose na modificação da parede celular vegetal, como importantes agentes de defesa primários contra patógenos.

Outras importantes vias ativadas através da expressão diferencial de genes oriundas de genótipos resistentes, principalmente a *Foc* TR4, foram descritas, como a biossíntese de flavonoides (Li et al. 2012; Wang et al. 2017; Sun et al. 2019), metabolismo do ácido alfa-linolênico (Wang et al. 2017; Wang et al. 2012), metabolismo de amido e sacarose (Wang et al. 2017), metabolismo da fenilalanina (Li et al. 2017; Wang et al. 2012), metabolismo de drogas - citocromo P450, metabolismo

de açúcares de aminoácidos e açúcares de nucleotídeos (Wang et al. 2012), fitoalexinas (Li et al., 2013), modificações da parede celular (Bai et al. 2013; Li et al. 2012; Niu et al. 2018; Sun et al. 2019; Zhang et al. 2019), biossíntese via sinalização de etileno (Li et al. 2013), fatores de transcrição (Bai et al. 2013; Li et al. 2012; Song et al. 2016; Zhang et al. 2019), morte celular programada (Li et al. 2012), atividade da oxidoredutase (Sun et al. 2019), via de sinalização ativada por auxina, processo catabólico de lignina (Cheng et al. 2019), resposta celular (Cheng et al. 2019; Fei et al. 2019), biossíntese de metabólitos secundários, metabolismo de ácidos graxos, metabolismo de glicerolípídios e glicerofosfolípídios (Li et al. 2017), processo metabólico de polissacarídeos celulares, metabolismo de frutose e manose, metabolismo de esfingolípídeos, metabolismo de butanoato, metabolismo de porfirina e clorofila, e biossíntese de carotenóides (Niu et al. 2018).

1.11 Transformação de *Musa*

Diferentes métodos têm sido utilizados na transformação de bananas, incluindo a transformação mediada por *Agrobacterium*, eletroporação e bombardeamento por micro projéteis (Tripathi et al. 2015). O principal alvo da transformação de bananas são as suspensões de células embrionárias (ECS), pois as plantas transgênicas são derivadas de uma única célula, diminuindo o risco de quimeras (Wang et al. 2021). Os primeiros estudos envolvendo a introdução de genes de resistência em cultivares de banana tinham como foco a resistência à Sigatoka Negra e Amarela e à Murcha de Fusarium (Remy et al. 2013).

Por desempenhar um importante papel na defesa de plantas contra o ataque de fitopatógenos, as proteínas PR têm se mostrado como candidatos para realização de engenharia genética em diversos cultivos, agindo como potenciais agentes de defesa de plantas resistentes a fungos. As estratégias incluem testes de um ou mais genes intraespecíficos que codificam “proteínas antifúngicas” que possuem funções de toxicidade ou efeito fungistático, além de genes que possuem como função um complexo de mecanismos de defesa como SAR, que possibilitam a criação de cultivares de bananas transgênicas pelo desenvolvimento de técnicas de engenharia genética. Assim, o incremento de genes *R* que codificam proteínas PR intra ou intercelular pode implicar na resistência de amplo espectro pela co-expressão de

várias proteínas PR direcionadas aos patógenos, em diversas partes da planta e suas progênes (Thangavelu et al. 2012 e todas as referências nele).

Estes genes podem, após identificados e caracterizados, serem utilizados em estratégias de melhoramento genético em diferentes cultivares de *Musa*. Uma diversidade de genes oriundos de organismos vegetais distintos, relacionados a respostas de defesas a ataques bióticos, foram introgrididos em cultivares de bananas e estão demonstrados na Tabela 1.

Tabela 1. Genes utilizados em estudos de transformação genética de *Musa* para obter variados níveis de resistência à diferentes raças de *Fusarium oxysporum* f. sp. *cubense* (*Foc*).

Gene	Promotor	Origem	<i>Foc</i>	Referência
Quitinase (<i>RCC2</i>)	CaMV35S	<i>Oriza sativa</i>	R1	Maziah et al. (2007)
Endoglucanase (β 1-3)	CaMV35S	<i>Glycine max</i>	R1	Maziah et al. (2007)
Genes anti-apoptose (<i>Bcl-Xl</i> e <i>Bcl-2</i>) e Morte celular (<i>Ced-9</i>)	pZmUbi	Animal	R1	Paul et al. (2011)
Defensinas florais (<i>PhDef1</i> , <i>PhDef2</i>)	pZmUbi	Petúnia	R1	Ghag et al. (2012)
Peptídeo antimicrobiano (<i>Ace-AMP1</i>)	CaMV35S	<i>Allium cepa</i>	R1	Mohandas et al. (2013)
Defensinas (<i>Sm-AMP-D1</i>)	pZmUbi	<i>Stellaria media</i>	R1	Ghag et al. (2014b)
<i>MusaDAD1</i> , <i>MusaBAG1</i> , <i>MusaBI1</i>	pZmUbi	<i>Musa</i>	R1	Ghag et al. (2014b)
Morte celular (<i>Ced-9</i>)	CaMV35S	Sintético	R1	Magambo et al. (2016)
Morte celular (<i>Ced-9</i>)	pZmUbi	<i>Caenorhabditis elegans</i>	R1	Sunisha et al. (2020)
Peptídeo antimicrobiano (<i>Ace-AMP1</i>) e Ferredoxina vegetal (<i>Ca-pflp</i>)	CaMV35S, pZmUbi	<i>Allium cepa</i> ; <i>Capsicum annum</i>	R1	Sunisha et al. (2020)
Análogo de magainina (<i>MSI99</i>)	pAtUbq3	<i>Xenopus laevis</i>	R2	Chakrabarti et al. (2003)
Lisozima humana	CaMV35S	Humano	TR4	Pei et al. (2005)
Gene da ferredoxina (<i>Atfd3</i>); Ferredoxina vegetal (<i>pflp</i>)	CaMV35S	<i>Arabidopsis</i>	TR4	Yip et al. (2011)
Proteína semelhante à taumatina (<i>tlp</i>)	CaMV35S	<i>Oryza sativa</i>	TR4	Mahdavi et al. (2012)
Endoquitinase (<i>ThChit42</i>)	CaMV35S	<i>Trichoderma harzianum</i>	TR4	Hu et al. (2013)
Gene de resistência Análogo 2 (<i>RGA2</i>)	Nos-P	<i>Musa acuminata</i> ssp. <i>malaccensis</i>	TR4	Dale et al. (2017)
Morte celular (<i>Ced-9</i>)	Ubi-P	<i>Caenorhabditis elegans</i>	TR4	Dale et al. (2017)
Gene relacionado a patogênese (<i>MaPR-10</i>)	CaMV35S	<i>Musa acuminata</i> ssp. <i>malaccensis</i>	TR4	Baharum et al. (2018)
Fator de transcrição (<i>MpbHLH</i>)	pZmUbi	<i>M. acuminata</i> 'Dajiao'	TR4	Li et al. (2022)

Muitos genes super expressos em *Musa* selvagens durante a interação com *Foc*, podem ser aliadas ao incremento de resistência em cultivares comerciais originalmente suscetíveis. Como demonstrado na Tabela 7, diversos genes oriundos de genótipos selvagens de *Musa* como *MusaDAD1*, *MusaBAG1*, e *MusaBI1*, responsáveis pela morte celular, são considerados eficientes após a cisgenia em cultivares comerciais de *Musa*. Entretanto, genes também podem ser oriundos de espécies distintas e, após a inserção no genótipo de *Musa*, apresentar características desejáveis de resistência à *Foc*, como em linhagens transgênicas que possuem o gene *Ced9* do nematoide *Caenorhabditis elegans*.

Os casos bem sucedidos na produção de cultivares comerciais de bananas resistentes a *Foc* R1, como a cv. 'Rasthali' (subgrupo Maçã AAB) (Ghag et al. 2014a) e TR4, como cv. 'GN' (subgrupo Cavendish AAA) (Dale et al. 2017), utilizando a introgressão de genes envolvidos em respostas de defesa, mostra a importância da técnica de transcritômica para o avanço na engenharia genética de *Musa*, especialmente através da cisgenia, que utiliza de genes intraespecíficos de espécies selvagens para ser introgrididos em cultivares comerciais.

1.12 Conclusão e justificativa de trabalho

Diante do exposto, fica evidente que a bananicultura apresenta grande importância social e econômica, entretanto sofre elevadas perdas devido à sua suscetibilidade a um grande número de patógenos que causam doenças de alto impacto econômico, como a Murcha de Fusarium.

Tendo em vista a persistência do patógeno *F. oxysporum* f. sp. *cubense* no solo, o desenvolvimento de plantas resistentes é uma medida de controle recomendável. Nos últimos anos, a análise massiva do transcrito de várias espécies vegetais tem possibilitado a prospecção e identificação de genes de interesse para o melhoramento genético. Entretanto, ainda é relativamente pequena a lista de genes envolvidos na resistência/tolerância a estresses que sejam comprovadamente efetivos e que já tenham sido introgrididos em variedades comerciais.

Neste contexto, na atual era pós-genômica, torna-se estratégico a prospecção de novos genes e moléculas utilizando modelos biológicos alternativos, além daqueles existentes nas culturas-alvo. Desta forma, a prospecção da diversidade

genética contida no germoplasma de plantas silvestres é um valioso recurso para a agricultura, e que ainda é muito pouco explorado pela comunidade científica brasileira. Genes envolvidos em resistência a *F. oxysporum* f. sp. *cubense* raça STR4 na espécie selvagem *M. acuminata* subsp. *burmannica* acesso 'Calcutta 4' poderão ser identificados nesse projeto através do sequenciamento dos transcritos resultantes das interações patógeno-hospedeiro.

O emprego de métodos de validação de função gênica de genes candidatos baseados em PCR quantitativo em tempo real possibilitará a seleção de genes de resistência apropriados para serem utilizados em transformação genética de plantas modelo e, posteriormente, para o desenvolvimento de cultivares comerciais de *Musa* mantendo as características agronômicas desejáveis e oferecendo a resistência contra o fungo *F. oxysporum* f. sp. *cubense* raça1, STR4 e possivelmente a TR4, organismo causador da Murcha de Fusarium de extrema importância para o Brasil e para o mundo.

Assim, o objetivo desse trabalho foi identificar e validar genes alvo envolvidos na resistência de *M. acuminata* subsp. *burmannica* acesso 'Calcutta 4' durante a interação com o patógeno *F. oxysporum* f. sp. *cubense* raça STR4, causador da Murcha de Fusarium.

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Capítulo II

Identificação molecular do patógeno *Fusarium oxysporum* f. sp. *cubense* isolado 218A, causador da Murcha de Fusarium

1. Introdução

A classificação atual de *Fusarium oxysporum* f. sp. *cubense*, causador da Murcha de Fusarium em bananeiras, é baseada em raças obtidas por testes de virulência em cultivares, análises de VCGs (Correll 1991; Edel-Hermann and Lecomte 2019; Leslie and Summerell 2006; Ploetz 2006a) e análises de diversidade genética e filogenética através de marcadores moleculares (Magdama et al. 2020; Maryani et al. 2019; Ploetz 2006a). A raça tropical 4 (TR4) é considerada como a mais destrutiva mundialmente, por seu potencial devastador em plantações de bananas do subgrupo 'Cavendish' (AAA), resistente à raça 1 (Pegg et al. 2019). Entretanto, em termos de patogenicidade, a única diferença entre as raças TR4 e subtropical 4 (STR4) é a capacidade de STR4 causar sintomas em 'Cavendish' apenas em regiões subtropicais (Dita et al. 2018). Em termos de VCGs é conhecido que TR4 possui apenas a VCG 01213/16, enquanto STR4 possui 8 VCGs conhecidas: 0120, 021, 0122, 0126, 0129, 01210, 01211, 01215. As raças 1 e 2 compartilham todas as VCGs descritas, exceto a VCG 01213/16. Isolados de STR4 pertencentes às VCGs 0120/15 já foram encontradas infectando 'Cavendish' em regiões subtropicais como Brasil, Austrália, Ilhas Canárias e África do Sul (Dita et al. 2020).

Somente no Brasil, três raças de *Foc* estão presentes em todas as regiões produtoras de bananas do país (raças 1, 2 e STR4), e a raça TR4 é considerada praga quarentenária regulamentada pelos órgãos de fiscalização regido pelo Ministério de Agricultura, Pecuária e Abastecimento (MAPA). Destes, 8 VCGs já foram encontradas no Brasil, sendo elas 0120, 0122, 0124, 0125, 0128, 01215, 01220, e 01222, sendo a mais predominante a VCG 0120 de STR4 (Batista et al. 2022).

A diagnose da Murcha de Fusarium se dá pela reconhecimento de sintomas típicos, como o amarelecimento e murcha de folhas, descoloração e necrose de pseudocaule e rizoma. Entretanto, os sintomas externos são visualizados cerca de 60 dias após o processo de infecção inicial, o que compromete a plantação visto que o

inóculo pode ter percorrido por toda a área plantada de bananas. O uso de primers específicos nas abordagens moleculares como a PCR, permite a identificação de raças de *Foc* em plantas assintomáticas. Essas abordagens de reconhecimento específico de DNA auxiliam programas de contingência e quarentena no controle preventivo da doença. Os primers *Foc*TR4-F e *Foc*TR4-R descrito por Dita et al. (2010) desenhado na região espaçadora intergênica (IGS), é utilizado na identificação de TR4 VCG 01213/16 adotado pela *International Regional Organization for Agricultural Health* (OIRSA) e *Food and Agriculture Organization of the United Nations* (FAO) nos programas de quarentena da América Latina e Caribe. Outros primers são capazes de identificar ambos TR4 e STR4, como *Foc*-1 e *Foc*-2 (Lin et al. 2009) e *Foc*-F e *Foc*-R (Li et al. 2013a). Entretanto, ao comparar as amplificações de isolados de *Foc*, Magdama et al. (2019) reportaram amplificações inespecíficas dos primers *Foc*TR4-F/*Foc*TR4-R, *Foc*-1/*Foc*-2 e *Foc*-F/*Foc*-R em isolados endofíticos de *F. oxysporum*.

Fusarium oxysporum f. sp. *cubense* pertence ao complexo de espécies de *Fusarium oxysporum* (FOSC). A diversidade genética de *Foc* tem sido estudada baseada também em análises filogenéticas com o uso de marcadores moleculares como RNA *polymerase II large subunit* (RPB1), RNA *polymerase II subunit* (RPB2), *elongation factor 1-alpha gene* (TEF) e *mitochondrial subunit rDNA* (mtssu). Em uma reclassificação das raças de *Foc* em novas espécies, Maryani et al. (2019) descreveram nove linhagens distintas em novas espécies, onde *Foc* Raça 4 pertencente à linhagem 1, foi reclassificada para *Fusarium odoratissimum*, sem a distinção de isolados de TR4 e STR4. Entretanto a reclassificação foi refutada por Bedoya et al. (2021) reportando discrepância nos dados, que por não serem robustos, não suportam os mesmos clados monofiléticos descritos. Outras estratégias no estudo de diversidade também incluem as VCGs, RAPDs, RFLPs, entre outros.

Para se fazer um estudo de interação entre patógeno e hospedeiro, é imprescindível que se tenha conhecimento sobre a biologia e taxonomia dos objetos de estudo. Conhecer a biologia do patógeno (necrotrófico, biotrófico ou hemibiotrófico) informa quais vias de sinalização da planta são mais relevantes durante a interação. Por exemplo, patógenos biotróficos geralmente induzem vias de defesa mediadas por ácido salicílico, enquanto necrotróficos frequentemente envolvem ácido jasmônico e etileno (Glazebrook 2005). Além disso, o conhecimento

acerca de efetores que regulam a patogenicidade de raças de *Foc* e modulam as respostas de defesa no hospedeiro. Em ‘Cavendish’ o estudo de transcriptômica envolvendo duas raças distintas de *Foc* mostraram que as respostas de defesa e as vias de sinalização são completamente diferentes, o que pode explicar os padrões de resistência diferenciais em ‘Cavendish’ em relação a *Foc* raça 1 e *Foc* TR4 (Dong et al. 2020; Li et al. 2023).

O isolado utilizado nos bioensaios constituintes deste trabalho foi *Fusarium oxysporum* f. sp. *cubense* CNPMF 218A coletado em 2014 no pseudocaule da cultivar ‘Nanica’ na cidade de Eldorado, São Paulo, cedido pelo laboratório de Fitopatologia da Embrapa Mandioca e Fruticultura. O isolado monospórico foi classificado anteriormente como raça STR4 baseado na classificação pelo grupo de compatibilidade vegetativa, resultante na VCG 0120, e através do teste de patogenicidade positivo para ‘Cavendish’ (Rocha et al. 2018). Atualmente é utilizado como isolado padrão nos estudos envolvendo a interação *Foc* e *Musa* sp. na Embrapa CNPMF.

Desta forma, o objetivo deste capítulo foi identificar o isolado 218A para a confirmação da espécie *Foc* e para a raça STR4, usando abordagens moleculares e genômicas.

2. Materiais e métodos

2.1 Caracterização do isolado

Análises microscópicas foram realizadas em isolados que permaneceram por 10 dias em placas com meio BDA a 28°C e 12h de fotoperíodo, a fim de visualizar a morfologia de esporos e hifas em microscópio estereoscópio.

2.2 Extração de DNA total e reação de PCR

Para confirmar a identidade molecular do isolado 218A utilizado no bioensaio da interação entre *Foc* STR4 x *Musa*, foi realizada a extração de DNA a partir do micélio fúngico produzido por 10 dias em placas com meio BDA, coletado com o auxílio de um palito estéril e armazenado em microtubos de 1,5 mL contendo 20 µl de tampão TE pH 8,0. A extração seguiu utilizando o protocolo e o kit Dneasy Plant Mini Kit (Qiagen). A verificação da qualidade da extração foi realizada via eletroforese em gel de agarose 1%.

Foram realizadas reações de PCR a partir do DNA extraído com pares de primers iniciadores das regiões RNA polimerase II, Beta-tubulina e Fator de alongação 1-alfa (Tabela 1). Para cada reação de PCR foram utilizados os seguintes reagentes: MyTaq Mix 2x (Bioline Meridian Bioscience), 0.2 mM de primers forward e reverse (10 uM), 1 µL de DNA genômico (25 ng/µL) e água Milli-q. O DNA do isolado 218A também foi testado utilizando os primers específicos para detecção de *Foc* TR4: *FOCTR4-F* e *FOCTR4-R* descrito por (Dita et al. 2010), oriundos da região IGS. Para essa reação foram utilizados os seguintes reagentes: PlatinumTaq 2 U (Invitrogen Thermo Fisher), 1 X de PCR buffer -MgCl₂ (10X), 1,5 mM de MgCl₂ (50 mM), 0,2 mM de cada dNTPs (10mM), 0.2 mM de primers *forward* e *reverse* (10 uM), 1 µL de DNA genômico (25 ng/µL) e 26,4 µL de água Milli-q. As amplificações foram realizadas com temperatura inicial de desnaturação de 95 °C por 1:30 min, seguida de 35 ciclos compostos por: desnaturação a 95 °C por 20 s, anelamento a 56 °C (TEF), 55 °C (para TUB e RPB2) e 62 °C (para IGS) por 45 s, e extensão a 72 °C por 45 s. Foi deixada uma extensão final a 72 °C por 5 min.

Tabela 1. Iniciadores (primers) utilizados na identificação molecular do isolado 218A.

Região gênica	Iniciador	Sequência (5'→3')	Sentido	Fragmento (pb)	Ref.
RNA polimerase II (RPB2)	RPB2-5f2	GGG GWG AYC AGA AGA AGG C	Senso	882	O'Donnell et al. (2010)
	RPB2-7cr	CCC ATR GCT TGY TTR CCC AT	Anti-senso		O'Donnell et al. (2010)
Beta-tubulina (TUB)	T1_P1	AAC ATG CGT GAG ATT GTA AGT	Senso	700-800	O'Donnell and Cigelnik (1997)
	Bt2b_P6	ACC CTC AGT GTA GTG ACC CTT GGC	Anti-senso		Glass and Donaldson (1995)
Fator de alongação (TEF)	EF1F	TGC GGT GGT ATC GAC AAG CGT	Senso	550-700	Jacobs et al. (2004)
	EF2R	AGC ATG TTG TCG CCG TTG AAG	Anti-senso		Jacobs et al. (2004)
Região intergênica (IGS)	<i>FOCTR4-F</i>	CACGTTTAAGGTGCCATGAGAG	Senso	463	Dita et al. (2010)
	<i>FOCTR4-R</i>	CGCACGCCAGGACTGCCTCGTGA	Anti-senso		Dita et al. (2010)

A confirmação do tamanho dos fragmentos e pureza da reação foi realizada através da eletroforese em gel de agarose 1%. Todos os produtos de PCR foram tratados, para a remoção de resíduos de dNTPs e iniciadores, com as enzimas *Exonuclease I* e *Shrimp Alkaline Phosphatase* (ExoSap - Cellco), seguindo o protocolo do fabricante, e posteriormente encaminhados para sequenciamento no Laboratório de Sequenciamento de DNA da Universidade Católica de Brasília, Brasília, Distrito Federal, Brasil.

2.3 Análise de sequências e filogenia

A qualidade da sequência foi analisada utilizando o software DNA Dragon (Hepperle 2011), e a sequência consenso foi montada utilizando as fitas senso e anti-

senso no software MEGA7 (Kumar et al. 2016). As ambiguidades foram resolvidas manualmente observando o a qualidade do nucleotídeo no eletroferograma. As sequências oriundas dos produtos de PCR das regiões TUB, TEF, RPB2 e IGS foram submetidas a identificação de identidade de nucleotídeos utilizando a ferramenta BLAST, nos bancos de dados do NCBI e Fusarium-ID (<https://www.fusarium.org/page/Pairwise%20ID>). Sequências de referência foram baixadas dos bancos de dados para comporem as comparações filogenéticas. O alinhamento das sequências foi realizado no MAFFT v.7 (Kato and Standley 2013) usando o algoritmo Muscle e analisado manualmente. A análise filogenética foi feita usando os critérios de Máxima Verossimilhança (MV) e Inferência Bayesiana (IB) a partir de cada sequência de genes parciais identificados, para verificar possíveis conflitos tipológicos com sequências de *Foc* depositadas no NCBI e Fusarium-ID. Foi escolhido o melhor modelo de substituição nucleotídica para cada conjunto de dados utilizando MrModeltest (Nylander 2004) e implementado na análise de IB. Os blocos de dados foram submetidos ao MrBayes v3.2.2 (Ronquist et al. 2012) disponível dentro do webportal CIPRES (Miller et al. 2010a), foi gerado um bloco de dados para cada gene analisado e um contendo a combinação deles. A análise de IB utilizou a cadeia de Markov Monte Carlo (MCMC) executada com um total de 10 milhões de gerações, com amostragens a cada 1000 gerações. A Probabilidade posterior foi calculada utilizando 75% das árvores amostradas. A análise MV foi realizada usando RAxML 8.2.9 (*Randomized Axelerated Maximum Likelihood*) (Stamatakis 2014) através do portal CIPRES (Miller et al. 2010a), começando com uma árvore de parcimônia de adição aleatória sob um modelo GTR+GAMMA e com 1000 *bootstrap* (BS) para cada conjunto de dados. Todas as árvores foram previamente editadas no FigTree 1.4 (Rambaut 2018). A árvore consenso da análise de IB foi usada como base para a anotação dos valores de PP (probabilidade posterior) e BS de cada ramo utilizando o TreeGraph (Stöver and Müller 2010). As espécies *F. foetens* (isolado NRRL31852) e *F. dimerum* (isolado NRRL 36140) foram utilizadas como grupo externo (*outgroup*).

2.4 Sequenciamento do genoma total

A partir do DNA extraído foi realizado o sequenciamento do genoma completo (WGS) utilizando o sequenciamento de nova geração DNBseq PE 150 pb (BGI Genomics, China). O controle de qualidade foi conduzido utilizando FastQC. A

montagem dos *contigs* foi realizada pelo software MaSuRCA v4.1.0 (Maryland Super Read Cabog Assembler) (Zimin et al. 2013). A anotação e predição de genes que codificam proteínas foi realizada com o Funannotate v1.8.15 (Palmer and Stajich 2020). Para avaliar a robustez da montagem do genoma e predição das proteínas, os resultados gerados por MaSuRCA e Funannotate foram avaliados no software BUSCO v5.8.0 (Benchmarking Universal Single-Copy Orthologue) (Manni et al. 2021).

2.5 Análise de ortogrupos

Uma análise comparativa de grupos de genes ortólogos e parálogos em múltiplos genomas e *formae speciales* de *Fusarium oxysporum* foi realizada utilizando o OrthoFinder (Emms and Kelly 2019). Os genomas usados para a análise foram obtidos da plataforma do NCBI: *F. falciforme* (GCA_027945495.1), *F. fujikuroi* (GCA_900079805.1), *F. graminearum* (GCA_000240135.3), *F. odoratissimum* (GCA_000260195.2), *F. odoratissimum* (GCA_007994515.1), *F. oxysporum* (GCA_000271745.2), *F. oxysporum* f. sp. *albedinis* (GCA_026229875.1), *F. oxysporum* f. sp. *cepa* (GCA_003615085.1), *F. oxysporum* f. sp. *conglutinans* (GCA_014154955.1), *F. oxysporum* f. sp. *cubense* raça 1 (GCA_000350345.1), *F. oxysporum* f. sp. *cubense* raça STR4 (GCA_016802205.1), *F. oxysporum* f. sp. *lycopersici* (GCA_000149955.2), *F. oxysporum* f. sp. *matthiolae* (GCA_020796175.1), *F. oxysporum* f. sp. *melonis* (GCA_000260495.2), *F. oxysporum* f. sp. *narcissi* (GCA_004141715.1), *F. oxysporum* f. sp. *pisi* (GCA_000260075.2), *F. oxysporum* f. sp. *radicis-cucumerinum* (GCA_001702695.2), *F. oxysporum* f. sp. *radicis-lycopersici* (GCA_000260155.3), *F. oxysporum* f. sp. *rapae* (GCA_019157295.1), *F. oxysporum* f. sp. *raphani* (GCA_000260235.2), *F. oxysporum* f. sp. *vasinfectum* (GCA_000260175.2), *F. solani* (GCA_020744495.1), *F. verticillioides* (GCA_000149555.1) e *Pyricularia oryzae* (GCA_000002495.2) como *outgroup*. A análise filogenética foi feita usando os critérios de MV com os valores de *bootstrap* gerados pelo software RAxML v.8.2.12 (Stamatakis 2014). O *web software* iTol (Letunic and Bork 2007) foi utilizado para a visualização da árvore filogenética.

3. Resultados

3.1 Caracterização do isolado 218A e identificação molecular

As características morfológicas típicas de *F. oxysporum* foram observados no isolado 218A, como a presença de esporos distintos, os macroconídios e

microconídios, e a presença de estruturas de sobrevivência denominadas de clamidósporos, formados nas hifas (Figura 1), conforme as mesmas características descritas por Leslie e Summerell (2006). Conidióforos com formações distintas também foram observadas, como o macroconidióforo, que produzem macroconídios (Figura 1C), e microconidióforo em formato doliforme, com pequenas fiálides, que produzem os microconídios (Figura 1D).

Foram observados clamidósporos em abundância, intercalados, submergidos ou localizados na porção final das hifas. Possuíam formatos de ovalado a redondo, com parede celular dupla (Figura 1E-F).

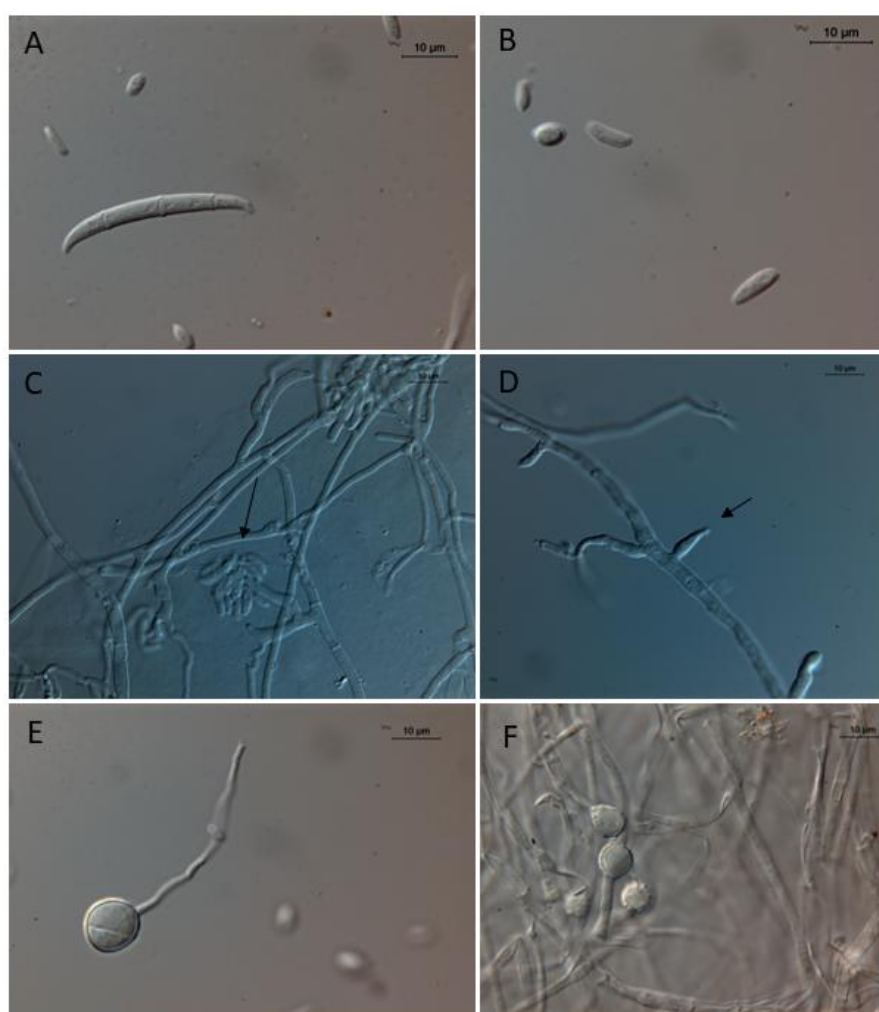


Figura 1. *Fusarium oxysporum* f. sp. *cubense* (isolado 218A). **A.** macroconídio, **B.** microconídios, **C.** conidióforo em formato de 'false heads' produzindo microconídios (seta), **D.** microconidióforo doliforme, **E.** clamidósporo emitindo hifa germinativa, **F.** clamidósporos formados em hifas. Bar = 10μm.

Através do sequenciamento dos produtos de PCR das regiões β -tubulina, RPB2 e TEF, foi possível realizar uma primeira identificação molecular usando a

ferramenta BLAST realizando pelas plataformas NCBI e Fusarium-ID, como demonstrado na Tabela 2. Os três marcadores apresentaram alta porcentagem de identidade do isolado 218A com *F. odoratissimum* (Maryani et al. 2019) (anteriormente denominado *F. oxysporum* f. sp. *cubense* raça tropical 4).

Tabela 2. Identificação molecular do isolado fúngico 218A, através das plataformas NCBI e Fusarium-ID.

Região	Tamanho do fragmento (pb)	Espécie	Região de pareamento (pb)	Identidade (%)	Acesso
β-tub	544	<i>Fusarium odoratissimum</i>	1 - 543	100%	MH485104
		<i>Fusarium odoratissimum</i>	1 - 505	100%	MW533953
		<i>Fusarium odoratissimum</i>	7 - 504	100%	MW533952
RPB2	775	<i>Fusarium oxysporum</i>	1512 - 2286	100%	MN457531
		<i>Fusarium odoratissimum</i>	44 - 818	100%	MW474585
		<i>Fusarium odoratissimum</i>	1959 - 2733	100%	XM_031209139
TEF	633	<i>Fusarium oxysporum</i>	23 - 655	100%	KU938999
		<i>Fusarium odoratissimum</i>	228 - 666	100%	MW594348
		<i>Fusarium oxysporum</i> f. sp. <i>cubense</i>	64 - 696	99,86%	KP964892
		<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	216 - 848	99,86%	KT323874
IGS	387	<i>Fusarium odoratissimum</i>	1414 - 1800	100%	MH484831
		<i>Fusarium oxysporum</i> f. sp. <i>cubense</i>	567 - 953	100%	MK754080

O conjunto de iniciadores específicos para a detecção de *Foc*TR4 descrito por Dita et al. (2010) foi utilizado no isolado 218A. Todos os testes realizados nas temperaturas de 61,4, 62,3 e 62,5 °C apresentaram amplificação com tamanho de *amplicon* menor que 500 pb. Conforme descrito, o tamanho do *amplicon* esperado para a região IGS desses iniciadores é de 463 pares de bases (pb) (Figura 2), indicando que todos os testes do isolado 218A foram positivos para *Foc* TR4.

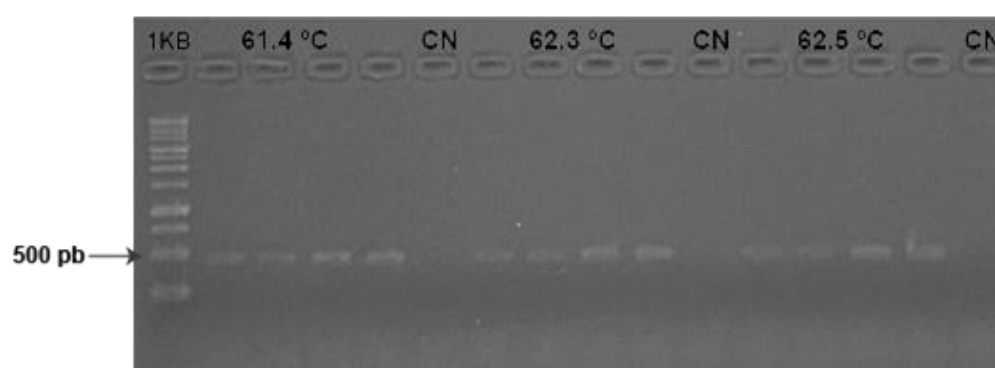


Figura 2. Produtos de PCR de *F. oxysporum* f. sp. *cubense* isolado 218A, com *amplicons* de aproximadamente 463 pb, utilizando iniciadores específicos de TR4

(*Foc*TR4-F/*Foc*TR4-R), com temperaturas de anelamento de 61,4, 62,3 e 62,5 °C, revelados através de eletroforese em gel de agarose a 1%. Marcador molecular 1KB (Kasvi), CN: controle negativo.

3.2 Análise filogenética

Uma análise filogenética mais robusta foi realizada utilizando, ao todo, 123 isolados de FOSC utilizados por Maryani et al. (2019), tais isolados foram selecionados para compor a análise filogenética concatenada dos marcadores RPB2 e TEF, utilizando análises de Máxima Verossimilhança (ML) e Inferência Bayesiana (IB). *F. dimerum* foi utilizado como “outgroup”.

Para o marcador TEF o alinhamento das sequências apresentou 429 pb, incluindo *gaps*, desses 294 sítios foram conservados, 106 sítios foram variáveis e 20 sítios foram informativos para parcimônia. Para o marcador RPB2 o alinhamento das sequências foi de 778 pb, incluindo *gaps*, desses 573 foram sítios conservados, 202 foram sítios variáveis e 18 sítios foram informativos para parcimônia. Para a análise de IB da árvore filogenética, os melhores modelos evolutivos escolhidos foram HKY+I para TEF e HKY para RPB2 (Tabela suplementar 1).

A análise filogenética com os marcadores TEF e RPB2 demonstrou que o isolado 218A está no clado 2 e é filogeneticamente próximo de *F. phialophorum*, *F. purpurascens*, e *F. odoratissimum* (Figura 3). A presença de politomia mostra que mesmo com a utilização concatenada envolvendo dois marcadores moleculares (TEF e RPB2), a resolução oferecida é baixa. As espécies *F. duoseptatum*, *F. tardichlamydosporum*, *F. tardicrescens*, *F. grosnichelii*, *F. kalimantanense*, *F. cugenangense* e *F. sangayamense* foram alocados em clados mais distantes que o isolado 218A (Figura 3).

O isolado 218A agrupou com as espécies de *F. phialophorum* e *F. purpurascens*, ambos isolados patogênicos a ‘Gros Michel’ e não patogênicos a ‘Cavendish’. Esses isolados foram anteriormente classificados como *Foc* raça 1. Os isolados da espécie *F. purpurascens* apresentam colônias de coloração roxa em meio BDA e não apresentam formação de clamidósporos, enquanto os isolados classificados em *F. odoratissimum*, anteriormente classificados como *Foc* TR4 (Maryani et al. 2019), apresentam formação rápida de clamidósporos, macro e microconídios septados, e são patogênicos a ‘Cavendish’. Por ser patogênico a

Para o marcador IGS, 78 táxons apresentaram alinhamento das sequências de 449 pb, incluindo *gaps*, desses 366 foram sítios conservados, 81 foram sítios variáveis e 65 foram informativos para parcimônia. Para o marcador TEF, 77 táxons apresentaram alinhamento das sequências de 413 pb, incluindo *gaps*, desses 363 conservados, 50 variáveis e 35 foram informativos para parcimônia. Para a análise de IB, os melhores modelos evolutivos escolhidos foram K80+G (Gamma = 0,1650) para IGS e GTR para TEF (Tabela suplementar 1).

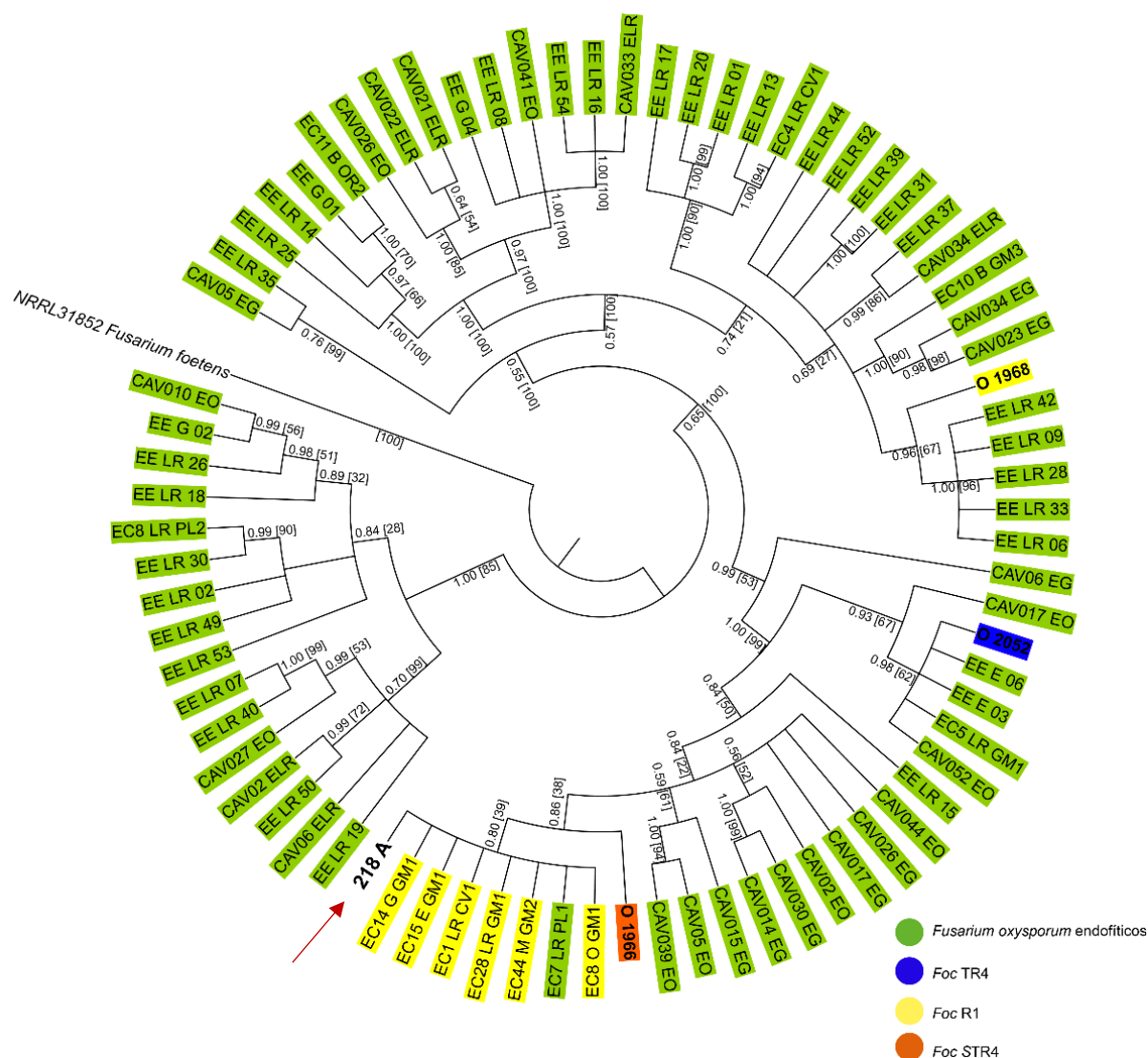


Figura 4. Árvore filogenética obtida por inferência bayesiana (IB) e máxima verossimilhança (MV) das sequências de dois genes concatenados, IGS e TEF. Os valores de *bootstrap* (BS) e probabilidade posterior (PP), são indicados nos nós. A seta indica o isolado 218A.

Os isolados endofíticos de *F. oxysporum* misturaram-se nos três maiores clados. O isolado 218A agrupou com isolados de *Foc* R1 e o isolado tipo de *Foc* STR4

(Figura 4). Entretanto os baixos valores de probabilidade posterior (PP) e *bootstrap* (BS) nos ramos pode indicar que o isolado tipo de *Foc* TR4 pode não apresentar alta diferença genética com esses isolados. Magdama et al. (2019) reporta que os isolados endofíticos de *F. oxysporum* testaram positivos na PCR utilizando os iniciadores específicos para *Foc* TR4 descritos por Dita et al. (2010), oriundos da região IGS, indicando que os isolados endofíticos possuem semelhança genética nessa região com isolados de *Foc* TR4.

Observando o alinhamento deste grupo pelo software MEGA, é possível notar que entre os isolados filogeneticamente próximos de *Foc* R1, *Foc* TR4 e 218A compartilham 91,7% e 94,9% dos sítios, e somente 15 e 8 sítios são informativos para parcimônia para a região IGS e TEF, respectivamente. A diferença é ainda menor na comparação entre os três isolados. Entre o isolado 218A e *Foc* TR4 existem 8 sítios variáveis (IGS=7 e TEF=1), enquanto, entre o isolado 218A e *Foc* R1 só 6 sítios são variáveis na região IGS e entre *Foc* R1 e *Foc* TR4 são 12 sítios (IGS=11 e TEF=1).

3.3 Montagem e anotação do genoma de *Foc* 218A

O tamanho do genoma do isolado 218A após a montagem foi de 43,5 Mb, com número total de 861 *contigs* e 11.689 sequências de proteínas anotadas (Tabela 3). A análise de termos genéticos esperados realizada pelo BUSCO, gerou 98,4% de robustez da montagem genoma e 86,3% da anotação de proteínas. Os genomas de referência disponíveis no NCBI de *Foc* TR4 (sin. *F. odoratissimum*) e R1 possuem 46,6 e 47,7 Mb, com 16.634 e 15.626 proteínas, respectivamente.

Tabela 3. Informação de genomas de *Fusarium oxysporum* f. sp. *cubense* raças TR4 (*Fusarium odoratissimum* NRRL54006), R1 (*Foc1_1.0*) e STR4 (isolado 218A).

Características	<i>Fusarium oxysporum</i> f. sp. <i>cubense</i> isolado 218A	<i>Fusarium odoratissimum</i> NRRL54006 (GCA_000260195.2)	<i>Fusarium oxysporum</i> f. sp. <i>cubense</i> raça 1 <i>Foc1_1.0</i> (GCA_000350345.1)
Tamanho do genoma (Mb)	43,5	46,6	47,7
Número de <i>scaffolds</i>	-	418	1341
Número de <i>contigs</i> (count)	861	716	2185
<i>Contig</i> N50 (kb)	175,8	348,6	146
Tamanho médio dos <i>contigs</i>	50.497	63.007	-
Quantidade de GC %	48,14	47,5	48
Proteínas codificadas por genes	11.689	16.634	15.438
Genes totais	-	16.975	15.626
BUSCO – genoma (%)	98,4	-	-
BUSCO – proteína (%)	86,3	-	-
Tecnologia de Sequenciamento	DNBseq	Illumina	Illumina GAIIX
Método de montagem	MaSuRCA	ALLPATHS v. R37753	SOAPdenovo v. 2.01

3.4 Análise de orthogrupos

Na análise filogenética gerada pelo Orthofinder, com o objetivo de investigar a relação evolutiva e funcional baseada nos ortogrupos entre o isolado 218A e as demais raças de *Foc*. Os isolados de raça 4, que incluíram TR4 (sin. *F. odoratissimum*) e STR4, formaram um clado maior e distinto de *Foc* R1 e demais espécies de *F. oxysporum*, indicando que há maior similaridade de ortogrupos entre os isolados de raça 4 à raça 1, mas que *F. odoratissimum* e *Foc* STR4 não são geneticamente idênticos. A separação de *F. odoratissimum* (IIV e UK0001) (*Foc* TR4) em um clado distinto, indica uma possível adaptação genômica que os diferenciam de *Foc* STR4 (Figura 5). Essa separação entre TR4 e STR4 indicam que agrupamentos de genes são exclusivos de cada raça, mostrando que determinados genes podem estar relacionados à especialização ou virulência.

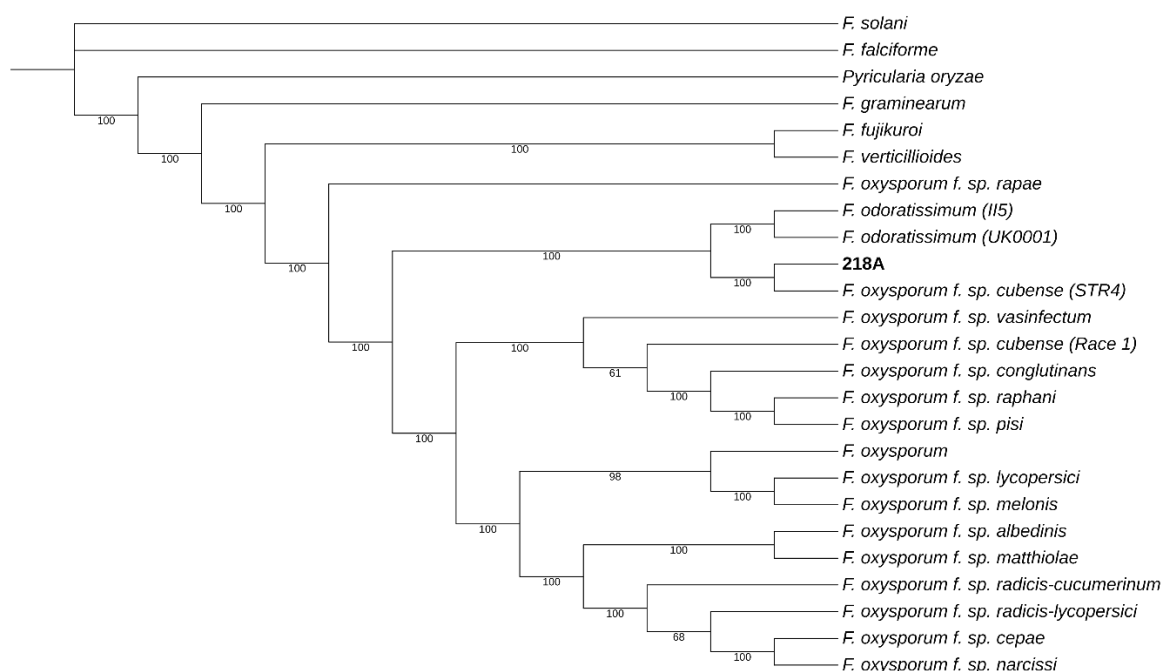


Figura 5. Análise filogenética de ortogrupos entre seis diferentes espécies de *Fusarium* e vinte *formae speciales* de *Fusarium oxysporum* realizada pelo Orthofinder. Os valores nos ramos indicam *bootstrap*.

Os genes ortólogos entre as raças de *Foc* foram ilustrados na Figura 6. 9533 genes são comuns entre todos os isolados de *Foc*, independente da raça, e sugerem que estes são genes altamente conservados que possivelmente possuem funções essenciais. 1864 genes são comuns apenas entre os dois isolados de TR4 (*F. odoratissimum*) e o isolado de R1. Outros 555 genes são comuns entre os isolados representantes de raça 4. Entretanto, o isolado 218A possui 261 genes exclusivos em comum com os isolados de TR4, uma quantidade de genes expressivamente superior quando comparado com os genes em comum entre o isolado 218A e *Foc* STR4 (65 genes). O isolado 218A possui apenas 29 genes únicos em comum com *Foc* R1, e 24 genes em comum entre STR4, 218A e *Foc* R1. Esses dados corroboram com a maior distância entre os clados representativos dos isolados de STR4 de *Foc* R1 mostrados na análise filogenética. O isolado 218A apresentou ainda, 105 genes únicos, não compartilhados com nenhuma outra raça.

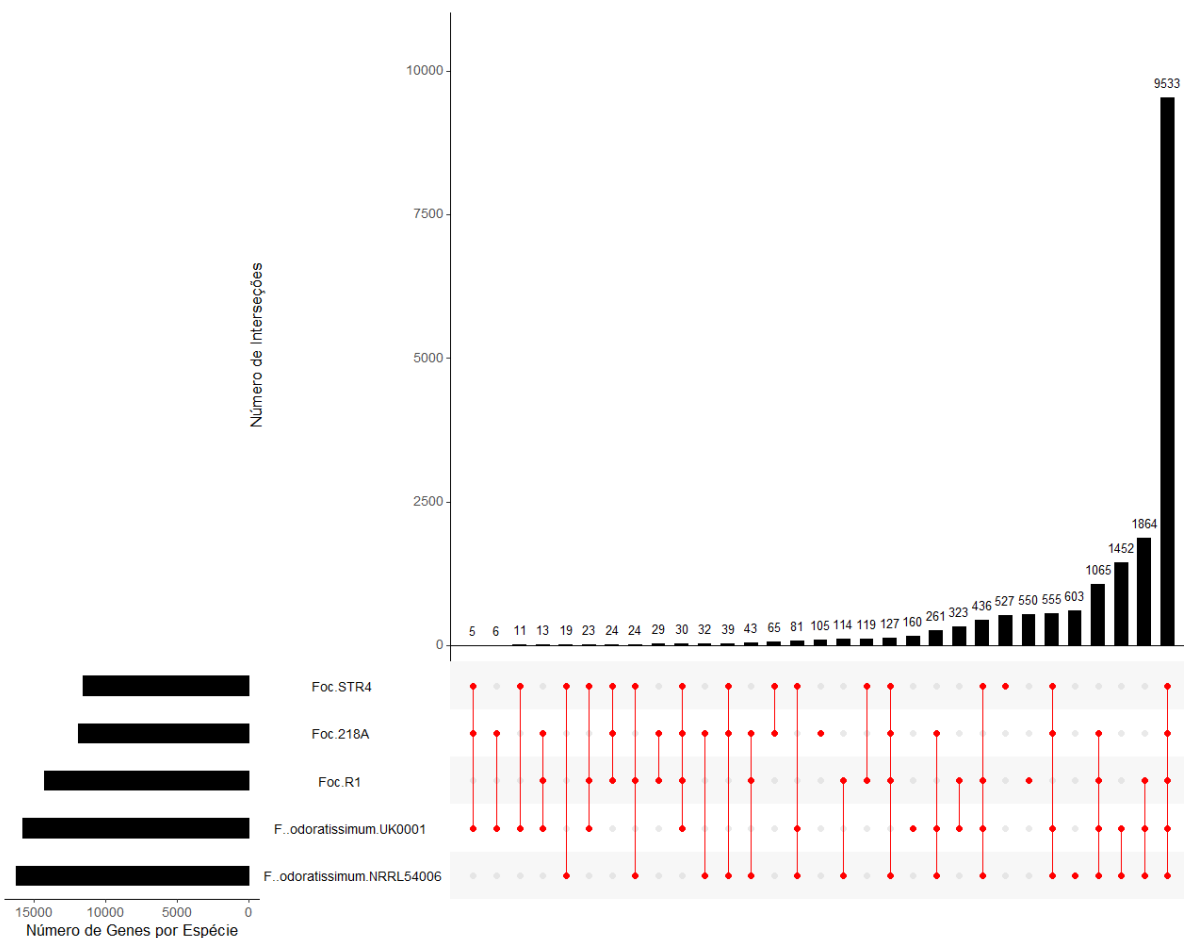


Figura 6. Análise comparativa entre genes ortólogos e parálogos compartilhados entre os genomas do isolado 218A e isolados referências de *Fusarium oxysporum* f. sp. *cubense* raças 1 (GCA_000350345.1), STR4 (GCA_016802205.1) e dois de TR4 (NRRL54006 - GCA_000260195.2 e UK0001 - GCA_007994515.1), disponíveis no NCBI.

4. Discussão

O movimento acelerado na dispersão de *Foc* TR4 desde o seu surgimento, têm preocupado pesquisadores e produtores de bananas no mundo todo, especialmente nos países da América Latina como o Brasil, que precisam proteger as fronteiras para prevenir a entrada do patógeno. Como a única diferença entre as raças TR4 e STR4, ambas pertencentes à raça 4, está na capacidade patogênica de STR4 em ‘Cavendish’ apenas em condições subtropicais, acrescenta a importância da caracterização dos isolados presentes no Brasil. Baseado em diversidade de populações de *Foc* em isolados encontrados em diversas regiões do país, 8 VCGs distintas foram encontradas em plantações de bananas dos grupos ‘Prata’, ‘Maçã’ e ‘Cavendish’ (Batista et al. 2022). Em países da Ásia, 17 VCGs de *Foc* foram encontradas (Mostert et al. 2017).

A presença de SIX genes (*Secreted in Xylem*) associados a patogenicidade, também é objeto de estudo na diferenciação de raças de *Foc*. A presença de SIX8 é responsável por conferir patogenicidade de isolados de *Foc* à 'Cavendish'. SIX8 possui dois homólogos, SIX8a e SIX8b. *Foc* STR4 possui ambos os homólogos SIX8, enquanto TR4 possui apenas SIX8a. Entretanto, alguns isolados com VCG 0120 encontrados em 'Cavendish' apresentam apenas a presença de SIX8 ou até mesmo, sem a presença de qualquer homólogo de SIX8 (Batista et al. 2022).

Mesmo que demais análises realizadas anteriormente por Rocha et al. (2018) concluíssem por meio de VCG e teste de patogenicidade que o isolado 218A pertencia à *Foc* STR4, ainda seria necessário o reconhecimento e caracterização molecular para o isolado, visto que as informações por VCGs podem ser inespecíficas entre as raças de *Foc*. Dessa forma, análises envolvendo o uso de marcadores moleculares específicos para a detecção de *Foc* TR4, *FocTR4-F/FocTR4-R* (Dita et al. 2010), análises filogenéticas concatenadas, foram realizadas. Apesar do teste com os primers específicos para a detecção de *Foc* TR4 amplificarem a região de interesse para o isolado 218A, foram necessários outros testes envolvendo análise genômica para uma classificação específica do isolado. Magdama et al. (2019) descrevem que os métodos de detecção devem levar em consideração a diversidade existente em isolados de FOSC, e que diversos métodos de detecção realizados por PCR falharam ao testar isolados patogênicos e não patogênicos, inclusive mostrando a inespecificidade dos primers *FocTR4-F/FocTR4-R* que amplificaram a região esperada em isolados de *F. oxysporum* endofíticos, elucidando a geração de falsos positivos.

As análises filogenéticas concatenadas realizadas com os marcadores moleculares IGS e TEF, comparadas com os isolados utilizados no trabalho de Magdama et al (2019) mostraram baixa quantidade de sítios variáveis entre o isolado 218A e *Foc* TR4 (IGS=7 e TEF=1), apesar de não haver diferenciação de clados entre raças de *Foc* e *F. oxysporum* endofíticos. Da mesma forma, não foi possível a classificação do isolado 218A baseada na análise filogenética com os marcadores TEF e RPB2, que agrupou juntamente com as espécies *F. phialophorum*, *F. purpurascens*, e *F. odoratissimum*, que são novas espécies oriundas de isolados envolvendo raças 1 e 4 de *Foc*, reclassificadas por Maryani et al. (2019). Assim,

mostramos que as análises filogenéticas utilizando apenas os marcadores moleculares em questão, não são suficientes para uma classificação de raças de *Foc*.

Na análise de ortologia dos genes foi possível verificar que o isolado 218A e *Foc* STR4 aparecem no mesmo clado, e, portanto, são idênticos do ponto de vista genético. A proximidade, porém, do clado pertencente à *Foc* TR4 (sin *F. odoratissimum*) revela divergências evolutivas entre os representantes de *Foc* raça 4, que podem estar relacionados a adaptações específicas ou a características únicas de virulência, como uma adaptação relacionada ao hospedeiro ou ambiente. Isso pode explicar o fato de ambas as raças STR4 e TR4 serem patogênicas ao subgrupo 'Cavendish', considerando, porém, que o ambiente influencia na agressividade de STR4 e no desenvolvimento da doença nessa cultivar.

5. Conclusão

As análises realizadas mostraram que não há diferenciação de raças de *Foc* baseadas no uso de marcadores moleculares para análises de identidade de sequências e filogenia, necessitando assim, análises mais robustas envolvendo a genômica. Mostramos ainda, que a reclassificação de *Foc* TR4 em *F. odoratissimum* não foi suportada quando conduzida a análise filogenética concatenada das regiões TEF e RPB2.

A análise de genes ortólogos demonstrou uma maior similaridade dos genes de 218A com a raça *Foc* STR4, reafirmando, entretanto, que o isolado 218A é classificado como *Foc* STR4. Os dados de genoma e proteínas servirão para apoiar a análise comparativa de genomas de diferentes raças de *Foc*, posteriormente. Tais dados são aplicáveis para a caracterização de efetores candidatos em relação à exclusividade e expressão nas raças de *Foc* durante a interação com cultivares de banana.

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Material Suplementar

Tabela suplementar 1. Estimativas de parâmetros para as regiões utilizadas nas análises filogenéticas de *Fusarium oxysporum*.

Parâmetro	Filogenia Maryani et al. (2019)		Filogenia Magdama et al. (2019)	
	Região Gênica			
	TEF	RPB2	TEF	IGS
Nº táxons	124	124	77	78
Modelo evolutivo	HKY+I	HKY	GTR	K80+G
Likelihood	-801,1636	-1321,6311	-887,6088	-1400,5936
Tamanho da matriz (pb)	429	778	413	449
Sítios variáveis	106	202	50	81
Sites informativos de parsimônia	20	18	35	65
Sítios conservados	294	573	363	366
Frequência de Bases				
Freq. A	0,2261	0,2896	0,2188	Equal
Freq. C	0,2818	0,2384	0,2860	
Freq. G	0,2151	0,2666	0,2131	
Freq. T	0,2770	0,2054	0,2820	
Taxas de transição				
[A-C]			1,4821	2,4328
[A-G]			1,2754	
[A-T]	1,2473	2,0066	0,7770	
[C-G]			0,3767	
[C-T]			3,3504	
[G-T]			1,0000	
Proporção de sítios invariáveis	0,6329	-	-	-
Gamma	-	-	-	0,1650

Capítulo III

Genes de referência para análise de RT-qPCR em genótipos de *Musa acuminata* contrastantes na resistência a *Fusarium oxysporum* f. sp. *cubense*, raça subtropical 4

(Reference genes for RT-qPCR analysis in *Musa acuminata* genotypes contrasting in resistance to *Fusarium oxysporum* f. sp. *cubense* subtropical race 4)

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RESUMO

A identificação de genótipos selvagens de *Musa* que possuem resistência ao Foc, como *Musa acuminata* subsp. *burmannica* acesso ‘Calcutta 4’ (sin. *Musa acuminata* subsp. *burmannicoides* var. ‘Calcutta 4’), é fundamental para o entendimento dos mecanismos de defesa do hospedeiro e para o desenvolvimento de cultivares resistentes. Neste contexto, a análise da expressão gênica por meio de PCR em tempo real (RT-qPCR) se destaca como uma ferramenta poderosa para investigar as vias moleculares envolvidas nas respostas de defesa ao patógeno. Contudo, a precisão dessas análises depende da utilização de genes de referência com expressão estável, especialmente em condições específicas de estresse biótico e em diferentes tipos de tecidos.

O artigo apresentado a seguir publicado na revista Scientific Reports em 2024 avaliou a estabilidade de 12 genes de referência potenciais em amostras de RNA extraídas de tecidos radiculares de ‘Calcutta 4’ (resistente) e da cultivar Prata-anã (suscetível), ambos submetidos à interação com Foc STR4 isolado 218A. Os genes mais estáveis identificados nesse estudo foram essenciais para normalizar as variações entre amostras e avaliar as análises de expressão gênica (RNAseq) no patossistema *Musa*-Foc STR4.

OPEN Reference genes for RT-qPCR analysis in *Musa acuminata* genotypes contrasting in resistance to *Fusarium oxysporum* f. sp. *cubense* subtropical race 4

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Banana (*Musa* spp.) is the most widely consumed fruit globally. *Fusarium* wilt, caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*), is a highly threatening disease to banana production. Resistance genes to *Foc* exist in wild *Musa* genotypes such as *Musa acuminata* subsp. *burmannicoides* var. Calcutta 4. Whilst real-time PCR (RT-qPCR) is appropriate for accurate analysis of gene expression in pathways involved in host defence responses, reference genes with stable expression under specific biotic stress conditions and host tissue types are necessary for normalization of sample variation. In this context, the stability in potential host reference genes *ACT1*, *APT*, *EF1 α* , *GAPDH*, *α TUB*, *RAN*, *UBIQ1*, *UBIQ2*, *β TUB1*, *β TUB3*, *L2* and *ACTA1* was evaluated in total RNA samples from root tissues in Calcutta 4 (resistant) and *Musa* sp. cultivar Prata-anã (susceptible) extracted during interaction with *Foc* subtropical race 4 (STR4). Expression stability was calculated using the algorithms geNorm, NormFinder and BestKeeper. *β TUB3* and *L2* were identified as the most stable in Calcutta 4, with *ACTA1* and *GAPDH* the most stable in Prata-anã. These reference genes for analysis of gene expression modulation in the *Musa-Foc* STR4 pathosystem are fundamental for advancing understanding of host defence responses to this important pathogen.

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Keywords *Musa*, *Fusarium oxysporum*, Gene expression normalization

Banana (*Musa* spp.) is one of the most widely traded and consumed fruits globally and, as a basic carbohydrate component of the diet in numerous developing countries, is highly relevant for global food security^{1,2}. With cultivated edible bananas and plantains derived from the fertile diploid progenitor species of *Musa acuminata* (AA) and *Musa balbisiana* (BB), many of today's commercial cultivars such as 'Cavendish' (AAA), 'Pome' (AAB), and 'Silk' (AAB) are sterile, with seedless fruits developing via parthenocarpy³. As genetic diversity is low across such important cultivars, resistance to evolving pathogens is limited⁴.

Fusarium wilt is currently the most devastating and widely disseminated fungal disease of banana globally^{2,5,6}. Vascular wilt due to pathogen advance in xylem vessels restricts water movement, resulting in leaf yellowing, pseudostem splitting and eventual plant death⁷. *Fusarium* wilt is caused by the soilborne fungus *Fusarium oxysporum* f. sp. *cubense* (*Foc*)^{6,8}, with three physiological races of *Foc* capable of infecting banana, namely race 1, race 2, and race 4, with the latter subdivided into tropical race 4 (TR4) and subtropical race 4 (STR4)⁸. Cultivars of the 'Gros Michel' group (AAA), 'Pome' (AAB), and 'Silk' (AAB) are susceptible to all races of *Foc*, while cultivars of the 'Cavendish' subgroup (AAA) are typically resistant to race 1 of *Foc* but susceptible to STR4 and TR4⁶. Breakdown of resistance to *Foc* race 1 (VCG0124⁹, 0125 and 0 1220¹⁰) in Cavendish cultivars has, however, been reported in certain growing regions^{11–14}.

The fungal pathogen *Foc* can survive in soils in the absence of the host plant, with chlamydospores, which are thick-walled survival spores, able to maintain viability for periods in excess of 20 years¹⁵. Given such pathogen persistence, the employment of resistant varieties is essential for disease management. In contrast to susceptible cultivars, fertile resistant wild diploid *Musa* genotypes, that have coevolved with pathogens at the centre of origin of the genus, represent a source of defence genes and alleles that are appropriate for genetic improvement of cultivars via genetic engineering or gene modification.

For candidate gene discovery, RNAseq-based high-throughput sequencing approaches for investigation of host transcriptome responses in *Musa* materials contrasting in resistance are advancing understanding of the cellular-level defence response involved in resistance to *Foc*^{16–19}. Following such investigations, in silico-derived candidate genes in the defence response require lab-based validation via the reverse transcription real-time polymerase chain reaction (RT-qPCR), which is widely employed to accurately quantify gene expression levels in cDNA derived from RNA^{20–22}. The guidelines of the Minimum Information for Publication of Real-Time Quantitative PCR Experiments (MIQE)²³ recommend that for accurate measurement of target gene expression, normalization is necessary to that of a reference gene which displays stable expression for any particular

tissue type or experimental treatment²⁴. As reference genes are subject to correction of the effect of cDNA quality^{25,26}, normalization of target gene expression to reference genes also corrects for variation between sample replicates that may occur during cDNA synthesis.

Several studies have been conducted in recent years for the development of stable reference genes for different genotypes and tissue types in *Musa* sp.^{27–30}, with data highlighting that genes involved in basal cell activities, such as *ACT*, *UBQ*, *GAPDH*, *EF*, *L2*, *TUB*, *GAPDH*, *RAN*, and *APT*, amongst others²⁸, may not necessarily show a stable constitutive expression under particular experimental conditions. As such, the identification of specific stable reference genes is required for each plant genotype, tissue type and experimental treatment.

Several mathematical algorithms are available for assessing the stability of potential reference genes, with three programs widely employed, namely geNorm²⁵, NormFinder²⁶ and BestKeeper³¹. Each offers distinct statistical parameters for the prediction of gene stability and the optimal number of reference genes for accurate normalization of RT-qPCR data.

Here, 12 candidate reference genes were compared for gene expression normalization in root tissues in *Musa* genotypes contrasting in resistance to the vascular wilt pathogen *F. oxysporum* f. sp. *cubense* STR4, namely *Musa acuminata* subsp. *burmannicoides* var. Calcutta 4 (C4) (resistant) and *Musa* sp. cultivar Prata-anã (PA) (susceptible). The stability of the candidate reference genes was validated through examination of the relative expression of a Thaumatin-like protein 1 (*TL1*) target gene, encoding a Pathogenesis-related protein 5 (PR5). The reference gene sets developed in this study will enable accurate analysis of expression of target genes involved in defence responses to this important pathogen.

Results

Analysis of expression levels and Cq values of candidate reference genes

Information regarding primer sequences and PCR amplification conditions for the 12 candidate reference genes is summarized in Table 1. Dissociation curve analysis confirmed amplification specificity for all the tested primers, with single peaks observed in melting curves with cDNA material from inoculated and non-inoculated treatments in C4 and PA (Fig. 1). Variations in cycle quantification (Cq) values across treatments were depicted in a Box-plot representation (Fig. 2), with intervals, means, medians, and outliers presented for the tested reference genes across the four treatments. Overall, Cq values were generally lower in C4 when compared to PA, indicating greater expression. The lowest Cq values were observed for the genes *ACTA1* and *bTUB3* in C4, and for *GAPDH* and *bTUB3* in PA. Highest Cq values, by contrast, were observed for the genes *APT*, *RAN* and *Ubiq1* in both genotypes, indicating lowest expression

levels. In the case of C4, greater variation in outlier Cq values was observed in the genes *bTUB1* and *Ubiq1*. No such variation was observed in the case of PA.

Determination of candidate reference genes stability

Stability analysis and rankings for the candidate reference genes were conducted utilizing the mathematical algorithms geNorm, NormFinder and BestKeeper. Data was analysed separately for both genotypes, with gene expression data originating from root tissue samples collected at 1, 2, and 4 days after inoculation (DAI) and similarly from mock-inoculated controls. The combined analysis of gene expression stability was based on the total datasets generated for the two genotypes, combining data for biotic stress treatments and non-stressed controls.

When considering all C4 samples, *L2* and *BTUB3* were ranked as the most stable genes according to analyses conducted with geNorm and NormFinder, exhibiting the lowest M (average expression stability) and SV (stability value). Based on BestKeeper, *UBIQ2* and *UBIQ1* genes were the most highly ranked in terms of stability, with the lowest SD (standard deviation) observed in expression values (Table 2). In the case of all PA samples, *GAPDH* and *ACTIN 1* were identified as the genes with the lowest M values according to analysis with geNorm. By contrast, NormFinder identified *TUBa* and *APT* as the most stable genes based on SV values, with BestKeeper indicating the greatest stability in *bTUB3* and *L2*, with these genes displaying the lowest SD. For both genotypes, following the $M \leq 0.5$ cutoff set by geNorm, the first 7 genes would qualify as stable and suitable for use (Table 2).

In analysis of the Global datasets, geNorm identified only *TUBa* and *ACTIN1* as stable genes, with M values ≤ 0.5 . NormFinder identified *TUBa* and *bTUB3*, based on SV values, while BestKeeper indicated *L2* and *bTUB3* as the most stable genes, based on SD values (Table 2).

Determination of the optimal number of reference genes for normalizing the expression of target genes was assessed using the algorithm geNorm (Fig. 3). Based on a $V \leq 0.15$ cutoff, two genes were considered sufficient for normalization in each of C4 and PA, with V values of 0.141 and 0.092, respectively. However, when considering the global sample, two genes would be insufficient ($V = 0.175$), with three genes required for data normalization ($V = 0.134$).

Gene	Description	Primer name	Primer sequence (5'-3')	Tm (°C)	Amplicon size (bp)
<i>ACTIN1</i>	Actin-3	Macu_Act1pp1-Fw1	CTG CGA CAA TGG TAC TGG AAT	81.95	146
		Macu_Act1pp1-Rv1	CCT CGT CAC CAA CAT AAG CAT		
<i>APT</i>	Adenine phosphoribosyltransferase	Macu_APT-Fw	TTG AAC TGC CAG AAT TGA AGG	80.47	125
		Macu_APT-Rv	TTG GGA AGA ACA GAG AAG CAG		
<i>EF1a</i>	Tr-type G domain-containing protein	Macu_EF1a-Fw	GCT ACA ACC CAG AGA AGA TAC CCT T	82.25	80
		Macu_EF1a-Rv	CAG GTT GGT AGA CCT CTC AAT CAT G		

<i>GAPDH</i>	Glyceraldehyde-3-phosphate dehydrogenase 2, cytosolic	Macu_GAPDH-Fw	CAT CAA GCA AGG ACT GGA GAG	80.91	99
		Macu_GAPDH-Rv	AAG CAG GGA GAA CTT TTC CAA		
<i>TUBa</i>	Tubulin alpha-3 chain	Macu_TubA-Fw	GGA AGA AGT CGA AGC TTG GTT	75.53	95
		Macu_TubA-Rv	GGA ATG GGT GGA TAG GAC ACT		
<i>RAN</i>	Translation factor GUF1, mitochondrial	Macu_RAN-Fw	AGC TGC AAT TGG ATC GAA AGT	78.82	90
		Macu_RAN-Rv	GTA ACA TCG CCA CCA TAG CAT		
<i>UBIQ1</i>	Ubiquitin 1	Macu_Ubq1-Fw	GGC AGG AGT AAC GAA CAA CAA	81.8	142
		Macu_Ubq1-Rv	CAT TTC TCG TAG CTG GGT CAG		
<i>UBIQ2</i>	Ubiquitin domain-containing protein DSK2a	Macu_Ubq2-Fw	AGA GAG ATG CTG CAA AAT CCA	78.82	140
		Macu_Ubq2-Rv	CCA GCT GTC TGC TCT TGT TCT		
<i>bTUB3</i>	Tubulin beta-3 chain	bTUB-F	TGT TGC ATC CTG GTA CTG CT	81.36	150
		bTUB-R	GGC TTT CTT GCA CTG GTA CAC		
<i>L2</i>	Ribosomal protein L2	L2-F	AGG GTT CAT AGC CAC ACC AC	81.06	100
		L2-R	CCG AAC TGA GAA GCC CCT AC		
<i>bTUB1</i>	Tubulin beta-1 chain	bTUB1-F	TGT TGG ACA ACG AAG CTC TCT	81.21	192
		BTUB1-R	GTG GCC GAA ATA AGA TGG TTC		
<i>ACTA1</i>	Actin-2	ACTA1-F	AGG AGC ATC ATC TCC AGC AAAG	78.37	78
		ACTA1-R	ACA CTG ACG ACA TTC AGC CTCTT		

Table 1. Supporting information for candidate reference genes, primers and amplification parameters for reverse transcription-quantitative PCR (RT-qPCR) normalization of gene expression data for root tissues in *Musa* spp. in the presence and absence of *Fusarium oxysporum* f. sp. *cubense* STR4.

Following analysis of stability values of gene pairs with the lowest SV values generated by NormFinder (Fig. 4), *L2-RAN*, *bTUB3-EF1a*, and *L2-EF1a* were identified as pairs with SV values ≤ 0.15 in the case of C4, and *APT-TUBa* and *GAPDH-TUBa* in the case of PA. No pairs were observed with SV ≤ 0.15 in the case of the global dataset.

Expression analysis of the *TL1* gene (PR5) using selected reference genes

To verify the stability of any potential reference gene, analysis of normalization of expression of a target marker gene for a specific experimental condition is required. Here, the relative expression of a *TL1* gene, which is a *PR5* gene involved in plant host resistance to various pathogens, including *Foc*^{32,33}, was evaluated in cDNA samples for the *Foc* STR4-resistant genotype C4, inoculated at 1, 2, and 4 DAI, as well as for corresponding non-inoculated controls. Evaluations were also conducted in the susceptible *Musa* genotype PA, under the same conditions.

The four most stable genes for relative expression normalization, based on rankings through geNorm and NormFinder analyses, were tested for each *Musa* genotype. In the case of C4, the most stable genes employed comprised *L2*, *bTUB3*, *EF1a*, and *TUBa*, while for PA, these comprised *GAPDH*, *ACTIN1*, *APT*, and *TUBa*. *Ubiq1* and *bTUB1* were also included in relative expression normalization analysis of the target gene *TL1* to

represent the least stable genes for employment in normalization for both genotypes.

In C4, differential expression of the *TL1* gene between inoculated and non-inoculated controls was observed when normalizing using the two pairs formed by the four most stable genes. This contrasted with the relative expression data normalized using the least stable genes, where no differential expression of the *TL1* gene between inoculated and non-inoculated controls was observed. In PA, where there was no differential expression of *TL1* between inoculated and non-inoculated controls, expression levels following normalization using the most or least stable genes did significantly differ, with the same expression trends observed (Fig. 5).

Discussion

The interaction between different *Musa* genotypes and *Foc* has been the subject of several transcriptome analyses, with a focus on host gene characterization during both the compatible and incompatible response²⁸. To verify in silico-derived gene expression data, RT-qPCR is widely employed to quantify the expression of a specific gene in cDNA samples originating from different treatments^{17,27,29,30,34–37}. Accurate analysis of gene expression, however, requires normalization to reference genes that display stable expression under the investigated experimental conditions. Such normalization will then correct for variation inherent between cDNA samples. Screening of potential reference genes for normalization thus requires consideration of specific experimental samples³⁰, whether originating from different organisms, tissue types, biotic or abiotic stresses or cDNA sample.

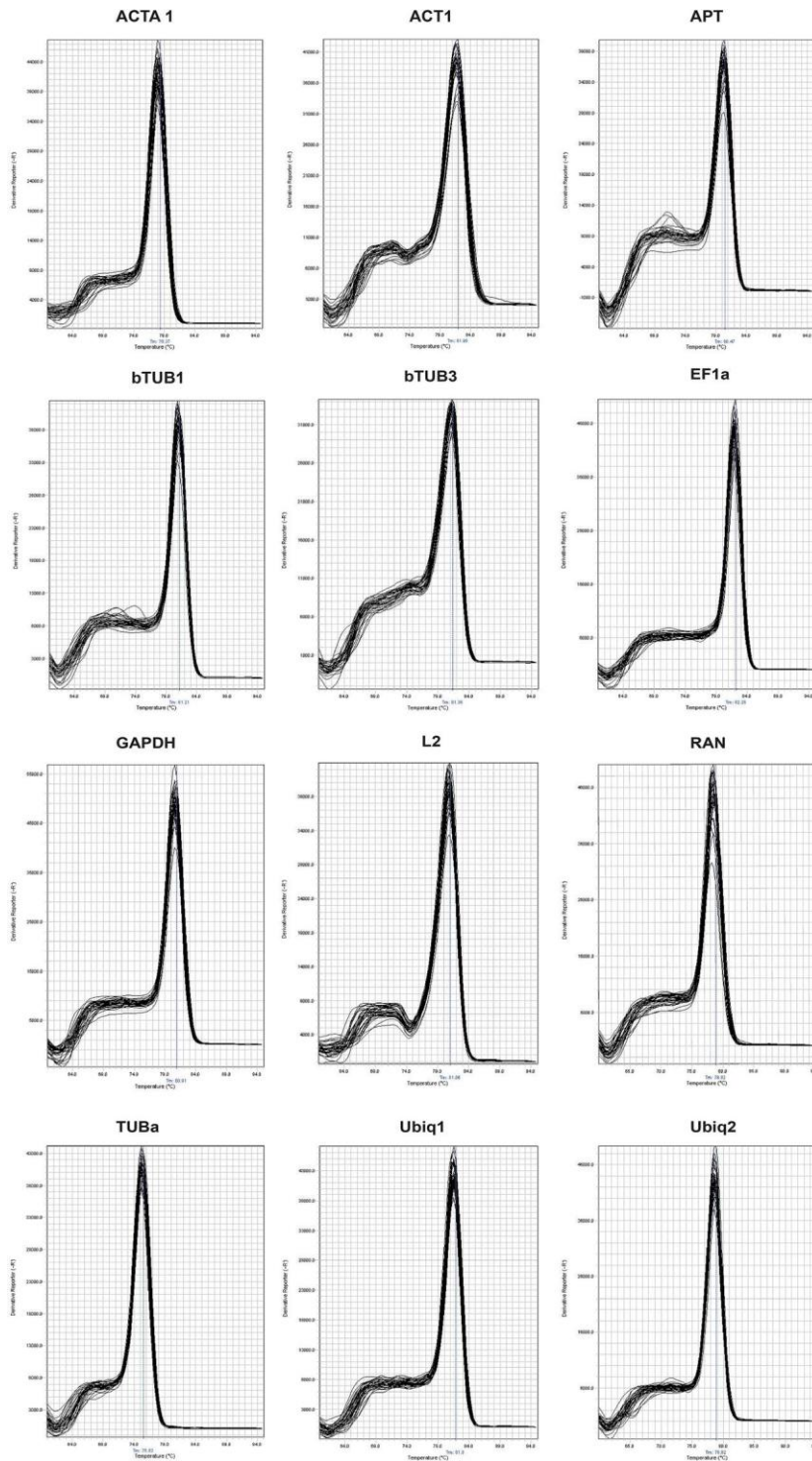


Figure 1. Dissociation curves for the 12 candidate reference genes evaluated in *Musa acuminata* Calcutta 4

Previous reference genes developed for *Musa* have included those encoding essential and constitutive proteins in plants. These have included the ribosomal protein 2 (*RPS2*), ribosomal protein L2 (*L2*), and actin (*Actin*), which constitutes an essential component of the cell cytoskeleton. Proteins responsible for translation and formation of other proteins, such as the elongation factor 1 α (*EF1 α*) and ubiquitin (*UBIQ1* and *UBIQ2*), have

also been employed, as well as proteins responsible for cellular metabolism, such as glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), among others^{27–30}. In all these cases, however, gene expression, even when considered



Figure 2. Representative boxplot of quantification cycle (Cq) values for 12 candidate reference genes tested in root material in *Musa acuminata* subsp. *burmannicoides* Calcutta 4 (C4) and *Musa* sp. cultivar Prata-anã (PA), both inoculated (inoc) with *Fusarium oxysporum* f. sp. *cubense* STR4 and non-inoculated (mock).

basal, is often variable, such that different genotypes, tissues, developmental stages, and responses to biotic and abiotic stresses must all be considered in the examination of gene expression stability.

Previously, Chen and colleagues²⁷ evaluated expression stability of 20 candidate genes in *M. acuminata* Cavendish subgroup fruit tissues, during different developmental stages, including post-harvest, different abiotic stresses (chilling and high temperature), biotic stress (*Colletotrichum musae*), and during ethylene hormone treatment. Stability ranking varied among all treatments, with only the *APT* gene being less stable in four out of the six tested conditions. Rego and coworkers²⁹ also characterized the expression of eight candidate genes in leaves of *M. acuminata* Cavendish Grand Naine and Calcutta 4 under biotic stress (*Pseudocercospora musae*). Data revealed *APT* and *UBQ2* as the most stable genes for

normalization in Calcutta 4, whilst *RAN* and α -*TUB* were more stable in Cavendish Grand Naine. Podevin and coworkers³⁰ similarly investigated the expression of six candidate reference genes in various tissues of *Musa* genotypes from the AAA, AA, and ABB groups, and under various experimental treatments that included physiological growth conditions, biotic (*Pseudocercospora fijiensis*) and abiotic (osmotic) stress conditions. Data demonstrated that for each experimental condition, an investigation of appropriate reference genes is necessary.

In our study on the stability of 12 candidate reference genes in *Musa* C4 and PA during the interaction with *Foc* STR4, data based on analyses of stability using geNorm were supported by NormFinder-based analysis. BestKeeper-derived rankings were not considered for determining genetic stability, with different stability rankings to the former algorithms based on high coefficient of variation values. We demonstrate that the employment of a set of distinct most stable genes is necessary for each genotype: *L2* and *bTUB3* for C4 and *GAPDH* and *ACTIN1* for PA. The least stable reference genes comprised *UBIQ1* and *bTUB1* for both C4 and PA. Rankings were also corroborated based on Cq value outlier numbers, as observed in Fig. 1. Whilst more outliers were apparent for *UBIQ1* and *bTUB1*, the opposite was observed for the most stable genes, with a low amplitude of Cq for *bTUB3* and *L2* between the inoculated and mock treatments in C4, and similarly for *GAPDH* and *ACTIN1* in the inoculated and mock treatments in PA. Previously, Zhang and colleagues²⁸ reported candidate reference genes for normalization of target gene expression in the *Musa* genotypes Guangfen No.1 (ABB) and Cavendish Brazilian (AAA) following inoculation with *Foc* TR4 and *Foc* R1, with *L2* and *TUB* most stable in Guangfen No. 1, and *ACT1* and *TUB* most stable in Brazilian. Whilst *L2* and *TUB* were also found to be the most stable genes for C4 in our study, indicating that certain genotypes from different groups (AA and ABB, respectively) may share stable reference genes appropriate for studies with *Foc*, comparisons also revealed differences between specific genotypes. Such data highlights that for each experimental condition and genotype examined, specific

Sample	Rank	geNorm		NormFinder		BestKeeper		
		Gene	M	Gene	SV	Gene	SD	CV
Calcutta 4	1	<i>L2</i>	0.28	<i>L2</i>	0.19	<i>UBIQ2</i>	0.54	2.22
	2	<i>bTUB3</i>	0.28	<i>bTUB3</i>	0.23	<i>UBIQ1</i>	0.61	2.31
	3	<i>EF1a</i>	0.39	<i>EF1a</i>	0.25	<i>APT</i>	0.67	2.50
	4	<i>TUBa</i>	0.42	<i>RAN</i>	0.26	<i>GAPDH</i>	0.70	3.11
	5	<i>GAPDH</i>	0.46	<i>ACTIN1</i>	0.27	<i>bTUB3</i>	0.71	3.48
	6	<i>ACTIN1</i>	0.48	<i>TUBa</i>	0.28	<i>RAN</i>	0.81	3.03
	7	<i>APT</i>	0.50	<i>GAPDH</i>	0.31	<i>L2</i>	0.85	3.67
	8	<i>ACTA1</i>	0.54	<i>APT</i>	0.32	<i>EF1a</i>	0.86	3.68
	9	<i>UBIQ2</i>	0.58	<i>ACTA1</i>	0.34	<i>Actin1</i>	0.91	3.78
	10	<i>RAN</i>	0.61	<i>UBIQ2</i>	0.55	<i>ACTA1</i>	0.94	4.80
	11	<i>UBIQ1</i>	0.65	<i>bTUB1</i>	0.56	<i>TUBa</i>	0.96	4.12

	12	<i>bTUB1</i>	0.74	<i>UBIQ1</i>	0.66	<i>bTUB1</i>	1.15	4.49
Prata-Anã	1	<i>GAPDH</i>	0.29	<i>TUBa</i>	0.15	<i>bTUB3</i>	0.95	4.34
	2	<i>ACTIN1</i>	0.29	<i>APT</i>	0.17	<i>L2</i>	0.97	4.07
	3	<i>ACTA1</i>	0.31	<i>GAPDH</i>	0.24	<i>ACTIN1</i>	1.09	4.34
	4	<i>TUBa</i>	0.36	<i>ACTIN1</i>	0.27	<i>UBIQ1</i>	1.10	3.88
	5	<i>APT</i>	0.40	<i>RAN</i>	0.29	<i>GAPDH</i>	1.11	5.71
	6	<i>bTUB3</i>	0.46	<i>ACTA1</i>	0.32	<i>APT</i>	1.13	3.86
	7	<i>L2</i>	0.50	<i>bTUB3</i>	0.34	<i>ACTA1</i>	1.16	4.75
	8	<i>RAN</i>	0.54	<i>L2</i>	0.35	<i>TUBa</i>	1.18	4.77
	9	<i>UBIQ1</i>	0.58	<i>UBIQ2</i>	0.35	<i>RAN</i>	1.22	4.50
	10	<i>UBIQ2</i>	0.63	<i>EF1a</i>	0.49	<i>EF1a</i>	1.28	5.09
	11	<i>EF1a</i>	0.70	<i>UBIQ1</i>	0.51	<i>bTUB1</i>	1.40	5.29
	12	<i>bTUB1</i>	0.81	<i>bTUB1</i>	0.56	<i>UBIQ2</i>	1.41	4.69
Global	1	<i>TUBa</i>	0.44	<i>TUBa</i>	0.30	<i>L2</i>	0.90	3.81
	2	<i>ACTIN1</i>	0.44	<i>bTUB3</i>	0.32	<i>bTUB3</i>	0.91	4.28
	3	<i>bTUB3</i>	0.54	<i>ACTIN1</i>	0.39	<i>RAN</i>	1.02	3.79
	4	<i>L2</i>	0.57	<i>EF1a</i>	0.48	<i>ACTIN1</i>	1.07	4.35
	5	<i>RAN</i>	0.66	<i>APT</i>	0.60	<i>TUBa</i>	1.09	4.55
	6	<i>EF1a</i>	0.73	<i>L2</i>	0.67	<i>EF1a</i>	1.15	4.74
	7	<i>UBIQ1</i>	0.78	<i>UBIQ1</i>	0.68	<i>UBIQ1</i>	1.16	4.23
	8	<i>APT</i>	0.83	<i>RAN</i>	0.78	<i>bTUB1</i>	1.31	5.03
	9	<i>bTUB1</i>	0.92	<i>bTUB1</i>	0.83	<i>APT</i>	1.37	4.88
	10	<i>ACTA1</i>	11.34	<i>ACTA1</i>	1.81	<i>GAPDH</i>	1.64	7.78
	11	<i>UBIQ2</i>	13.46	<i>UBIQ2</i>	2.28	<i>ACTA1</i>	2.48	11.28
	12	<i>GAPDH</i>	15.77	<i>GAPDH</i>	2.49	<i>UBIQ2</i>	2.89	10.65

Table 2. Analysis of expression stability of 12 candidate genes for root tissues of *Musa acuminata* subsp. *burmannicoides* Calcutta 4 and *Musa* sp. cultivar Prata-anã during interaction with *Fusarium oxysporum* f. sp. *cubeense* STR4 and non-inoculated controls. Analyses were conducted using the geNorm, NormFinder and BestKeeper algorithms. M: average expression stability, SV: stability value, Std: standard deviation, CV: variation coefficient. Lowest M and V values indicate greater gene stability. Standard deviation values with BestKeeper greater than 1 indicate gene stability inconsistency.

reference genes are essential and that disregarding biological differences is not suitable for correct normalization, potentially interfering with accurate analysis of target gene expression via RT-qPCR.

Validation of the stability of the reference genes was conducted through analysis of the relative expression of the *TL1* gene during the interaction of each genotype with *Foc* STR4. This gene encodes a thaumatin-like protein 1, which is a PR-5 protein involved in the defence response to biotic stress. Previously, Mahdavi and coworkers³³ demonstrated that insertion of a thaumatin-like protein (*TLP*) gene in susceptible Pisang Nangka bananas (AAB) conferred resistance to *Foc* TR4. Here, expression analysis of the *TL1* gene in C4 when performed with the four most highly ranked stable reference genes based on geNorm (Table 2) demonstrated

that all four genes were appropriate for normalization of target gene expression when there was differential expression of the gene in response to *Foc* STR4. As expected, the *TL1* gene did not show differential expression in response to infection by *Foc* STR4 in the susceptible genotype PA, with low relative expression even after infection. No difference in the normalization pattern was observed between the most and least stable reference genes in this genotype.

In summary, given the importance of reference genes for accurate normalization of target gene expression in the *Musa-Foc* SRT4 pathosystem, this study developed reference gene sets for application in candidate gene

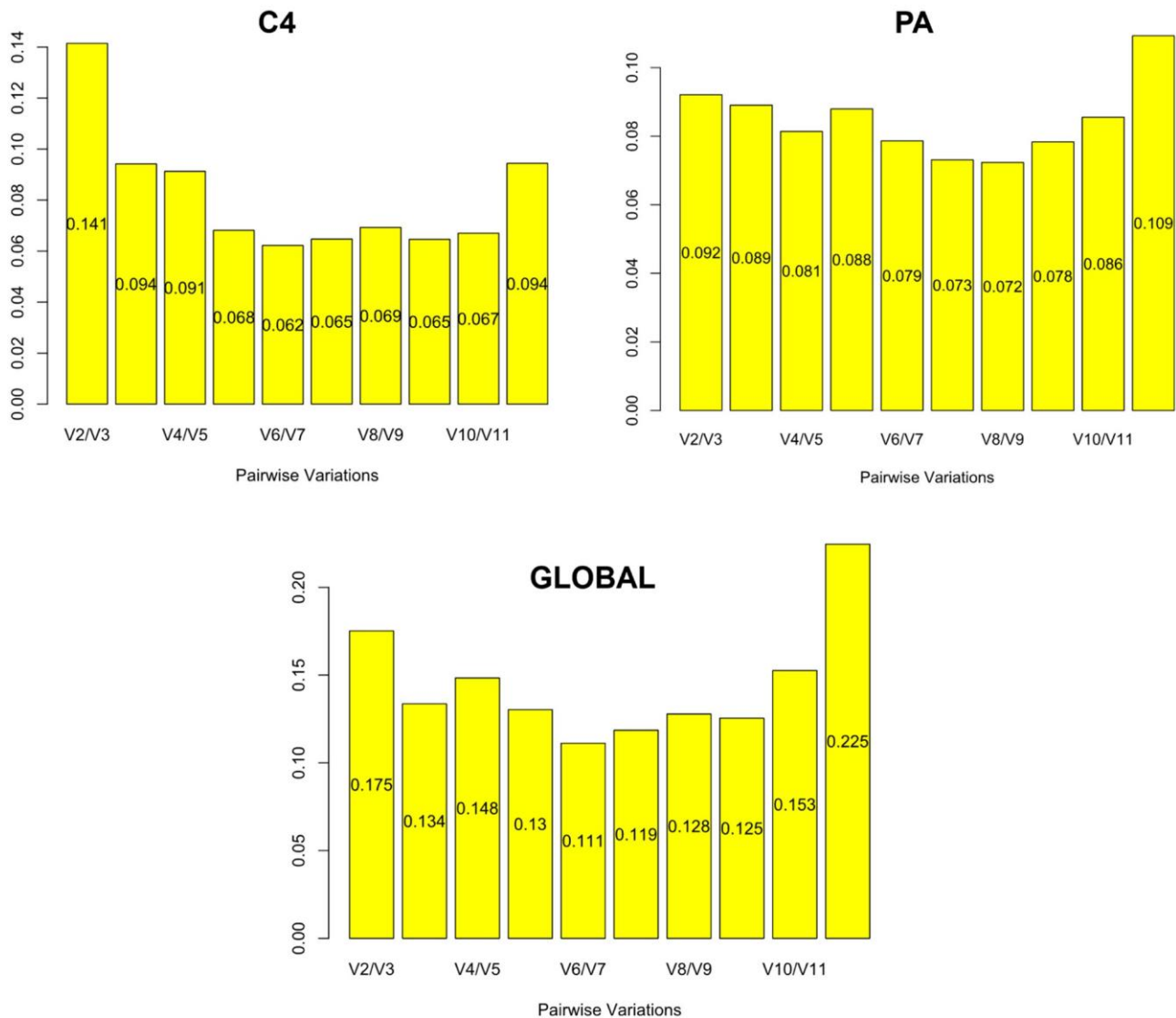


Figure 3. Pairwise variation values obtained using geNorm to determine the optimal number of reference genes for accurate RT-qPCR normalization, for both genotypes *Musa acuminata* subsp. *burmannicoides* Calcutta 4 (C4) and *Musa* sp. cultivar Prata-anã (PA), conditions, and the global analysis. When values are below the cutoff of 0.15, the inclusion of an additional reference gene is not necessary.

discovery in genotypes contrasting in resistance to this important pathogen. Based on algorithm ranking, *L2* and *bTUB3* should be prioritized for use in C4 root materials both inoculated with *Foc* STR4 or non-inoculated, and similarly with *GAPDH* and *ACTIN1* in PA. These reference genes will contribute to advancing understanding of the host defence responses to this important pathogen.

Methods

Plant material

In vitro propagated plantlets of the susceptible *Musa* sp. cultivar 'Prata-anã' (subgroup 'Pome' AAB) and the resistant wild genotype 'Calcutta 4' (*Musa acuminata* subsp. *burmannicoides*, subgroup AA) were provided by Embrapa Cassava and Tropical Fruits. Plantlets were acclimatized and subsequently grown in pots containing coconut fibre substrate. Eighteen plants of each genotype were maintained in a greenhouse at an average temperature of 24 °C, with watering at 48-h intervals for 60 days prior to inoculation.

Root inoculation with *Foc* STR4

Plants of each genotype were inoculated with *F. oxysporum* f. sp. *ubense* STR4 (isolate CNPMF 218A from Embrapa Cassava and Tropical Fruits). Inoculation was conducted according to Rocha and colleagues³⁸. Inoculum comprised 50 g of rice, previously infested with the pathogen at a concentration of 10⁶ CFU/g of rice, added directly to the substrate. Controls comprised non-infested rice, with application to the substrate as above. Following a complete randomized design, with three distinct biological replicates per treatment, root samples

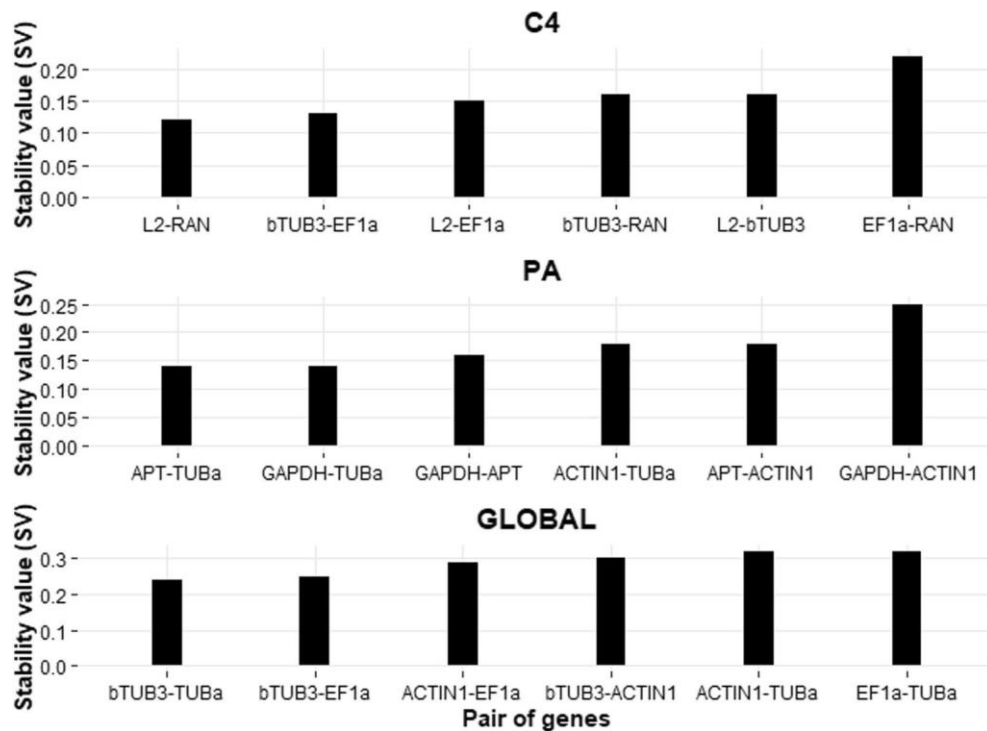


Figure 4. NormFinder-based analysis of stability values of combinations of reference gene pairs for normalizing RT-qPCR gene expression data. Lower SV values for each gene combination indicate greater expression stability.

were collected from Calcutta 4 (C4) and Prata-Anã (PA) at 1, 2, and 4 DAI and similarly from mock-inoculated controls. Samples were immediately flash-frozen in liquid nitrogen and stored at -80°C .

RNA extraction and cDNA synthesis

Total RNA was extracted from frozen 1 g root samples using the Plant RNA Reagent (Invitrogen ThermoFisher Scientific, MA, USA) and purified using the Directzol Plant RNA Purification kit (Zymo Research, CA, USA) following the manufacturer's protocol. RNA quality was verified using 1% agarose gels and quantified using a NanoDrop One spectrophotometer (Thermo Fisher Scientific, MA, USA). A 1 μg aliquot of RNA from each treatment and replicate was used for cDNA (complementary DNA) synthesis via the Super Script IV Reverse Transcriptase kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturer's protocol.

Selection of candidate reference genes

A total of 12 candidate genes were selected for testing as potential reference genes based on recent literature for genes with stable expression in *Musa spp.* and involvement in basal cell activities. Actin-3

(*ACTIN1*), *APT*, *EF1 α* , *GAPDH*, α -TUB, *RAN*, β -TUB (*bTUB1*), *Ubiq1*, and *Ubiq2* were selected from Rego et al. (2019), *L2* and β -TUB (*bTUB3*) from Zhang and coworkers²⁸, and Actin-2 (*ACTA1*) from Castañeda and coworkers³⁹ (Table 1).

Real-time quantitative PCR

Analysis of gene expression was conducted using the iTaq™ Universal SYBR® Green kit (Bio-Rad, Hercules, CA, USA). Each qPCR reaction was conducted in a final volume of 10 μ L, containing 2 μ L of a 1:20 dilution of each cDNA stock, 0.2 μ M of each primer, and 5 μ L of the iTaq™ Universal SYBR® Green kit. PCR amplifications were carried out on an ABI StepOne™ Real-Time PCR thermocycler (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) according to the following parameters: an initial two-step phase of 50 °C for 2 min and 95 °C for 10 min, 40 cycles of denaturation at 95 °C for 15 s, and primer annealing and extension at 60 °C for 60 s. Three biological and three technical replicates were included for each gene. Melting temperatures (T_m) were determined using the StepOne Software v2.3 (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA).

Determination of candidate reference gene stability

Expression analysis of each of the 12 candidate genes was measured for all C4 and PA replicate cDNA samples based on quantification cycle (Cq) values. Gene expression stability was determined using the statistical algorithms geNorm²⁵, NormFinder²⁶ and BestKeeper³¹, according to default parameters. Analyses were conducted for expression data from each individual genotype, as well as based on a global analysis of data for both genotypes, both inoculated and non-inoculated. In the case of geNorm, the lower the average M value, below a threshold of ≤ 0.5 , the greater the stability for a candidate reference gene. This algorithm also estimates a V value ($V_n/n + 1$)

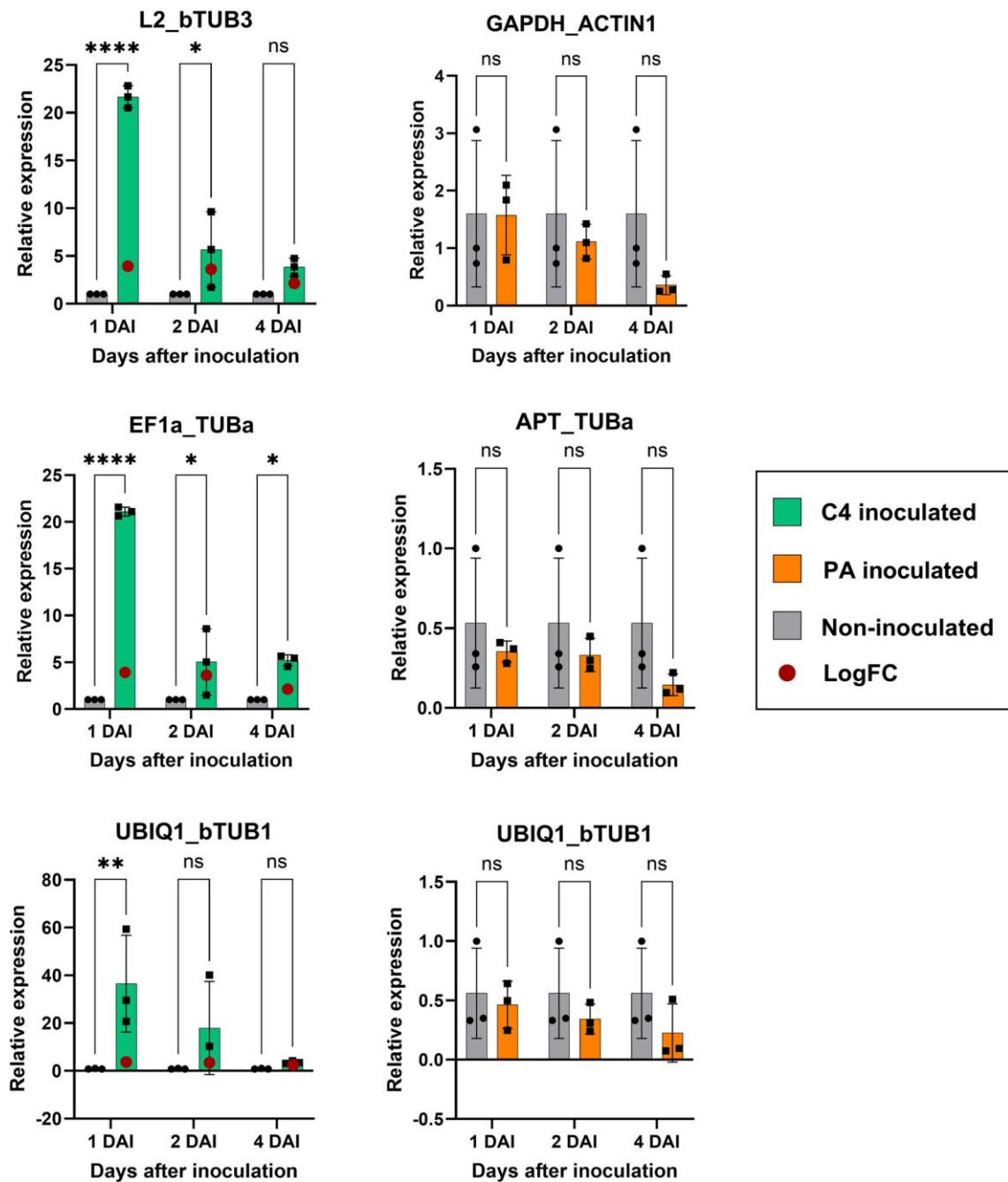


Figure 5. Relative expression levels of Thaumatin-like protein 1 (TL1) in *Musa acuminata* subsp. *burmannicoides* var. Calcutta 4 (C4) and *Musa* sp. cultivar Prata-anã (PA), under infection with *Fusarium oxysporum* f. sp. *cubense* STR4 isolate 218A 1, 2 and 4 days after inoculation. Normalization was examined using the two most stable gene pairs for C4 (*L2* and *bTUB3*; *EF1a* and *TUBa*), and for PA (*GAPDH* and *ACTIN1*; *APT* and *TUBa*), with each compared with the least stable pair for C4 and PA (*UBIQ1* and *bTUB1*). **** indicated significance at $p < 0.0001$, ** indicated significance at $p < 0.001$ and * indicated significance at $p < 0.05$.

to determine the optimal number of reference genes for use in normalization. NormFinder calculates expression stability based on the stability value (SV), where lower values indicate higher gene stability. This algorithm also identifies the most appropriate combinations of pairs of stable genes. In the case of BestKeeper, lower coefficient of variation (CV) and standard deviation (SD) values indicate greater expression stability. R software⁴⁰ was employed for analysis of data generated with all algorithms and for preparation of figures.

Expression analysis of a PR-5-encoding gene

Combinations of the most stable genes in C4 (*L2* and *bTUB3*; *EF1a* and *TUBa*) and PA (*GAPDH* and *ACTIN1*; *APT* and *TUBa*), together with the least stable genes in both C4 and PA (*Ubiq1* and *bTUB1*), were all compared in terms of efficiency in normalization of expression of a gene in C4 encoding a Thaumatin-like protein 1 (*TLP1*) (Macma4_05_g17130), which is member of the Pathogenesis-related-5 protein family (PR-5). In a previous transcriptome study by the group (data not shown), the Log2 fold-change (LogFC) expression of this gene in C4 in inoculated root materials compared to non-inoculated controls was 3.86, 3.46 and 2.37 for 1, 2 and 4 DAI, respectively. Specific primers designed for this gene comprised TL1-F (GAT GCG ACG CTG ATG AAA) and TL1-R (AGA CCG GCC ATA AGA TAC A). RNA extraction, cDNA synthesis and RT-qPCR were conducted as described above.

Data availability

All data generated and analysed during the study is included in the published article.

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Author contributions

R.N.G.M. conceived and designed the study. E.C.C. conducted the biotic assays and RT-qPCR analysis. E.C.C.,

L.S.B. and T.G.G. analysed the data. R.N.G.M., E.C.C. and T.G.G. provided intellectual input. E.C.C. and

R.N.G.M. wrote and revised the manuscript. All authors have read and approved the final manuscript.

Competing interests

The authors declare that they have no competing financial and/or non-financial interests in relation to the work described.

Additional information

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Capítulo IV

Uma análise transcriptômica da resposta de resistência em *Musa acuminata* subsp. *burmannica* acesso 'Calcutta 4' a *Fusarium oxysporum* f. sp. *cubense* Raça Subtropical 4

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RESUMO

A banana (*Musa spp.*) é uma cultura de grande importância mundial e um alimento básico na dieta de milhões de pessoas. No entanto, os cultivares comerciais são altamente suscetíveis à Murcha de Fusarium, uma doença devastadora causada por *Fusarium oxysporum* f. sp. *cubense* (*Foc*). As raças tropical 4 (TR4) e subtropical 4 (STR4) representam ameaças significativas à produção de banana, sendo a STR4 menos agressiva e patogênica para cultivares 'Cavendish' apenas em regiões subtropicais. A resistência genética continua sendo a estratégia mais eficaz para combater o *Foc*, destacando a necessidade de compreender os mecanismos de resistência. Este estudo teve como objetivo identificar e validar genes envolvidos na resposta de resistência à STR4 por meio de análises de RNA-seq. Dois genótipos foram avaliados: 'Calcutta 4' (genótipo selvagem resistente, AA) e 'Prata-Anã' (genótipo comercial suscetível, AAB). Mudanças de 'Calcutta 4' e 'Prata-anã' foram inoculadas com o isolado 218A de *Foc* STR4, e amostras de raízes de 'Calcutta 4' foram coletadas em 1, 2 e 4 dias após a inoculação (DAI) para análises de RNAseq. Estudos histológicos comparativos entre os genótipos revelaram respostas de defesa, como deposição de calose e produção de compostos fenólicos, exclusivamente em 'Calcutta 4' nos tempos de 1 e 2 DAI, enquanto a colonização por *Foc* STR4 foi observada apenas em 'Prata-Anã' em 8 e 15 DAI. A análise de RNAseq identificou 1.416 genes diferencialmente expressos (DEGs) em 'Calcutta 4', com rápida regulação positiva, em relação ao controle não inoculado, de 752 DEGs em 2 DAI, incluindo genes associados a PRRs, quitinases, fitormônios, genes de resistência (pertencentes à família NLR), fatores de transcrição e resistência sistêmica adquirida. A análise de vias funcionais destacou respostas de defesa coordenadas no processo de resistência de 'Calcutta 4' à *Foc* STR4. Esses achados fornecem uma base para a identificação de genes candidatos para melhoramento genético por meio de introgressão ou técnicas de edição genômica, como CRISPR, visando o desenvolvimento de variedades de banana resistentes à Murcha de Fusarium, mitigando os impactos dessa doença na produção global.

Palavras-chave: Banana, Murcha de Fusarium, transcriptômica, resistência genética, doença.

A transcriptome analysis of the resistance response in *Musa acuminata* subsp. *burmannica* accession ‘Calcutta 4’ to *Fusarium oxysporum* f. sp. *cubense* Subtropical Race 4

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ABSTRACT

Banana (*Musa spp.*) is a globally significant crop and a staple food in the diet of millions of people. However, commercial cultivars are highly susceptible to Fusarium wilt, a devastating disease caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*). Tropical race 4 (TR4) and subtropical race 4 (STR4) pose significant threats to banana production, with STR4 less aggressive and pathogenic to ‘Cavendish’ cultivars only in subtropical regions. Genetic resistance remains the most effective strategy to combat *Foc*, highlighting the importance of advancing understanding of resistance mechanisms. This study aimed to identify and validate genes involved in the resistance response to *Foc* STR4 through RNA-seq analyses. Two genotypes were evaluated: ‘Calcutta 4’ (a resistant wild genotype, AA) and ‘Prata-Anã’ (a susceptible commercial genotype, AAB). Seedlings of ‘Calcutta 4’ and ‘Prata-Anã’ were inoculated with *Foc* STR4 isolate 218A, and root samples from ‘Calcutta 4’ were collected at 1, 2, and 4 days after inoculation (DAI) for RNA-seq analysis. Comparative histological studies between the genotypes revealed defence responses, such as callose deposition and phenolic compound production, occurring exclusively in ‘Calcutta 4’ at 1 and 2 DAI, while colonization by *Foc* STR4 was observed only in ‘Prata-Anã’ at 8 and 15 DAI. RNA-seq analysis identified 1,416 differentially expressed genes (DEGs) in ‘Calcutta 4’, with a rapid upregulation of 752 DEGs at 2 DAI, including genes associated with PRRs, chitinases, phytohormones, resistance genes (from the NLR family), transcription factors, and systemic acquired resistance. Functional pathway analysis highlighted coordinated defence responses in the resistance response of ‘Calcutta 4’ to *Foc* STR4. These findings provide a foundation for identifying candidate genes for genetic improvement through introgression or genome-editing techniques such as CRISPR, aiming to develop Fusarium wilt-resistant banana varieties and mitigate the impacts of this disease on global production.

Keywords: Banana, Fusarium wilt, transcriptomic, genetic resistance, disease.

INTRODUCTION

Banana is globally the most widely cultivated, consumed, and exported fruit crops, with an annual production of 125 million tons (FAO, 2024). Together with plantains, they represent a staple food for over 400 million people globally (Voora et al. 2023), providing a source of carbohydrates, essential nutrients, vitamins A, B1, B2, B3, B6, and C, and minerals such as iron, potassium, phosphorus and calcium (Chandler 1995).

Banana cultivation faces numerous challenges that prevent further expansion of the international market (FAO 2024b). Diseases caused by phytopathogens are the primary cause of yield and quality losses, affecting plants at planting through to post-harvest and storage (Ghag et al. 2015). Among the fungal diseases, Yellow Sigatoka, caused by *Pseudocercospora musae* (Zimm.) Deighton, Black Sigatoka, caused by *P. fijiensis* (Morelet) Deighton, and Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*) (E. F. Smith) Snyder & H.N. Hansen, are particularly destructive and can decimate entire banana plantations (Cordeiro et al. 2016; Ploetz et al. 2015).

Fusarium wilt is a classic vascular wilt disease, where the pathogen enters the plant via direct penetration of the root epidermal cells, eventually reaching the vascular tissues. The first visible symptoms of disease include a discoloration of the rhizome and subsequent necrosis, with fungal colonization of vascular bundles in the pseudostem leading to xylem blockage. At this stage, the plant typically exhibits yellowing of the younger leaves and wilt symptoms. The time period for appearance of symptoms can vary between two to six months, complicating early diagnosis of the disease (Cordeiro et al. 2016; Dita et al. 2018; Rishbeth 1957). Disease progression will eventually cause death of the plant.

Foc is subdivided into three pathogenic races based on pathogenicity to different *Musa* cultivars. Race 1 affects 'Gros Michel' (AAA group), 'Silk', and 'Pome' (AAB group) cultivars; race 2 affects 'Bluggoe' (ABB group) (Ploetz 2006a); and race 4 is pathogenic to all cultivars, including 'Cavendish' (AAA group). Race 4 is further subdivided into Tropical Race 4 (TR4) and Subtropical Race 4 (STR4), with STR4 being less aggressive and capable of causing disease in 'Cavendish' only in subtropical regions (Buddenhagen 2009). Conversely, TR4 comprises highly virulent isolates that cause disease in 'Cavendish' in both tropical and subtropical regions. Historically, Fusarium wilt was first reported in 1874 in Brisbane, Australia, affecting the banana cultivar 'Sugar' (AAB, Silk sub-group) (Bancroft, 1876). The disease subsequently spread across the Americas, with cases reported in Panama and Costa Rica in 1890, Suriname in 1906, Cuba in 1908, Trinidad in 1909, Jamaica in 1911, Honduras in 1916, and Guatemala in 1919, indicating its early continental distribution (Vézina 2022a). During this period, an epidemic caused by *Foc* race 1 devastated plantations of the susceptible 'Gros Michel' cultivar (AAA group), which was extensively cultivated in Central America (Zheng et al. 2018). In Brazil, the first record of Fusarium Wilt occurred in 1930 in Piracicaba, São Paulo, in the 'Silk' cultivar (AAB group), rapidly spreading throughout the country and decimating entire plantations (Cordeiro et al. 2016). The advance of the pathogen in banana production, especially in 'Gros Michel', prompted extensive research into the genetic improvement of *Musa* genotypes for resistance, resulting in the development of *Foc* race 1 - resistant 'Cavendish' cultivars (Buddenhagen 1990). In 1967, however, symptoms of Fusarium wilt were observed in 'Cavendish' cultivars in Taiwan, with approximately half a million infected plants covering 1200 hectares by 1976. Pathogenicity tests identified the isolates as *Foc* (Su et al. 1986). This *Foc* pathogen, referred to as Tropical Race 4 (TR4), spread to 'Cavendish' plantations in Indonesia and Malaysia during the mid-

1990s (Pin. 1996), and later appeared in China by the late 1990s (Vézina 2022b). It then reached Australia, Oman, Jordan, and Mozambique in 2015, Pakistan and Lebanon in 2018, and Vietnam, Laos, Myanmar, and Israel shortly thereafter (Maymon et al. 2020; Nadiyah Jamil et al. 2019). The first case of TR4 in Latin America was reported in Colombia in 2019 (García-Bastidas et al. 2019), followed by Peru in 2021 (Acuña et al. 2022), and Venezuela in 2023 (Mejías Herrera et al. 2023). STR4, meanwhile, infecting Cavendish bananas, has been reported in Australia, Brazil, the Canary Islands, China, South Africa, and Taiwan (Munhoz et al. 2024).

Banana production has declined over the last decade, primarily due to abiotic factors such as drought, heavy rains, and hurricanes in certain regions, as well as pest and disease infestations. Despite the replacement of 'Gros Michel' with 'Cavendish' after *Foc* Race 1 devastation, one of the greatest challenges for the industry, exacerbated by climate change, is the increased spread of diseases (Voorra et al. 2023). Salvacion et al. (2019) reported that climate change could expand areas affected by *Foc* by 67% in the Philippines. Globally, *Fusarium* wilt caused by all pathogenic races result in an annual yield loss of 60–90% (Sankari et al. 2023). In Brazil, disease incidence in Silk fields has reached up to 41.42%, with a production impact of 1856.7 kg ha⁻¹ year⁻¹, translating to an average loss of USD 109.8 ha⁻¹ year⁻¹ (Heck et al. 2021).

Considering that *Foc* chlamydospores can persist in the soil for over 30 years in the absence of a host (Pegg et al. 2019; Ploetz 2006a; Stover 1962), the most effective management strategy to control *Fusarium* wilt is via exclusion, preventing the introduction of the pathogen into banana-producing areas. Chemical, cultural, and biological control methods for managing *Fusarium* wilt, by contrast, have not yet proven to be sufficiently effective to protect susceptible cultivars (Ploetz et al. 2015). Given this scenario, the only effective control method, when the pathogen is present in production fields, is to use resistant cultivars against *Foc* (Kema et al. 2021; Ploetz 2015a; Xu et al. 2011). Whilst vegetative propagation of sterile commercial bananas equates to a low genetic variability (Silva et al. 2013, 2016; Simmonds 1953), greater genetic variability can be found in wild subspecies of *M. acuminata* and their varieties. The *M. acuminata* subsp. *burmannica* accession 'Calcutta 4', which originated from Southeast Asia and the Western Pacific, the *Musa* centre of origin, has co-evolved with several pathogens and, as a result, has developed an arsenal of defence mechanisms to biotic stress. This accession is widely used in breeding programs to develop disease-resistant cultivars (Miller et al. 2010c; Sachter-Smith 2023).

Different molecular approaches are advancing our understanding of the molecular basis of host resistance responses to *Foc*, with *Focus* on candidate gene discovery including in resistant wild bananas such as *M. acuminata* 'Pahang' and *M. acuminata* subsp. *burmannica* 'Calcutta 4' (Chen et al. 2019; Kotari et al. 2018a; Miller et al. 2008; Zhang et al. 2018, 2019). A number of genes expressed in wild *Musa* genotypes during interactions with *Foc* have been applied to enhance resistance in commercial cultivars. Genes such as *MusaDAD1*, *MusaBAG1*, and *MusaBI1*, which regulate cell death, are considered efficient after cisgenesis in banana cv. 'Rasthali' (AAB subgroup), with minimum symptoms after inoculation with *Foc* race 1 (Ghag et al. 2014a). The introgression of the *SM-AMP-d1* gene, a defensin with antimicrobial activity present in *Stellaria media* seeds, into the cv. 'Rasthali' also resulted in enhanced resistance to infection by *Foc* Race 1 (Ghag et al. 2014b). Transformation of cv. 'Rasthali' using the *Ace-AMP1* gene, involved in pathogen recognition and signaling, and the *pflp* gene involved in hypersensitive response and the development of systemic acquire resistance (SAR), increased peroxidase and superoxide

dismutase activity, indicating improved tolerance to oxidative stress and resulting in greater resistance to *Foc* race 1 (Sunisha et al. 2020). Through introgression of an NLR *RGA2* gene from a wild species, together with the *Ced9* gene from *Caenorhabditis elegans*, which plays a crucial role in the regulation of apoptosis, Dale et al. (2017) also transformed Cavendish cv. 'Grande Naine', with cisgenic plants exhibiting high resistance to *Foc* TR4, including in field trials.

Cultivars such as Pome (AAB), Silk (AAB) and Gros Michel, all of which are susceptible to *Foc* races 1 STR4 and TR4, predominate in local Latin American and Caribbean markets (Munhoz et al. 2024). Given the widespread presence of STR4 in the region, the aim of this study was to identify genes involved in the defence response of *M. acuminata* subsp. *burmannica* accession 'Calcutta 4' (AA), during interaction with the pathogen *F. oxysporum* f. sp. *cubense* race STR4. RNAseq-based analysis of differential gene expression together with investigation of structural defence responses provided insights into the immune response in this resistant wild diploid. Data will serve as a basis for future introgression of defence genes into commercial varieties such as Prata, Silk and Cavendish.

METHODS

Preparation of *Musa* Seedlings

In vitro propagated seedlings of the wild accession 'Calcutta 4' (*Musa acuminata* subsp. *burmannica*, subgroup AA), resistant to *Fusarium oxysporum* f. sp. *cubense* race 1 and race 4 (STR4 and TR4), and the susceptible cultivar 'Prata-anã' (*Musa* sp., subgroup 'Pome', AAB) to both races, were grown in sterile coconut fiber substrate, with acclimatization in a greenhouse at 24°C with irrigation every 48 hours. Fertilization was applied 30 days after transplanting, using 100 mL of a 1g/L monoammonium phosphate (MAP) solution per plant. After 45 days, plants were transferred to 2L pots containing sterile coconut fiber substrate. After further 30 days, a second fertilization was applied using a mixture of urea, magnesium sulphate, MAP, calcium, and potassium sulphate, with a further application after 10 days, adding zinc sulphate, with concentrations also applied at 1g / L).

Molecular Identification of the Fungal Isolate

Fusarium oxysporum f. sp. *cubense* isolate 218A, belonging to the culture collection at the Plant Pathology Laboratory at Embrapa Cassava and Fruits, was employed in interaction assays. This isolate, originally isolated in 2014 from a Cavendish subgroup cultivar in São Paulo state, Brazil, was previously identified as Subtropical Race 4 (STR4), based on vegetative compatibility group testing (VCG 0120) (Rocha et al. 2020). For molecular identification, total DNA was extracted from fungal mycelium using a DnEasy Plant Mini Kit (Qiagen), following the manufacturer's instructions. Whole-genome sequencing was performed using DNBseq PE 150 bp next-generation sequencing (BGI Genomics, China). Quality control was conducted using FastQC, and contig assembly was performed with the Platanus and Bwise software, followed by sequence validation using BUSCO. Protein annotation was performed with Funannotate, and a comparative analysis of orthologous and paralogous gene groups in multiple genomes and *formae speciales* of *F. oxysporum* was conducted using OrthoFinder (Emms and Kelly 2019). Orthologous gene analysis revealed a higher similarity of genes from isolate 218A with STR4, based on

comparison with sequence data for *Foc* STR4 (GCA_016802205.1) (Raman et al. (2021) (Supplementary Figure 1).

Inoculum Preparation

Foc STR4 218A was incubated on PDA plates for 7 days under a 12-hour photoperiod at 28°C. After colony growth, a spore suspension was prepared without calibration, with 20 mL inoculated onto 500g of autoclaved rice. Inoculated rice bags were incubated at 28°C with a 12-hour photoperiod for 14 days. Serial dilutions were performed to obtain spore concentrations at 10⁶ CFU/g of infested rice, with quantification conducted using a Neubauer chamber (Santana et al. 2024).

Bioassays

Calcutta 4 and Prata-anã plants were individually inoculated through addition of 50g of *Foc* STR4 218A-infested rice to the coconut fibre substrate. Non-inoculated control plants were prepared through addition of non-infested rice to the substrate. The experimental design followed a complete randomized design, with each treatment comprising three independent biological replicates. For histological analyses, root samples from 'Calcutta 4' and 'Prata-anã' were collected at 1, 2, 4, 8, and 15 days after inoculation (DAI). Total roots from inoculated and non-inoculated control plants were collected at 1, 2, and 4 DAI for both RNAseq and RT-qPCR analyses, with samples flash-frozen in liquid nitrogen then stored at -80 °C.

Histological Analyses

Root fragments collected at 1, 2, and 4 DAI from both plant materials were washed and stored in Karnovsky's solution (0.05M, pH 7.2) [2% glutaraldehyde, 2% paraformaldehyde, 5 mM calcium chloride in 0.05M sodium cacodylate buffer] at 4°C for 24 hours. Samples were then dehydrated through successive washes in 40 - 100%, alcohol, embedded in historesin (Leica), sectioned using a microtome (Leitz 1516) to a thickness of 10 µm then mounted on slides.

To visualize phenolic compounds, sections were stained with ferric chloride (sodium carbonate, ferric chloride hexahydrate, distilled water), washed three times with distilled water, then observed under a fluorescence microscope (Olympus model BX51).

For visualization of callose deposition, Lugol's reagent was applied to fragments for 3 minutes, washed with distilled water, then stained with a 0.01% aniline blue solution for 10 minutes in total darkness. Stained fragments were analyzed under a fluorescence microscope (Olympus model BX51), with callose deposition detected by the presence of bright yellow fluorescence under blue light (excitation 450-490 nm, with a maximum wavelength of 515 nm).

For scanning electron microscopy (SEM), samples were post-fixed in 1% osmium tetroxide for 1 hour, dehydrated in an acetone gradient (30%, 50%, 70%, 90%, and 100%), then dried in a critical point dryer (Blazers CPD 030). Samples were coated in gold using a sputter coater (Leica EM SCD 500) and visualized using a scanning electron microscope (JEOL JSM 7001F).

To assess *F. oxysporum* colonization of host tissues, root fragments collected at 1, 2, 4, 8, and 15 DAI from 'Calcutta 4' and 'Prata-anã' were cleared and stained. Fragments were placed in a 10% KOH solution for 48 hours and washed with distilled

water. HCl (1%) was added for 5 minutes, the solution was discarded, and roots then stained with Trypan Blue in a 0.05% lactoglycerol solution (2:1:1; v:v;lactic acid:glycerol) at room temperature for 1 hour. After discarding the stain, a solution of lactoglycerol was added to the roots which were then visualized and photographed under light microscopy (Olympus model BX51).

Symptom Evaluation

Seedlings of 'Calcutta 4' and 'Prata-anã' were inoculated with *Foc* STR4 218A at a concentration of 10^6 CFU/g of rice, with non-inoculated controls for each genotype prepared according to Santana et al. (2024) protocol. Symptoms of wilt, leaf yellowing, and rhizome necrosis were evaluated in both genotypes, with the assessment limited to their presence or absence at 90 and 120 DAI.

Total RNA extraction

Roots collected at 1, 2, and 4 DAI from 'Calcutta 4' and 'Prata-anã' were immediately frozen in liquid nitrogen and stored in an ultrafreezer at -80°C. Root fragments were ground in liquid nitrogen, and total RNA extraction was performed using 1 mL of Plant RNA Reagent (Invitrogen – ThermoFisher Scientific, Waltham, MA, USA) per sample for 5 minutes. After centrifugation for 10 minutes at 15,000 rpm at 4°C, the supernatant was purified using the Directzol Plant RNA Purification Kit (Zymo Research) following the manufacturer's instructions for transcriptomic analysis and gene expression validation via RT-qPCR. RNA integrity and quantification were verified following agarose gel electrophoresis and via NanoDrop One spectrophotometry (ThermoFisher Technologies, Waltham, MA, USA).

Sequencing and RNAseq

Total RNA samples from 'Calcutta 4' at 1, 2, and 4 DAI, and from non-inoculated controls, were transported using RNA stable™ (Biomatrica, San Diego, CA, USA) according to the manufacturer's instructions. Sequencing was performed at the Genome Quebec Innovation Center, Canada. RNA-Seq libraries were constructed using the mRNA stranded Library Prep Kit (Illumina Inc., San Diego, CA, USA), with paired-end sequencing (2x100 bases) conducted using the Illumina NovaSeq 6000 PE100 technology (Illumina Inc., San Diego, CA, USA).

RNAseq Data Processing and Differential Expression Analysis

Raw sequence reads obtained from RNAseq were quality-checked using FastQC (FastQC -<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Reads were quality filtered (Fastq QC>30 and minimum length $\geq$ 100 bp) using Trimmomatic (Bolger et al. 2014). High-quality sequences were aligned to the *M. acuminata* DH-Pahang v.4 reference genome (available on the Banana Genome HUB - <https://banana-genome-hub.southgreen.fr/organism/Musa/acuminata>) in batch mode, allowing individualized analysis using the STAR software in two-pass Basic Mode (Dobin et al. 2013). The number of reads mapped to gene modules was counted using HTSeq-count (Python Software Foundation, Portland, OR, USA) (Anders et al. 2015).

Differential expression analysis was performed using the exact test from the EdgeR package in Rstudio (Robinson et al. 2010). Differentially expressed genes (DEGs) were identified based on statistical significance (adjusted p-value for FDR) using the Benjamini-Hochberg method, with the log2 Fold Change (log2FC) used for determining expression changes between treatments (Calcutta 4-inoculated (I) vs. non-inoculated (NI)).

Functional Analysis of DEGs

Different functional annotation strategies were employed to determine the key processes and metabolic pathways affected by biotic stress. These included Gene Ontology (GO), the Kyoto Encyclopedia of Genes and Genomes (KEGG - <https://www.genome.jp>) (Kanehisa et al. 2016a), EuKaryotic Orthologous Groups (KOG) (Tatusov et al. 2003), and MapMan (Thimm et al. 2004). GO annotation of DEGs was carried out by homology using GO enrichment software (<https://banana-genome-hub.southgreen.fr/content/go-enrichment>), with parameters set to "Merge similar GO-term: sensitive", cutoff p-value ≤ 0.05 , and q-value ≤ 0.01 (Droc et al. 2013). For the mapping of metabolic pathways, DEG protein sequences were analyzed using BlastKOALA (<https://www.kegg.jp/blastkoala/>) to generate KEGG identifiers (KOs) based on sequence similarity to the KEGG gene database subset (Kanehisa et al. 2016b). Generated KOs were then used to construct KEGG pathway maps (<https://www.genome.jp/kegg/mapper/>) (Kanehisa et al. 2022; Kanehisa and Sato 2020). Functional annotation using KOG was performed on the basis of sequence similarity using the eggNOG v5.0 software (<http://eggnog5.embl.de>) (Huerta-Cepas et al. 2019). For metabolic pathway analysis via MapMan v. 3.5.1 (Thimm et al. 2004), DEG protein sequences were hierarchically annotated using Mercator4 v.5 (LOHSE et al. 2014) and mapped to the *M. acuminata* gene matrix, together with corresponding expression values (Log2FC).

Additionally, the InterPro database (<https://www.ebi.ac.uk/interpro/>) (Mitchell et al. 2019) and the InterProScan package (Jones et al. 2014) were employed to assign functional domains to the protein products of DEGs, providing an overview of the protein families and domains involved.

Validation of Candidate Genes via RT-qPCR

A total of 15 DEGs were selected for expression validation via the Reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) (Table 1). Primers targeting the transcript sequence of each gene were designed using the PrimerQuest Tool (IDT - <https://www.idtdna.com/pages/tools/primerquest>), with application of the following parameters: amplicon size between 80 to 150 bp, primer length between 18 to 25 nt, G/C content between 40 to 60%, and melting temperature between 57 to 63°C (ideally 60°C). Primer specificity was verified using NCBI Primer-BLAST for eukaryotic organisms (<https://www.ncbi.nlm.nih.gov/tools/primer-blast>). For each treatment, for both 'Calcutta 4' and 'Prata-anã', extracted RNAs were purified and treated with DNase as described previously (Section Total RNA extraction). cDNA synthesis was conducted using SuperScript IV and Oligo(DT) primers (Invitrogen Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's protocol. Specificity of primer pairs (sense and antisense) was tested using a pool of cDNA samples from 'Calcutta 4' and 'Prata-anã' via PCR. Amplicons were sequenced and analysed via BLAST against the reference *M. acuminata* 'DH-Pahang' genome

sequence. For RT-qPCR validation of target genes, cDNA was diluted 1:20 from stock solutions. Each reaction was conducted in a final volume of 10 µL, comprising 0.2 µM of each primer (forward and reverse), 5 µL of iTaq Universal SYBR Green (BioRad), 2 µL of diluted cDNA, and 2.6 µL of ddH₂O. Stable reference genes L2 and bTUB3 were employed for 'Calcutta 4', and ACTIN1 and GAPDH for 'Prata-anã' (Costa et al. 2024). Thermocycling was performed using an ABI StepOne Plus Real-Time Thermocycler (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) with three independent biological replicates and three technical replicates per amplification. The cycling conditions were as follows: initial denaturation at 50°C for 2 minutes, followed by 95°C for 10 minutes; 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds. Melting curves were obtained in three steps: 95°C for 15 seconds, 60°C for 60 seconds, and 95°C for 15 seconds.

Statistical Analysis of RT-qPCR Data

The qPCR data were analyzed according to the linear mixed model below, following the strategy proposed by Steibel et al. (2009). Data on the target and endogenous control genes are analyzed simultaneously:

$$CT_{ijklm} = \mu + G_i + P(G)_{(i)j} + T_k + (GT)_{ik} + [P(G)T]_{(i)jk} + D_l + (GD)_{il} + [P(G)D]_{(i)jl} + (TD)_{kl} + (GTD)_{ikl} + [P(G)TD]_{(i)jkl} + S_m + e_{ijklm}$$

where CT_{ijklm} is the CT value; μ is the overall mean, G_i is the fixed effect of the i^{th} level of Genotype, with $i = 1$ or 2 ; $P(G)_{(i)j}$ is the fixed effect of the j^{th} level of Primer (target/endogenous) within Genotype, with $j = 1$ to 3 ; T_k is the fixed effect of the k^{th} level of Treatment, with $k = 1$ or 2 ; D_l is the fixed effect of the l^{th} level of Day, with $l = 1$ to 3 ; $(GT)_{ik}$, $[P(G)T]_{(i)jk}$, $(GD)_{il}$, $[P(G)D]_{(i)jl}$, $(TD)_{kl}$, $(GTD)_{ikl}$, and $[P(G)TD]_{(i)jkl}$, represent the fixed effect interactions among the previously described parameters; S_m is the random effect of the m^{th} Sample, assuming $S \sim N(0, I_s \sigma_s^2)$, where I_s represents the identity matrix with dimensions equal to the number of samples and σ_s^2 the sample variance; and e_{ijklm} is the random error associated with CT_{ijklm} , assuming $e \sim N(0, I_e \sigma_e^2)$, where I_e represents the identity matrix with dimensions equal to the total number of observations.

Orthogonal contrasts were constructed for model terms including the effect of *Primer* to obtain P -values for effect of interest: $P(G)_{(i)j}$ for the main effect of Genotype, $[P(G)T]_{(i)jk}$ for the main effect of Treatment and Genotype-Treatment interaction, $[P(G)D]_{(i)jl}$ for the main effect of Day and Genotype-Day interaction, and $[P(G)TD]_{(i)jkl}$ for the Genotype-Treatment-Day interaction. Similarly, contrasts were used to compute $-\Delta adjCT$ as the difference in CT s between the target and the average of the two endogenous genes, and $-\Delta\Delta CT$ for pairwise comparisons of interest. This analysis enables the appropriate modelling of all sources of variation, based on the assumption of a Gaussian distribution for log-transformed expression levels, utilizing heteroscedastic models that allow for heterogeneous variances and providing a more realistic and precise fit for gene expression data. Additionally, the inclusion of sample-specific effects, representing the total mRNA level, enhances the accuracy and applicability of the results obtained. All analyses were performed in SAS Studio 3.81 (Enterprise Edition, SAS Institute Inc., Cary, NC, USA). The $-\Delta\Delta CT$ and Log2FC values were used to calculate relative expression values using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). These values were then employed in graphical

representations generated using the R software (R Core Team 2023), specifically the ggplot2 package (Wickham 2016).

RESULTS

Histological analysis of the host-pathogen interactions

Root sections from 'Calcutta 4' inoculated with *Foc* STR4 218 A at 1 DAI showed the presence of callose deposition, indicated by a strong fluorescence in the root cortex cells (Figure 1A). At 2 and 4 DAI, fluorescence was observed with lower intensity in the epidermal and parenchymal cells (Figure 1B-C). No fluorescence was observed in the control treatment (Figure 1G). In the case of 'Prata-anã', both inoculated and non-inoculated control samples showed no evidence for callose deposition in the stained root sections (Figures 1D-F, 1H).

A similar pattern of physiological responses was observed with regard to the formation of phenolic compounds. Parenchymal cells in 'Calcutta 4' root fragments revealed deposition of phenolic compounds at 1 and 2 DAI (Figure 2A-B), followed by a reduction at 4 DAI (Figure 2C). No deposition was observed in non-inoculated controls (Figure 2G). In 'Prata-anã', no phenolic compounds were detected in inoculated samples and non-inoculated controls (Figure 2H).

Clearing and subsequent staining of root samples revealed *Foc* STR4 colonization in the root tissues of 'Prata-anã', particularly at 4, 8, and 15 DAI (Figures 3H-J). In 'Calcutta 4', spore germination, hyphae formation, and accumulation of chlamydospores were observed on the outer root surface (Figures 3A-E), without colonization of internal root tissues. Non-inoculated controls showed an absence of fungal spores or hyphae (Figures 3K-L).

Scanning electron microscopy (SEM) analysis showed the presence of spores, potentially chlamydospores, on the root surface of 'Calcutta 4' at all the examined time points (Figures 4A-C). At 4 DAI, spores exhibited deformations in the cell wall, when compared to 1 and 2 DAI (Figure 4C). In 'Prata-anã', spores (Figure 4D-E), abundant hyphae and conidiophores were observed on the root surface at 4 DAI (Figure 4F). No pathogen-related structures were found in the non-inoculated controls.

In summary, the histological analyses of callose and phenolic compound deposition in 'Calcutta 4' indicated their exclusive presence in the root cells of this genotype, with no such responses observed in the susceptible 'Prata-anã' genotype. Considering the differences in pathogen growth between these contrasting genotypes, these compounds likely represent defence responses that hinder *Foc* STR4 colonization in 'Calcutta 4' root tissues.

Fusarium Wilt Symptoms

Fusarium wilt symptoms were compared at 90 and 120 DAI in inoculated and control plants of 'Calcutta 4' and 'Prata-anã' (Figure 5). In the case of inoculated 'Prata anã' plants, symptoms comprised a yellowing of older leaves, with progression to younger leaves (Figure 5I-J), and rhizome tissues presenting necrosis symptoms in both timepoints (Figures 5M-N). Non-inoculated control plants (Figures 5K-L and 5O P) did not display any disease symptoms. Similarly, for 'Calcutta 4' plants, no Fusarium wilt symptoms were observed in inoculated or non-inoculated control plants (Figures 5A-H).

RNAseq Data Overview

Illumina NovaSeq 6000 PE100 sequencing resulted in a total of 447,843,971 reads from the cDNA libraries representing 1, 2, and 4 DAI treatments and non-inoculated controls in 'Calcutta 4'. Following filtering, the total number of reads was reduced to 437,740,123, with 326,407,220 originating from the inoculated treatments and 111,332,903 from the controls. Between 77% and 92% of the 'Calcutta 4' reads were correctly mapped to 36,769 gene models in the *M. acuminata* subsp. *malaccensis* DH-Pahang v.4 reference genome (Table 2). Illumina RNA-Seq raw sequence data were deposited in the NCBI Sequence Read Archive (SRA) database as a BioProject, accessible under SRA submission number SUB14923683 and BioProject accession number PRJNA1198017.

Differential Gene Expression Analysis

DEGs with significant fold change (at least ≥ 2 -fold and at a probability level of $p \leq 0.05$) between inoculated and non-inoculated control treatments were identified following comparison of mapped read counts. A total of 1,416 DEGs were identified in total (Supplementary table 1). Of these, 1,223 were upregulated, with 270, 872, and 81 DEGs at 1, 2, and 4 DAI, respectively. Conversely, 193 DEGs were downregulated, with 150, 33, and 10 identified at 1, 2, and 4 DAI, respectively (Figure 6). The greatest number of DEGs was observed at 2 DAI, with 905, followed by 1 DAI with 420, and 4 DAI with 91 DEGs. Comparison of DEGs across treatments revealed a greater number of shared DEGs 1 DAI and 2 DAI (Figure 6), indicating a higher expression of certain genes in the early stages of *Foc* STR4 infection in 'Calcutta 4'. The larger number of upregulated DEGs suggests that the defence response in 'Calcutta 4' to STR4 may require an early positive regulation of genes, particularly at 2 DAI. Changes in expression of shared DEGs across treatments are shown in Figure 7, where shades of red represent upregulation, and shades of blue represent downregulation. Most genes were highly expressed at 2 DAI compared to non-inoculated controls, with a different expression pattern observed at 1 and 4 DAI. These data highlight the significance of the 2 DAI time point in modulating the defence responses of 'Calcutta 4' to *Foc* STR4 (Figure 7).

Functional Analysis of DEGs

Gene ontology

Gene Ontology (GO) analysis revealed that the upregulated DEGs were enriched in 81 biological process, seven molecular function, and 40 cellular component categories, with the majority of terms related to the 2 DAI treatment. Downregulated DEGs were enriched in 20 biological processes, 3 molecular functions, and 14 cellular component categories, again mostly associated with the 2 DAI treatment, with no enrichment observed for the 4 DAI treatment. Key processes related to biotic stress were identified among the upregulated DEGs, including pathways associated with early pathogen recognition, chitin response, oxidative stress, membrane receptor signaling, chitin binding, and several signaling pathways related to plant hormones such as ethylene, jasmonic acid (JA), and abscisic acid (ABA) (Figure 8). Differential modulation of cellular components, particularly related to cell wall and apoplast modifications were observed between 1 and 2 DAI, potentially indicating structural alterations, such as callose deposition, hindering pathogen colonization of host tissues. Negative regulation of secondary root formation, which is the primary entry point for *Foc* STR4 in banana roots, was also observed at 2 DAI.

KEGG pathway analysis

KEGG pathway mapping revealed important signaling pathways related to MAPK (mitogen-activated protein kinase) signaling (Supplementary Figure 2), hormone signal transduction (Supplementary Figure 3), and plant-pathogen interaction (Supplementary Figure 4). At 1 DAI, upregulated genes were involved in MAPK signaling pathways related to early responses to pathogen attack, cell death, and signaling of ethylene and ABA (Supplementary Figure 2). At 2 DAI, MAPK, MAPKK, and MAPKKK pathways were highly activated, with DEGs related to late defence responses to pathogen attack, hydrogen peroxide production involved in programmed cell death, ethylene signaling, and ROS (reactive oxygen species) production. At 4 DAI, the responses were mainly related to late defence signaling against pathogens (Supplementary Figure 2). The hormone signal transduction pathway map also showed that DEGs, particularly at 2 DAI, were involved in the activation of cell expansion and elongation, ubiquitin-mediated proteolysis, and disease resistance via the auxin, ABA, ethylene, brassinosteroid, JA, and SA pathways. Only one downregulated DEG at 1 DAI was observed in the disease resistance pathway in SA signaling (Supplementary Figure 3). In the plant-pathogen interaction map, several upregulated DEGs were observed that are involved in the initial recognition of pathogen-associated molecular patterns (PAMPs), triggering PAMP-triggered immunity (PTI). At 1 DAI, WRKY transcription factors were observed that typically activate PTI and defence-related genes, leading to the production and accumulation of phytoalexins. A similar pattern was observed at 2 DAI, with additional MAPK signaling and the activation of PTI5 (pathogenesis-related genes transcriptional activator), which recognizes pathogens through surface pattern recognition receptors (PRRs) (Supplementary Figure 4). At 2 DAI, genes involved in effector-triggered immunity (ETI) were observed, such as NLRs RIN4 and RPS2, which play a central role in plant resistance following pathogen infection (Supplementary Figure 4).

KOG annotation

Of the 1,416 DEGs, 1,177 were functionally predicted and annotated in the KOG database, with 233, 712, and 67 upregulated at 1, 2, and 4 DAI, respectively, and 131, 25, and 9 downregulated at 1, 2, and 4 DAI, respectively. Approximately 35% were annotated in the “S” category (no known function). Around 20% of DEGs from all treatments were mapped to the “K” category – Transcription (Figure 9). Categories E, I, O, P, and U displayed similar gene proportions across all three treatments, except for categories Q, C, and G, which showed a higher number of functional genes related to secondary metabolism, energy production, carbohydrate metabolism, and transport at 4 DAI compared to 1 DAI. Categories A, B, M, T, V, and Z only contained genes from 1 and 2 DAI, representing functions related to RNA processing and modification, chromatin structure, cell wall biogenesis, signal transduction, defence mechanisms, and the cytoskeleton (Figure 9). Of the 15 DEGs in the KOG V category (defence mechanisms), five were upregulated at 1 DAI, namely *Macma4_05_g11220* (Abhydrolase3 domain-containing protein), *Macma4_05_g23430*, *Macma4_08_g11580*, *Macma4_11_g03200* (Protein DETOXIFICATION), and *Macma4_09_g11990* (putative Protein DETOXIFICATION 40). Two DEGs were downregulated at 1 DAI: *Macma4_03_g03150* (3Beta_HSD domain-containing protein) and *Macma4_07_g04620* (Protein DETOXIFICATION). The remaining eight DEGs were upregulated at 2 DAI, comprising *Macma4_02_g12060* (conserved hypothetical protein), *Macma4_02_g13270*, *Macma4_08_g28030* (Protein

DETOXIFICATION), *Macma4_06_g14580* (1-aminocyclopropane-1-carboxylate oxidase), *Macma4_06_g27140* (3Beta_HSD domain-containing protein), *Macma4_06_g29370* (putative Phosphatidylglycerophosphate phosphatase PTPMT1), *Macma4_06_g32160*, and *Macma4_08_g05580* (Abhydrolase_3 domain-containing protein).

Mapman metabolic pathway analysis

Functional annotation through MapMan analysis revealed differential modulation of genes involved in key signaling pathways and defence mechanisms in 'Calcutta 4' in response to *Foc* STR4 infection over the time course investigated. Of the 1,416 DEGs, 1,370 functional categories (bins) were generated, with 231 related to regulation, 219 to biotic stress, 142 to transcription, 44 to metabolism, 32 to cellular response, and 13 to secondary metabolism. Overall, signaling pathways involving plant hormones, transcription factors, and secondary metabolites were activated by a significant number of upregulated DEGs at 2 DAI in 'Calcutta 4', compared to 1 and 4 DAI (Figure 10). These DEGs, with Log2FC values ranging from 2 to 4 at 2 DAI, were responsible for auxin, ethylene, and brassinosteroid signaling, activation of transcription factors such as ERF (ethylene response factor), WRKY (which regulates transcription by activating a signaling network in response to biotic and abiotic stress), and DOF (DNA-binding with one finger), which plays a key role in plant growth, development, and stress responses. Moreover, secondary metabolite pathways were activated at 1 and 2 DAI, indicating that these compounds were produced following the initial pathogen attack by STR4. Other pathways, such as cell wall, proteolysis, signaling, and abiotic stress-related pathways, also showed greater activation at 2 DAI (Figure 10).

Validation of *in silico* data via RT-qPCR

Validation of *in silico*-derived differential gene expression data for 'Calcutta 4' and equivalent genes in 'Prata-anã' was conducted by RT-qPCR, with expression compared in inoculated samples relative to non-inoculated controls (Figure 11). A total of 15 genes, selected from up-regulated DEGs in 'Calcutta 4' RNAseq data, were analyzed by RT-qPCR, with all displaying relative expression trends similar to those obtained by RNAseq (Figure 11). Thaumatin-like protein 1 (*Macma4_05_g17130*) exhibited a significant relative expression at 1, 2, and 4 DAI in 'Calcutta 4' in comparison with the non-inoculated control. Overexpression of genes Leucine-rich repeat receptor-like serine/threonine/tyrosine-protein kinase SOBIR1 (*Macma4_02_g09450*), Lipoxygenase 3-2C Chloroplastic (*Macma4_03_g08040*), putative Disease resistance protein RPS5 (*Macma4_04_g37480*), Peroxidase 4 (*Macma4_06_g16370*), NDR1/HIN1-like protein 10 (*Macma4_09_g31980*), Nematode resistance protein-like HSPRO2 (*Macma4_10_g08320*), Stilbene synthase 1 (*Macma4_10_g24620*), and Protein SAR DEFICIENT 1 (*Macma4_08_g16850*) was significant at 1 and 2 DAI in 'Calcutta 4'. For the genes Salicylic acid-binding protein 2 (*Macma4_04_g03250*), Premnaspirodien oxygenase (*Macma4_07_g21750*), and Pathogenesis-related protein 1 (*Macma4_03_g08420*), significant overexpression occurred only at 1 DAI, although positive trends were also observed in other treatments, aligning with RNAseq data. In contrast to data for Calcutta 4, where both RNAseq and RT-qPCR confirmed upregulation of gene expression at 1, 2 and 4 DAI, Stilbene synthase 1 (*Macma4_10_g24620*) and Premnaspirodien oxygenase (*Macma4_07_g21750*), which are involved in phytoalexin biosynthesis, showed no relative expression data by RT-qPCR in any treatments in 'Prata-anã'. Other genes,

including Acid endochitinase (*Macma4_01_g21300*), Leucine-rich repeat receptor-like serine/threonine/tyrosine-protein kinase SOBIR1 (*Macma4_02_g09450*), Lipoxygenase 3-2C Chloroplastic (*Macma4_03_g08040*), NDR1/HIN1-like protein 10 (*Macma4_09_g31980*), Nematode resistance protein-like HSPRO2 (*Macma4_10_g08320*), Pathogenesis-related protein 1 (*Macma4_03_g08420*), Peroxidase 4 (*Macma4_06_g16370*), Protein SAR DEFICIENT 1 (*Macma4_08_g16850*), putative Disease resistance protein RPS5 (*Macma4_04_g37480*), putative L-type lectin-domain containing receptor kinase IX.1 (*Macma4_06_g30880*), Salicylic acid-binding protein 2 (*Macma4_04_g03250*), and Thaumatin-like protein 1 (*Macma4_05_g17130*), also showed higher relative expression in 'Calcutta 4' than in 'Prata-anã'.

These results indicate a trend towards increased expression of genes associated with resistance to *Foc* in 'Calcutta 4', especially in the early stages of the interaction at 2 DAI, contrasting to an absence of upregulation of expression in the susceptible genotype 'Prata-anã'. Figure 11 also highlights expression trends observed through RT-qPCR analysis supporting those observed by *in silico* RNAseq analysis.

DISCUSSION

Bananas are among the world's most important fruits, with approximately 80% of production consumed domestically and 20% destined for global export markets. With regard to the latter, 90% of production occurs in Central America, South America, and the Philippines (FAO 2024b). Further expansion of banana cultivation is limited as a result of certain abiotic and biotic stresses, with the greatest threat to production today imposed by *Foc*. Whilst *Foc* Race 1 devastated global production of the 'Gros Michel' variety in the middle of the 20th Century, the emergence of Race 4 is now threatening the 'Gros Michel' replacement cultivars within the 'Cavendish' subgroup, with around 80% of the cultivars grown globally considered susceptible to *Foc* TR4 (Bermúdez-Caraballoso et al. 2020). Losses are considerable, particularly across much of Asia, Australia, Mozambique, Oman, Turkey, Venezuela, Colombia, and Peru, where TR4 is present. Entry of TR4 into Brazil also now appears to be only a matter of time, as is the case for other banana-producing nations in the Americas (Munhoz et al. 2024; Ploetz et al. 2015). In addition to TR4, STR4 is also widely disseminated and now present in China, Australia, Taiwan, South Africa, Morocco, and Brazil (Munhoz et al. 2024).

Given the limited genetic diversity in commercial *Musa* cultivars and the constraints to crop improvement in conventional *Musa* breeding programs, research efforts have focused on candidate resistance gene discovery via transcriptome analysis of the interaction between resistant genotypes and diverse pathogens (Anuradha et al. 2024; Castañeda et al. 2017; Lantican et al. 2023; Pinheiro et al. 2022; Tripathi et al. 2019).

With regard to *Foc*, given the impact of TR4, a greater number of studies have so far focused on this pathogen race. In an analysis of the compatible response in Cavendish subgroup 'Brazilian' to *Foc* TR4, Wang et al. (2012) demonstrated that pathogen recognition and initial activation of defence signalling pathways (such as SA, JA, and ET) were strongly induced at 1 and 2 days post-inoculation, with expression of pathogenesis-related (PR) proteins and lignification enzymes at 4 DAI. Additionally, peroxidase overexpression was detected at the early stages of the infection, suggesting that ROS activation occurs after TR4 infection. These findings indicated

that while susceptible cultivars may activate certain defence mechanisms against *Foc*, these are insufficient to confer resistance to the pathogen. A comparative study in the Cavendish cultivar ‘Baxi’, which is resistant to R1 and susceptible to TR4, during early stage responses to infection by each race, revealed similar expression patterns at 2DAI to both races, with the exception of suppression of the *allene oxide synthase* gene following challenge by TR4, suggesting that *Foc* TR4 infection may reduce JA synthesis (Li et al. 2013b). The authors suggest that cultivar susceptibility patterns may be influenced by longer-term infection responses than those investigated in their study (Li et al. 2013). In a similar study with the Cavendish cultivar ‘Brazilian’ (resistant to R1 and susceptible to TR4), Dong et al. (2020) showed that *Foc* Race 1 infection induced significant changes in RLK receptor recognition, TFs, metabolic pathway activation, lignin and flavonoid synthesis, and secondary metabolite accumulation, such as glucosinolates, alkaloids, and terpenoids. There was also unique activation of PR-1-like and PR-4 chitinase genes. In contrast, gene expression and pathway changes after *Foc* TR4 infection were either unaffected or only weakly affected, highlighting contrasting early-stage resistance responses (48 HAI) to both *Foc* races. Li et al. (2023) further demonstrated suppression of plant hormone pathways such as SA during early TR4 infection in the cultivar ‘Brazilian’ when compared to Race 1 infection. Notably, the RIN4 gene, which is part of the NLR family that confers resistance when interacting with the membrane receptor RPM1, was overexpressed only after *Foc* Race 1 infection, potentially explaining the susceptibility of ‘Brazilian’ to *Foc* TR4.

Several studies have also focused on differential gene expression in wild genotypes or resistant mutants for identification of genes involved in the resistance response to *Foc* TR4. Nine pathways, comprising PAMP perception via PRRs, ETI, ion flux, TFs, oxidative burst, PRs, programmed cell death, plant hormones, and cell wall modification, were identified as immunity-related in resistant cultivar ‘Nongke No1’ (Li et al. 2012). Enhanced signalling in SA and JA metabolism pathways, along with glycolysis and glyoxylate cycle-related DEGs, were also observed in ‘Nongke No1’ when compared to expression in susceptible ‘Baxi’ (Li et al. 2019b).

In ‘Yueyoukang 1’ (Cavendish), genes associated with CeBiP, BAK1, NLR, PR proteins, TFs, glucanases, and endochitinases were expressed more strongly compared to ‘Brazilian’ following TR4 infection (Bai et al. 2013). Activation of genes such as *MaWRKY50* and other TFs, PR-3, and PR-4, along with genes involved in cell wall biosynthesis or degradation, including pectinesterases, β -glucosidases, xyloglucan endotransglucosylase/hydrolase, and endoglucanase, were also identified as possible resistance-related genes in ‘Yueyoukang 1’ against TR4 (Niu et al. 2018). In ‘Guijiao 9’, overexpression of RPM1 (disease resistance proteins), PTI6 (pathogenesis-related transcriptional activator gene), CeBiP (chitin elicitor-binding protein), CDPK (calcium-dependent protein kinase), RCHY1 (RING finger and CHY zinc finger domain-containing protein 1), and UBE2H (ubiquitin-conjugating enzyme E2 H) were considered resistance candidate genes to *Foc* TR4 at 2, 4, and 6 DPI in comparison with the susceptible ‘Williams’ cultivar (Cavendish) (Sun et al. 2019). Zhang et al. (2019) also observed a constitutive defence response in ‘Pahang’ (*Musa acuminata* subsp. *malaccensis* AA), involving 7 WRKY TFs engaged in biotic stress resistance, along with 12 TIFY and 4 putative disease resistance genes. After TR4 inoculation, key genes such as PR1, CHIT, ASP, GLIP, POD, PPO, cytochrome P450, and WRKY TFs were identified as encoding proteins that confer resistance in ‘Pahang’ to the pathogen. In a study involving the *Foc*-resistant ‘Calcutta 4’ (AA) in contrast with the susceptible ‘Kadali’ (AA) cultivar, 18 defence-related genes (CLP-1, Defensin,

Lectin, CeBiP, RPS2, RPM1, POX, CERK-6, GCL, SBP18, MAPKKK 11, RPK2, PPO, PR-1A, PR-1, PAL, PR-TLP, and Oxistress-3), involved in signalling, ROS, lignification, and PR pathways, were identified as playing roles in the defense response in 'Calcutta 4's against *Foc* Race 1 (Kotari et al. 2018a). In the case of a study on 'Grand Naine' 72 hours post-infection by *Foc* Race 1 and biological control agents, in contrast to the susceptible 'Mchare' genotype, numerous defense pathways displayed modulation, with activation of MYB, bHLH, and NAC TFs, PR-1, secondary metabolism pathways, plant hormone signal transduction, starch and sucrose metabolism, cysteine and methionine metabolism, flavonoid biosynthesis, seleno compound metabolism, phenylpropanoid biosynthesis, mRNA surveillance pathway, mannose-type O-glycan biosynthesis, amino acid and nucleotide sugar metabolism, and cyanoamino acid metabolism (Kaushal et al. 2021).

Our study provides a significant perspective on the wild accession 'Calcutta 4', which is resistant to all *Foc* races. With *Focus* on the interaction with *Foc* STR4, which is genetically the closest race to TR4 and widespread in Brazil, Australia, the Canary Islands, China, South Africa, and Taiwan (Munhoz et al. 2024), histological analyses showed a rapid defence response following *Foc* STR4 challenge, with callose formation and phenolic compound deposition at 1 and 2 DAI. When comparing *Foc* STR4 colonization in resistant 'Calcutta 4' and susceptible 'Prata-anã', the presence of chlamydospores and hyphae on the root exterior was similar in both genotypes up to 4 DAI. However, at 8 and 15 DAI, hyphae were more prevalent in 'Prata-anã', both externally and internally within root cells, with only chlamydospores observed in 'Calcutta 4'. Previously, Li et al. (2019b) similarly demonstrated a greater abundance of *Foc* TR4 hyphae and spores in susceptible 'Baxi' in comparison to resistant 'Nongke No.1' at 27 and 51 hpi.

Transcriptome data aligned with these findings, with a greater differential expression of genes at 2 DAI. Among the total of 1,416 DEGs identified in RNAseq analysis, 270 and 872 were overexpressed at 1 and 2 DAI, respectively, with only 81 at 4 DAI. Of these, 752 DEGs were regulated exclusively at 2 DAI, suggesting a swift response of 'Calcutta 4' to the onset of *Foc* STR4 infection, as has been shown in other pathosystems. Seventy-five globally upregulated DEGs were identified as possible agents in 'Calcutta 4's defence response to *Foc* STR4, associated with GO biological processes categorized under response to stimulus.

PTI responses

Resistance responses to PAMPs involve recognition by pattern recognition receptors (PRRs), including RLK and RLP receptors, triggering signaling pathways such as calcium ion (Ca^{2+}) influx, ROS production, and MAPK activation (Ding et al. 2022; Ngou et al. 2022b). Here, numerous such membrane receptors were modulated during the incompatible response in Calcutta 4 (Supplementary Table 3). These included receptors encoded by the genes Protein LYK5 (*Macma4_04_g09660*), which is a primary chitin receptor that binds to the putative Chitin elicitor receptor kinase 1 - CERK1 (*Macma4_10_g11450*) receptor, forming a strong chitin recognition complex that induces plant immunity (Cao et al. 2014), and involved in non-host resistance signaling (Huaping et al. 2017), together with a Leucine-rich repeat receptor-like serine/threonine/tyrosine-protein kinase SOBIR1 (*Macma4_02_g09450*) that activates cytoplasmic signaling pathways leading to rapid cellular responses that include positive regulation of cell death, often as an oxidative burst and activation of mitogen-activated protein kinase (MAPK) (Gao et al. 2009; Liebrand et al. 2013; Wei et al. 2022), and putative LysM domain receptor-like kinase 4 (*Macma4_04_g01380*),

which is involved in recognition of fungal chitin, PRR activation and PTI. Previously, Kotari et al. 2018 also observed upregulation of genes related to PAMP recognition and defence signaling in the resistance response of Calcutta 4 to *Foc* R1, including CeBiP, CERK-6, RPK-2, LECTIN, and MAPKKK-11, were overexpressed following *Foc* race 1 infection. Following PAMP recognition, PRR activation was noted for Thaumatin-like protein 1 (*Macma4_05_g17130*) at 1 and 2 DAI. Mahdavi et al. (2012) previously demonstrated a lower incidence of fusarium wilt caused by TR4 in transgenic 'Pisang Nangka' bananas (AAB) expressing a rice Thaumatin-Like Protein gene. Upregulation of a PR-protein 1C (*Macma4_02_g16620*), as well as a TL1, which belongs to the PR5 class, was also significant in 'Calcutta 4' during the early stages of the interaction with *Foc* STR4, in contrast to 'Prata-anã' (Dixon et al. 1991; Hakim et al. 2018; Han et al. 2023). The nematode resistance protein-like HSPRO2 (*Macma4_05_g32600*, *Macma4_10_g08320*, *Macma4_10_g08320*, *Macma4_11_g23440*) (Supplementary Table 3) was upregulated at 1 and 2 DAI. HSPRO2 is a protein containing an imperfect LRR domain without the canonical nucleotide-binding site, typical of NLR R proteins. This gene has been described as a component of basal resistance in plants under pathogen attack, including bacteria (Murray et al. 2007; Nemchinov et al. 2017).

Chitinases

Plant chitinases play a crucial role in resistance to biotic stress. In this study, seven distinct chitinases were overexpressed in 'Calcutta 4' following interaction with *Foc* STR4: Hevamine-A (*Macma4_08_g34260*), Acidic endochitinase (*Macma4_01_g21300*), Chitinase (*Macma4_03_g29600*), Basic endochitinase C (*Macma4_03_g29630*), Chitinase 6 (*Macma4_05_g18850* and *Macma4_05_g18860*), Endochitinase EP3 (*Macma4_06_g23390*), and Chitin-inducible gibberellin-responsive protein 1 (*Macma4_09_g12390*). Given their ability to hydrolyze chitin, a major component of fungal cell walls, chitinases can serve as a primary defence response against different fungal pathogens. Previously, chitinases have been described in the defence response of 'Cavendish' to *Foc* R1 (Dong et al. 2020), in conferred resistance to black leaf streak disease in 'Gros Michel' transformants harboring the rice chitinase (RCC2) (Kovács et al. 2013), and in engineered resistance to *Foc* race 4 in Furenzhi (AA), following introgression of a *Trichoderma harzianum* endochitinase gene (*chit42*) (Hu et al. 2013).

Reactive oxygen species (ROS) and Systemic acquired resistance (SAR)

ROS and SAR activation follows recognition of pathogen Avr proteins by R proteins. ROS production can act directly by thickening cell walls to prevent pathogen colonisation (Huang et al. 2019), and also serves as a conserved PRR signalling mechanism for chitin perception, as well as in MAPK signalling (Kawasaki et al. 2017). Key genes associated with ROS and SAR activation were predominantly identified in Calcutta 4 at 1 and 2 DAI (Supplementary Table 3), such as Protein SAR DEFICIENT 1 (SARD1) (*Macma4_08_g16850*) and numerous peroxidases. In *Arabidopsis*, salicylic acid (SA) synthesis is promoted by transcription factors SARD1 and CBP60g (*Calmodulin-binding protein 60g*), which regulate ICS1 (*Isochorismate Synthase*) expression (Wang et al. 2011; Zhang et al. 2010). Besides ICS1, SARD1 and CBP60g modulate numerous other immunity-related genes, suggesting their broad role in plant defence (Sun et al. 2015). The presence of SARD1 (*Macma4_08_g16850*) in our study reinforcing its role as a key immune response regulator. Thus, systemic signalling

involving PTI and ETI activation is closely linked to ROS burst, calcium influx, and electrical signals (Ding et al. 2022).

Effector triggered immunity

ETI signaling is initiated by the direct or indirect recognition of race-specific pathogen effectors by NLR R proteins, with a cell death response / hypersensitivity response (HR) common following pathogen infection (Jones and Dangl 2006b). Previously, 52 partial NLR genes were sequenced in 'Calcutta 4' (Miller et al. 2008). In 'Pahang', the *RGA2* gene (resistance gene analogue 2) and four other putative disease resistance genes were highly expressed upon *Foc* TR4 attack (Zhang et al. 2019). Using the *RGA2* gene, Dale et al. (2017) also successfully developed the first transgenic *Foc* TR4-resistant 'Cavendish' cultivar. In this study, we identified an upregulation of an NDR1/HIN1-like protein 10 gene, involved in CC-NBS-LRR-type NLR activation (*Macma4_09_g31980*), along with the activation of several other NLR genes at 2 DAI, such as NB-ARC domain-containing proteins (*Macma4_01_g04350*, *Macma4_01_g04360*, *Macma4_01_g04380*) and RPS5 (*Macma4_04_g37480*).

Transcription factors

Transcription factors (TFs) play a crucial role in signaling and regulating various plant defence pathways under biotic stress conditions. Of the upregulated TFs in 'Calcutta 4' during interaction with STR4, most belonged to the TIFY, WRKY, MYB, and MYC families. Two critical WRKY genes were identified in the defense response: putative WRKY transcription factor WRKY28 (*Macma4_11_g08360*) and WRKY transcription factor WRKY51 (*Macma4_09_g24850*). WRKY51 participates in NPR1 signalling, required for SA-induced PR gene expression and resistance against pathogens (Ding et al. 2018). WRKY50 and WRKY51 proteins also mediate both SA- and low-dependent repression of JA signalling (Gao et al. 2011). TFs in the TIFY family are plant-specific and involved in various processes, including development and responses to both biotic and abiotic stresses. JAZ proteins within the TIFY family are essential in JA signalling pathways, acting as co-receptors and repressors of downstream TFs, and they interact with MYC2, which positively regulates JA-responsive gene expression. (Zhang et al. 2020). Notable members of the TIFY family were found in our study, such as putative protein TIFY 5A (*Macma4_02_g16390*, *Macma4_06_g34540*, *Macma4_09_g10730*) and Protein TIFY 10b (*Macma4_03_g01960*).

Plant hormones

The activation of plant hormone pathways plays a significant role in pathogen defence. The salicylic acid (SA) pathway is activated by the recognition of elicitors (Avr) by R genes, triggering various downstream signalling events that stimulate the induction of SA and the systemic acquired resistance (SAR) response against biotrophic pathogens. SA activation occurs through the expression of EDS1, PAD4, and GDG1, as well as the signalling from the MAPK cascade (Ding et al. 2022). When activated locally, SA stimulates hypersensitive response (HR) and pathogenesis-related (PR) gene expression, while the systemic response is characterised by SAR. In this study, we identified Salicylic acid-binding protein 2 (*Macma4_04_g03250*) and NDR1/HIN1-like protein 10 (*Macma4_09_g31980*) associated with the SA pathway, acting at the onset of infection by *Foc* STR4 in 'Calcutta 4.' Salicylic acid-binding protein 2, or SABP2, is an SA receptor required for immune responses in plants, involved in the induction of PR1 and the development of SAR (Kumar et al. 2006;

Kumar and Klessig 2003; Vlot et al. 2008). NDR1 is positively regulated for the accumulation of SA (Ding et al. 2022; Shapiro and Zhang 2001), with its overexpression capable of enhancing disease resistance in *Arabidopsis* (Lu et al. 2013).

Ethylene (ET) and jasmonic acid (JA) pathways are activated in response to necrotrophic pathogen attacks and mediate induced systemic resistance (ISR) (Ding et al. 2022; Li et al. 2019a). JA can induce the expression of PR proteins, peroxidases, alkaloids, volatile compounds, and the formation of defence structures. MYC2 transcription factors promote the activation of JA-responsive genes (Du et al. 2017). The JA-responsive transcription factor JAMYB increase the resistance of rice to viral diseases (Yang et al. 2020). Here, we observed both Transcription factor MYC2 (*Macma4_07_g04870*) and Transcription factor JAMYB (*Macma4_09_g16730*) positively regulated at 2 DAI in 'Calcutta 4' after *Foc* STR4 infection, confirming the role of the JA pathway in the resistance of this wild genotype to the pathogen. Several ethylene-responsive transcription factors were activated during the interaction of 'Calcutta 4' with *Foc* STR4 (Supplementary table 3). ET receptors activate the EIN2 protein, which positively regulates the ET pathway by signalling the nuclear protein EIN3 that binds to ERF1 to activate ET production (Berrocal-Lobo and Molina 2004). In 'Calcutta 4,' Protein ETHYLENE-INSENSITIVE 3-like 1a (EIL1) (*Macma4_08_g22960*) and Ethylene-responsive transcription factor 1B (ERF1B) (*Macma4_11_g21380*) were positively regulated following *Foc* STR4 attack, involved in ET signalling translation. Downstream defence responses arising from the activation of JA/ET pathways involve the activation of PR genes, where ERFs represent the final layer of regulation that can turn on or off the expression of JA/ET-responsive defence genes (Huang et al. 2016). ERFs are involved in immune responses in *Arabidopsis* to pathogens (Huang et al. 2016); however, further investigation into other ERFs, such as those identified in our study, is warranted to ascertain their roles in the resistance of 'Calcutta 4' to *Foc* STR4.

Secondary metabolism

The hormonal signalling pathways of salicylic acid (SA), ethylene (ET), and jasmonic acid (JA) regulate the distribution and production of secondary metabolites during plant stress (Yang et al. 2019). Phytoalexins and flavonoids produced by plant roots exhibit inhibitory or toxic effects on fungi and can directly influence spore germination, playing a crucial role in the induction of plant defence against phytopathogenic fungi (Agrios, 2005). The overexpression of Premnaspirodiene oxygenase, an enzyme encoded by Cytochrome P450 (CYP71), confers resistance in rice to *Xanthomonas oryzae* pv. *oryzae* (Niño et al. 2016). This enzyme catalyses regio- and stereospecific hydroxylations of various sesquiterpene substrates, resulting in solavetivone, a potent antifungal phytoalexin (Takahashi et al. 2007). Stilbenes, another group of phytoalexins within the flavonoid family, are catalysed by Stilbene synthase (STS) in the final step of the biosynthetic pathway (Jeandet et al. 2002). Stilbene genes have been cloned in several plant species such as peanut (*Arachis hypogaea*), Scots pine (*Pinus sylvestris*), eastern white pine (*Pinus strobus*), Japanese red pine (*Pinus densiflora*), grapevine (*Vitis vinifera*), and sorghum (*Sorghum bicolor*) (Qayyum et al. 2022). STS enzymes are responsive to external signals triggered by abiotic stresses and biotic signalling from fungal cells (Jeandet et al. 2002, 2010). The genes Premnaspirodiene oxygenase (*Macma4_07_g21750*) and Stilbene synthase 1 (*Macma4_10_g24620*) were positively regulated in 'Calcutta 4' during infection with *Foc* STR4 compared to controls, at the early stages of defence

at 1 and 2 DAI, and remained absent in 'Prata-anã', as confirmed by RT-qPCR analysis. These results demonstrate the importance of phytoalexins as defence agents in 'Calcutta 4' against *Foc* STR4.

Plants defend themselves against pathogens using a variety of chemical and physical mechanisms. Callose is a polymer deposited in plant cells as a result of signalling triggered following PRR recognition of PAMPs. Serving as a mechanism of cell wall strengthening, this polymer also serves as a matrix for antimicrobial compound deposition, contributing to plant defence against pathogen attack (Luna et al. 2011). Callose deposition in *Arabidopsis* is known to be induced by the regulation of the EXO70B1 or EXO70B2 genes, and activated by the bacterial flagellin PAMP peptide flg22 (Redditt et al. 2019). Callose deposition can also be influenced by signalling from abscisic acid (ABA) pathways in response to infection by fungi and oomycetes, as demonstrated by the ABA-dependent stimulation of callose deposition during *Botrytis cinerea* infection in *Arabidopsis* (You et al. 2010). We confirmed through comparative histological analyses in inoculated and non-inoculated Calcutta 4 and 'Prata-anã' that callose deposition occurred exclusively in 'Calcutta 4' at 1 and 2 DAI, following interaction with *Foc* STR4. We also observed the positive regulation of the gene Exocyst complex component EXO70B1 (*Macma4_10_g10790*). Notably, there was also overexpression of the gene Absciscic acid receptor PYL2 (*Macma4_06_g02820*) at 1 and 2 DAI, which is a regulatory component of the ABA receptor family (García-Andrade et al. 2020), potentially involved in the signaling pathway for callose deposition in 'Calcutta 4' in response to *Foc* STR4 attack.

CONCLUSIONS

In the present study, we identified numerous DEGs positively regulated and involved in the immediate defence response of 'Calcutta 4' to attack by *Foc* STR4, including PRRs, PR proteins, chitinases, NLR resistance genes, TFs, genes associated with ROS and SAR, phytoalexins, genes related to callose production, and extensive regulation of phytohormone pathways such as ET and JA (Supplementary table 3), showed in our 'Calcutta 4' and *Foc* STR4 interaction model (Figure 12). Through histological analyses, we observed defence responses to *Foc* STR4 of callose deposition and accumulation of phenolic compounds which are likely responsible for the rapid resistance response in this wild genotype. In contrast, 'Prata-anã' exhibited a limited response to the pathogen, with histopathology and RT-qPCR data revealing an absence of phenolic compounds and phytoalexins, suggesting that it lacks genetic machinery to activate resistance genes against *Foc* STR4.

Functional analysis of DEGs reinforces the conclusion that the wild diploid 'Calcutta 4' activates a robust and immediate defence response against *Foc* STR4, including genes that participate in functional pathways related to stimulus responses, plant-pathogen interactions, secondary metabolism, plant hormones, and biotic stress. These findings will assist in genetic modification via integration of defence genes from wild genotypes into commercial cultivars, such as candidate defence genes of 'Calcutta 4' against *Foc* STR4 described in supplementary table 3, particularly those in the 'Prata' group, which are widely consumed in South America. Given the close phylogenetic relationship between *Foc* STR4 and TR4, this strategy holds promise for developing banana varieties that are resistant to both race 4 pathogens through introgression of candidate genes, relevant for reducing the impact of Fusarium wilt epidemics globally.

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Author contributions

R.N.G.M. conceived and designed the study. E.C.C., A.P.S.R. and L.S.R. conducted the biotic assays and microscopy analysis. E.C.C. and L.S.B. conducted the RT-qPCR analysis. E.C.C., R.C.T. and P.G. conducted the bioinformatic analysis. E.C.C. and N.V.L.S. analysed the qPCR data. R.N.G.M., E.P.A., C.F.F. and E.C.C. provided intellectual input. E.C.C. and R.N.G.M. wrote and revised the manuscript. All authors have read and approved the final manuscript.

Data availability statement (mandatory)

All data generated and analysed during the study is included in the published article.

Additional Information

Competing Interests: The authors declare that they have no competing financial and/or non-financial interests in relation to the work described.

Figure legends

Figure 1. Histological analyses of callose deposition in root tissues in Calcutta 4 (CA) and Prata-anã (PA). **A-C.** Callose deposition in C4 at 1, 2, and 4 DAI, respectively; **D-F.** Callose deposition in PA at 1, 2, and 4 DAI, respectively; **G-H.** Non-inoculated controls for C4 and PA. Arrows highlight fluorescence, indicative of callose deposition.

Figure 2. Histological analyses of phenolic compound formation in root tissues in 'Calcutta 4' (C4) and 'Prata-anã' (PA). **A-C.** Callose deposition in C4 at 1, 2, and 4 days after inoculation (DAI), respectively; **D-F.** Callose deposition in PA at 1, 2, and 4 DAI, respectively; **G-H.** Non-inoculated controls for C4 and PA. Arrows indicate the presence of phenolic compounds.

Figure 3. Root clearing of 'Calcutta 4' (**A-E**) and 'Prata-anã' (**F-J**) at 1, 2, 4, 8, and 15 days after inoculation (DAI), respectively. Arrows indicate chlamydo spores and hyphae. **K.** non-inoculated C4 control; **L.** non-inoculated PA control (Bar = 100 μ m)

Figure 4. Scanning microscopy analysis of roots from Calcutta 4 (**A-C**) and Prata-anã (**D-F**) inoculated at 1, 2, and 4 days after inoculation (DAI), respectively. **G.** non-inoculated C4 control; **H.** non-inoculated PA control.

Figure 5. Fusarium Wilt symptoms in contrasting cultivars 'Calcutta 4' resistant and 'Prata-anã' susceptible to *Fusarium oxysporum* f. sp. *cubense* race STR4 at 90 and 120 DAI (days after inoculation). **A-D.** external symptoms in 'Calcutta 4': **A.** 90 DAI, **B.** 120 DAI, **C.** control at 90 DAI, **D.** control at 120 DAI, **E-H.** internal symptoms in 'Calcutta 4' rhizome, **E.** 90 DAI, **F.** 120 DAI, **G.** control at 90 DAI, **H.** control at 120 DAI. **I-L.** external symptoms in 'Prata-anã'. **I.** 90 DAI, **J.** 120 DAI, **K.** control at 90 DAI, **L.** control at 120 DAI, **M-P.** internal symptoms in 'Prata-anã' rhizome, **M.** 90 DAI, **N.** 120 DAI, **O.** control at 90 DAI, **P.** control at 120 DAI.

Figure 6. Venn diagram summarizing the numbers of differentially expressed genes (DEGs) observed in RNAseq data in *Musa acuminata* subsp. *burmannica* accession 'Calcutta 4' at 1, 2, and 4 days after inoculation (DAI) with *Fusarium oxysporum* f. sp. *cubense* STR4. Red arrows indicate positively regulated genes, while blue arrows indicate negatively regulated genes. DEGs were considered significant when relative gene expression between an inoculated and corresponding control treatments showed at least a ≥ 2 -fold (FC) and considering a false discovery rate (FDR)-adjusted P-value (padj) of $p \leq 0.05$. Overlapping regions indicate DEGs common to different time points.

Figure 7. Heatmap of differentially expressed genes in *Musa acuminata* subsp. *burmannica* accession 'Calcutta 4' at 1, 2, and 4 days after inoculation (DAI) with *Fusarium oxysporum* f. sp. *cubense* subtropical race 4. The red color indicates positive regulation while the blue color indicates a negative regulation of DEGs in relation to non-inoculated control samples.

Figure 8. Gene ontology analysis of differentially expressed genes (DEGs) in *Musa acuminata* subsp. *burmannica* accession 'Calcutta 4', both positively and negatively regulated at 1, 2, and 4 days after inoculation (DAI).

Figure 9. Classification of genes by eukaryotic orthologous groups (KOG) of the differentially expressed genes (DEGs) that are positively regulated in *Musa acuminata* subsp. *burmannica* accession 'Calcutta 4' after infection with *Fusarium oxysporum* f. sp. *cubense* STR4, at 1, 2, and 4 days after inoculation.

Figure 10. Mapman-based functional prediction mapping of differentially expressed genes (DEGs) in *Musa acuminata* subsp. *burmannica* accession 'Calcutta 4' after infection with *Fusarium oxysporum* f. sp. *cubense* STR4, at 1, 2, and 4 days after inoculation.

Figure 11. RT-qPCR analysis of relative expression of 15 differentially expressed upregulated genes identified following RNAseq analysis of the interaction between *Musa acuminata* subsp. *burmannica* accession 'Calcutta 4' and *Fusarium oxysporum* f. sp. *cubense* STR4, at 1, 2, and 4 days after inoculation. RT-qPCR data was analysed using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). The expression of the control treatments is equal to 1 and marked by a red line. Both values for RT-qPCR and RNAseq were positive.

Figure 12. A schematic model of the innate immune responses observed in *Musa acuminata* subsp. *burmannica* accession 'Calcutta 4' following infection by *Fusarium oxysporum* f. sp. *cubense* subtropical race 4 (STR4). Upregulated candidate resistance genes included PRRs (transmembrane pattern recognition receptors) for recognition of fungal PAMPs (Pathogen associated molecular pattern) and NLR proteins (*R* genes) for recognition of pathogen effectors, triggering ETI (Effector-triggered immunity) and induction of a hypersensitive cell death (HR) response. Downstream defense response-associated genes were also observed amongst upregulated genes, including those encoding Pathogenesis-related (PRs) proteins, phytohormone pathway-associated proteins, chitinases, proteins involved in systemic acquired resistance (SAR), reactive oxygen species (ROS), and secondary metabolites such as phytoalexins.

Supplementary material

Supplementary figure 1. Orthofinder-based phylogenetic analysis of orthogroups among six different *Fusarium* species and twenty *formae speciales* of *Fusarium oxysporum*, including *F. oxysporum* f. sp. *cubense* isolate 218A from the cultivar 'Nanica' (Cavendish subgroup, AAA), isolated in the state of São Paulo. Numbers in branches indicate bootstrap values.

Supplementary figure 2. Kegg mapping of the MAPK (mitogen-activated protein kinase) signaling pathway based on differentially expressed genes (DEGs) observed in *Musa acuminata* subsp. *burmannica* accession 'Calcutta 4' after infection with *Fusarium oxysporum* f. sp. *cubense* STR4, at 1, 2, and 4 days after inoculation. Different shades of red indicate positively regulated DEGs (UP), while shades of blue indicate negatively regulated DEGs in the three treatments.

Supplementary figure 3. Kegg mapping of the signaling translation pathway based on differentially expressed genes (DEGs) observed in *Musa acuminata* subsp. *burmannica* accession 'Calcutta 4' after infection with *Fusarium oxysporum* f. sp. *cubense* STR4, at 1, 2, and 4 days after inoculation. Different shades of red indicate

positively regulated DEGs (UP), while shades of blue indicate negatively regulated DEGs in the three treatments.

Supplementary figure 4. Kegg mapping of the plant-pathogen interaction pathway highlighting the presence of differentially expressed genes (DEGs) observed in *Musa acuminata* subsp. *burmannica* accession 'Calcutta 4' after infection with *Fusarium oxysporum* f. sp. *cubense* STR4, at 1, 2, and 4 days after inoculation. Different shades of red indicate positively regulated DEGs (UP), while shades of blue indicate negatively regulated DEGs in the three treatments.

Supplementary table 1. Differentially expressed genes identified following RNAseq analysis of the interaction between *Musa acuminata* subsp. *burmannica* accession 'Calcutta 4' and *Fusarium oxysporum* f. sp. *cubense* STR4, at 1, 2, and 4 days after inoculation.

Supplementary table 2. Statistical analysis of RT-qPCR data. The table presents the mean $\Delta\Delta Ct$ values per treatment, the corresponding p-values from ANOVA, the standard error (SE) associated with the means, and the degrees of freedom (DF) for each gene analyzed for Calcutta 4 (C4) and Prata-anã (PA) at 1, 2 and 4 days after inoculation (DAI) with *Fusarium oxysporum* f. sp. *cubense* STR4. The $\Delta\Delta Ct$ values were calculated relative to the control and normalized using appropriate reference genes.

Supplementary table 3. Differentially expressed genes (DEGs) selected from *Musa acuminata* subsp. *burmannica* accession 'Calcutta 4' during infection with *Fusarium oxysporum* f. sp. *cubense* STR4, categorized by their functions in defence pathways against biotic stresses. Each gene group is described according to the role in the plant immune response to pathogens.

Table 1. Differentially expressed genes (DEGs) identified from the *in silico* RNAseq analysis of ‘Calcutta 4’ following infection with *Foc STR4* at 1, 2, and 4 DAI, selected for validation via RT-qPCR.

Gene	Product	Primer F	Primer R
Macma4_01_g21300	Acidic endochitinase	GGACTAGATACACGACAGA	AGACTTCACCGGCATAGA
Macma4_02_g09450	Leucine-rich repeat receptor-like serine/threonine/tyrosine-protein kinase SOBIR1	TACGGATCGCCATCTTCT	GGCTTTGGCTGTTAGTGT
Macma4_03_g08040	Lipoxygenase 3-2C chloroplastic	CCTCCTGTGAGTAAGCTAGA	GCAGAGACATGCCATTAAGA
Macma4_03_g29630	Basic endochitinase C	GGACTGCTACAACCAGAAAC	CGGAGTCGCTATCTCAGTAA
Macma4_04_g03250	Salicylic acid-binding protein 2	TGTCATCGAGACCTCCTTT	ACACATCTGCGGCATTAC
Macma4_04_g37480	putative Disease resistance protein RPS5	CACTATGGGATCTCGGAATTG	GACGGCTTGGGAGTATATTG
Macma4_06_g16370	Peroxidase 4	GACACTACTGTCCCTTGATTAC	GTGGCAGTATCATCCAGAAG
Macma4_06_g30880	putative L-type lectin-domain containing receptor kinase IX.1	CGGGTTTGCCTTCTTCTT	CGTGGTGTCTGTTGGATAAA
Macma4_07_g21750	Prenaspirodienase oxygenase	GAACAAAGGACGGCAAGTA	CAGGAAGTAGCACTCACAAG
Macma4_09_g31980	NDR1/HIN1-like protein 10	GTGTGATGTGGATCTCTTCTG	GAGACAGTCTGCTGCTAAAC
Macma4_10_g08320	Nematode resistance protein-like HSPRO2	AATCGAGCGGAGGATACA	TCTATCAGCATGTGGAGGT
Macma4_10_g17470	Cysteine-rich repeat secretory protein 55	GGTGCGCTACGAGATTTAT	AACAAGATGGCCTATGGATG
Macma4_10_g24620	Stilbene synthase 1	GATCGAGTCAGCAAGCATAG	AAGCGAGGAAACAGAAAGG
Macma4_08_g16850	Protein SAR DEFICIENT 1	CTAACGGAGAGGTTCTTTATC	GGTCCACTCATTTCCATCTC
Macma4_03_g08420	Pathogenesis-related protein 1	GGGTACGTGAAGGAAAGATTGG	TCGGCTCGAACTTGAAATGG
Macma4_05_g17130	Thaumatococcus-like protein 1	GATGCGACGCTGATGAAA	AGACCGGCCATAAGATACA

Table 2. Statistics following mRNA sequencing and initial analysis of sequence reads from the interaction between *Musa acuminata* subsp. *burmannica* accession 'Calcutta 4' and *Fusarium oxysporum* f. sp. *cubense* STR4.

Organism	Treatment	Reads sequenced	Reads Trimmomatic	Reads Mapped (%)
<i>M. acuminata</i> subsp. <i>burmannica</i> accession 'Calcutta 4'	1DAI	42.533.774	41.569.176	85,24%
	1DAI	38.180.151	37.335.221	85,10%
	1DAI	31.101.530	30.430.312	85,40%
	2DAI	34.312.404	33.473.876	90,73%
	2DAI	35.681.837	34.866.391	89,86%
	2DAI	40.301.477	39.464.423	77,82%
	4DAI	46.284.431	45.209.609	90,66%
	4DAI	33.534.412	32.867.273	91,13%
	4DAI	31.879.599	31.190.939	90,29%
	Control 1DAI	34.727.260	33.867.962	91,24%
	Control 2DAI	35.592.084	34.707.254	84,81%
	Control 4DAI	43.715.012	42.757.687	88,40%

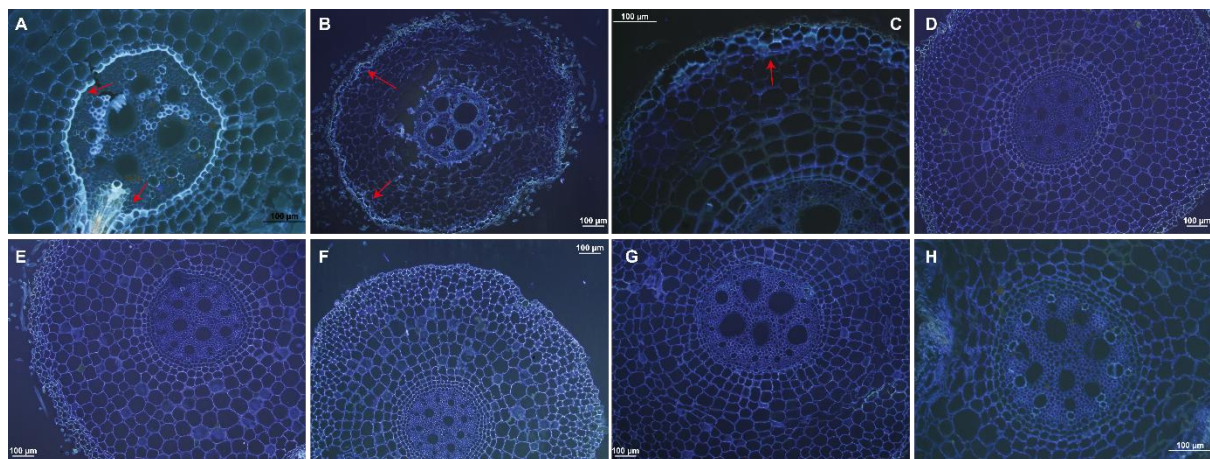


Figure 1. Histological analyses of callose deposition in root tissues in Calcutta 4 (CA) and Prata-anã (PA). **A-C.** Callose deposition in C4 at 1, 2, and 4 DAI, respectively; **D-F.** Callose deposition in PA at 1, 2, and 4 DAI, respectively; **G-H.** Non-inoculated controls for C4 and PA. Arrows highlight fluorescence, indicative of callose deposition.

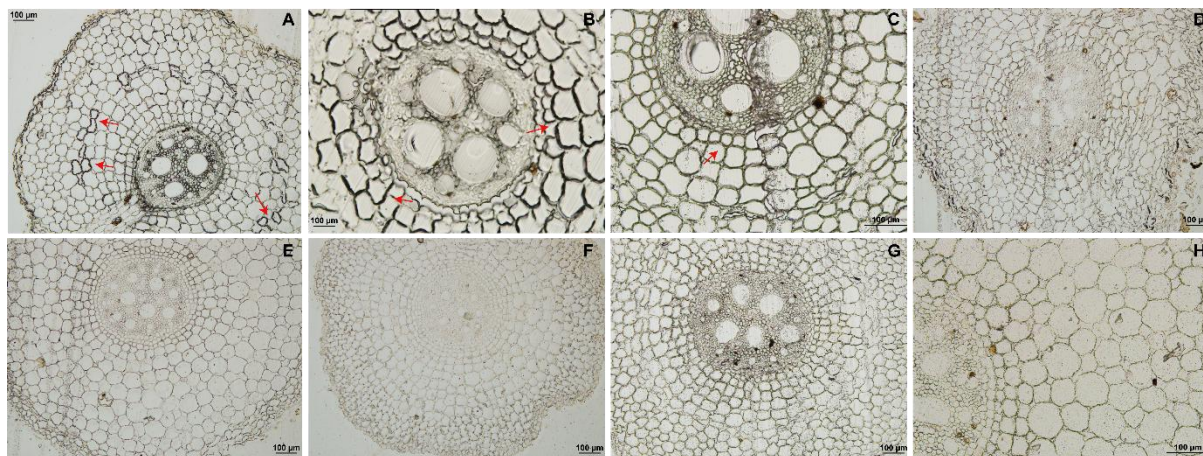


Figure 2. Histological analyses of phenolic compound formation in root tissues in 'Calcutta 4' (C4) and 'Prata-anã' (PA). **A-C.** Callose deposition in C4 at 1, 2, and 4 days after inoculation (DAI), respectively; **D-F.** Callose deposition in PA at 1, 2, and 4 DAI, respectively; **G-H.** Non-inoculated controls for C4 and PA. Arrows indicate the presence of phenolic compounds.

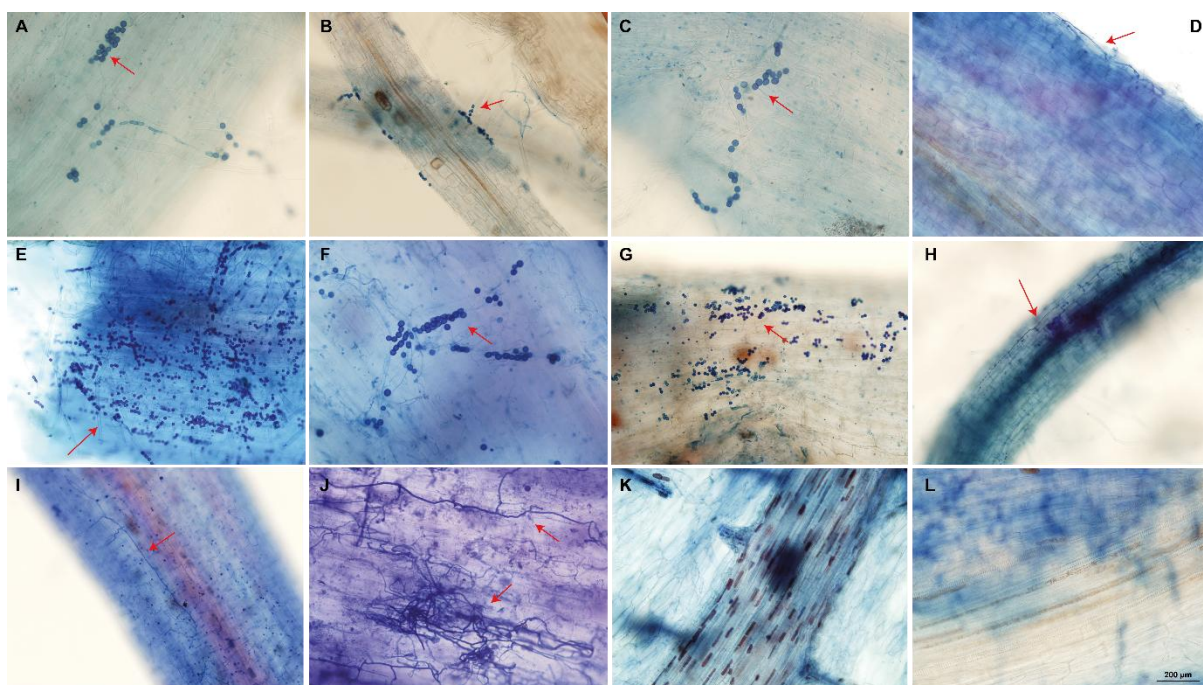


Figure 3. Root clearing of 'Calcutta 4' (**A-E**) and 'Prata-anã' (**F-J**) at 1, 2, 4, 8, and 15 days after inoculation (DAI), respectively. Arrows indicate chlamydospores and hyphae. **K.** non-inoculated C4 control; **L.** non-inoculated PA control (Bar = 100 µm)

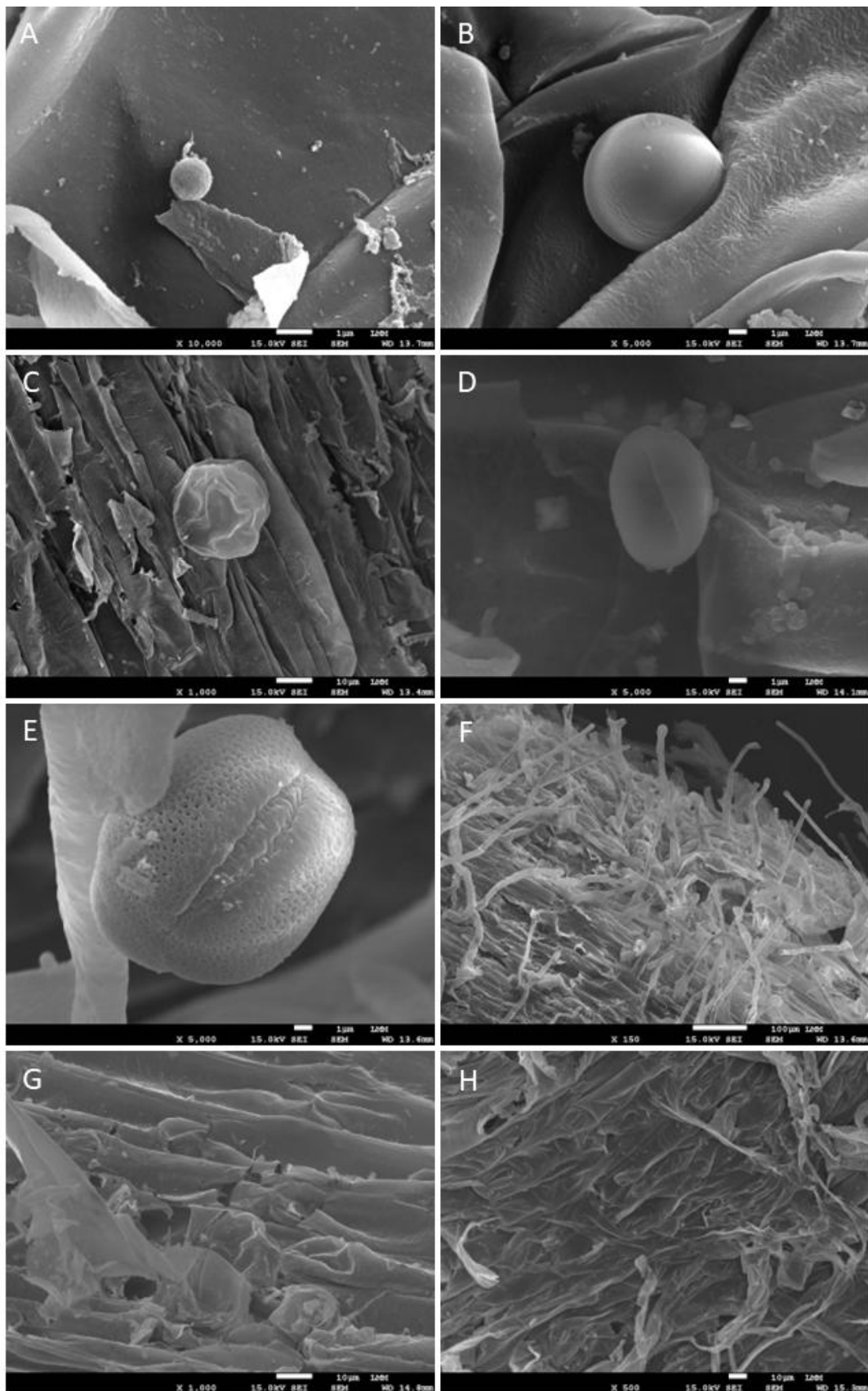


Figure 4. Scanning microscopy analysis of root tissues from Calcutta 4 (**A-C**) and Prata-anã (**D-F**) at 1, 2, and 4 days after inoculation (DAI), respectively. **G.** non-inoculated C4 control; **H.** non-inoculated PA control.



Figure 5. Fusarium Wilt symptoms in contrasting cultivars 'Calcutta 4' resistant and 'Prata-anã' susceptible to *Fusarium oxysporum* f. sp. *cubense* race STR4 at 90 and 120 DAI (days after inoculation). **A-D.** external symptoms in 'Calcutta 4': **A.** 90 DAI, **B.** 120 DAI, **C.** control at 90 DAI, **D.** control at 120 DAI, **E.H.** internal symptoms in 'Calcutta 4' rhizome, **E.** 90 DAI, **F.** 120 DAI, **G.** control at 90 DAI, **H.** control at 120 DAI. **I-L.** external symptoms in 'Prata-anã'. **I.** 90 DAI, **J.** 120 DAI, **K.** control at 90 DAI, **L.** control at 120 DAI, **M-P.** internal symptoms in 'Prata-anã' rhizome, **M.** 90 DAI, **N.** 120 DAI, **O.** control at 90 DAI, **P.** control at 120 DAI.

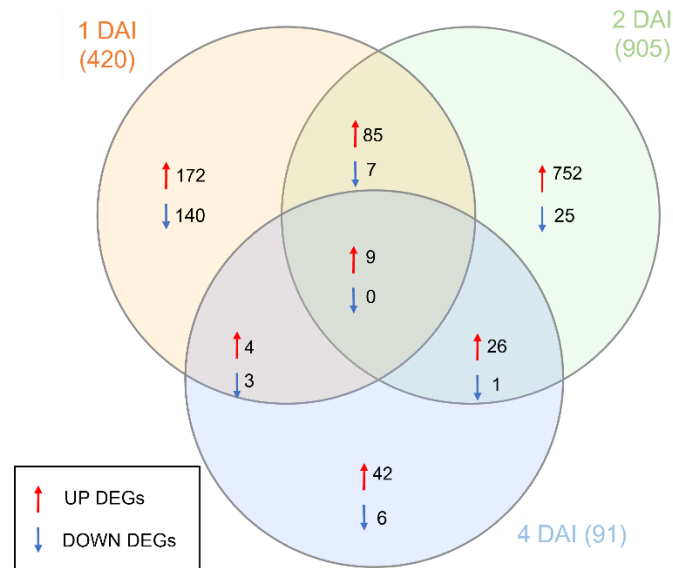


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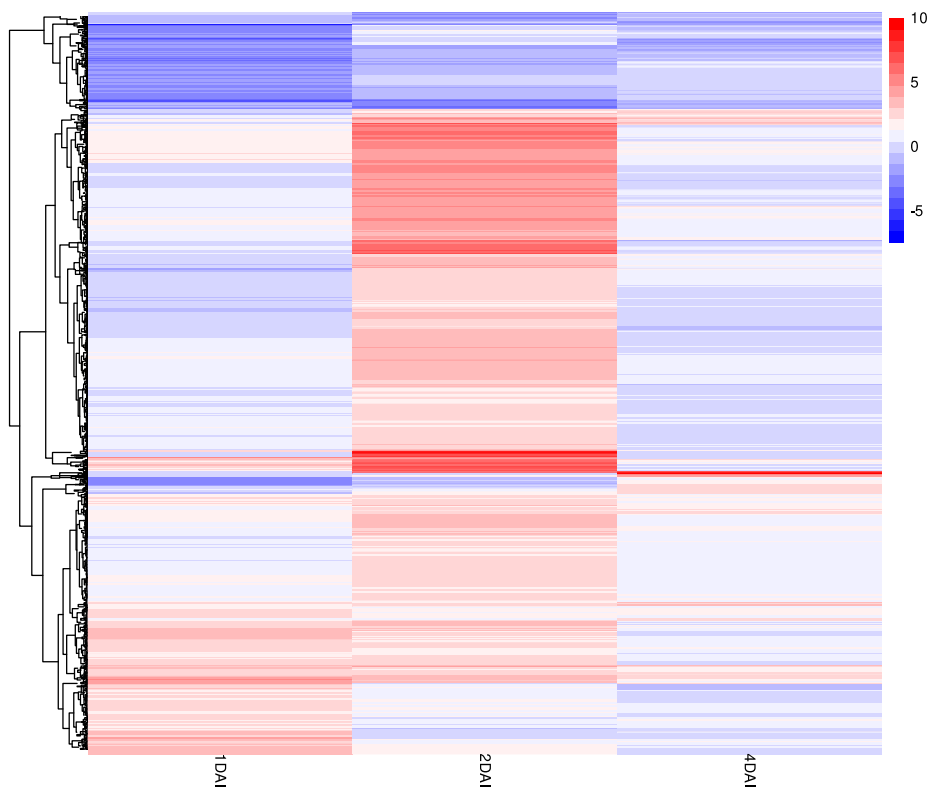


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regulation while the blue color indicates a negative regulation of DEGs in relation to non-inoculated control samples.

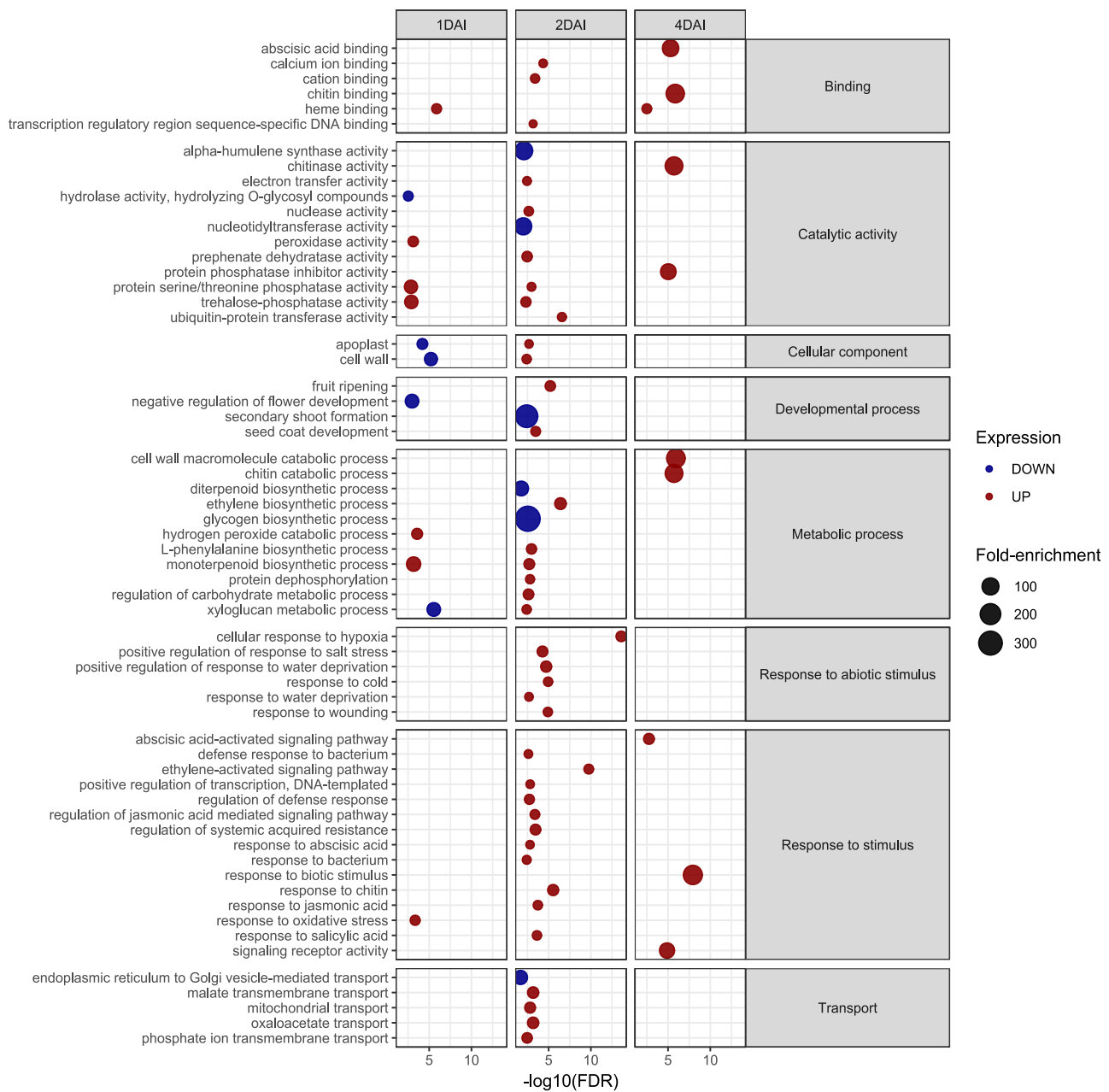


Figure 8. Gene ontology analysis of differentially expressed genes (DEGs) in *Musa acuminata* subsp. *burmannica* accession 'Calcutta 4', both positively and negatively regulated at 1, 2, and 4 days after inoculation (DAI).

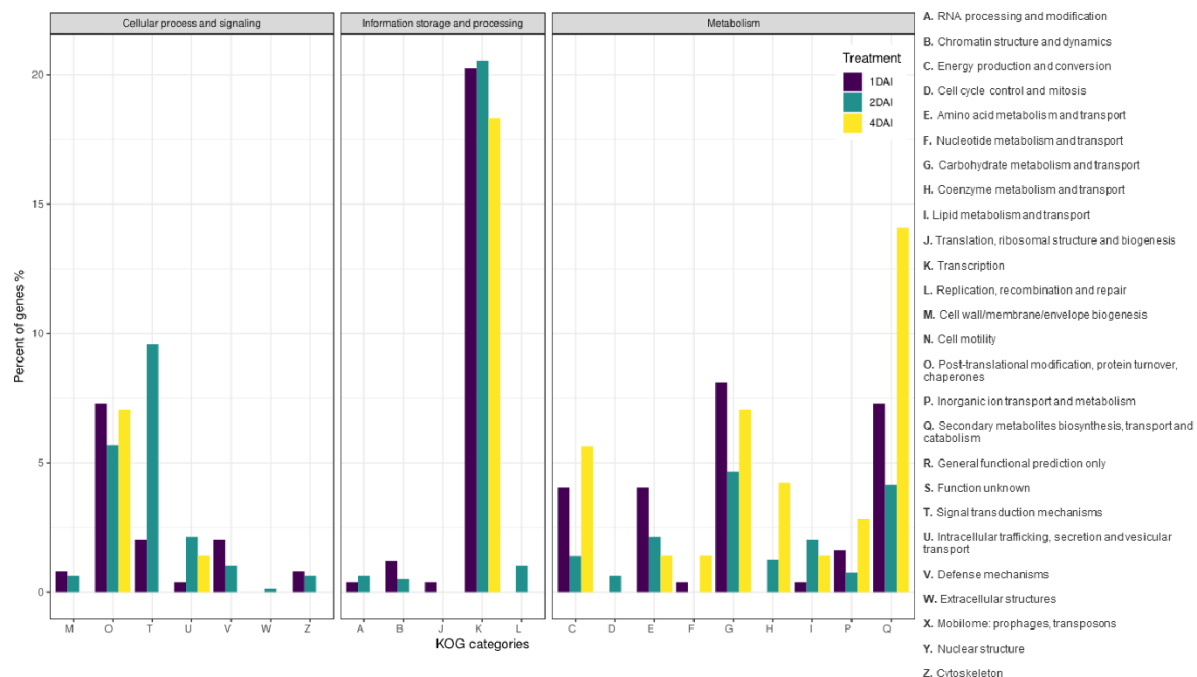


Figure 9. Classification of genes by eukaryotic orthologous groups (KOG) of the differentially expressed genes (DEGs) that are positively regulated in *Musa acuminata* subsp. *burmannica* accession 'Calcutta 4' after infection with *Fusarium oxysporum* f. sp. *cubense* STR4, at 1, 2, and 4 days after inoculation.

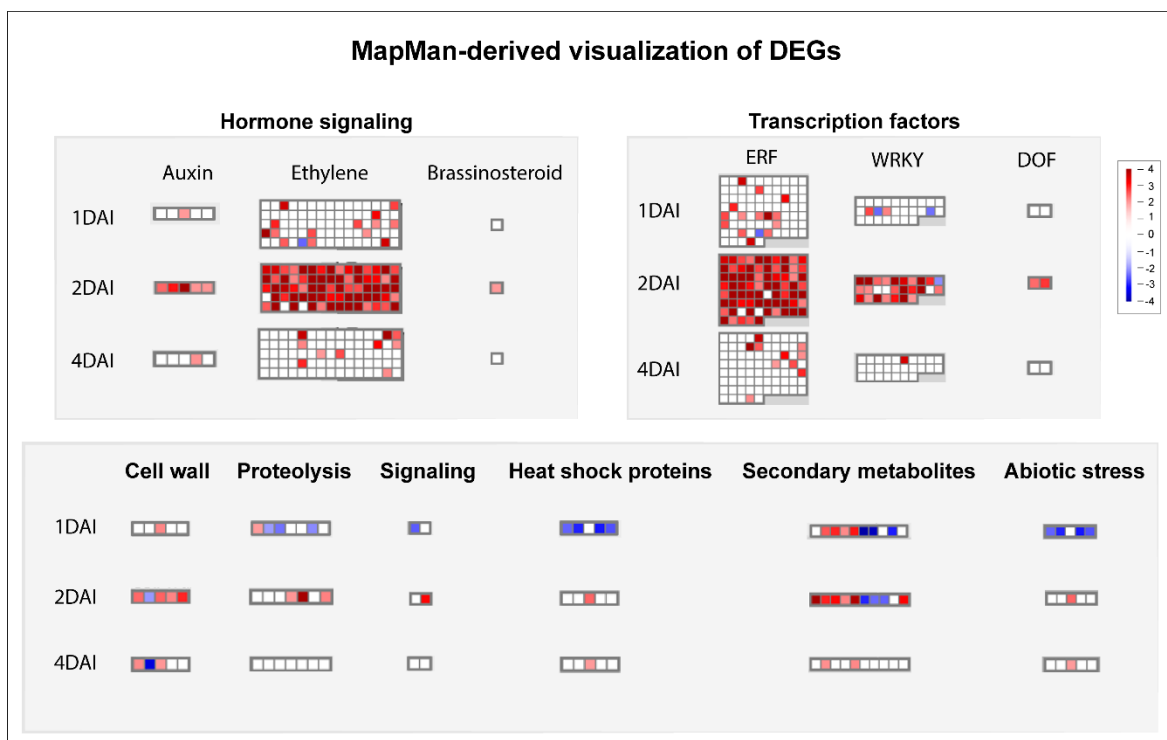


Figure 10. Mapman-based functional prediction mapping of differentially expressed genes (DEGs) in *Musa acuminata* subsp. *burmannica* accession 'Calcutta 4' after infection with *Fusarium oxysporum* f. sp. *cubense* STR4, at 1, 2, and 4 days after inoculation.

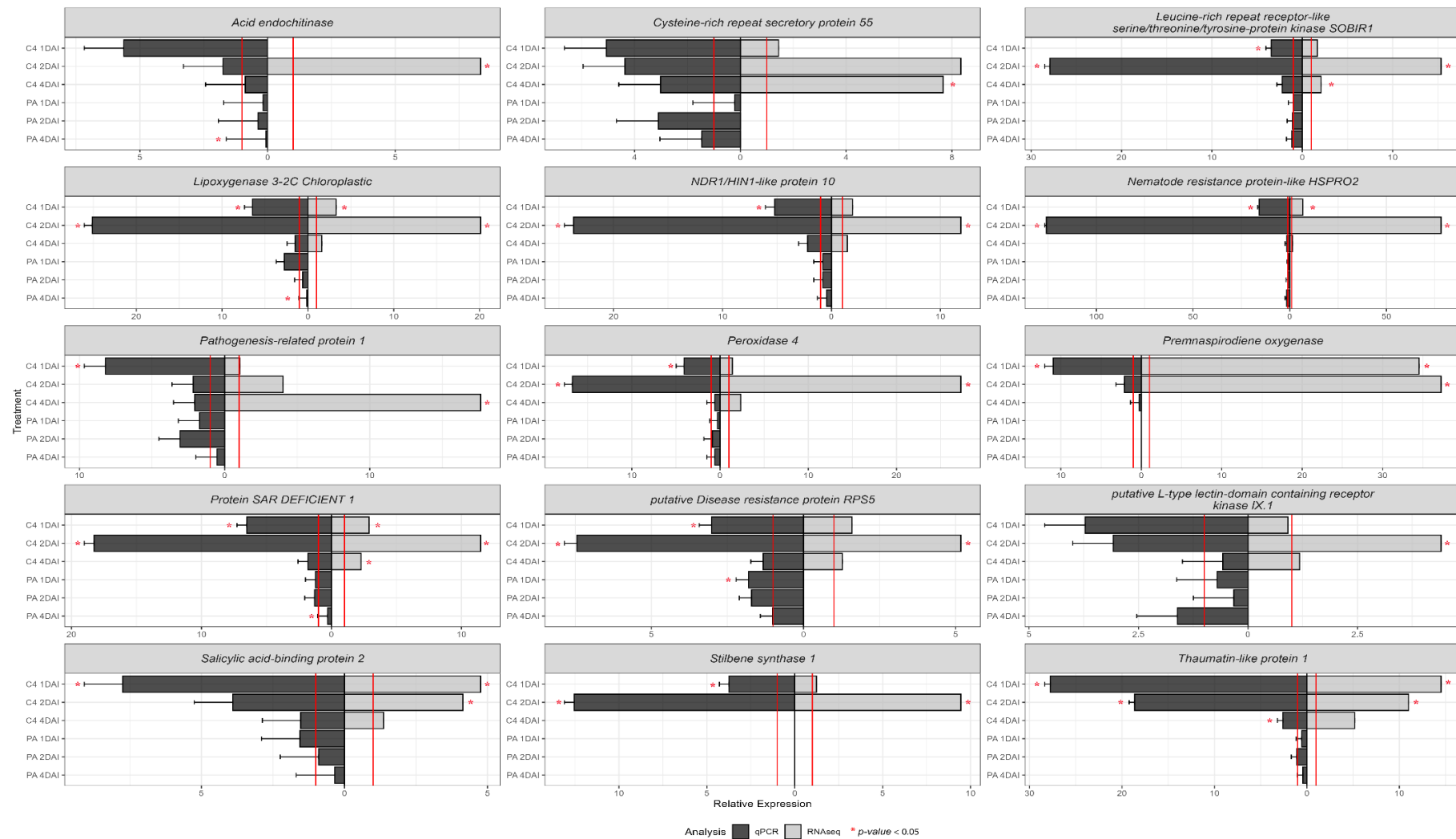


Figure 11. RT-qPCR analysis of relative expression of 15 differentially expressed upregulated genes identified following RNAseq analysis of the interaction between *Musa acuminata* subsp. *burmannica* accession 'Calcutta 4' and *Fusarium oxysporum* f. sp. *cubeense* STR4, at 1, 2, and 4 days after inoculation. RT-qPCR data was analysed using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). The expression of the control treatments is equal to 1 and marked by a red line. Both values for RT-qPCR and RNAseq were positive.

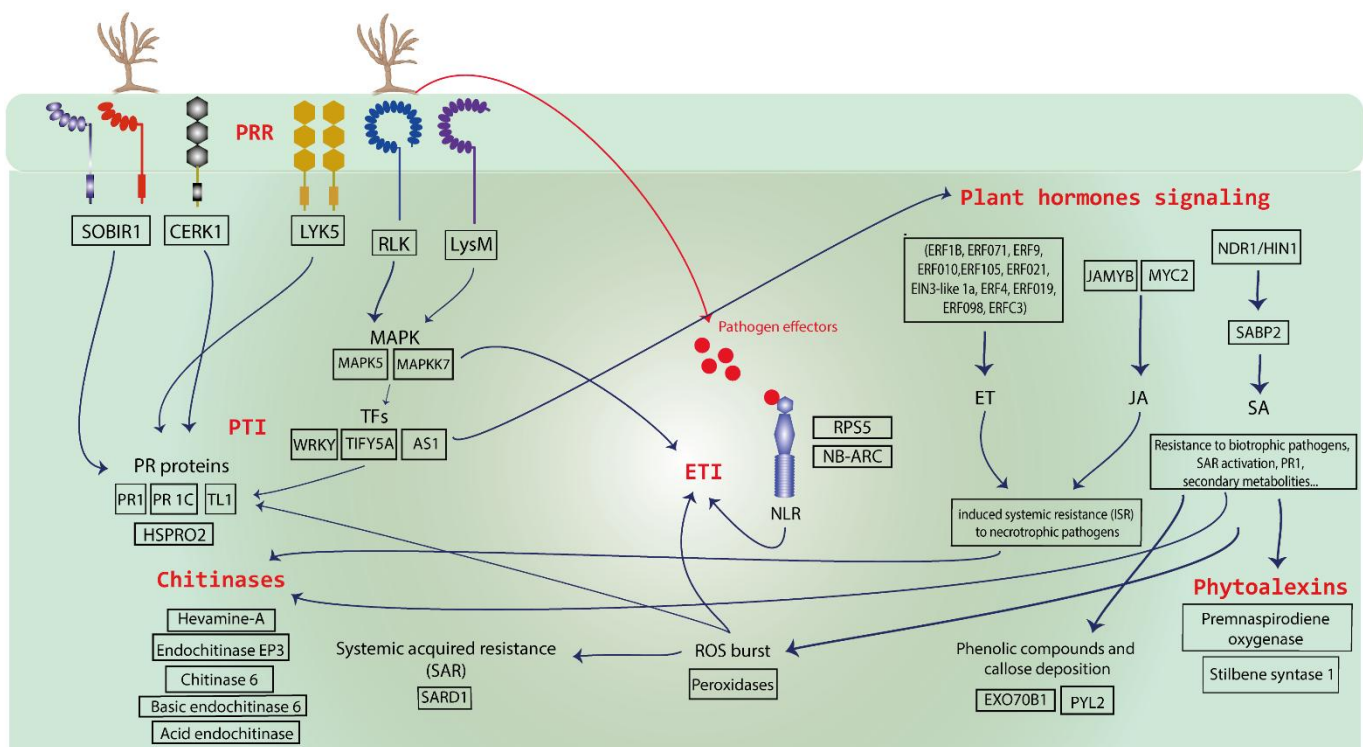
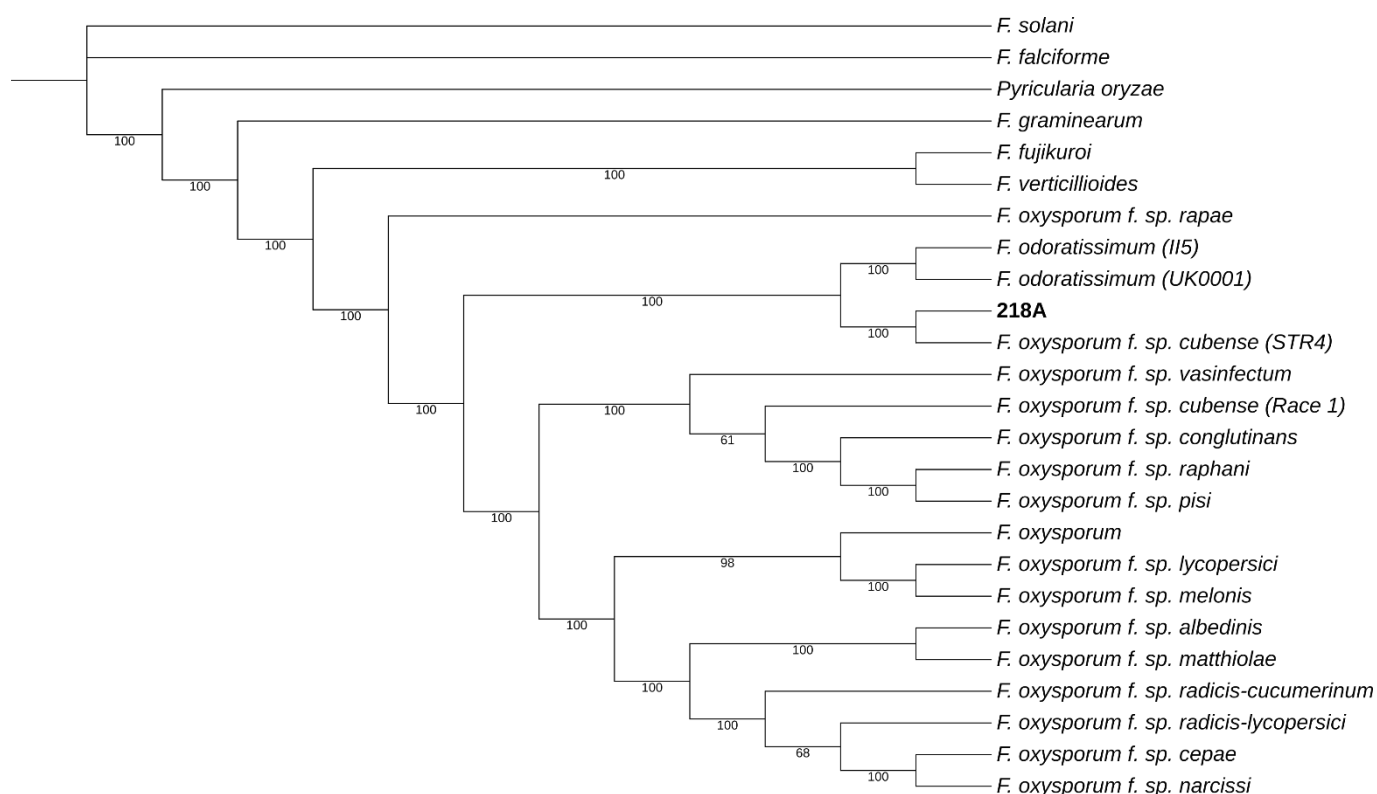
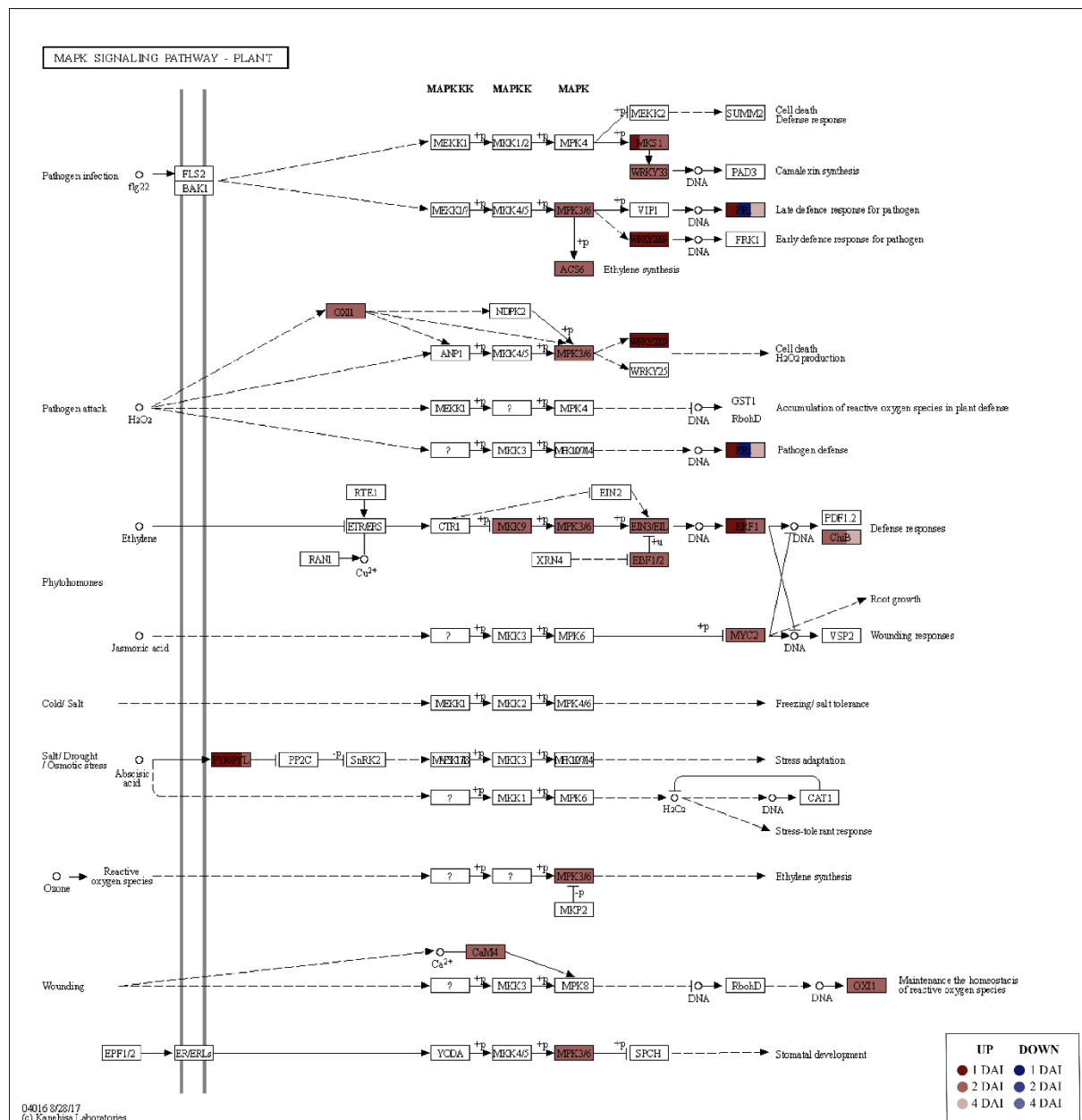


Figure 12. A schematic model of the innate immune responses observed in *Musa acuminata* subsp. *burmannica* accession 'Calcutta 4' following infection by *Fusarium oxysporum* f. sp. *cubense* subtropical race 4 (STR4). Upregulated candidate resistance genes included PRRs (transmembrane pattern recognition receptors) for recognition of fungal PAMPs (Pathogen associated molecular pattern) and NLR proteins (*R* genes) for recognition of pathogen effectors, triggering ETI (Effector-triggered immunity) and induction of a hypersensitive cell death (HR) response. Downstream defense response-associated genes were also observed amongst upregulated genes, including those encoding Pathogenesis-related (PRs) proteins, phytohormone pathway-associated proteins, chitinases, proteins involved in systemic acquired resistance (SAR), reactive oxygen species (ROS), and secondary metabolites such as phytoalexins.

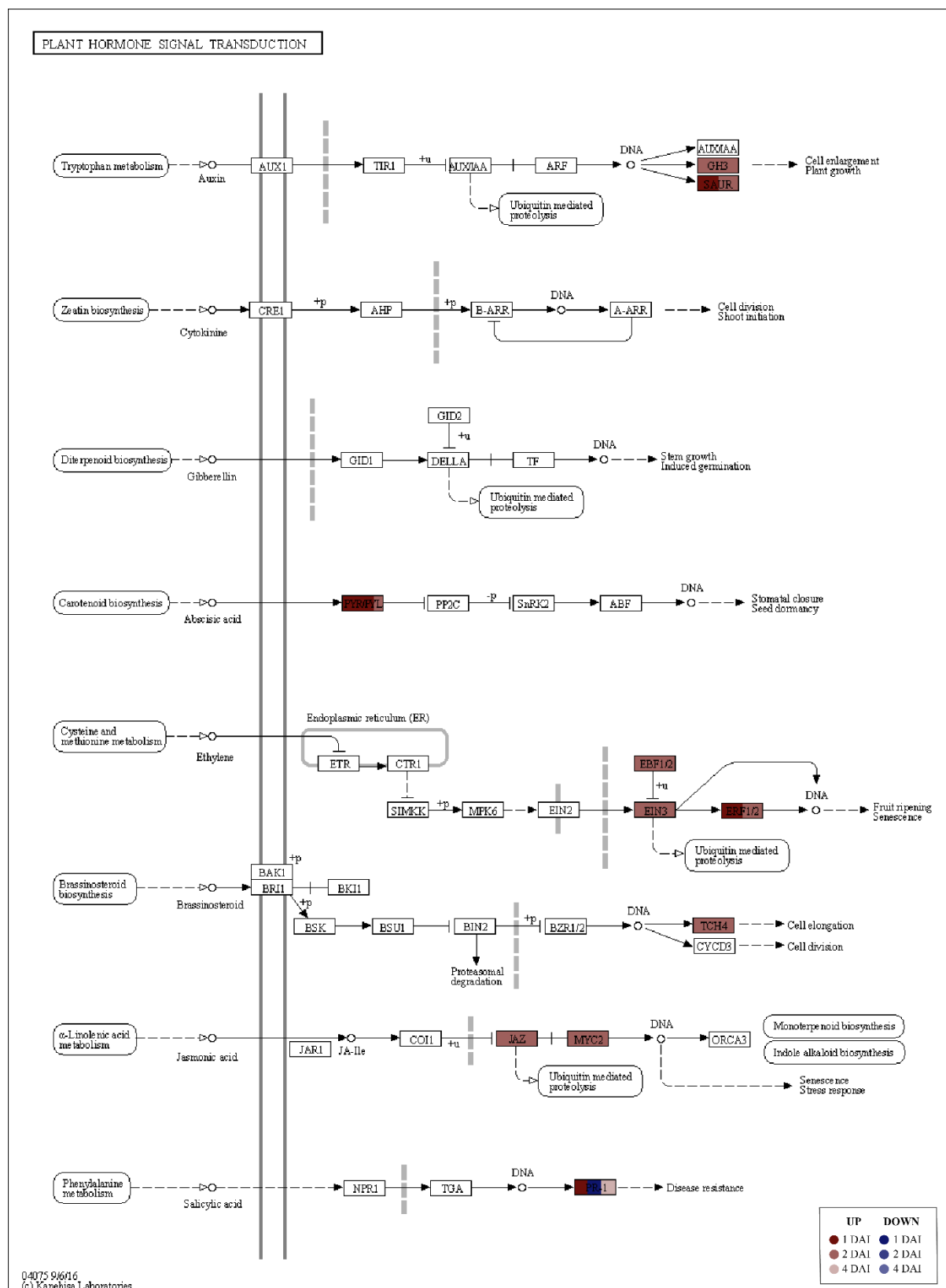
Supplementary material



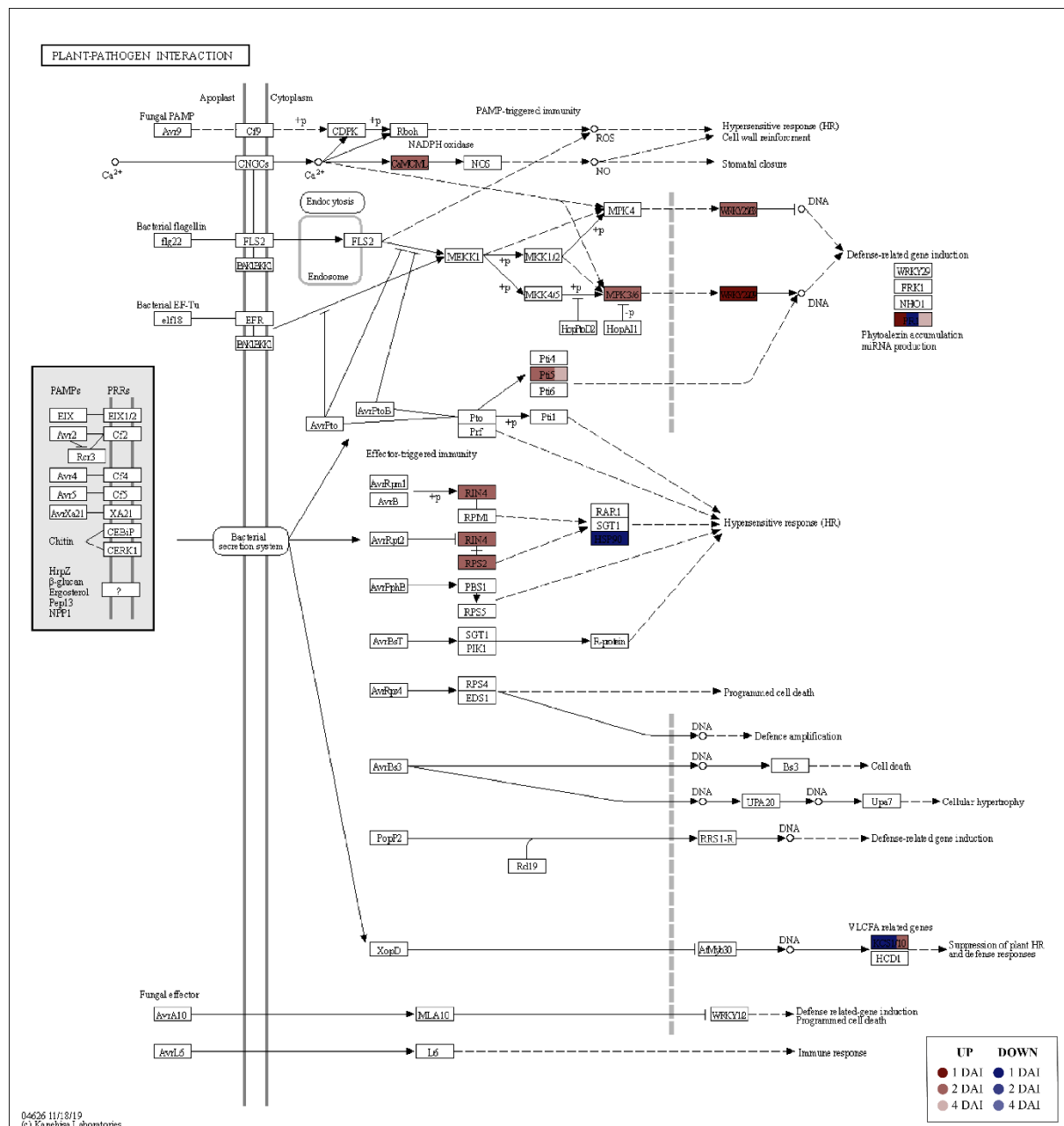
Supplementary figure 1. Orthofinder-based phylogenetic analysis of orthogroups among six different *Fusarium* species and twenty *formae speciales* of *Fusarium oxysporum*, including *F. oxysporum* f. sp. *cubense* isolate 218A from the cultivar 'Nanica' (Cavendish subgroup, AAA), isolated in the state of São Paulo. Numbers in branches indicate bootstrap values.



Supplementary figure 2. Kegg mapping of the MAPK (mitogen-activated protein kinase) signaling pathway based on differentially expressed genes (DEGs) observed in *Musa acuminata* subsp. *burmannica* accession 'Calcutta 4' after infection with *Fusarium oxysporum* f. sp. *cubense* STR4, at 1, 2, and 4 days after inoculation. Different shades of red indicate positively regulated DEGs (UP), while shades of blue indicate negatively regulated DEGs in the three treatments.



Supplementary figure 3. Kegg mapping of the signaling translation pathway based on differentially expressed genes (DEGs) observed in *Musa acuminata* subsp. *burmannica* accession 'Calcutta 4' after infection with *Fusarium oxysporum* f. sp. *cubense* STR4, at 1, 2, and 4 days after inoculation. Different shades of red indicate positively regulated DEGs (UP), while shades of blue indicate negatively regulated DEGs in the three treatments.



Supplementary figure 4. Kegg mapping of the plant-pathogen interaction pathway highlighting the presence of differentially expressed genes (DEGs) observed in *Musa acuminata* subsp. *burmannica* accession 'Calcutta 4' after infection with *Fusarium oxysporum* f. sp. *cubense* STR4, at 1, 2, and 4 days after inoculation. Different shades of red indicate positively regulated DEGs (UP), while shades of blue indicate negatively regulated DEGs in the three treatments.

Supplementary table 1. Differentially expressed genes identified following RNAseq analysis of the interaction between *Musa acuminata* subsp. *burmannica* accession 'Calcutta 4' and *Fusarium oxysporum* f. sp. *cubense* STR4, at 1, 2, and 4 days after inoculation.

Gene	Log2FC	Treatment	Expression	Gene Product
Macma4_02_g23870	10.01582	2DAI	UP	Dehydration-responsive element-binding protein 1C
Macma4_10_g04260	9.492266	4DAI	UP	hypothetical protein
Macma4_11_g04840	8.879213	2DAI	UP	Transcription factor MYB78
Macma4_11_g20940	8.664698	2DAI	UP	hypothetical protein
Macma4_06_g08720	8.306125	2DAI	UP	putative Galactolipase DONGLE%2C chloroplastic
Macma4_04_g00380	8.303026	4DAI	UP	putative auxin efflux carrier component 9
Macma4_07_g21080	8.25739	2DAI	UP	conserved hypothetical protein
Macma4_08_g01360	7.775426	2DAI	UP	NAC domain-containing protein 90
Macma4_06_g06690	7.527127	2DAI	UP	putative protein ANTAGONIST OF LIKE HETEROCHROMATIN PROTEIN 1
Macma4_11_g19210	7.440152	2DAI	UP	hypothetical protein
Macma4_03_g21730	7.409999	2DAI	UP	Cytochrome P450 94B3
Macma4_10_g19460	7.389632	2DAI	UP	Dehydration-responsive element-binding protein 1C
Macma4_06_g27380	7.370363	2DAI	UP	VQ domain-containing protein
Macma4_10_g33770	7.319173	2DAI	UP	conserved hypothetical protein
Macma4_04_g04680	7.306969	4DAI	UP	Ethylene-responsive transcription factor ERF098
Macma4_09_g10730	7.303584	2DAI	UP	putative protein TIFY 5A
Macma4_02_g16390	7.258106	2DAI	UP	putative protein TIFY 5A
Macma4_05_g30420	7.161932	2DAI	UP	putative Ethylene-responsive transcription factor 9
Macma4_08_g11600	7.087686	2DAI	UP	Dehydration-responsive element-binding protein 1E
Macma4_11_g03180	7.084934	2DAI	UP	putative Ethylene-responsive transcription factor ERF024
Macma4_08_g23810	7.059274	2DAI	UP	Glucan endo-1%2C3-beta-glucosidase
Macma4_06_g02680	6.960256	2DAI	UP	conserved hypothetical protein
Macma4_05_g05690	6.943673	2DAI	UP	hypothetical protein
Macma4_06_g04150	6.918999	2DAI	UP	conserved hypothetical protein
Macma4_09_g19510	6.887521	2DAI	UP	Nudix hydrolase 17%2C mitochondrial
Macma4_05_g26170	6.842658	4DAI	UP	Gamma-glutamyl peptidase 5
Macma4_05_g25610	6.769677	2DAI	UP	putative protein ANTAGONIST OF LIKE HETEROCHROMATIN PROTEIN 1

Macma4_04_g04680	6.768885	2DAI	UP	Ethylene-responsive transcription factor ERF098
Macma4_06_g10890	6.75785	2DAI	UP	hypothetical protein
Macma4_07_g11400	6.751698	2DAI	UP	AP2/ERF domain-containing protein
Macma4_05_g31780	6.742533	2DAI	UP	conserved hypothetical protein
Macma4_11_g10410	6.694448	2DAI	UP	putative Dehydration-responsive element-binding protein 1D
Macma4_05_g16240	6.647853	2DAI	UP	Expansin-like B1
Macma4_06_g32160	6.555354	2DAI	UP	putative carboxylesterase 18
Macma4_06_g19720	6.548632	2DAI	UP	conserved hypothetical protein
Macma4_10_g17380	6.513627	2DAI	UP	conserved hypothetical protein
Macma4_07_g06140	6.508426	2DAI	UP	Dehydration-responsive element-binding protein 1D
Macma4_03_g30650	6.46617	2DAI	UP	WRKY domain-containing protein
Macma4_05_g05140	6.455247	2DAI	UP	AP2/ERF domain-containing protein
Macma4_02_g06900	6.364056	2DAI	UP	Chaperone protein dnaJ 11%2C chloroplastic
Macma4_10_g08320	6.295824	2DAI	UP	Nematode resistance protein-like HSPRO2
Macma4_06_g34540	6.26558	2DAI	UP	putative protein TIFY 5A
Macma4_05_g11360	6.224373	2DAI	UP	hypothetical protein
Macma4_05_g16240	6.145109	4DAI	UP	Expansin-like B1
Macma4_08_g31110	6.103248	2DAI	UP	Cytochrome P450 94C1
Macma4_05_g21040	6.093772	4DAI	UP	hypothetical protein
Macma4_01_g17980	6.062273	2DAI	UP	hypothetical protein
Macma4_04_g20980	6.061472	2DAI	UP	conserved hypothetical protein
Macma4_11_g03170	6.036247	2DAI	UP	Dehydration-responsive element-binding protein 1E
Macma4_03_g33650	6.001008	2DAI	UP	hypothetical protein
Macma4_04_g25600	5.973224	2DAI	UP	putative Ethylene-responsive transcription factor ERF025
Macma4_04_g28270	5.970069	2DAI	UP	putative Dehydration-responsive element-binding protein 1D
Macma4_04_g17700	5.958912	2DAI	UP	putative Ethylene-responsive transcription factor 9
Macma4_03_g22900	5.952041	2DAI	UP	putative Transcription factor bHLH162
Macma4_07_g00600	5.930788	2DAI	UP	hypothetical protein
Macma4_04_g11680	5.901551	2DAI	UP	putative Transcription factor bHLH92
Macma4_11_g08360	5.896637	2DAI	UP	putative WRKY transcription factor WRKY28
Macma4_02_g00170	5.868346	2DAI	UP	putative Calmodulin-binding protein 25
Macma4_04_g40350	5.86666	2DAI	UP	putative protein TIFY 5A
Macma4_09_g15670	5.866415	2DAI	UP	putative Auxin-responsive protein SAUR32

Macma4_03_g34820	5.854431	2DAI	UP	hypothetical protein
Macma4_06_g21200	5.850782	2DAI	UP	putative CCR4-associated factor 1
Macma4_01_g26050	5.790572	2DAI	UP	putative calcium-binding protein CML31
Macma4_01_g26040	5.781009	2DAI	UP	Calcium-binding protein CML38
Macma4_02_g00160	5.778199	2DAI	UP	putative Calmodulin-binding protein 25
Macma4_09_g07430	5.743861	2DAI	UP	putative protein JINGUBANG
Macma4_09_g19150	5.741423	2DAI	UP	hypothetical protein
Macma4_09_g29440	5.736143	2DAI	UP	Nudix hydrolase domain-containing protein
Macma4_10_g09650	5.72703	2DAI	UP	hypothetical protein
Macma4_06_g14850	5.704704	2DAI	UP	putative protein ROH1
Macma4_09_g23580	5.70376	2DAI	UP	hypothetical protein
Macma4_03_g06050	5.697207	2DAI	UP	AP2/ERF domain-containing protein
Macma4_09_g19140	5.695716	2DAI	UP	Zinc finger A20 and AN1 domain-containing stress-associated protein 1
Macma4_06_g04380	5.68183	2DAI	UP	putative U-box domain-containing protein 21
Macma4_02_g04950	5.667856	2DAI	UP	VQ domain-containing protein
Macma4_06_g38980	5.658324	2DAI	UP	Amino acid transporter AVT11
Macma4_07_g13840	5.637088	2DAI	UP	BHLH domain-containing protein
Macma4_02_g10700	5.605107	2DAI	UP	conserved hypothetical protein
Macma4_05_g16190	5.598754	2DAI	UP	putative Anthocyanidin-3-O-glucoside rhamnosyltransferase
Macma4_07_g03360	5.58474	2DAI	UP	E3 ubiquitin-protein ligase PUB23
Macma4_07_g09990	5.573046	2DAI	UP	Mitochondrial uncoupling protein 5
Macma4_02_g18260	5.525745	4DAI	UP	Transcription factor ILI5
Macma4_08_g07740	5.501923	2DAI	UP	AP2/ERF domain-containing protein
Macma4_01_g18540	5.490752	2DAI	UP	hypothetical protein
Macma4_09_g14540	5.486312	2DAI	UP	putative Ethylene-responsive transcription factor ERF015
Macma4_07_g15130	5.480165	2DAI	UP	putative protein PSK SIMULATOR 3
Macma4_04_g07870	5.473761	2DAI	UP	Alpha-terpineol synthase%2C chloroplastic
Macma4_03_g08910	5.466974	2DAI	UP	Cytokinin dehydrogenase 4
Macma4_01_g00810	5.461234	2DAI	UP	WRKY domain-containing protein
Macma4_10_g25320	5.448797	1DAI	UP	Nudix hydrolase domain-containing protein
Macma4_07_g18500	5.436326	2DAI	UP	hypothetical protein
Macma4_10_g10890	5.402511	2DAI	UP	conserved hypothetical protein
Macma4_05_g19410	5.395895	2DAI	UP	Cytochrome P450 94C1

Macma4_04_g22990	5.376592	2DAI	UP	AP2/ERF domain-containing protein
Macma4_04_g33600	5.362949	2DAI	UP	putative Ethylene-responsive transcription factor ERF024
Macma4_05_g06000	5.348352	2DAI	UP	Nudix hydrolase domain-containing protein
Macma4_11_g05120	5.346197	2DAI	UP	hypothetical protein
Macma4_08_g13950	5.341478	2DAI	UP	hypothetical protein
Macma4_08_g00550	5.323421	2DAI	UP	conserved hypothetical protein
Macma4_07_g15980	5.317173	2DAI	UP	Transcription factor bHLH35
Macma4_07_g29990	5.306051	2DAI	UP	hypothetical protein
Macma4_07_g01260	5.299798	2DAI	UP	conserved hypothetical protein
Macma4_10_g29730	5.2928	2DAI	UP	putative Monothiol glutaredoxin-S3
Macma4_10_g19810	5.250464	2DAI	UP	putative protein ROH1
Macma4_06_g25370	5.243673	2DAI	UP	Fe2OG dioxygenase domain-containing protein
Macma4_07_g21750	5.22496	2DAI	UP	Premnaspirodiene oxygenase
Macma4_02_g21150	5.222574	2DAI	UP	conserved hypothetical protein
Macma4_05_g21040	5.208468	2DAI	UP	hypothetical protein
Macma4_11_g11520	5.177422	2DAI	UP	LOB domain-containing protein 41
Macma4_06_g22320	5.138699	2DAI	UP	putative protein TIFY 9
Macma4_05_g01640	5.122986	2DAI	UP	hypothetical protein
Macma4_07_g21750	5.1191	1DAI	UP	Premnaspirodiene oxygenase
Macma4_10_g18810	5.118876	2DAI	UP	hypothetical protein
Macma4_04_g38480	5.095687	2DAI	UP	putative protein BPS1%2C chloroplastic
Macma4_03_g24180	5.081032	2DAI	UP	conserved hypothetical protein
Macma4_02_g14120	5.066777	2DAI	UP	Sugar transport protein 10
Macma4_11_g19990	5.059227	2DAI	UP	hypothetical protein
Macma4_11_g21920	5.059181	2DAI	UP	hypothetical protein
Macma4_02_g13270	5.057788	2DAI	UP	Protein DETOXIFICATION 51
Macma4_04_g39190	5.043048	2DAI	UP	hypothetical protein
Macma4_07_g01290	5.040809	2DAI	UP	9-cis-epoxycarotenoid dioxygenase 1%2C chloroplastic
Macma4_03_g32130	5.026853	2DAI	UP	putative Transcription factor bHLH92
Macma4_06_g03410	5.009662	2DAI	UP	hypothetical protein
Macma4_06_g34820	4.998673	2DAI	UP	hypothetical protein
Macma4_09_g24390	4.987018	2DAI	UP	hypothetical protein
Macma4_09_g00280	4.972602	2DAI	UP	VOC domain-containing protein

Macma4_11_g08880	4.93845	2DAI	UP	hypothetical protein
Macma4_08_g26680	4.936273	2DAI	UP	putative WRKY transcription factor 46
Macma4_01_g04350	4.931387	2DAI	UP	NB-ARC domain-containing protein
Macma4_07_g28080	4.928357	2DAI	UP	putative Zinc finger CCCH domain-containing protein 49
Macma4_10_g09800	4.928306	2DAI	UP	putative Ethylene-responsive transcription factor ERF019
Macma4_06_g06230	4.927999	2DAI	UP	conserved hypothetical protein
Macma4_04_g11230	4.927495	2DAI	UP	Calcium-transporting ATPase 7%2C plasma membrane-type
Macma4_07_g14220	4.916597	2DAI	UP	WRKY domain-containing protein
Macma4_02_g18160	4.90438	2DAI	UP	putative trehalose-phosphate phosphatase 6
Macma4_03_g17230	4.872539	2DAI	UP	Type IV inositol polyphosphate 5-phosphatase 11
Macma4_10_g01630	4.871481	4DAI	UP	Cationic peroxidase 1
Macma4_10_g25760	4.865833	2DAI	UP	conserved hypothetical protein
Macma4_07_g03750	4.85946	2DAI	UP	hypothetical protein
Macma4_01_g27360	4.859047	2DAI	UP	putative WRKY transcription factor 41
Macma4_01_g18450	4.84936	2DAI	UP	hypothetical protein
Macma4_07_g17990	4.847033	2DAI	UP	putative RING-H2 finger protein ATL2
Macma4_07_g21330	4.842772	1DAI	UP	hypothetical protein
Macma4_05_g00970	4.841413	2DAI	UP	hypothetical protein
Macma4_01_g08720	4.840548	2DAI	UP	putative UPF0496 protein 3
Macma4_05_g21530	4.838076	2DAI	UP	hypothetical protein
Macma4_06_g02820	4.807098	1DAI	UP	Absciscic acid receptor PYL2
Macma4_09_g24400	4.805149	2DAI	UP	hypothetical protein
Macma4_07_g20730	4.796897	2DAI	UP	putative U-box domain-containing protein 21
Macma4_05_g16180	4.795264	2DAI	UP	Brassinosteroid-responsive RING protein 1
Macma4_06_g12260	4.780734	2DAI	UP	hypothetical protein
Macma4_06_g16370	4.776774	2DAI	UP	Peroxidase 4
Macma4_05_g07170	4.774219	2DAI	UP	putative protein PSK SIMULATOR 1
Macma4_08_g16730	4.765171	1DAI	UP	conserved hypothetical protein
Macma4_06_g38260	4.747936	2DAI	UP	Protein JINGUBANG
Macma4_07_g21330	4.74611	2DAI	UP	hypothetical protein
Macma4_04_g22980	4.745361	2DAI	UP	AP2/ERF domain-containing protein
Macma4_06_g13100	4.737224	2DAI	UP	hypothetical protein
Macma4_10_g19020	4.725333	2DAI	UP	hypothetical protein

Macma4_06_g05710	4.69679	2DAI	UP	hypothetical protein
Macma4_03_g31990	4.695383	2DAI	UP	hypothetical protein
Macma4_03_g13720	4.686602	2DAI	UP	conserved hypothetical protein
Macma4_02_g12160	4.685068	1DAI	UP	Non-symbiotic hemoglobin 2
Macma4_09_g00910	4.682017	2DAI	UP	C2 domain-containing protein
Macma4_10_g29480	4.67624	2DAI	UP	putative Ethylene-responsive transcription factor ERF017
Macma4_07_g29520	4.67233	2DAI	UP	putative Nodulin-related protein 1
Macma4_06_g02870	4.668403	2DAI	UP	hypothetical protein
Macma4_05_g15560	4.667522	2DAI	UP	putative Auxin-responsive protein SAUR15
Macma4_08_g07100	4.650634	2DAI	UP	hypothetical protein
Macma4_10_g09530	4.64039	2DAI	UP	putative NAC domain-containing protein 61
Macma4_08_g06530	4.63711	2DAI	UP	putative Ethylene-responsive transcription factor 4
Macma4_05_g03760	4.636997	2DAI	UP	Transcription factor MYB4
Macma4_09_g32020	4.61655	2DAI	UP	putative CCR4-associated factor 1
Macma4_06_g14950	4.604126	2DAI	UP	hypothetical protein
Macma4_08_g09740	4.597719	2DAI	UP	C2 domain-containing protein
Macma4_07_g05580	4.593978	2DAI	UP	Ethylene-responsive transcription factor ERF010
Macma4_11_g23440	4.571433	2DAI	UP	Nematode resistance protein-like HSPRO2
Macma4_06_g06670	4.566112	2DAI	UP	hypothetical protein
Macma4_10_g26570	4.565509	2DAI	UP	putative protein PSK SIMULATOR 1
Macma4_11_g09170	4.560534	2DAI	UP	putative Transcription factor DIVARICATA
Macma4_01_g17120	4.553702	2DAI	UP	hypothetical protein
Macma4_06_g24480	4.552578	2DAI	UP	hypothetical protein
Macma4_03_g25680	4.551996	2DAI	UP	putative Calcium-binding protein PBP1
Macma4_03_g05100	4.549111	2DAI	UP	Xyloglucan endotransglucosylase/hydrolase protein 22
Macma4_02_g07440	4.548489	2DAI	UP	Oxalate--CoA ligase
Macma4_03_g28120	4.544781	1DAI	UP	Chlorophyllase
Macma4_03_g19210	4.533253	2DAI	UP	Calmodulin-like protein 5
Macma4_05_g14530	4.532986	2DAI	UP	putative calcium-binding protein CML31
Macma4_01_g18440	4.530551	2DAI	UP	hypothetical protein
Macma4_05_g32600	4.528188	2DAI	UP	Nematode resistance protein-like HSPRO2
Macma4_09_g28760	4.524566	2DAI	UP	C2H2-type domain-containing protein
Macma4_10_g09240	4.520219	2DAI	UP	hypothetical protein

Macma4_11_g01230	4.520018	2DAI	UP	NAC domain-containing protein 22
Macma4_06_g35670	4.518184	2DAI	UP	conserved hypothetical protein
Macma4_07_g26990	4.517945	2DAI	UP	conserved hypothetical protein
Macma4_06_g34510	4.511465	2DAI	UP	9-cis-epoxycarotenoid dioxygenase NCED3%2C chloroplastic
Macma4_03_g03410	4.506993	2DAI	UP	LEA_2 domain-containing protein
Macma4_09_g07420	4.502789	2DAI	UP	putative Zinc finger A20 and AN1 domain-containing stress-associated protein 1
Macma4_11_g22910	4.502658	2DAI	UP	conserved hypothetical protein
Macma4_09_g23920	4.497626	1DAI	UP	putative Mitogen-activated protein kinase kinase kinase 17
Macma4_05_g29140	4.497617	2DAI	UP	Premnaspirodiene oxygenase
Macma4_04_g22930	4.487637	2DAI	UP	Lipase_3 domain-containing protein
Macma4_04_g07080	4.486249	2DAI	UP	putative Photosystem II 5 kDa protein%2C chloroplastic
Macma4_06_g35340	4.48113	2DAI	UP	putative Calmodulin-binding protein 25
Macma4_11_g23270	4.480904	2DAI	UP	putative Zinc finger protein ZAT18
Macma4_08_g06470	4.464196	2DAI	UP	hypothetical protein
Macma4_03_g18330	4.460024	2DAI	UP	conserved hypothetical protein
Macma4_11_g20160	4.451792	2DAI	UP	conserved hypothetical protein
Macma4_09_g23470	4.438829	2DAI	UP	conserved hypothetical protein
Macma4_07_g28680	4.433373	2DAI	UP	Calcium-transporting ATPase 7%2C plasma membrane-type
Macma4_10_g20670	4.43194	2DAI	UP	putative E3 ubiquitin-protein ligase XERICO
Macma4_07_g11650	4.4315	2DAI	UP	Trehalose 6-phosphate phosphatase
Macma4_04_g08390	4.420634	2DAI	UP	putative protein DOG1-like 3
Macma4_02_g24330	4.411553	2DAI	UP	putative Cell wall / vacuolar inhibitor of fructosidase 2
Macma4_10_g26580	4.410592	2DAI	UP	hypothetical protein
Macma4_06_g28400	4.403557	2DAI	UP	NAC domain-containing protein
Macma4_06_g24470	4.397427	2DAI	UP	putative WRKY transcription factor 41
Macma4_06_g09440	4.395024	2DAI	UP	putative Mitogen-activated protein kinase kinase kinase 18
Macma4_03_g27380	4.387276	2DAI	UP	conserved hypothetical protein
Macma4_06_g16330	4.373982	2DAI	UP	Peroxidase 4
Macma4_07_g18770	4.373693	1DAI	UP	putative Dehydration-responsive element-binding protein 2C
Macma4_10_g34050	4.372907	2DAI	UP	hypothetical protein
Macma4_03_g00640	4.370013	2DAI	UP	hypothetical protein
Macma4_07_g20520	4.36866	2DAI	UP	putative galacturonosyltransferase-like 9
Macma4_08_g22210	4.365772	2DAI	UP	putative E3 ubiquitin-protein ligase RDUF1

Macma4_06_g22160	4.361834	1DAI	UP	Late embryogenesis abundant protein Lea5-D
Macma4_04_g37860	4.35579	2DAI	UP	1-aminocyclopropane-1-carboxylate synthase
Macma4_05_g31830	4.35102	1DAI	UP	Asparagine synthetase [glutamine-hydrolyzing]
Macma4_10_g28470	4.346547	2DAI	UP	putative E3 ubiquitin-protein ligase ATL6
Macma4_09_g21130	4.345349	2DAI	UP	putative F-box protein At1g47056
Macma4_03_g07940	4.340985	2DAI	UP	hypothetical protein
Macma4_08_g15790	4.340772	2DAI	UP	1-aminocyclopropane-1-carboxylate oxidase
Macma4_03_g08040	4.338849	2DAI	UP	Lipoxygenase 3%2C chloroplastic
Macma4_10_g13470	4.331265	2DAI	UP	conserved hypothetical protein
Macma4_05_g30440	4.329777	2DAI	UP	hypothetical protein
Macma4_09_g11200	4.329193	1DAI	UP	putative Horcolin
Macma4_03_g32150	4.315199	2DAI	UP	putative PRA1 family protein B5
Macma4_06_g07560	4.286389	2DAI	UP	hypothetical protein
Macma4_11_g10210	4.280296	2DAI	UP	putative Ethylene-responsive transcription factor ERF021
Macma4_09_g12380	4.266445	1DAI	UP	hypothetical protein
Macma4_05_g08840	4.241646	2DAI	UP	putative Zinc finger protein ZAT10
Macma4_05_g06290	4.234557	2DAI	UP	Arabinogalactan protein 16
Macma4_05_g11940	4.221372	1DAI	UP	RING-H2 finger protein ATL8
Macma4_03_g29630	4.218355	2DAI	UP	Basic endochitinase C
Macma4_04_g18820	4.213101	2DAI	UP	putative Cation/H(+) antiporter 15
Macma4_04_g29530	4.212983	4DAI	UP	hypothetical protein
Macma4_09_g14670	4.200107	2DAI	UP	IAA-amino acid hydrolase ILR1-like 1
Macma4_02_g10380	4.194032	2DAI	UP	hypothetical protein
Macma4_10_g14170	4.190432	2DAI	UP	hypothetical protein
Macma4_04_g06850	4.182691	2DAI	UP	conserved hypothetical protein
Macma4_09_g06440	4.180118	2DAI	UP	hypothetical protein
Macma4_08_g10480	4.178124	2DAI	UP	putative Transcription factor MYB2
Macma4_09_g11250	4.151019	1DAI	UP	putative Horcolin
Macma4_07_g17580	4.147381	2DAI	UP	hypothetical protein
Macma4_05_g17770	4.146058	1DAI	UP	putative Cytochrome c1-2%2C heme protein%2C mitochondrial
Macma4_03_g08420	4.145096	4DAI	UP	Pathogenesis-related protein 1
Macma4_03_g31710	4.143414	2DAI	UP	putative Mitogen-activated protein kinase kinase kinase 18
Macma4_07_g23970	4.141361	2DAI	UP	hypothetical protein

Macma4_09_g13430	4.12539	2DAI	UP	hypothetical protein
Macma4_04_g34350	4.12004	2DAI	UP	3-ketoacyl-CoA synthase 1
Macma4_09_g19890	4.111077	2DAI	UP	1-aminocyclopropane-1-carboxylate synthase
Macma4_06_g07550	4.109401	2DAI	UP	hypothetical protein
Macma4_03_g26520	4.105484	2DAI	UP	hypothetical protein
Macma4_09_g12390	4.091945	2DAI	UP	Chitin-inducible gibberellin-responsive protein 1
Macma4_05_g07860	4.088281	2DAI	UP	VQ domain-containing protein
Macma4_05_g09240	4.084536	2DAI	UP	hypothetical protein
Macma4_06_g30120	4.074062	2DAI	UP	hypothetical protein
Macma4_10_g28600	4.071144	2DAI	UP	Mitochondrial uncoupling protein 5
Macma4_03_g01960	4.063008	2DAI	UP	Protein TIFY 10b
Macma4_02_g20560	4.058012	2DAI	UP	hypothetical protein
Macma4_09_g28150	4.043123	2DAI	UP	Tify domain-containing protein
Macma4_01_g04340	4.034639	2DAI	UP	putative NB-ARC domain-containing protein
Macma4_05_g07680	4.030997	2DAI	UP	NAC domain-containing protein
Macma4_10_g12030	4.030774	2DAI	UP	RING-H2 finger protein ATL80
Macma4_03_g22360	4.029996	2DAI	UP	conserved hypothetical protein
Macma4_09_g11240	4.029224	1DAI	UP	putative Horcolin
Macma4_08_g10220	4.023844	1DAI	UP	hypothetical protein
Macma4_02_g08910	4.023294	2DAI	UP	conserved hypothetical protein
Macma4_10_g10810	4.009362	4DAI	UP	Peroxidase 4
Macma4_10_g21750	4.007427	2DAI	UP	hypothetical protein
Macma4_02_g06980	4.004575	2DAI	UP	putative UPF0496 protein 2
Macma4_09_g11860	4.002336	2DAI	UP	Ethylene-responsive transcription factor 4
Macma4_06_g18840	4.001509	2DAI	UP	putative Mitogen-activated protein kinase kinase kinase 18
Macma4_06_g35580	4.001196	2DAI	UP	NAC domain-containing protein 68
Macma4_02_g24100	3.989557	2DAI	UP	putative protein ROH1
Macma4_06_g10180	3.985981	2DAI	UP	hypothetical protein
Macma4_01_g04380	3.966552	2DAI	UP	NB-ARC domain-containing protein
Macma4_03_g18410	3.95923	2DAI	UP	putative WRKY transcription factor 41
Macma4_02_g09450	3.949231	2DAI	UP	Leucine-rich repeat receptor-like serine/threonine/tyrosine-protein kinase SOBIR1
Macma4_04_g26950	3.94703	1DAI	UP	putative Bowman-Birk type proteinase inhibitor
Macma4_05_g25280	3.944171	2DAI	UP	hypothetical protein

Macma4_01_g14850	3.943681	2DAI	UP	hypothetical protein
Macma4_03_g32610	3.930752	2DAI	UP	Oleosin H2
Macma4_06_g37980	3.923618	2DAI	UP	putative Zinc finger protein ZAT8
Macma4_07_g28770	3.913432	2DAI	UP	Terpene synthase 10
Macma4_05_g24740	3.911099	2DAI	UP	Ethylene-responsive transcription factor ERF071
Macma4_04_g07080	3.903177	1DAI	UP	putative Photosystem II 5 kDa protein%2C chloroplastic
Macma4_07_g07090	3.900853	2DAI	UP	Ethylene-responsive transcription factor ERF105
Macma4_09_g07310	3.891602	2DAI	UP	hypothetical protein
Macma4_08_g19660	3.888352	2DAI	UP	Ethylene-responsive transcription factor ERF021
Macma4_10_g03310	3.888255	4DAI	UP	hypothetical protein
Macma4_06_g13640	3.885504	1DAI	UP	hypothetical protein
Macma4_03_g28730	3.884447	2DAI	UP	conserved hypothetical protein
Macma4_07_g30550	3.881078	2DAI	UP	Calcium-binding protein PBP1
Macma4_10_g22190	3.870495	2DAI	UP	Cytochrome P450 81Q32
Macma4_09_g13750	3.866971	2DAI	UP	UDP-glucuronate 4-epimerase 1
Macma4_07_g15680	3.86538	2DAI	UP	putative protein phosphatase 2C 25
Macma4_05_g17130	3.862014	1DAI	UP	Thaumatococcus-like protein 1
Macma4_09_g14030	3.85559	2DAI	UP	Xyloglucan endotransglucosylase/hydrolase protein 22
Macma4_08_g05580	3.851719	2DAI	UP	putative carboxylesterase 17
Macma4_10_g19190	3.848859	2DAI	UP	putative Aspartic proteinase nepenthesin-2
Macma4_06_g15470	3.843943	2DAI	UP	putative GDSL esterase/lipase At4g10955
Macma4_06_g34090	3.843051	1DAI	UP	Cytochrome P450 81Q32
Macma4_05_g27400	3.839002	2DAI	UP	putative RING-H2 finger protein ATL3
Macma4_03_g26750	3.830803	2DAI	UP	putative protein LURP-one-related 11
Macma4_07_g25180	3.829335	2DAI	UP	PRA1 family protein B4
Macma4_04_g02820	3.828172	1DAI	UP	hypothetical protein
Macma4_02_g08700	3.818643	2DAI	UP	F-box protein SKIP2
Macma4_07_g12240	3.801626	2DAI	UP	DUF569 domain-containing protein
Macma4_05_g31670	3.799538	2DAI	UP	AP2/ERF domain-containing protein
Macma4_04_g30480	3.799277	2DAI	UP	Transcription factor MYB4
Macma4_05_g31830	3.796536	2DAI	UP	Asparagine synthetase [glutamine-hydrolyzing]
Macma4_04_g21980	3.792301	2DAI	UP	putative glucuronosyltransferase PGSIP8
Macma4_09_g14870	3.781554	1DAI	UP	putative Basic leucine zipper 43

Macma4_09_g33040	3.779615	2DAI	UP	hypothetical protein
Macma4_04_g28760	3.771013	2DAI	UP	Transcription factor MYB44
Macma4_07_g25010	3.770868	2DAI	UP	U-box domain-containing protein 16
Macma4_09_g15660	3.770139	2DAI	UP	hypothetical protein
Macma4_02_g25340	3.75722	2DAI	UP	Protein TIFY 10b
Macma4_06_g12960	3.753028	2DAI	UP	putative U-box domain-containing protein 21
Macma4_02_g04360	3.743749	2DAI	UP	conserved hypothetical protein
Macma4_03_g34540	3.736246	2DAI	UP	hypothetical protein
Macma4_02_g21150	3.735636	4DAI	UP	conserved hypothetical protein
Macma4_03_g29620	3.730569	2DAI	UP	conserved hypothetical protein
Macma4_07_g21950	3.725549	1DAI	UP	putative Alpha-humulene 10-hydroxylase
Macma4_03_g25360	3.723431	2DAI	UP	putative Zinc finger A20 and AN1 domain-containing stress-associated protein 4
Macma4_02_g18550	3.719958	2DAI	UP	Monothiol glutaredoxin-S9
Macma4_06_g26910	3.714014	2DAI	UP	GRAS domain-containing protein
Macma4_11_g19170	3.711173	2DAI	UP	hypothetical protein
Macma4_10_g20830	3.710879	2DAI	UP	hypothetical protein
Macma4_06_g01640	3.703659	2DAI	UP	hypothetical protein
Macma4_09_g11220	3.69461	2DAI	UP	putative Horcolin
Macma4_06_g40710	3.68883	2DAI	UP	Phospholipase A1-lbeta2%2C chloroplastic
Macma4_05_g00280	3.675605	2DAI	UP	putative protein PHLOEM PROTEIN 2-LIKE A1
Macma4_02_g06440	3.675202	1DAI	UP	Peroxidase 1
Macma4_01_g14800	3.672901	2DAI	UP	hypothetical protein
Macma4_11_g09540	3.6716	2DAI	UP	putative U-box domain-containing protein 21
Macma4_03_g12730	3.667864	2DAI	UP	hypothetical protein
Macma4_02_g04330	3.657349	1DAI	UP	AP2/ERF domain-containing protein
Macma4_06_g34330	3.657311	2DAI	UP	putative RING-H2 finger protein ATL3
Macma4_02_g08010	3.651971	2DAI	UP	putative B-box zinc finger protein 32
Macma4_01_g24450	3.648033	1DAI	UP	hypothetical protein
Macma4_11_g21380	3.647522	1DAI	UP	Ethylene-responsive transcription factor 1B
Macma4_06_g19010	3.642666	2DAI	UP	Arogenate dehydratase 3
Macma4_07_g20820	3.642157	2DAI	UP	conserved hypothetical protein
Macma4_04_g24110	3.640997	1DAI	UP	putative Chaperone protein dnaJ 20%2C chloroplastic
Macma4_03_g07690	3.638328	2DAI	UP	Nudix hydrolase 17%2C mitochondrial

Macma4_09_g05690	3.62644	2DAI	UP	hypothetical protein
Macma4_01_g14930	3.621014	2DAI	UP	conserved hypothetical protein
Macma4_10_g09250	3.619131	2DAI	UP	Calcium-binding protein CML42
Macma4_07_g21960	3.61436	1DAI	UP	Premnaspirodiene oxygenase
Macma4_01_g20190	3.614164	2DAI	UP	putative Ethylene-responsive transcription factor 4
Macma4_03_g32300	3.613096	2DAI	UP	hypothetical protein
Macma4_01_g21080	3.60507	2DAI	UP	Ethylene-responsive transcription factor ERF010
Macma4_06_g09060	3.603472	4DAI	UP	putative Monooxygenase 2
Macma4_03_g22540	3.601379	4DAI	UP	conserved hypothetical protein
Macma4_06_g14590	3.594298	2DAI	UP	1-aminocyclopropane-1-carboxylate oxidase
Macma4_09_g31120	3.593827	2DAI	UP	U-box domain-containing protein 17
Macma4_01_g03620	3.592718	2DAI	UP	C2 domain-containing protein
Macma4_05_g31800	3.592715	2DAI	UP	Protein kinase domain-containing protein
Macma4_04_g03970	3.589753	2DAI	UP	putative ribose-5-phosphate isomerase 2
Macma4_11_g17590	3.579296	2DAI	UP	Late embryogenesis abundant protein Lea5
Macma4_10_g28290	3.578321	2DAI	UP	Protein EXORDIUM
Macma4_09_g22180	3.578029	2DAI	UP	Oxalate--CoA ligase
Macma4_06_g14610	3.576455	2DAI	UP	1-aminocyclopropane-1-carboxylate oxidase
Macma4_09_g31980	3.574249	2DAI	UP	NDR1/HIN1-like protein 10
Macma4_09_g08880	3.573788	2DAI	UP	Stress-response A/B barrel domain-containing protein At5g22580
Macma4_03_g22540	3.572961	2DAI	UP	conserved hypothetical protein
Macma4_06_g25090	3.557721	2DAI	UP	conserved hypothetical protein
Macma4_04_g09620	3.547488	2DAI	UP	putative Heavy metal-associated isoprenylated plant protein 47
Macma4_03_g27040	3.525201	2DAI	UP	Ocs element-binding factor 1
Macma4_06_g36370	3.523059	2DAI	UP	Mitogen-activated protein kinase 5
Macma4_08_g16850	3.522391	2DAI	UP	Protein SAR DEFICIENT 1
Macma4_04_g26950	3.516454	2DAI	UP	putative Bowman-Birk type proteinase inhibitor
Macma4_04_g23730	3.51282	1DAI	UP	putative RING-H2 finger protein ATL80
Macma4_09_g01470	3.511855	2DAI	UP	conserved hypothetical protein
Macma4_10_g31460	3.507106	2DAI	UP	Cationic peroxidase 1
Macma4_08_g19310	3.50357	2DAI	UP	Mitogen-activated protein kinase kinase 7
Macma4_11_g05130	3.501506	2DAI	UP	hypothetical protein
Macma4_09_g11250	3.498132	2DAI	UP	putative Horcolin

Macma4_09_g12750	3.487377	2DAI	UP	putative 3-oxoacyl-[acyl-carrier-protein] reductase%2C chloroplastic
Macma4_06_g21160	3.486004	2DAI	UP	putative Transcription factor DIVARICATA
Macma4_03_g19300	3.482495	2DAI	UP	hypothetical protein
Macma4_09_g04460	3.482042	4DAI	UP	putative 2-oxoglutarate-dependent dioxygenase At5g05600
Macma4_04_g13610	3.480693	2DAI	UP	Protein C2-DOMAIN ABA-RELATED 11
Macma4_11_g17390	3.48007	2DAI	UP	Xyloglucan endotransglucosylase/hydrolase
Macma4_09_g11230	3.477546	1DAI	UP	putative Horcolin
Macma4_07_g21950	3.476275	2DAI	UP	putative Alpha-humulene 10-hydroxylase
Macma4_05_g02490	3.475341	2DAI	UP	putative F-box protein At5g46170
Macma4_04_g25720	3.470512	2DAI	UP	Calcium-binding protein KRP1
Macma4_02_g16390	3.470275	1DAI	UP	putative protein TIFY 5A
Macma4_05_g17130	3.469943	2DAI	UP	Thaumatococcus-like protein 1
Macma4_07_g19750	3.462259	1DAI	UP	conserved hypothetical protein
Macma4_05_g14850	3.460237	2DAI	UP	Transcription factor MYB4
Macma4_11_g01490	3.449313	1DAI	UP	hypothetical protein
Macma4_05_g12190	3.44393	2DAI	UP	NDR1/HIN1-like protein 6
Macma4_03_g11310	3.442366	2DAI	UP	Zinc finger A20 and AN1 domain-containing stress-associated protein 1
Macma4_07_g05370	3.439005	2DAI	UP	putative WRKY transcription factor WRKY71
Macma4_10_g31060	3.438083	2DAI	UP	hypothetical protein
Macma4_06_g09050	3.432923	2DAI	UP	putative protein ROH1
Macma4_10_g29350	3.432903	1DAI	UP	conserved hypothetical protein
Macma4_06_g25100	3.427084	2DAI	UP	hypothetical protein
Macma4_06_g11460	3.417626	2DAI	UP	Transcription factor MYB78
Macma4_04_g24300	3.416918	2DAI	UP	hypothetical protein
Macma4_04_g35230	3.41537	2DAI	UP	putative Brassinosteroid-responsive RING protein 1
Macma4_03_g01680	3.412246	2DAI	UP	putative E3 ubiquitin-protein ligase MPSR1
Macma4_01_g22550	3.409467	2DAI	UP	putative Zinc finger protein ZAT10
Macma4_08_g31910	3.407088	2DAI	UP	putative U-box domain-containing protein 27
Macma4_06_g00850	3.39757	2DAI	UP	LEA_2 domain-containing protein
Macma4_10_g14030	3.391379	2DAI	UP	conserved hypothetical protein
Macma4_02_g24330	3.386895	4DAI	UP	putative Cell wall / vacuolar inhibitor of fructosidase 2
Macma4_08_g28030	3.384069	2DAI	UP	Protein DETOXIFICATION 48
Macma4_03_g32110	3.382878	2DAI	UP	putative Ethylene-responsive transcription factor 9

Macma4_05_g10640	3.382435	2DAI	UP	putative Ethylene-responsive transcription factor ERF024
Macma4_02_g14050	3.373508	2DAI	UP	putative ripening-related protein 2
Macma4_09_g11240	3.371822	2DAI	UP	putative Horcolin
Macma4_01_g18300	3.362067	2DAI	UP	conserved hypothetical protein
Macma4_03_g15930	3.357736	2DAI	UP	Dehydration-responsive element-binding protein 1C
Macma4_09_g32030	3.351751	2DAI	UP	hypothetical protein
Macma4_07_g18350	3.34973	2DAI	UP	S-type anion channel SLAH1
Macma4_05_g11940	3.342689	2DAI	UP	RING-H2 finger protein ATL8
Macma4_08_g34950	3.342409	2DAI	UP	AP2/ERF domain-containing protein
Macma4_07_g13690	3.34198	2DAI	UP	putative F-box protein At4g22030
Macma4_05_g06540	3.333788	2DAI	UP	C2 NT-type domain-containing protein
Macma4_11_g20440	3.328823	2DAI	UP	putative CBS domain-containing protein CBSX5
Macma4_03_g07920	3.325149	1DAI	UP	Homeobox-leucine zipper protein HOX11
Macma4_01_g04360	3.323512	2DAI	UP	NB-ARC domain-containing protein
Macma4_05_g10120	3.321935	2DAI	UP	AP2/ERF and B3 domain-containing transcription repressor RAV2
Macma4_09_g20910	3.321188	2DAI	UP	hypothetical protein
Macma4_07_g12930	3.320127	2DAI	UP	Mitochondrial uncoupling protein 5
Macma4_08_g29550	3.315951	1DAI	UP	Glutamate synthase [NADH] ₂ C amyloplastic
Macma4_05_g22840	3.315146	1DAI	UP	putative Ethylene-responsive transcription factor 1B
Macma4_04_g41200	3.310963	2DAI	UP	hypothetical protein
Macma4_09_g16220	3.308311	2DAI	UP	Linoleate 13S-lipoxygenase 3-1 ₂ C chloroplastic
Macma4_08_g01350	3.301858	2DAI	UP	Transcription factor MYB94
Macma4_06_g14580	3.294796	2DAI	UP	1-aminocyclopropane-1-carboxylate oxidase
Macma4_10_g35070	3.293547	2DAI	UP	putative Exocyst complex component EXO70B1
Macma4_11_g03200	3.288442	1DAI	UP	Protein DETOXIFICATION 51
Macma4_08_g27870	3.287653	2DAI	UP	Transcription factor MTB1
Macma4_05_g22840	3.2836	2DAI	UP	putative Ethylene-responsive transcription factor 1B
Macma4_06_g26620	3.282305	1DAI	UP	AB hydrolase-1 domain-containing protein
Macma4_02_g04370	3.282249	2DAI	UP	LEA_2 domain-containing protein
Macma4_05_g23510	3.276964	2DAI	UP	bZIP transcription factor 29
Macma4_08_g19860	3.274258	1DAI	UP	putative protein PSK SIMULATOR 1
Macma4_05_g18860	3.27087	4DAI	UP	Chitinase 6
Macma4_10_g26600	3.267318	2DAI	UP	putative E3 ubiquitin-protein ligase RDUF1

Macma4_02_g22660	3.26629	4DAI	UP	18.2 kDa class I heat shock protein
Macma4_08_g08310	3.266205	2DAI	UP	RING-type domain-containing protein
Macma4_06_g34090	3.843051	1DAI	UP	Cytochrome P450 81Q32
Macma4_06_g38200	3.261821	1DAI	UP	Phytosulfokines 3
Macma4_02_g20960	3.261296	1DAI	UP	hypothetical protein
Macma4_04_g39580	3.260734	2DAI	UP	hypothetical protein
Macma4_02_g12060	3.260412	2DAI	UP	hypothetical protein
Macma4_03_g29610	3.259065	2DAI	UP	conserved hypothetical protein
Macma4_04_g30100	3.257666	2DAI	UP	putative Cyclin-dependent protein kinase inhibitor SMR4
Macma4_08_g22120	3.256927	1DAI	UP	putative Bowman-Birk type trypsin inhibitor
Macma4_04_g15430	3.255306	1DAI	UP	hypothetical protein
Macma4_07_g30720	3.247932	2DAI	UP	CBL-interacting protein kinase 6
Macma4_01_g07720	3.245448	1DAI	UP	hypothetical protein
Macma4_06_g38060	3.244077	2DAI	UP	Ankyrin repeat-containing protein ITN1
Macma4_03_g34550	3.243058	2DAI	UP	conserved hypothetical protein
Macma4_10_g24620	3.242737	2DAI	UP	Stilbene synthase 1
Macma4_09_g15670	3.242208	1DAI	UP	putative Auxin-responsive protein SAUR32
Macma4_06_g11380	3.234749	2DAI	UP	hypothetical protein
Macma4_01_g18900	3.231525	1DAI	UP	Linoleate 9S-lipoxygenase 6
Macma4_01_g15060	3.231502	2DAI	UP	Protein SAR DEFICIENT 1
Macma4_11_g13390	3.231366	2DAI	UP	hypothetical protein
Macma4_01_g12390	3.225602	2DAI	UP	conserved hypothetical protein
Macma4_08_g08590	3.22324	4DAI	UP	ABC transporter C family member 3
Macma4_05_g22840	3.221854	4DAI	UP	putative Ethylene-responsive transcription factor 1B
Macma4_02_g06900	3.218501	1DAI	UP	Chaperone protein dnaJ 11%2C chloroplastic
Macma4_05_g26000	3.218321	1DAI	UP	hypothetical protein
Macma4_10_g22160	3.217251	2DAI	UP	Cytochrome P450 81Q32
Macma4_09_g23920	3.213307	2DAI	UP	putative Mitogen-activated protein kinase kinase kinase 17
Macma4_05_g28240	3.212147	1DAI	UP	F-box/kelch-repeat protein At1g80440
Macma4_10_g14980	3.210497	2DAI	UP	Protein NRT1/ PTR FAMILY 4.3
Macma4_10_g32270	3.209771	2DAI	UP	hypothetical protein
Macma4_03_g20970	3.207504	2DAI	UP	Mitogen-activated protein kinase kinase kinase 18
Macma4_05_g33350	3.206958	2DAI	UP	Root phototropism protein 2

Macma4_05_g03210	3.203559	2DAI	UP	conserved hypothetical protein
Macma4_01_g26330	3.203227	2DAI	UP	hypothetical protein
Macma4_04_g02630	3.202676	2DAI	UP	NAC domain-containing protein 68
Macma4_09_g12960	3.201833	2DAI	UP	hypothetical protein
Macma4_05_g17770	3.199966	2DAI	UP	putative Cytochrome c1-2%2C heme protein%2C mitochondrial
Macma4_08_g20090	3.198664	2DAI	UP	putative Ethylene-responsive transcription factor 4
Macma4_07_g27510	3.198231	2DAI	UP	hypothetical protein
Macma4_10_g30510	3.197025	1DAI	UP	putative Zinc finger protein CONSTANS-LIKE 16
Macma4_04_g07910	3.19589	2DAI	UP	Alpha-terpineol synthase%2C chloroplastic
Macma4_03_g13230	3.195439	2DAI	UP	putative RPM1-interacting protein 4
Macma4_04_g35440	3.195118	1DAI	UP	Noroxomaritidine synthase
Macma4_09_g23380	3.191411	1DAI	UP	putative AP2/ERF and B3 domain-containing transcription factor RAV1
Macma4_09_g11260	3.190188	1DAI	UP	putative Horcolin
Macma4_07_g08650	3.189219	2DAI	UP	Acyl transferase 7
Macma4_07_g19250	3.189116	2DAI	UP	hypothetical protein
Macma4_09_g02860	3.18117	2DAI	UP	F-box domain-containing protein
Macma4_03_g34540	3.179053	1DAI	UP	hypothetical protein
Macma4_11_g09760	3.176033	1DAI	UP	Protein S-acyltransferase 8
Macma4_10_g23810	3.175088	2DAI	UP	putative calcium-binding protein CML18
Macma4_05_g04910	3.171132	2DAI	UP	O-fucosyltransferase family protein
Macma4_05_g18850	3.17104	4DAI	UP	Chitinase 6
Macma4_08_g24050	3.163443	2DAI	UP	Lipoxygenase 3%2C chloroplastic
Macma4_02_g16840	3.156376	2DAI	UP	Zinc finger protein 1
Macma4_06_g09530	3.1559	4DAI	UP	Basic blue protein
Macma4_11_g25310	3.155516	2DAI	UP	hypothetical protein
Macma4_04_g14220	3.154685	1DAI	UP	putative Transcription factor HRS1
Macma4_09_g11210	3.149198	1DAI	UP	putative Horcolin
Macma4_06_g01050	3.14867	2DAI	UP	AP2/ERF domain-containing protein
Macma4_07_g15080	3.145276	1DAI	UP	Protein NRT1/ PTR FAMILY 5.7
Macma4_08_g19160	3.132382	2DAI	UP	putative protein TIFY 10a
Macma4_05_g28010	3.130778	2DAI	UP	putative Ethylene-responsive transcription factor 2
Macma4_03_g01480	3.129931	2DAI	UP	putative Ethylene-responsive transcription factor RAP2-4
Macma4_03_g28340	3.125452	2DAI	UP	conserved hypothetical protein

Macma4_06_g34370	3.125111	2DAI	UP	putative protein TIFY 10b
Macma4_05_g11550	3.124399	2DAI	UP	hypothetical protein
Macma4_02_g12160	3.12345	4DAI	UP	Non-symbiotic hemoglobin 2
Macma4_09_g24850	3.123442	2DAI	UP	WRKY transcription factor WRKY51
Macma4_07_g25660	3.119931	1DAI	UP	hypothetical protein
Macma4_04_g24110	3.118157	2DAI	UP	putative Chaperone protein dnaJ 20%2C chloroplastic
Macma4_02_g08850	3.116557	1DAI	UP	hypothetical protein
Macma4_05_g26790	3.114824	2DAI	UP	hypothetical protein
Macma4_11_g13560	3.114189	1DAI	UP	hypothetical protein
Macma4_01_g06230	3.111945	2DAI	UP	hypothetical protein
Macma4_04_g25360	3.110653	2DAI	UP	hypothetical protein
Macma4_07_g00920	3.108622	2DAI	UP	conserved hypothetical protein
Macma4_08_g21520	3.107923	1DAI	UP	hypothetical protein
Macma4_06_g40010	3.107594	2DAI	UP	hypothetical protein
Macma4_03_g29710	3.106825	1DAI	UP	Molybdate transporter 1
Macma4_10_g04930	3.105547	2DAI	UP	hypothetical protein
Macma4_07_g28770	3.103722	1DAI	UP	Terpene synthase 10
Macma4_03_g29600	3.102108	2DAI	UP	Chitinase
Macma4_05_g00260	3.096606	2DAI	UP	putative Auxin-responsive protein SAUR71
Macma4_07_g09370	3.096186	1DAI	UP	Peroxidase 52
Macma4_09_g06170	3.085609	1DAI	UP	Aquaporin TIP4-4
Macma4_04_g15190	3.075175	2DAI	UP	hypothetical protein
Macma4_05_g11580	3.072118	1DAI	UP	conserved hypothetical protein
Macma4_01_g21300	3.069267	2DAI	UP	Acidic endochitinase
Macma4_04_g11960	3.066006	2DAI	UP	hypothetical protein
Macma4_06_g02820	3.064549	2DAI	UP	Absciscic acid receptor PYL2
Macma4_06_g14640	3.056832	2DAI	UP	1-aminocyclopropane-1-carboxylate oxidase
Macma4_08_g11440	3.053213	2DAI	UP	AAA-ATPase At2g46620
Macma4_02_g20630	3.049433	2DAI	UP	putative LRR receptor-like serine/threonine-protein kinase At2g16250
Macma4_07_g26810	3.041712	2DAI	UP	hypothetical protein
Macma4_03_g28540	3.039016	2DAI	UP	Aminotran_1_2 domain-containing protein
Macma4_09_g11230	3.037948	2DAI	UP	putative Horcolin
Macma4_11_g01110	3.034629	2DAI	UP	hypothetical protein

Macma4_06_g03600	3.030089	2DAI	UP	conserved hypothetical protein
Macma4_06_g14800	3.028077	2DAI	UP	conserved hypothetical protein
Macma4_08_g16030	3.024014	4DAI	UP	putative auxin efflux carrier component 9
Macma4_02_g16390	3.022714	4DAI	UP	putative protein TIFY 5A
Macma4_05_g30420	3.020654	1DAI	UP	putative Ethylene-responsive transcription factor 9
Macma4_06_g08730	3.019427	4DAI	UP	Amino acid transporter AVT1H
Macma4_10_g01310	3.019162	2DAI	UP	putative Calmodulin-binding protein 25
Macma4_11_g04340	3.019021	1DAI	UP	2-oxoglutarate-dependent dioxygenase 11
Macma4_06_g17680	3.018888	2DAI	UP	conserved hypothetical protein
Macma4_10_g29750	3.018783	2DAI	UP	Signaling peptide TAXIMIN 2
Macma4_11_g00520	3.016184	1DAI	UP	hypothetical protein
Macma4_04_g21250	3.011092	2DAI	UP	conserved hypothetical protein
Macma4_07_g15580	3.009907	2DAI	UP	LOB domain-containing protein 1
Macma4_02_g16730	3.009493	2DAI	UP	hypothetical protein
Macma4_01_g19560	3.00484	2DAI	UP	putative U-box domain-containing protein 35
Macma4_08_g28220	3.000265	2DAI	UP	hypothetical protein
Macma4_09_g11210	2.998107	2DAI	UP	putative Horcolin
Macma4_03_g29620	2.997822	4DAI	UP	conserved hypothetical protein
Macma4_06_g17460	2.997601	2DAI	UP	putative 2-oxoglutarate-dependent dioxygenase At3g111800
Macma4_05_g09760	2.997495	1DAI	UP	putative Bowman-Birk type proteinase inhibitor
Macma4_04_g07910	2.9974	1DAI	UP	Alpha-terpineol synthase%2C chloroplastic
Macma4_04_g18160	2.996725	2DAI	UP	hypothetical protein
Macma4_02_g14140	2.990939	2DAI	UP	Dihydroceramide fatty acyl 2-hydroxylase FAH1
Macma4_10_g18750	2.989958	1DAI	UP	putative Dynein 8 kDa light chain%2C flagellar outer arm
Macma4_03_g30210	2.984447	2DAI	UP	putative VQ domain-containing protein
Macma4_08_g07740	2.983838	4DAI	UP	AP2/ERF domain-containing protein
Macma4_02_g06980	2.981422	1DAI	UP	putative UPF0496 protein 2
Macma4_08_g03390	2.98	2DAI	UP	putative Ethylene-responsive transcription factor 4
Macma4_08_g06770	2.979869	1DAI	UP	conserved hypothetical protein
Macma4_11_g19910	2.979484	2DAI	UP	putative pectinesterase/pectinesterase inhibitor 41
Macma4_05_g18860	2.97542	2DAI	UP	Chitinase 6
Macma4_10_g06580	2.975189	1DAI	UP	hypothetical protein
Macma4_08_g25440	2.975035	2DAI	UP	Glycerol-3-phosphate dehydrogenase [NAD(+)] GPDHC1%2C cytosolic

Macma4_04_g04980	2.974333	1DAI	UP	putative Cocosin 1
Macma4_10_g32500	2.967294	2DAI	UP	hypothetical protein
Macma4_08_g01820	2.962771	2DAI	UP	hypothetical protein
Macma4_04_g39570	2.960755	1DAI	UP	hypothetical protein
Macma4_06_g29330	2.959211	2DAI	UP	conserved hypothetical protein
Macma4_03_g22440	2.956692	2DAI	UP	Patatin-like protein 2
Macma4_05_g00740	2.955804	2DAI	UP	Hexosyltransferase GAUT11
Macma4_06_g31310	2.953644	2DAI	UP	hypothetical protein
Macma4_03_g34100	2.950076	2DAI	UP	conserved hypothetical protein
Macma4_03_g32670	2.949525	2DAI	UP	conserved hypothetical protein
Macma4_04_g39450	2.946355	4DAI	UP	putative Zinc finger protein CONSTANS-LIKE 16
Macma4_10_g17470	2.94197	4DAI	UP	Cysteine-rich repeat secretory protein 55
Macma4_06_g03260	2.938713	1DAI	UP	AP2/ERF and B3 domain-containing transcription factor RAV1
Macma4_01_g17850	2.933413	1DAI	UP	S-type anion channel SLAH1
Macma4_11_g19580	2.928398	2DAI	UP	hypothetical protein
Macma4_11_g20260	2.928274	2DAI	UP	putative Ethylene-responsive transcription factor 2
Macma4_02_g12160	2.926947	2DAI	UP	Non-symbiotic hemoglobin 2
Macma4_11_g15640	2.925305	2DAI	UP	Transcription factor MYB78
Macma4_05_g31890	2.925015	2DAI	UP	Zinc finger CCCH domain-containing protein 33
Macma4_06_g34190	2.924564	2DAI	UP	conserved hypothetical protein
Macma4_05_g32850	2.923131	2DAI	UP	conserved hypothetical protein
Macma4_09_g06550	2.922536	2DAI	UP	Monothiol glutaredoxin-S5
Macma4_05_g12740	2.920531	2DAI	UP	hypothetical protein
Macma4_04_g18780	2.919025	4DAI	UP	Non-specific lipid-transfer protein Lac s 1
Macma4_07_g18100	2.9153	2DAI	UP	CBL-interacting protein kinase 1
Macma4_06_g16490	2.914624	2DAI	UP	hypothetical protein
Macma4_10_g14350	2.912047	2DAI	UP	putative U-box domain-containing protein 27
Macma4_06_g19820	2.91134	2DAI	UP	putative U-box domain-containing protein 27
Macma4_09_g12600	2.908963	2DAI	UP	hypothetical protein
Macma4_03_g02680	2.908145	2DAI	UP	1-aminocyclopropane-1-carboxylate oxidase
Macma4_05_g09040	2.90555	1DAI	UP	Cytochrome P450 78A5
Macma4_04_g22110	2.904049	2DAI	UP	AP2/ERF domain-containing protein
Macma4_11_g24760	2.904041	1DAI	UP	hypothetical protein

Macma4_04_g32580	2.903914	1DAI	UP	Oxygen-evolving enhancer protein 3-2%2C chloroplastic
Macma4_09_g03610	2.901913	4DAI	UP	hypothetical protein
Macma4_10_g28150	2.89927	2DAI	UP	conserved hypothetical protein
Macma4_06_g19440	2.891477	1DAI	UP	NAC domain-containing protein 2
Macma4_08_g24170	2.890144	2DAI	UP	putative Heavy metal-associated isoprenylated plant protein 14
Macma4_10_g28300	2.888629	2DAI	UP	Protein PHOSPHATE-INDUCED 1
Macma4_10_g22700	2.886421	2DAI	UP	putative calcium-binding protein CML47
Macma4_11_g17100	2.883886	2DAI	UP	MADS-box domain-containing protein
Macma4_04_g30040	2.880987	2DAI	UP	hypothetical protein
Macma4_01_g12760	2.880664	2DAI	UP	hypothetical protein
Macma4_05_g07440	2.879891	2DAI	UP	Protein-serine/threonine phosphatase
Macma4_04_g13910	2.877863	2DAI	UP	putative Mitogen-activated protein kinase kinase kinase 20
Macma4_07_g12660	2.876984	2DAI	UP	Protein PHOSPHATE-INDUCED 1
Macma4_07_g20710	2.876593	2DAI	UP	putative clathrin assembly protein At1g25240
Macma4_08_g11420	2.873652	2DAI	UP	hypothetical protein
Macma4_06_g02810	2.869478	2DAI	UP	Protein NDL1
Macma4_03_g26200	2.867753	2DAI	UP	hypothetical protein
Macma4_11_g21060	2.863291	2DAI	UP	putative Dof zinc finger protein 1
Macma4_06_g38980	2.863107	1DAI	UP	Amino acid transporter AVT1I
Macma4_03_g29610	2.862228	4DAI	UP	conserved hypothetical protein
Macma4_06_g06690	2.860773	1DAI	UP	putative protein ANTAGONIST OF LIKE HETEROCHROMATIN PROTEIN 1
Macma4_09_g08980	2.859872	2DAI	UP	putative Calmodulin-like protein 7
Macma4_07_g26250	2.85888	1DAI	UP	NAC domain-containing protein 48
Macma4_06_g01230	2.84379	2DAI	UP	peptide-N4-(N-acetyl-beta- glucosaminyI)asparagine amidase A
Macma4_03_g28540	2.84283	1DAI	UP	Aminotran_1_2 domain-containing protein
Macma4_11_g12690	2.84149	1DAI	UP	Transcription factor HHO3
Macma4_08_g22340	2.841073	1DAI	UP	ACT domain-containing protein ACR9
Macma4_03_g28600	2.839973	2DAI	UP	IQ domain-containing protein IQM1
Macma4_04_g28650	2.837648	2DAI	UP	hypothetical protein
Macma4_08_g03670	2.835704	2DAI	UP	hypothetical protein
Macma4_06_g16590	2.831813	2DAI	UP	Dehydration-responsive element-binding protein 3
Macma4_05_g29870	2.828926	2DAI	UP	hypothetical protein
Macma4_06_g16710	2.82669	1DAI	UP	hypothetical protein

Macma4_11_g09370	2.821336	2DAI	UP	Protein TIFY 10a
Macma4_09_g18450	2.82105	4DAI	UP	conserved hypothetical protein
Macma4_03_g17360	2.819847	2DAI	UP	hypothetical protein
Macma4_04_g14090	2.819124	1DAI	UP	putative ribose-5-phosphate isomerase 2
Macma4_10_g06410	2.818997	2DAI	UP	putative protein phosphatase 2C 2
Macma4_05_g16150	2.814832	2DAI	UP	putative Brassinosteroid-responsive RING protein 1
Macma4_04_g20430	2.81452	2DAI	UP	Transcription factor MYB15
Macma4_03_g08410	2.813187	4DAI	UP	Pathogenesis-related protein 1
Macma4_09_g04840	2.810639	2DAI	UP	Calcium-binding protein CML42
Macma4_10_g26190	2.807385	1DAI	UP	Aquaporin TIP2-1
Macma4_09_g06400	2.806436	2DAI	UP	Gibberellin 2-beta-dioxygenase 3
Macma4_10_g04930	2.803487	1DAI	UP	hypothetical protein
Macma4_04_g05760	2.792543	1DAI	UP	Ethylene-response factor C3
Macma4_06_g16120	2.791384	1DAI	UP	hypothetical protein
Macma4_06_g34090	2.787436	2DAI	UP	Cytochrome P450 81Q32
Macma4_09_g11200	2.786036	2DAI	UP	putative Horcolin
Macma4_03_g27960	2.784275	2DAI	UP	MADS-box transcription factor 18
Macma4_05_g18610	2.783879	2DAI	UP	hypothetical protein
Macma4_07_g13490	2.783859	2DAI	UP	Protein kinase domain-containing protein
Macma4_03_g02750	2.774301	1DAI	UP	Peroxidase
Macma4_10_g15870	2.77367	2DAI	UP	Glycerophosphodiester phosphodiesterase GDPD1%2C chloroplastic
Macma4_04_g12350	2.772098	1DAI	UP	hypothetical protein
Macma4_07_g22600	2.768405	2DAI	UP	putative xyloglucan glycosyltransferase 5
Macma4_06_g29990	2.76524	2DAI	UP	conserved hypothetical protein
Macma4_09_g12470	2.765091	2DAI	UP	conserved hypothetical protein
Macma4_08_g23260	2.764832	2DAI	UP	PsbP domain-containing protein 7%2C chloroplastic
Macma4_03_g25090	2.764775	2DAI	UP	conserved hypothetical protein
Macma4_07_g02730	2.760614	2DAI	UP	putative Cyclin-B1-2
Macma4_08_g29490	2.759991	4DAI	UP	Acidic endochitinase
Macma4_04_g07010	2.758699	1DAI	UP	hypothetical protein
Macma4_04_g05760	2.756075	2DAI	UP	Ethylene-response factor C3
Macma4_07_g03290	2.75564	1DAI	UP	conserved hypothetical protein
Macma4_03_g12420	2.754545	2DAI	UP	Mannan endo-1%2C4-beta-mannosidase 1

Macma4_10_g08320	2.7534	1DAI	UP	Nematode resistance protein-like HSPRO2
Macma4_07_g12670	2.752828	2DAI	UP	Protein PHOSPHATE-INDUCED 1
Macma4_02_g04330	2.748433	2DAI	UP	AP2/ERF domain-containing protein
Macma4_04_g26750	2.746713	1DAI	UP	putative WRKY transcription factor 22
Macma4_09_g01130	2.746297	2DAI	UP	GRAM domain-containing protein
Macma4_07_g08920	2.743907	1DAI	UP	putative Beta-galactosidase
Macma4_11_g09170	2.743355	1DAI	UP	putative Transcription factor DIVARICATA
Macma4_06_g34080	2.743218	1DAI	UP	Cytochrome P450 81Q32
Macma4_08_g01810	2.741851	2DAI	UP	putative Transcription factor MYB77
Macma4_07_g19180	2.733117	2DAI	UP	hypothetical protein
Macma4_09_g01730	2.725438	2DAI	UP	putative calcium-binding protein CML47
Macma4_01_g21150	2.72218	2DAI	UP	Transcription factor MYB73
Macma4_01_g04370	2.720673	2DAI	UP	DUF4220 domain-containing protein
Macma4_08_g15520	2.720277	1DAI	UP	Pentatricopeptide repeat-containing protein At1g31920
Macma4_07_g03290	2.717802	2DAI	UP	conserved hypothetical protein
Macma4_04_g02280	2.713962	2DAI	UP	hypothetical protein
Macma4_04_g14100	2.712969	1DAI	UP	putative ribose-5-phosphate isomerase 2
Macma4_05_g02420	2.709868	2DAI	UP	putative Phospholipase A1-Igamm2%2C chloroplastic
Macma4_09_g05700	2.709509	2DAI	UP	hypothetical protein
Macma4_03_g05110	2.708766	2DAI	UP	Xyloglucan endotransglucosylase/hydrolase
Macma4_09_g30650	2.708265	2DAI	UP	Transcription factor AS1
Macma4_06_g19720	2.705064	4DAI	UP	conserved hypothetical protein
Macma4_06_g12820	2.7045	2DAI	UP	putative protein phosphatase 2C 78
Macma4_03_g21080	2.70293	2DAI	UP	NAC domain-containing protein 2
Macma4_07_g25630	2.701581	2DAI	UP	conserved hypothetical protein
Macma4_03_g03580	2.697056	4DAI	UP	conserved hypothetical protein
Macma4_03_g24590	2.695934	2DAI	UP	Amino acid permease 2
Macma4_06_g17200	2.693536	2DAI	UP	putative Type I inositol polyphosphate 5-phosphatase 8
Macma4_02_g18580	2.692439	2DAI	UP	hypothetical protein
Macma4_05_g20960	2.689357	2DAI	UP	hypothetical protein
Macma4_08_g08590	2.687961	1DAI	UP	ABC transporter C family member 3
Macma4_09_g23340	2.686122	2DAI	UP	conserved hypothetical protein
Macma4_05_g02840	2.682157	4DAI	UP	hypothetical protein

Macma4_05_g08790	2.681626	2DAI	UP	hypothetical protein
Macma4_04_g27230	2.678915	2DAI	UP	hypothetical protein
Macma4_11_g23980	2.676434	1DAI	UP	Transcription factor MYB59
Macma4_05_g06260	2.676425	1DAI	UP	conserved hypothetical protein
Macma4_03_g29600	2.675691	4DAI	UP	Chitinase
Macma4_02_g25790	2.667327	2DAI	UP	hypothetical protein
Macma4_07_g04870	2.664359	2DAI	UP	Transcription factor MYC2
Macma4_11_g00370	2.663925	2DAI	UP	hypothetical protein
Macma4_09_g23270	2.662352	1DAI	UP	LOB domain-containing protein 41
Macma4_04_g14430	2.661679	1DAI	UP	hypothetical protein
Macma4_04_g12650	2.660606	2DAI	UP	Ethylene-response factor C3
Macma4_01_g23280	2.655481	1DAI	UP	SNF1-related protein kinase regulatory subunit beta-2
Macma4_02_g10120	2.6554	4DAI	UP	putative Pectinesterase inhibitor 9
Macma4_08_g11840	2.649152	2DAI	UP	hypothetical protein
Macma4_07_g06880	2.647026	2DAI	UP	L-lactate dehydrogenase A
Macma4_08_g14860	2.646129	1DAI	UP	hypothetical protein
Macma4_07_g08170	2.645112	2DAI	UP	hypothetical protein
Macma4_11_g25210	2.645092	2DAI	UP	Calcium-binding protein PBP1
Macma4_02_g25270	2.642019	1DAI	UP	Peroxidase 59
Macma4_05_g32830	2.639478	2DAI	UP	zinc finger protein ZAT11
Macma4_10_g25340	2.638489	1DAI	UP	DUF4005 domain-containing protein
Macma4_10_g28400	2.635908	2DAI	UP	hypothetical protein
Macma4_08_g33810	2.635251	2DAI	UP	hypothetical protein
Macma4_07_g16150	2.632909	2DAI	UP	hypothetical protein
Macma4_04_g07880	2.631913	1DAI	UP	(-)-alpha-terpineol synthase
Macma4_07_g06880	2.629471	4DAI	UP	L-lactate dehydrogenase A
Macma4_02_g16660	2.626004	1DAI	UP	Elongation of fatty acids protein 3-like
Macma4_04_g31710	2.623071	2DAI	UP	hypothetical protein
Macma4_08_g16030	2.622887	1DAI	UP	putative auxin efflux carrier component 9
Macma4_06_g11550	2.619719	2DAI	UP	putative LEAF RUST 10 DISEASE-RESISTANCE LOCUS RECEPTOR-LIKE PROTEIN KINASE-like 2.1
Macma4_06_g01180	2.618608	2DAI	UP	putative WRKY transcription factor 33
Macma4_02_g04790	2.618198	2DAI	UP	PNPLA domain-containing protein

Macma4_09_g06170	2.616847	2DAI	UP	Aquaporin TIP4-4
Macma4_11_g04340	2.615246	2DAI	UP	2-oxoglutarate-dependent dioxygenase 11
Macma4_03_g27040	2.614297	1DAI	UP	Ocs element-binding factor 1
Macma4_06_g20570	2.612417	2DAI	UP	hypothetical protein
Macma4_05_g11460	2.611919	2DAI	UP	putative PR5-like receptor kinase
Macma4_06_g22160	2.611007	2DAI	UP	Late embryogenesis abundant protein Lea5-D
Macma4_05_g11220	2.607457	1DAI	UP	2-hydroxyisoflavanone dehydratase
Macma4_02_g05980	2.605693	2DAI	UP	VQ domain-containing protein
Macma4_04_g02680	2.602116	2DAI	UP	hypothetical protein
Macma4_04_g05760	2.600752	4DAI	UP	Ethylene-response factor C3
Macma4_05_g24580	2.599668	2DAI	UP	hypothetical protein
Macma4_08_g29160	2.598614	2DAI	UP	putative Receptor-like protein kinase HSL1
Macma4_09_g32170	2.598061	1DAI	UP	Ferredoxin-3%2C chloroplastic
Macma4_03_g02690	2.595707	2DAI	UP	1-aminocyclopropane-1-carboxylate oxidase
Macma4_10_g22900	2.59388	2DAI	UP	conserved hypothetical protein
Macma4_09_g26070	2.588161	1DAI	UP	Plastocyanin
Macma4_10_g17930	2.586932	2DAI	UP	putative Ethylene-responsive transcription factor TINY
Macma4_06_g20630	2.585262	2DAI	UP	putative Early nodulin-like protein 1
Macma4_10_g23040	2.584495	1DAI	UP	AP2/ERF domain-containing protein
Macma4_02_g26240	2.584037	2DAI	UP	Ribulose biphosphate carboxylase small chain%2C chloroplastic
Macma4_01_g15870	2.583105	1DAI	UP	SPX domain-containing protein 5
Macma4_03_g35240	2.582841	2DAI	UP	conserved hypothetical protein
Macma4_09_g14290	2.580981	4DAI	UP	Cytochrome P450 CYP73A100
Macma4_01_g20920	2.580341	1DAI	UP	hypothetical protein
Macma4_04_g27920	2.580085	1DAI	UP	conserved hypothetical protein
Macma4_10_g30660	2.579178	1DAI	UP	putative EG45-like domain containing protein
Macma4_04_g24130	2.576927	2DAI	UP	hypothetical protein
Macma4_08_g11580	2.572914	1DAI	UP	Protein DETOXIFICATION 51
Macma4_06_g00510	2.572473	2DAI	UP	Delta(12)-acyl-lipid-desaturase
Macma4_04_g25520	2.56982	4DAI	UP	Glutathione S-transferase U18
Macma4_04_g15440	2.566296	2DAI	UP	putative protein ELC-like
Macma4_07_g12680	2.565895	2DAI	UP	Protein PHOSPHATE-INDUCED 1
Macma4_09_g00870	2.565785	4DAI	UP	Aluminum-activated malate transporter 10

Macma4_06_g29370	2.563169	2DAI	UP	putative Phosphatidylglycerophosphate phosphatase PTPMT1
Macma4_02_g19830	2.561984	1DAI	UP	putative leucine-rich repeat receptor-like protein kinase At2g19210
Macma4_05_g12740	2.561771	1DAI	UP	hypothetical protein
Macma4_05_g09040	2.561144	2DAI	UP	Cytochrome P450 78A5
Macma4_02_g07160	2.559768	1DAI	UP	hypothetical protein
Macma4_01_g21740	2.558917	2DAI	UP	hypothetical protein
Macma4_08_g09340	2.558896	2DAI	UP	putative calcium-binding protein CML36
Macma4_02_g02610	2.557914	2DAI	UP	conserved hypothetical protein
Macma4_05_g06590	2.557286	1DAI	UP	putative Transcription repressor MYB5
Macma4_10_g03350	2.556212	2DAI	UP	hypothetical protein
Macma4_06_g32640	2.550885	2DAI	UP	hypothetical protein
Macma4_03_g08680	2.550609	1DAI	UP	putative Cytochrome P450 709B2
Macma4_09_g03240	2.547887	2DAI	UP	conserved hypothetical protein
Macma4_05_g25610	2.544997	1DAI	UP	putative protein ANTAGONIST OF LIKE HETEROCHROMATIN PROTEIN 1
Macma4_07_g29270	2.544865	2DAI	UP	putative LOB domain-containing protein 37
Macma4_03_g33300	2.544162	2DAI	UP	hypothetical protein
Macma4_04_g11680	2.542541	1DAI	UP	putative Transcription factor bHLH92
Macma4_07_g22600	2.54214	1DAI	UP	putative xyloglucan glycosyltransferase 5
Macma4_05_g14920	2.542079	2DAI	UP	hypothetical protein
Macma4_10_g33860	2.539658	1DAI	UP	AT-hook motif nuclear-localized protein 17
Macma4_04_g16150	2.53899	2DAI	UP	putative Late embryogenesis abundant protein At1g64065
Macma4_09_g11260	2.538876	2DAI	UP	putative Horcolin
Macma4_01_g02990	2.537834	2DAI	UP	Glycosyltransferase family 92 protein
Macma4_02_g23700	2.53716	2DAI	UP	WRKY transcription factor 6
Macma4_11_g24760	2.535227	2DAI	UP	hypothetical protein
Macma4_04_g16320	2.533592	2DAI	UP	hypothetical protein
Macma4_09_g21760	2.533271	4DAI	UP	Tricetin 3'-O-trimethyltransferase
Macma4_03_g29160	2.532581	4DAI	UP	Protein trichome birefringence-like 1
Macma4_09_g30140	2.531398	1DAI	UP	Premnaspirodiene oxygenase
Macma4_04_g11960	2.530682	1DAI	UP	hypothetical protein
Macma4_10_g32330	2.528776	2DAI	UP	hypothetical protein
Macma4_04_g35940	2.528703	2DAI	UP	Salt tolerance receptor-like cytoplasmic kinase 1
Macma4_08_g23970	2.525365	2DAI	UP	putative Formin-like protein 8

Macma4_07_g12460	2.525046	2DAI	UP	Nudix hydrolase domain-containing protein
Macma4_08_g04850	2.523978	2DAI	UP	putative xyloglucan endotransglucosylase/hydrolase protein 7
Macma4_11_g21260	2.523817	2DAI	UP	putative Expansin-A4
Macma4_05_g30230	2.521872	1DAI	UP	putative germin-like protein 12-3
Macma4_09_g08520	2.520676	2DAI	UP	U-box domain-containing protein 25
Macma4_02_g06390	2.511713	2DAI	UP	hypothetical protein
Macma4_03_g20960	2.51105	2DAI	UP	putative U-box domain-containing protein 3
Macma4_03_g31010	2.509503	2DAI	UP	Cellulase
Macma4_02_g19990	2.509375	2DAI	UP	Heat shock factor protein HSF30
Macma4_10_g27000	2.508081	2DAI	UP	putative Trihelix transcription factor GT-3b
Macma4_10_g05630	2.506661	2DAI	UP	hypothetical protein
Macma4_04_g19660	2.50541	2DAI	UP	AAA-ATPase ASD%2C mitochondrial
Macma4_10_g35110	2.50452	2DAI	UP	hypothetical protein
Macma4_04_g24220	2.504082	2DAI	UP	putative Thaumatin-like protein 1
Macma4_10_g03310	2.503228	2DAI	UP	hypothetical protein
Macma4_05_g22090	2.500476	2DAI	UP	conserved hypothetical protein
Macma4_04_g41220	2.500362	2DAI	UP	Allene oxide synthase%2C chloroplastic
Macma4_05_g04900	2.498653	2DAI	UP	conserved hypothetical protein
Macma4_02_g15750	2.498487	2DAI	UP	Phosphoenolpyruvate carboxylase kinase 2
Macma4_10_g11450	2.497997	2DAI	UP	putative Chitin elicitor receptor kinase 1
Macma4_05_g00320	2.497143	2DAI	UP	Protein SRC2
Macma4_08_g21580	2.495474	1DAI	UP	putative Transcription factor bHLH162
Macma4_08_g16030	2.492424	2DAI	UP	putative auxin efflux carrier component 9
Macma4_03_g33040	2.491902	2DAI	UP	putative indole-3-acetic acid-amido synthetase GH3.8
Macma4_09_g06180	2.49006	1DAI	UP	hypothetical protein
Macma4_08_g09260	2.489629	2DAI	UP	putative U-box domain-containing protein 4
Macma4_03_g07770	2.486524	1DAI	UP	Alpha%2Calpha-trehalose-phosphate synthase [UDP-forming] 6
Macma4_06_g14560	2.485603	2DAI	UP	Amino acid permease 2
Macma4_04_g12350	2.48498	2DAI	UP	hypothetical protein
Macma4_04_g19660	2.481874	4DAI	UP	AAA-ATPase ASD%2C mitochondrial
Macma4_03_g02680	2.480855	4DAI	UP	1-aminocyclopropane-1-carboxylate oxidase
Macma4_10_g23730	2.478715	2DAI	UP	HTH myb-type domain-containing protein
Macma4_08_g24200	2.47758	2DAI	UP	conserved hypothetical protein

Macma4_02_g22860	2.476158	1DAI	UP	putative Transcription repressor MYB5
Macma4_05_g11940	2.476126	4DAI	UP	RING-H2 finger protein ATL8
Macma4_03_g07850	2.475268	4DAI	UP	Non-functional NADPH-dependent codeinone reductase 2
Macma4_04_g21230	2.472863	2DAI	UP	conserved hypothetical protein
Macma4_06_g31730	2.470931	1DAI	UP	conserved hypothetical protein
Macma4_04_g15760	2.47037	1DAI	UP	Absciscic acid receptor PYL7
Macma4_08_g01770	2.468119	1DAI	UP	putative protein LIGHT-DEPENDENT SHORT HYPOCOTYLS 5
Macma4_11_g01980	2.464638	2DAI	UP	putative RING-H2 finger protein ATL1
Macma4_09_g03130	2.461565	2DAI	UP	putative Exocyst complex component EXO70H1
Macma4_09_g27000	2.460773	2DAI	UP	Dof-type domain-containing protein
Macma4_06_g29970	2.460537	2DAI	UP	Monothiol glutaredoxin-S5
Macma4_04_g37500	2.459492	2DAI	UP	hypothetical protein
Macma4_04_g10710	2.459069	4DAI	UP	UDP-glycosyltransferase 88B1
Macma4_03_g33130	2.458852	2DAI	UP	UDP-glucose 6-dehydrogenase 4
Macma4_04_g24500	2.456557	1DAI	UP	putative Oxygen-evolving enhancer protein 3-1%2C chloroplastic
Macma4_02_g16620	2.456543	4DAI	UP	Pathogenesis-related protein 1C
Macma4_04_g28870	2.456489	2DAI	UP	Ethylene-responsive transcription factor ERF011
Macma4_03_g15650	2.456257	2DAI	UP	Receptor-like cytoplasmic kinase 176
Macma4_09_g26490	2.45441	2DAI	UP	MFS domain-containing protein
Macma4_09_g17890	2.453749	2DAI	UP	hypothetical protein
Macma4_06_g11520	2.453556	2DAI	UP	Calcium-binding protein CML24
Macma4_10_g20830	2.44696	1DAI	UP	hypothetical protein
Macma4_05_g02490	2.443138	1DAI	UP	putative F-box protein At5g46170
Macma4_10_g09430	2.442332	4DAI	UP	hypothetical protein
Macma4_02_g20700	2.441082	2DAI	UP	conserved hypothetical protein
Macma4_08_g01720	2.438815	4DAI	UP	hypothetical protein
Macma4_08_g14340	2.43687	2DAI	UP	putative protein PSK SIMULATOR 1
Macma4_04_g02820	2.436783	2DAI	UP	hypothetical protein
Macma4_03_g04330	2.432954	2DAI	UP	F-box/LRR-repeat protein 14
Macma4_05_g32750	2.431077	2DAI	UP	Protein phosphatase 2C 35
Macma4_06_g27140	2.430435	2DAI	UP	3Beta_HSD domain-containing protein
Macma4_06_g03740	2.42947	1DAI	UP	hypothetical protein
Macma4_05_g06260	2.425433	2DAI	UP	conserved hypothetical protein

Macma4_02_g10730	2.425	2DAI	UP	putative Ethylene-responsive transcription factor 1
Macma4_01_g17630	2.424985	1DAI	UP	putative Glutathione S-transferase U18
Macma4_09_g25990	2.423385	1DAI	UP	Geraniol 8-hydroxylase
Macma4_03_g13220	2.420785	2DAI	UP	Ethylene-responsive transcription factor 3
Macma4_01_g18520	2.416704	1DAI	UP	putative RING-H2 finger protein ATL2
Macma4_01_g21420	2.414261	2DAI	UP	G-type lectin S-receptor-like serine/threonine-protein kinase At2g19130
Macma4_03_g34980	2.414032	2DAI	UP	Ras-related protein RIC1
Macma4_07_g04060	2.410918	1DAI	UP	Serine/threonine-protein kinase STY46
Macma4_08_g00220	2.410363	2DAI	UP	putative EID1-like F-box protein 3
Macma4_02_g18590	2.408859	2DAI	UP	hypothetical protein
Macma4_10_g07360	2.408203	1DAI	UP	putative trehalose-phosphate phosphatase 6
Macma4_08_g22980	2.40718	2DAI	UP	putative Calmodulin-binding protein 25
Macma4_03_g22360	2.404701	1DAI	UP	conserved hypothetical protein
Macma4_03_g10060	2.402566	1DAI	UP	Abscisic acid receptor PYL4
Macma4_10_g15410	2.402083	2DAI	UP	putative N-acetyltransferase HLS1-like
Macma4_03_g22960	2.40004	2DAI	UP	hypothetical protein
Macma4_02_g16620	2.398406	1DAI	UP	Pathogenesis-related protein 1C
Macma4_02_g24050	2.394116	2DAI	UP	conserved hypothetical protein
Macma4_10_g06180	2.393007	4DAI	UP	Glucan endo-1%2C3-beta-glucosidase
Macma4_09_g28320	2.392774	1DAI	UP	PsbP domain-containing protein
Macma4_09_g11990	2.392018	1DAI	UP	putative protein DETOXIFICATION 40
Macma4_11_g04380	2.388507	2DAI	UP	2-oxoglutarate-dependent dioxygenase 11
Macma4_07_g11400	2.387177	1DAI	UP	AP2/ERF domain-containing protein
Macma4_06_g30040	2.386609	2DAI	UP	conserved hypothetical protein
Macma4_06_g38690	2.384796	2DAI	UP	putative Calmodulin-like protein 7
Macma4_10_g22190	2.383538	1DAI	UP	Cytochrome P450 81Q32
Macma4_11_g23440	2.382996	1DAI	UP	Nematode resistance protein-like HSPRO2
Macma4_11_g03940	2.382202	2DAI	UP	Remorin
Macma4_05_g16830	2.380749	2DAI	UP	putative Expansin-like A1
Macma4_04_g37480	2.379741	2DAI	UP	putative Disease resistance protein RPS5
Macma4_05_g25330	2.37962	2DAI	UP	hypothetical protein
Macma4_03_g31040	2.379438	2DAI	UP	Transcription factor MYB44
Macma4_03_g08580	2.378457	1DAI	UP	putative Polygalacturonase At1g48100

Macma4_05_g08830	2.375911	2DAI	UP	hypothetical protein
Macma4_03_g14100	2.373568	2DAI	UP	putative Gamma-glutamylcyclotransferase 2-2
Macma4_11_g15700	2.372654	2DAI	UP	AP2/ERF domain-containing protein
Macma4_01_g18510	2.372155	1DAI	UP	putative 60S ribosomal protein L19-2
Macma4_07_g07760	2.37111	1DAI	UP	putative bZIP transcription factor RISBZ2
Macma4_08_g08820	2.370578	1DAI	UP	Glycerophosphodiester phosphodiesterase
Macma4_01_g22540	2.368782	1DAI	UP	hypothetical protein
Macma4_11_g16940	2.366757	2DAI	UP	conserved hypothetical protein
Macma4_07_g15690	2.366512	2DAI	UP	hypothetical protein
Macma4_09_g09510	2.36439	2DAI	UP	Protein JINGUBANG
Macma4_04_g07010	2.362596	2DAI	UP	hypothetical protein
Macma4_06_g20640	2.355832	2DAI	UP	putative Early nodulin-like protein 1
Macma4_07_g09990	2.355693	1DAI	UP	Mitochondrial uncoupling protein 5
Macma4_09_g08760	2.347792	2DAI	UP	B3 domain-containing protein At3g11580
Macma4_07_g19900	2.347509	2DAI	UP	conserved hypothetical protein
Macma4_11_g03530	2.34707	2DAI	UP	putative B-box zinc finger protein 20
Macma4_03_g30210	2.34559	1DAI	UP	putative VQ domain-containing protein
Macma4_08_g26680	2.343141	4DAI	UP	putative WRKY transcription factor 46
Macma4_09_g21650	2.342494	4DAI	UP	Tricetin 3'-O-trimethyltransferase
Macma4_04_g15590	2.342292	2DAI	UP	putative WRKY transcription factor 28
Macma4_08_g18490	2.337648	2DAI	UP	hypothetical protein
Macma4_06_g02090	2.336394	2DAI	UP	AP2/ERF domain-containing protein
Macma4_10_g34090	2.335414	2DAI	UP	putative Ethylene-responsive transcription factor ERF060
Macma4_09_g00600	2.334684	2DAI	UP	conserved hypothetical protein
Macma4_05_g06390	2.331157	1DAI	UP	Protein kinase domain-containing protein
Macma4_10_g02460	2.330448	2DAI	UP	putative protein ELC-like
Macma4_07_g30600	2.330432	4DAI	UP	Sugar transport protein MST4
Macma4_05_g17740	2.328443	1DAI	UP	conserved hypothetical protein
Macma4_07_g25020	2.326438	2DAI	UP	CBL-interacting protein kinase 2
Macma4_08_g21580	2.323301	2DAI	UP	putative Transcription factor bHLH162
Macma4_11_g23790	2.323063	2DAI	UP	conserved hypothetical protein
Macma4_07_g24670	2.322844	2DAI	UP	NAC domain-containing protein 68
Macma4_07_g01100	2.322323	4DAI	UP	hypothetical protein

Macma4_04_g33320	2.319702	1DAI	UP	hypothetical protein
Macma4_04_g07680	2.316713	1DAI	UP	hypothetical protein
Macma4_10_g03230	2.31444	2DAI	UP	hypothetical protein
Macma4_09_g05680	2.313633	2DAI	UP	putative VQ motif-containing protein 19
Macma4_07_g30190	2.311958	2DAI	UP	hypothetical protein
Macma4_06_g20740	2.311332	1DAI	UP	putative Transcription factor bHLH112
Macma4_08_g22120	2.309396	2DAI	UP	putative Bowman-Birk type trypsin inhibitor
Macma4_07_g30120	2.30868	2DAI	UP	conserved hypothetical protein
Macma4_11_g04090	2.308462	2DAI	UP	putative Ankyrin repeat-containing protein BDA1
Macma4_01_g16100	2.308086	1DAI	UP	putative Non-functional NADPH-dependent codeinone reductase 2
Macma4_06_g30180	2.307974	2DAI	UP	hypothetical protein
Macma4_01_g19090	2.30699	1DAI	UP	hypothetical protein
Macma4_09_g23580	2.306895	1DAI	UP	hypothetical protein
Macma4_03_g28470	2.306584	2DAI	UP	ACT domain-containing protein ACR1
Macma4_10_g25310	2.305709	1DAI	UP	C3H1-type domain-containing protein
Macma4_06_g13760	2.305322	2DAI	UP	Adenine/guanine permease AZG2
Macma4_08_g26700	2.303977	2DAI	UP	U-box domain-containing protein 16
Macma4_03_g27760	2.302478	2DAI	UP	hypothetical protein
Macma4_07_g12500	2.30244	2DAI	UP	MYB-like transcription factor ODO1
Macma4_07_g26720	2.301173	1DAI	UP	Crocetin glucosyltransferase 2
Macma4_07_g26510	2.300856	2DAI	UP	conserved hypothetical protein
Macma4_01_g02850	2.300393	1DAI	UP	conserved hypothetical protein
Macma4_05_g14270	2.299912	1DAI	UP	hypothetical protein
Macma4_10_g03580	2.297679	2DAI	UP	hypothetical protein
Macma4_05_g11110	2.297136	1DAI	UP	hypothetical protein
Macma4_11_g19160	2.295631	2DAI	UP	hypothetical protein
Macma4_06_g34130	2.295055	2DAI	UP	conserved hypothetical protein
Macma4_10_g03530	2.294635	2DAI	UP	hypothetical protein
Macma4_05_g23620	2.291539	4DAI	UP	Peroxidase 5
Macma4_07_g08530	2.291254	2DAI	UP	3-ketoacyl-CoA synthase 11
Macma4_10_g14460	2.288696	2DAI	UP	conserved hypothetical protein
Macma4_04_g13200	2.287195	2DAI	UP	hypothetical protein
Macma4_05_g21250	2.287181	2DAI	UP	conserved hypothetical protein

Macma4_04_g21240	2.286772	2DAI	UP	conserved hypothetical protein
Macma4_10_g21900	2.283628	2DAI	UP	putative U-box domain-containing protein 20
Macma4_03_g31280	2.281745	1DAI	UP	conserved hypothetical protein
Macma4_03_g15260	2.278225	2DAI	UP	NDR1/HIN1-like protein 6
Macma4_09_g13860	2.277183	2DAI	UP	hypothetical protein
Macma4_06_g36130	2.276566	2DAI	UP	Zinc finger CCCH domain-containing protein 33
Macma4_09_g16730	2.276297	2DAI	UP	Transcription factor JAMYB
Macma4_09_g14750	2.274809	2DAI	UP	putative U-box domain-containing protein 19
Macma4_10_g02170	2.273674	2DAI	UP	Protein PLASTID TRANSCRIPTIONALLY ACTIVE 16%2C chloroplastic
Macma4_10_g33810	2.273562	2DAI	UP	hypothetical protein
Macma4_07_g28230	2.272138	1DAI	UP	putative Dynein 8 kDa light chain%2C flagellar outer arm
Macma4_10_g15210	2.270459	2DAI	UP	Arogenate dehydratase 3
Macma4_10_g22240	2.269887	2DAI	UP	conserved hypothetical protein
Macma4_03_g02690	2.269408	4DAI	UP	1-aminocyclopropane-1-carboxylate oxidase
Macma4_04_g08420	2.267513	2DAI	UP	(-)-alpha-terpineol synthase
Macma4_11_g15380	2.267233	2DAI	UP	Flowering-promoting factor 1-like protein 1
Macma4_03_g14190	2.266534	2DAI	UP	putative Trihelix transcription factor GT-3b
Macma4_08_g29320	2.266303	2DAI	UP	putative WRKY transcription factor 65
Macma4_11_g25310	2.264814	1DAI	UP	hypothetical protein
Macma4_10_g02580	2.263926	2DAI	UP	Ubiquitin-conjugating enzyme E2 10
Macma4_05_g23430	2.263015	1DAI	UP	Protein DETOXIFICATION 51
Macma4_04_g03940	2.262883	1DAI	UP	putative Transcription factor HHO3
Macma4_07_g00710	2.262384	1DAI	UP	conserved hypothetical protein
Macma4_07_g25170	2.262076	2DAI	UP	putative protein phosphatase 2C 73
Macma4_07_g13120	2.260573	2DAI	UP	Glucan endo-1%2C3-beta-D-glucosidase
Macma4_03_g06330	2.259822	1DAI	UP	putative alpha%2Calpha-trehalose-phosphate synthase [UDP-forming] 9
Macma4_01_g15390	2.259613	2DAI	UP	putative E3 ubiquitin-protein ligase ATL6
Macma4_05_g20560	2.258966	1DAI	UP	putative WRKY transcription factor WRKY71
Macma4_05_g32440	2.258382	2DAI	UP	Mannose-specific lectin 1
Macma4_10_g09590	2.256399	1DAI	UP	putative protein MKS1
Macma4_04_g03250	2.251685	1DAI	UP	Salicylic acid-binding protein 2
Macma4_08_g21580	2.251007	4DAI	UP	putative Transcription factor bHLH162
Macma4_06_g23390	2.250553	4DAI	UP	Endochitinase EP3

Macma4_08_g31920	2.249446	1DAI	UP	RING-H2 finger protein ATL78
Macma4_09_g07910	2.249215	1DAI	UP	putative F-box/kelch-repeat protein At2g44130
Macma4_01_g05440	2.247312	2DAI	UP	putative protein EARLY RESPONSIVE TO DEHYDRATION 15
Macma4_04_g19290	2.247017	2DAI	UP	hypothetical protein
Macma4_04_g18740	2.245299	2DAI	UP	putative Pathogenesis-related genes transcriptional activator PTI6
Macma4_07_g00660	2.245145	2DAI	UP	hypothetical protein
Macma4_03_g13600	2.244949	2DAI	UP	WRKY transcription factor WRKY28
Macma4_07_g12860	2.241296	2DAI	UP	FSH1 domain-containing protein
Macma4_04_g32590	2.240478	2DAI	UP	Cysteine-rich repeat secretory protein 38
Macma4_07_g15730	2.239753	2DAI	UP	Pectinesterase/pectinesterase inhibitor PPE8B
Macma4_07_g27510	2.238505	1DAI	UP	hypothetical protein
Macma4_04_g35510	2.237227	2DAI	UP	Kiwelin-1
Macma4_08_g21650	2.236714	1DAI	UP	putative RING-H2 finger protein ATL32
Macma4_01_g23290	2.236515	1DAI	UP	putative BAHD acyltransferase DCR
Macma4_09_g07690	2.236141	2DAI	UP	Serine/threonine-protein kinase OXI1
Macma4_06_g15320	2.23592	2DAI	UP	putative Mitogen-activated protein kinase kinase kinase 18
Macma4_02_g19820	2.235851	1DAI	UP	hypothetical protein
Macma4_02_g19630	2.234532	2DAI	UP	hypothetical protein
Macma4_10_g12290	2.233883	2DAI	UP	Arginine decarboxylase
Macma4_07_g18150	2.233745	2DAI	UP	hypothetical protein
Macma4_04_g34310	2.232435	1DAI	UP	Thaumatococcus-like protein 1
Macma4_05_g23280	2.232415	2DAI	UP	CEN-like protein 1
Macma4_11_g25310	2.230908	4DAI	UP	hypothetical protein
Macma4_04_g22110	2.229952	4DAI	UP	AP2/ERF domain-containing protein
Macma4_04_g07050	2.229224	2DAI	UP	hypothetical protein
Macma4_04_g21710	2.228118	2DAI	UP	VOC domain-containing protein
Macma4_02_g19730	2.225249	2DAI	UP	hypothetical protein
Macma4_11_g24010	2.224976	2DAI	UP	Zinc finger CCCH domain-containing protein 33
Macma4_11_g21260	2.224959	1DAI	UP	putative Expansin-A4
Macma4_06_g20740	2.224528	2DAI	UP	putative Transcription factor bHLH112
Macma4_09_g17820	2.224255	1DAI	UP	conserved hypothetical protein
Macma4_11_g01230	2.223511	1DAI	UP	NAC domain-containing protein 22
Macma4_02_g16650	2.219908	4DAI	UP	hypothetical protein

Macma4_10_g15510	2.218964	2DAI	UP	hypothetical protein
Macma4_04_g20200	2.218942	2DAI	UP	START domain-containing protein
Macma4_09_g30970	2.218833	1DAI	UP	putative Peroxidase 24
Macma4_05_g24390	2.216724	2DAI	UP	putative Ethylene-responsive transcription factor 2
Macma4_06_g31240	2.215503	2DAI	UP	putative Leucine-rich repeat receptor-like serine/threonine/tyrosine-protein kinase SOBIR1
Macma4_06_g26620	2.215039	2DAI	UP	AB hydrolase-1 domain-containing protein
Macma4_09_g33020	2.214155	1DAI	UP	4-hydroxyphenylpyruvate dioxygenase
Macma4_04_g29350	2.212794	2DAI	UP	hypothetical protein
Macma4_08_g35460	2.210257	2DAI	UP	conserved hypothetical protein
Macma4_07_g02340	2.208452	2DAI	UP	Serine/threonine-protein kinase RIPK
Macma4_02_g24720	2.208122	2DAI	UP	putative NAC domain-containing protein 83
Macma4_01_g16300	2.20739	1DAI	UP	Glutamine synthetase nodule isozyme
Macma4_10_g27360	2.205248	2DAI	UP	Xyloglucan endotransglucosylase/hydrolase protein 24
Macma4_02_g25120	2.203369	2DAI	UP	putative protein phosphatase 2C 78
Macma4_03_g21080	2.200439	1DAI	UP	NAC domain-containing protein 2
Macma4_09_g30140	2.200006	2DAI	UP	Premnaspirodiene oxygenase
Macma4_01_g26200	2.1967	2DAI	UP	AP2/ERF and B3 domain-containing transcription factor RAV1
Macma4_11_g11100	2.195693	2DAI	UP	conserved hypothetical protein
Macma4_01_g18500	2.194207	2DAI	UP	conserved hypothetical protein
Macma4_03_g21120	2.190586	2DAI	UP	Aspartic proteinase Asp1
Macma4_06_g39400	2.188298	1DAI	UP	conserved hypothetical protein
Macma4_03_g32180	2.188092	2DAI	UP	AP2/ERF and B3 domain-containing transcription repressor RAV2
Macma4_08_g30520	2.186978	1DAI	UP	hypothetical protein
Macma4_04_g08420	2.186736	1DAI	UP	(-)-alpha-terpineol synthase
Macma4_02_g26240	2.186679	1DAI	UP	Ribulose biphosphate carboxylase small chain%2C chloroplastic
Macma4_10_g06270	2.186542	1DAI	UP	conserved hypothetical protein
Macma4_04_g21230	2.186293	4DAI	UP	conserved hypothetical protein
Macma4_05_g23620	2.185795	2DAI	UP	Peroxidase 5
Macma4_07_g13510	2.184524	2DAI	UP	Protein kinase domain-containing protein
Macma4_03_g09390	2.183846	1DAI	UP	putative Sigma factor binding protein 1%2C chloroplastic
Macma4_03_g08590	2.183527	2DAI	UP	putative protein phosphatase 2C 2
Macma4_04_g04980	2.182832	2DAI	UP	putative Cocosin 1
Macma4_11_g21380	2.175611	4DAI	UP	Ethylene-responsive transcription factor 1B

Macma4_07_g06140	2.174106	1DAI	UP	Dehydration-responsive element-binding protein 1D
Macma4_06_g19220	2.172889	2DAI	UP	putative protein phosphatase 2C 77
Macma4_06_g34080	2.171245	2DAI	UP	Cytochrome P450 81Q32
Macma4_11_g01060	2.166268	2DAI	UP	NEP1-interacting protein 2
Macma4_06_g31300	2.166241	2DAI	UP	hypothetical protein
Macma4_01_g11020	2.165885	1DAI	UP	Metallothionein-like protein 4A
Macma4_11_g13880	2.16343	2DAI	UP	hypothetical protein
Macma4_02_g25270	2.163278	2DAI	UP	Peroxidase 59
Macma4_02_g07240	2.162779	2DAI	UP	hypothetical protein
Macma4_04_g24800	2.162202	1DAI	UP	Amino acid transporter AVT1C
Macma4_02_g16630	2.159979	4DAI	UP	hypothetical protein
Macma4_08_g27010	2.159919	2DAI	UP	Galactan beta-1%2C4-galactosyltransferase GALS1
Macma4_04_g23580	2.159112	2DAI	UP	conserved hypothetical protein
Macma4_07_g06270	2.158052	2DAI	UP	conserved hypothetical protein
Macma4_07_g07610	2.157185	2DAI	UP	Zinc finger AN1 and C2H2 domain-containing stress-associated protein 16
Macma4_11_g22850	2.153881	2DAI	UP	putative UDP-glycosyltransferase 73B3
Macma4_04_g05880	2.153428	1DAI	UP	conserved hypothetical protein
Macma4_06_g23170	2.15229	1DAI	UP	conserved hypothetical protein
Macma4_03_g26200	2.152087	4DAI	UP	hypothetical protein
Macma4_04_g09660	2.151758	2DAI	UP	Protein LYK5
Macma4_10_g17610	2.151465	2DAI	UP	hypothetical protein
Macma4_04_g16240	2.150177	2DAI	UP	S-type anion channel SLAH3
Macma4_02_g10060	2.149866	2DAI	UP	Heptahelical transmembrane protein 4
Macma4_06_g30880	2.148166	2DAI	UP	putative L-type lectin-domain containing receptor kinase IX.1
Macma4_03_g23340	2.147949	2DAI	UP	putative AP2/ERF and B3 domain-containing transcription repressor RAV2
Macma4_11_g15380	2.147695	4DAI	UP	Flowering-promoting factor 1-like protein 1
Macma4_06_g11200	2.145294	1DAI	UP	Auxin-induced protein 5NG4
Macma4_06_g10890	2.145108	1DAI	UP	hypothetical protein
Macma4_08_g06470	2.145088	4DAI	UP	hypothetical protein
Macma4_09_g02660	2.144259	2DAI	UP	conserved hypothetical protein
Macma4_11_g21000	2.143826	2DAI	UP	RHOMBOID-like protein 2
Macma4_11_g07340	2.142712	1DAI	UP	Myb-related protein 308
Macma4_02_g25610	2.14227	1DAI	UP	hypothetical protein

Macma4_05_g24740	2.141997	4DAI	UP	Ethylene-responsive transcription factor ERF071
Macma4_03_g13840	2.141952	2DAI	UP	Tetraspanin-8
Macma4_10_g02630	2.141511	1DAI	UP	Hydroquinone glucosyltransferase
Macma4_07_g09690	2.140478	2DAI	UP	putative WRKY transcription factor 50
Macma4_08_g02880	2.14	2DAI	UP	Syntaxin-124
Macma4_11_g25020	2.13951	1DAI	UP	NAC domain-containing protein 21/22
Macma4_10_g14100	2.139087	2DAI	UP	hypothetical protein
Macma4_09_g23470	2.133476	1DAI	UP	conserved hypothetical protein
Macma4_08_g01770	2.133238	4DAI	UP	putative protein LIGHT-DEPENDENT SHORT HYPOCOTYLS 5
Macma4_09_g29760	2.132553	1DAI	UP	conserved hypothetical protein
Macma4_10_g24430	2.131678	2DAI	UP	conserved hypothetical protein
Macma4_10_g26490	2.130705	2DAI	UP	Protein NRT1/ PTR FAMILY 5.7
Macma4_03_g08400	2.130504	4DAI	UP	Pathogenesis-related protein 1
Macma4_04_g15450	2.12914	1DAI	UP	conserved hypothetical protein
Macma4_08_g19850	2.12906	1DAI	UP	hypothetical protein
Macma4_02_g06030	2.125784	1DAI	UP	MADS-box transcription factor 29
Macma4_09_g04600	2.125325	2DAI	UP	hypothetical protein
Macma4_01_g23290	2.1249	2DAI	UP	putative BAHD acyltransferase DCR
Macma4_03_g19910	2.124647	2DAI	UP	Protein NRT1/ PTR FAMILY 8.1
Macma4_06_g13640	2.122906	2DAI	UP	hypothetical protein
Macma4_05_g04690	2.11971	2DAI	UP	NAD(P)-bd_dom domain-containing protein
Macma4_06_g23360	2.117227	2DAI	UP	hypothetical protein
Macma4_08_g34260	2.116792	1DAI	UP	Hevamine-A
Macma4_08_g22960	2.115808	2DAI	UP	Protein ETHYLENE-INSENSITIVE 3-like 1a
Macma4_07_g20980	2.11199	4DAI	UP	putative Auxin-responsive protein SAUR32
Macma4_01_g06100	2.111586	2DAI	UP	putative glycosyltransferase At3g07620
Macma4_06_g36180	2.111323	2DAI	UP	putative serine/threonine-protein kinase-like protein CCR3
Macma4_01_g15210	2.111023	4DAI	UP	Cytochrome P450 71A1
Macma4_04_g26440	2.110813	2DAI	UP	Calmodulin-like protein 8
Macma4_10_g30580	2.110261	2DAI	UP	EIN3-binding F-box protein 1
Macma4_04_g22960	2.108628	2DAI	UP	hypothetical protein
Macma4_04_g02650	2.107925	1DAI	UP	Protein LURP-one-related 8
Macma4_07_g09480	2.105866	1DAI	UP	hypothetical protein

Macma4_05_g30230	2.10512	2DAI	UP	putative germin-like protein 12-3
Macma4_08_g29440	2.103455	1DAI	UP	Acidic endochitinase
Macma4_10_g02170	2.102659	1DAI	UP	Protein PLASTID TRANSCRIPTIONALLY ACTIVE 16%2C chloroplastic
Macma4_08_g29480	2.10238	1DAI	UP	Acidic endochitinase
Macma4_05_g26860	2.101509	1DAI	UP	hypothetical protein
Macma4_04_g41830	2.101164	2DAI	UP	conserved hypothetical protein
Macma4_04_g14720	2.100945	2DAI	UP	LOB domain-containing protein 37
Macma4_04_g36040	2.100111	2DAI	UP	F-box protein PP2-A13
Macma4_02_g06050	2.1	2DAI	UP	hypothetical protein
Macma4_04_g33300	2.099716	1DAI	UP	hypothetical protein
Macma4_06_g19420	2.097314	1DAI	UP	Aldehyde dehydrogenase family 3 member H1
Macma4_02_g14460	2.092765	2DAI	UP	putative L-type lectin-domain containing receptor kinase S.5
Macma4_04_g18780	2.090896	2DAI	UP	Non-specific lipid-transfer protein Lac s 1
Macma4_04_g31850	2.090408	2DAI	UP	hypothetical protein
Macma4_08_g32450	2.085616	2DAI	UP	hypothetical protein
Macma4_10_g21820	2.084823	1DAI	UP	Type IV inositol polyphosphate 5-phosphatase 9
Macma4_06_g00130	2.081837	1DAI	UP	DNA-directed RNA polymerase subunit
Macma4_10_g28710	2.081182	1DAI	UP	hypothetical protein
Macma4_11_g19120	2.080754	2DAI	UP	conserved hypothetical protein
Macma4_11_g12690	2.080129	2DAI	UP	Transcription factor HHO3
Macma4_07_g03340	2.07946	2DAI	UP	putative protein MKS1
Macma4_06_g37760	2.078949	2DAI	UP	Scarecrow-like protein 9
Macma4_11_g15110	2.07752	2DAI	UP	putative Auxin-responsive protein SAUR50
Macma4_07_g13040	2.077211	1DAI	UP	EF-hand domain-containing protein
Macma4_07_g13170	2.075748	1DAI	UP	WAT1-related protein
Macma4_03_g01160	2.075219	2DAI	UP	hypothetical protein
Macma4_10_g02120	2.075015	2DAI	UP	hypothetical protein
Macma4_10_g02180	2.074748	2DAI	UP	conserved hypothetical protein
Macma4_11_g21260	2.073864	4DAI	UP	putative Expansin-A4
Macma4_09_g28390	2.073793	1DAI	UP	PSI-F
Macma4_01_g22830	2.073602	2DAI	UP	hypothetical protein
Macma4_07_g22700	2.073501	2DAI	UP	Metalloendoproteinase 2-MMP
Macma4_02_g16110	2.070972	2DAI	UP	putative Leucine-rich repeat extensin-like protein 6

Macma4_03_g12230	2.069732	2DAI	UP	putative UPF0481 protein At3g47200
Macma4_09_g29510	2.068813	2DAI	UP	Cell number regulator 10
Macma4_02_g16730	2.066461	1DAI	UP	hypothetical protein
Macma4_07_g22140	2.065612	2DAI	UP	Tetraspanin-8
Macma4_11_g02710	2.063084	2DAI	UP	Receptor-like protein kinase HERK 1
Macma4_09_g18300	2.061005	1DAI	UP	putative protein EARLY RESPONSIVE TO DEHYDRATION 15
Macma4_07_g22620	2.059862	1DAI	UP	Sucrose synthase 1
Macma4_04_g32580	2.05935	2DAI	UP	Oxygen-evolving enhancer protein 3-2%2C chloroplastic
Macma4_03_g32870	2.058451	2DAI	UP	hypothetical protein
Macma4_02_g21500	2.057816	1DAI	UP	hypothetical protein
Macma4_04_g32390	2.057168	2DAI	UP	Sugar transport protein MST3
Macma4_03_g13720	2.05569	1DAI	UP	conserved hypothetical protein
Macma4_07_g13350	2.055476	1DAI	UP	F-box/kelch-repeat protein At1g80440
Macma4_04_g03250	2.052511	2DAI	UP	Salicylic acid-binding protein 2
Macma4_06_g34470	2.05223	2DAI	UP	Syntaxin-124
Macma4_10_g10790	2.051235	2DAI	UP	Exocyst complex component EXO70B1
Macma4_05_g16170	2.049945	2DAI	UP	putative Scarecrow-like protein 8
Macma4_02_g19810	2.049798	1DAI	UP	putative LRR receptor-like serine/threonine-protein kinase At1g51860
Macma4_03_g22960	2.049011	1DAI	UP	hypothetical protein
Macma4_04_g08000	2.044728	2DAI	UP	conserved hypothetical protein
Macma4_05_g15560	2.042476	1DAI	UP	putative Auxin-responsive protein SAUR15
Macma4_04_g23880	2.040962	2DAI	UP	hypothetical protein
Macma4_09_g05560	2.040759	2DAI	UP	Protein BZR1
Macma4_04_g19590	2.038351	2DAI	UP	Galectin domain-containing protein
Macma4_11_g02940	2.038191	1DAI	UP	conserved hypothetical protein
Macma4_06_g35570	2.036987	1DAI	UP	NAC domain-containing protein 2
Macma4_01_g23280	2.035909	2DAI	UP	SNF1-related protein kinase regulatory subunit beta-2
Macma4_04_g01380	2.034397	2DAI	UP	putative LysM domain receptor-like kinase 4
Macma4_03_g14790	2.02542	2DAI	UP	putative RING-H2 finger protein ATL1
Macma4_10_g08770	2.024123	2DAI	UP	putative serine/threonine-protein kinase-like protein CCR3
Macma4_05_g11770	2.022763	1DAI	UP	hypothetical protein
Macma4_Mt_g01430	2.020558	1DAI	UP	hypothetical protein
Macma4_09_g14080	2.020034	2DAI	UP	hypothetical protein

Macma4_03_g18640	2.019827	1DAI	UP	Chloride channel protein CLC-b
Macma4_08_g33430	2.016721	2DAI	UP	putative glucose-6-phosphate 1-epimerase
Macma4_09_g14080	2.016538	1DAI	UP	hypothetical protein
Macma4_11_g24620	2.01592	1DAI	UP	putative bZIP transcription factor 53
Macma4_07_g01420	2.012685	2DAI	UP	Calmodulin-like protein 8
Macma4_02_g26360	2.005328	1DAI	UP	putative Vignain
Macma4_07_g18110	2.004252	2DAI	UP	putative Diacylglycerol O-acyltransferase 3
Macma4_06_g03000	2.00336	1DAI	UP	Ubiquinol oxidase 1a%2C mitochondrial
Macma4_11_g25870	2.001328	1DAI	UP	LOB domain-containing protein 6
Macma4_07_g13680	-2.00551	1DAI	DOWN	Inorganic pyrophosphatase 2
Macma4_07_g26000	-2.00588	1DAI	DOWN	conserved hypothetical protein
Macma4_06_g10310	-2.00868	2DAI	DOWN	putative COP9 signalosome complex subunit 2
Macma4_06_g15630	-2.01089	1DAI	DOWN	CASP-like protein 1C1
Macma4_01_g21760	-2.01365	1DAI	DOWN	Aspartic proteinase oryzasin-1
Macma4_06_g20660	-2.01923	2DAI	DOWN	putative Kinesin-like protein KIN-14H
Macma4_05_g03680	-2.0195	1DAI	DOWN	Patatin-like protein 7
Macma4_03_g06480	-2.02301	1DAI	DOWN	Peroxidase 11
Macma4_03_g02650	-2.02475	2DAI	DOWN	conserved hypothetical protein
Macma4_10_g21440	-2.03091	1DAI	DOWN	3-ketoacyl-CoA synthase 10
Macma4_03_g03150	-2.03099	1DAI	DOWN	3Beta_HSD domain-containing protein
Macma4_04_g10350	-2.03113	2DAI	DOWN	hypothetical protein
Macma4_05_g07850	-2.03129	1DAI	DOWN	Protein kinase domain-containing protein
Macma4_04_g09770	-2.0347	1DAI	DOWN	GDSL esterase/lipase At2g04570
Macma4_09_g04860	-2.0382	1DAI	DOWN	putative 2%2C3-bisphosphoglycerate-independent phosphoglycerate mutase
Macma4_04_g22940	-2.05574	1DAI	DOWN	conserved hypothetical protein
Macma4_03_g10480	-2.05612	1DAI	DOWN	hypothetical protein
Macma4_01_g13900	-2.05633	2DAI	DOWN	conserved hypothetical protein
Macma4_04_g31640	-2.05661	1DAI	DOWN	putative Pathogenesis-related protein 1
Macma4_04_g06430	-2.05887	1DAI	DOWN	Protein trichome birefringence-like 19
Macma4_03_g19010	-2.06367	1DAI	DOWN	Very-long-chain 3-oxoacyl-CoA reductase 1
Macma4_02_g24240	-2.06591	4DAI	DOWN	conserved hypothetical protein
Macma4_04_g37170	-2.06591	1DAI	DOWN	Leucine-rich repeat receptor-like serine/threonine-protein kinase At1g17230
Macma4_07_g08620	-2.0664	1DAI	DOWN	putative ABC transporter G family member 31

Macma4_10_g30310	-2.07201	1DAI	DOWN	hypothetical protein
Macma4_06_g10700	-2.07323	1DAI	DOWN	conserved hypothetical protein
Macma4_06_g00740	-2.07447	1DAI	DOWN	Glucan endo-1%2C3-beta-glucosidase
Macma4_08_g35860	-2.07542	1DAI	DOWN	LEA_2 domain-containing protein
Macma4_03_g03040	-2.07648	1DAI	DOWN	CASP-like protein
Macma4_06_g15580	-2.07794	1DAI	DOWN	hypothetical protein
Macma4_06_g02150	-2.07852	2DAI	DOWN	conserved hypothetical protein
Macma4_09_g04910	-2.08436	1DAI	DOWN	NAC domain-containing protein 1
Macma4_06_g04550	-2.08507	1DAI	DOWN	GDSL esterase/lipase At1g74460
Macma4_09_g18850	-2.08921	1DAI	DOWN	Chaperonin 60 subunit beta 4%2C chloroplastic
Macma4_06_g21090	-2.09318	1DAI	DOWN	hypothetical protein
Macma4_01_g00010	-2.09564	2DAI	DOWN	conserved hypothetical protein
Macma4_03_g03970	-2.09948	1DAI	DOWN	Hexosyltransferase
Macma4_07_g18390	-2.10211	4DAI	DOWN	hypothetical protein
Macma4_01_g05780	-2.11073	1DAI	DOWN	Glucose-1-phosphate adenylyltransferase large subunit 1%2C chloroplastic
Macma4_01_g04970	-2.11155	2DAI	DOWN	conserved hypothetical protein
Macma4_05_g30490	-2.11619	1DAI	DOWN	hypothetical protein
Macma4_07_g18390	-2.1209	2DAI	DOWN	hypothetical protein
Macma4_08_g33070	-2.13229	1DAI	DOWN	conserved hypothetical protein
Macma4_08_g32340	-2.13357	1DAI	DOWN	3-ketoacyl-CoA synthase 6
Macma4_01_g24620	-2.13673	1DAI	DOWN	hypothetical protein
Macma4_04_g25470	-2.13843	1DAI	DOWN	putative lysophospholipase BODYGUARD 3
Macma4_04_g23560	-2.13945	1DAI	DOWN	Cytochrome P450 86B1
Macma4_07_g29110	-2.14747	1DAI	DOWN	Nucleobase-ascorbate transporter LPE1
Macma4_03_g10470	-2.15285	1DAI	DOWN	hypothetical protein
Macma4_09_g27830	-2.15397	1DAI	DOWN	AAI domain-containing protein
Macma4_02_g25170	-2.15691	1DAI	DOWN	3-ketoacyl-CoA synthase 6
Macma4_04_g34050	-2.1642	1DAI	DOWN	conserved hypothetical protein
Macma4_04_g02130	-2.16584	2DAI	DOWN	putative WRKY transcription factor WRKY76
Macma4_06_g04940	-2.1718	1DAI	DOWN	putative F-box protein At4g18380
Macma4_04_g03120	-2.17203	1DAI	DOWN	3-ketoacyl-CoA synthase 11
Macma4_10_g20920	-2.17321	1DAI	DOWN	Omega-hydroxypalmitate O-feruloyl transferase
Macma4_10_g18410	-2.17445	1DAI	DOWN	hypothetical protein

Macma4_10_g19020	-2.17724	4DAI	DOWN	hypothetical protein
Macma4_10_g33570	-2.18015	1DAI	DOWN	Actin
Macma4_09_g18600	-2.18192	1DAI	DOWN	Metalloendoproteinase 2-MMP
Macma4_09_g15930	-2.19092	1DAI	DOWN	putative Transcription factor MYB52
Macma4_07_g06620	-2.19439	1DAI	DOWN	hypothetical protein
Macma4_01_g24320	-2.19493	1DAI	DOWN	Laccase-3
Macma4_01_g12170	-2.19681	1DAI	DOWN	ABC transporter B family member 15
Macma4_08_g30420	-2.19682	1DAI	DOWN	hypothetical protein
Macma4_01_g10370	-2.20444	1DAI	DOWN	putative Zinc finger protein WIP2
Macma4_11_g02730	-2.20953	1DAI	DOWN	hypothetical protein
Macma4_09_g04470	-2.22225	1DAI	DOWN	hypothetical protein
Macma4_03_g01640	-2.22567	1DAI	DOWN	WAT1-related protein At1g21890
Macma4_03_g04390	-2.22606	1DAI	DOWN	Plasma membrane ATPase
Macma4_04_g34950	-2.22646	4DAI	DOWN	hypothetical protein
Macma4_11_g09960	-2.22905	1DAI	DOWN	Omega-hydroxypalmitate O-feruloyl transferase
Macma4_06_g29250	-2.23325	2DAI	DOWN	Carotenoid cleavage dioxygenase 8%2C chloroplastic
Macma4_10_g27420	-2.23355	1DAI	DOWN	hypothetical protein
Macma4_10_g22100	-2.23433	1DAI	DOWN	conserved hypothetical protein
Macma4_05_g02880	-2.23453	1DAI	DOWN	putative Non-specific lipid transfer protein GPI-anchored 21
Macma4_11_g09700	-2.23661	1DAI	DOWN	GDGL esterase/lipase At1g28570
Macma4_11_g19760	-2.23681	1DAI	DOWN	GDGL esterase/lipase At2g23540
Macma4_05_g16370	-2.24666	1DAI	DOWN	putative Uclacyanin 1
Macma4_05_g22930	-2.25712	2DAI	DOWN	Kinesin-like protein KIN-UA
Macma4_04_g28200	-2.27092	1DAI	DOWN	conserved hypothetical protein
Macma4_10_g21060	-2.27555	1DAI	DOWN	hypothetical protein
Macma4_10_g24740	-2.27781	1DAI	DOWN	TLC domain-containing protein
Macma4_03_g13240	-2.27831	1DAI	DOWN	GDGL esterase/lipase At4g26790
Macma4_09_g22110	-2.28172	1DAI	DOWN	Xyloglucan endotransglucosylase/hydrolase protein 31
Macma4_09_g14820	-2.28184	1DAI	DOWN	Peroxidase 11
Macma4_03_g08470	-2.28318	1DAI	DOWN	hypothetical protein
Macma4_02_g14870	-2.2849	1DAI	DOWN	Cytochrome P450 86B1
Macma4_11_g22730	-2.2855	1DAI	DOWN	conserved hypothetical protein
Macma4_09_g20150	-2.29001	1DAI	DOWN	Xyloglucan endotransglucosylase/hydrolase protein 31

Macma4_02_g18340	-2.29015	2DAI	DOWN	conserved hypothetical protein
Macma4_03_g25350	-2.29495	1DAI	DOWN	CEN-like protein 2
Macma4_10_g06280	-2.30179	1DAI	DOWN	Hydroxyproline O-arabinosyltransferase PLENTY
Macma4_08_g00470	-2.30622	1DAI	DOWN	conserved hypothetical protein
Macma4_09_g15070	-2.32244	1DAI	DOWN	conserved hypothetical protein
Macma4_06_g39250	-2.32599	1DAI	DOWN	Laccase-3
Macma4_09_g25750	-2.32758	1DAI	DOWN	Lignin-forming anionic peroxidase
Macma4_05_g33250	-2.3279	1DAI	DOWN	Peroxidase
Macma4_04_g31210	-2.32813	1DAI	DOWN	hypothetical protein
Macma4_03_g21840	-2.34477	2DAI	DOWN	hypothetical protein
Macma4_08_g14640	-2.35127	1DAI	DOWN	Heat shock protein 83
Macma4_03_g08450	-2.35693	1DAI	DOWN	hypothetical protein
Macma4_03_g35330	-2.35815	2DAI	DOWN	hypothetical protein
Macma4_04_g35410	-2.36141	1DAI	DOWN	hypothetical protein
Macma4_05_g19000	-2.36239	1DAI	DOWN	putative Transcription factor E2FB
Macma4_04_g13790	-2.37751	1DAI	DOWN	High-affinity nitrate transporter 2.3
Macma4_09_g21710	-2.37759	4DAI	DOWN	Tricetin 3'-O-trimethyltransferase
Macma4_07_g12040	-2.38802	4DAI	DOWN	Dirigent protein
Macma4_07_g12040	-2.39408	1DAI	DOWN	Dirigent protein
Macma4_11_g10320	-2.39528	1DAI	DOWN	putative serine protease EDA2
Macma4_06_g37560	-2.40438	1DAI	DOWN	Laccase-25
Macma4_06_g24540	-2.41025	1DAI	DOWN	putative WRKY transcription factor 43
Macma4_05_g02960	-2.42718	1DAI	DOWN	hypothetical protein
Macma4_10_g31380	-2.43151	1DAI	DOWN	conserved hypothetical protein
Macma4_10_g34030	-2.45341	1DAI	DOWN	Heat shock 22 kDa protein%2C mitochondrial
Macma4_08_g19940	-2.45774	1DAI	DOWN	Plastidic glucose transporter 4
Macma4_06_g25290	-2.45948	2DAI	DOWN	putative Homeobox-DDT domain protein RLT3
Macma4_01_g02520	-2.46061	1DAI	DOWN	Xyloglucan endotransglucosylase/hydrolase
Macma4_09_g20030	-2.47654	2DAI	DOWN	Alpha-humulene synthase
Macma4_04_g26760	-2.48153	1DAI	DOWN	putative WRKY transcription factor 43
Macma4_03_g09470	-2.51311	2DAI	DOWN	putative RINT1-like protein MAG2L
Macma4_10_g30630	-2.51438	1DAI	DOWN	Ethylene-responsive transcription factor WIN1
Macma4_01_g21850	-2.51869	1DAI	DOWN	Heat stress transcription factor A-2c

Macma4_09_g06760	-2.522	1DAI	DOWN	Glucose-1-phosphate adenylyltransferase large subunit 2%2C chloroplastic
Macma4_06_g22620	-2.52728	1DAI	DOWN	hypothetical protein
Macma4_10_g34570	-2.54236	2DAI	DOWN	putative (S)-beta-bisabolene synthase
Macma4_09_g33410	-2.54891	2DAI	DOWN	hypothetical protein
Macma4_02_g22630	-2.55199	1DAI	DOWN	Mannose-1-phosphate guanylyltransferase 1
Macma4_09_g00500	-2.56842	1DAI	DOWN	putative 17.7 kDa class I heat shock protein
Macma4_10_g26790	-2.57876	1DAI	DOWN	Heat shock cognate 70 kDa protein
Macma4_09_g18790	-2.58159	1DAI	DOWN	hypothetical protein
Macma4_10_g30300	-2.58554	1DAI	DOWN	putative ADP-ribosylation factor GTPase-activating protein AGD6
Macma4_05_g04670	-2.58664	1DAI	DOWN	AP2-like ethylene-responsive transcription factor At1g16060
Macma4_07_g04620	-2.58982	1DAI	DOWN	Protein DETOXIFICATION 34
Macma4_08_g21990	-2.59255	1DAI	DOWN	putative dynamin-related protein 4A
Macma4_04_g29860	-2.59291	1DAI	DOWN	putative Non-specific lipid transfer protein GPI-anchored 15
Macma4_07_g11950	-2.59593	1DAI	DOWN	Multicopper oxidase LPR1
Macma4_01_g04450	-2.59603	1DAI	DOWN	DUF4408 domain-containing protein
Macma4_07_g15710	-2.60664	2DAI	DOWN	putative Mitogen-activated protein kinase kinase kinase 18
Macma4_08_g07470	-2.61446	1DAI	DOWN	MCN1
Macma4_05_g24570	-2.61654	1DAI	DOWN	hypothetical protein
Macma4_01_g03790	-2.62131	1DAI	DOWN	ABC transporter G family member 45
Macma4_06_g27020	-2.66838	1DAI	DOWN	Heat stress transcription factor A-2c
Macma4_04_g19120	-2.6764	1DAI	DOWN	ABC transporter B family member 2
Macma4_03_g14640	-2.68632	1DAI	DOWN	putative Transcription factor MYB39
Macma4_07_g15050	-2.68833	1DAI	DOWN	SHSP domain-containing protein
Macma4_01_g07930	-2.69013	1DAI	DOWN	Beta-carotene isomerase D27%2C chloroplastic
Macma4_05_g33060	-2.7245	1DAI	DOWN	conserved hypothetical protein
Macma4_05_g02950	-2.74234	1DAI	DOWN	putative Perakine reductase
Macma4_03_g09870	-2.76967	1DAI	DOWN	hypothetical protein
Macma4_11_g14490	-2.81195	2DAI	DOWN	putative UDP-galactose transporter 1
Macma4_05_g28280	-2.8212	1DAI	DOWN	Polyadenylate-binding protein 5
Macma4_05_g33010	-2.82585	4DAI	DOWN	conserved hypothetical protein
Macma4_08_g11110	-2.84228	1DAI	DOWN	putative DUF3741 domain-containing protein
Macma4_04_g34000	-2.84645	1DAI	DOWN	Xyloglucan endotransglucosylase/hydrolase protein 31
Macma4_11_g04730	-2.87286	1DAI	DOWN	putative receptor-like protein kinase At2g42960

Macma4_07_g15040	-2.89901	1DAI	DOWN	Os03g0267100 protein
Macma4_06_g17540	-2.93097	2DAI	DOWN	AMP deaminase
Macma4_06_g30790	-2.95995	1DAI	DOWN	Peptidyl-prolyl cis-trans isomerase FKBP65
Macma4_07_g19350	-2.96503	1DAI	DOWN	hypothetical protein
Macma4_07_g28790	-2.986	2DAI	DOWN	(-)-alpha-terpineol synthase
Macma4_06_g20150	-2.991	2DAI	DOWN	hypothetical protein
Macma4_02_g15290	-3.01131	1DAI	DOWN	Heat stress transcription factor B-2a
Macma4_10_g16190	-3.04774	1DAI	DOWN	Chalcone synthase 2
Macma4_01_g07930	-3.04984	2DAI	DOWN	Beta-carotene isomerase D27%2C chloroplastic
Macma4_04_g20990	-3.05623	2DAI	DOWN	Peroxidase 60
Macma4_09_g06760	-3.06108	2DAI	DOWN	Glucose-1-phosphate adenylyltransferase large subunit 2%2C chloroplastic
Macma4_01_g25130	-3.06609	1DAI	DOWN	hypothetical protein
Macma4_01_g14440	-3.06887	1DAI	DOWN	conserved hypothetical protein
Macma4_07_g03200	-3.0873	2DAI	DOWN	Stearoyl-[acyl-carrier-protein] 9-desaturase 1%2C chloroplastic
Macma4_05_g02940	-3.09021	1DAI	DOWN	Heat stress transcription factor A-2b
Macma4_01_g01910	-3.09301	2DAI	DOWN	Transmembrane 9 superfamily member
Macma4_10_g34340	-3.11441	2DAI	DOWN	hypothetical protein
Macma4_11_g24060	-3.15798	1DAI	DOWN	Pyruvate decarboxylase 2
Macma4_06_g04000	-3.24786	1DAI	DOWN	putative Transcription factor bHLH94
Macma4_02_g06370	-3.27881	1DAI	DOWN	Transcription factor MYB102
Macma4_05_g09500	-3.30505	4DAI	DOWN	BURP domain protein RD22
Macma4_03_g01490	-3.35164	1DAI	DOWN	putative trehalose-phosphate phosphatase 6
Macma4_10_g15840	-3.45704	1DAI	DOWN	conserved hypothetical protein
Macma4_01_g24620	-3.49157	2DAI	DOWN	hypothetical protein
Macma4_04_g23330	-3.547	4DAI	DOWN	Expansin
Macma4_06_g11190	-3.69334	2DAI	DOWN	hypothetical protein
Macma4_06_g34200	-3.69566	1DAI	DOWN	putative AP2-like ethylene-responsive transcription factor At1g16060
Macma4_06_g29250	-3.7692	1DAI	DOWN	Carotenoid cleavage dioxygenase 8%2C chloroplastic
Macma4_09_g20030	-3.81321	1DAI	DOWN	Alpha-humulene synthase
Macma4_09_g21710	-3.89096	1DAI	DOWN	Tricetin 3'%2C4'%2C5'-O-trimethyltransferase
Macma4_09_g06800	-4.01966	1DAI	DOWN	hypothetical protein
Macma4_06_g04860	-4.17724	1DAI	DOWN	conserved hypothetical protein
Macma4_07_g28790	-4.28273	1DAI	DOWN	(-)-alpha-terpineol synthase

Macma4_07_g03200	-4.74242	1DAI	DOWN	Stearoyl-[acyl-carrier-protein] 9-desaturase 1%2C chloroplastic
Macma4_10_g03540	-5.2814	4DAI	DOWN	hypothetical protein
Macma4_06_g06370	-6.05301	1DAI	DOWN	Early nodulin-93
Macma4_05_g09500	-7.42973	1DAI	DOWN	BURP domain protein RD22

Supplementary table 2. Statistical analysis of RT-qPCR data. The table presents the mean $\Delta\Delta Ct$ values per treatment, the corresponding p-values from ANOVA, the standard error (SE) associated with the means, and the degrees of freedom (DF) for each gene analyzed for Calcutta 4 (C4) and Prata-anã (PA) 1, 2 and 4 days after inoculation (DAI) with *Fusarium oxysporum* f. sp. *cubense* STR4. The $\Delta\Delta Ct$ values were calculated relative to the control and normalized using appropriate reference genes.

Gene	Treatment	$\Delta\Delta Ct$	P-value	Standard error	DF
Acid endochitinase (Magma4_01_g21300)	C4 1DAI	-2.4925	0.1208	1.5495	24
Acid endochitinase (Magma4_01_g21300)	C4 2DAI	-0.8025	0.6093	1.5495	24
Acid endochitinase (Magma4_01_g21300)	C4 4DAI	0.1941	0.9014	1.5495	24
Acid endochitinase (Magma4_01_g21300)	PA 1DAI	2.5482	0.1131	1.5495	24
Acid endochitinase (Magma4_01_g21300)	PA 2DAI	1.4069	0.3729	1.5495	24
Acid endochitinase (Magma4_01_g21300)	PA 4DAI	3.9890	0.0166	1.5495	24
Leucine-rich repeat receptor-like serine/threonine/tyrosine-protein kinase SOBIR1 (Magma4_02_g09450)	C4 1DAI	-1.7845	0.0055	0.5845	24
Leucine-rich repeat receptor-like serine/threonine/tyrosine-protein kinase SOBIR1 (Magma4_02_g09450)	C4 2DAI	-4.8029	<.0001	0.5845	24
Leucine-rich repeat receptor-like serine/threonine/tyrosine-protein kinase SOBIR1 (Magma4_02_g09450)	C4 4DAI	-1.1575	0.0592	0.5845	24
Leucine-rich repeat receptor-like serine/threonine/tyrosine-protein kinase SOBIR1 (Magma4_02_g09450)	PA 1DAI	0.0522	0.9296	0.5845	24
Leucine-rich repeat receptor-like serine/threonine/tyrosine-protein kinase SOBIR1 (Magma4_02_g09450)	PA 2DAI	-0.1290	0.8272	0.5845	24
Leucine-rich repeat receptor-like serine/threonine/tyrosine-protein kinase SOBIR1 (Magma4_02_g09450)	PA 4DAI	-0.2353	0.6908	0.5845	24
Lipoxygenase 3-2C Chloroplastic (Magma4_03_g08040)	C4 1DAI	-2.6930	0.0083	0.9361	24
Lipoxygenase 3-2C Chloroplastic (Magma4_03_g08040)	C4 2DAI	-4.6510	0.9361	0.9361	24
Lipoxygenase 3-2C Chloroplastic (Magma4_03_g08040)	C4 4DAI	-0.5687	<.0001	0.9361	24
Lipoxygenase 3-2C Chloroplastic (Magma4_03_g08040)	PA 1DAI	-1.4608	0.5492	0.9361	24
Lipoxygenase 3-2C Chloroplastic (Magma4_03_g08040)	PA 2DAI	0.7100	0.4556	0.9361	24
Lipoxygenase 3-2C Chloroplastic (Magma4_03_g08040)	PA 4DAI	3.0033	0.0038	0.9361	24
Salicylic acid-binding protein 2 (Magma4_04_g03250)	C4 1DAI	-2.9541	0.0377	1.3428	24
Salicylic acid-binding protein 2 (Magma4_04_g03250)	C4 2DAI	-1.9651	0.1563	1.3428	24
Salicylic acid-binding protein 2 (Magma4_04_g03250)	C4 4DAI	-0.6108	0.6533	1.3428	24
Salicylic acid-binding protein 2 (Magma4_04_g03250)	PA 1DAI	-0.6404	0.6377	1.3428	24
Salicylic acid-binding protein 2 (Magma4_04_g03250)	PA 2DAI	0.1489	0.9126	1.3428	24

Salicylic acid-binding protein 2 (Macma4_04_g03250)	PA 4DAI	1.5468	0.2607	1.3428	24
putative Disease resistance protein RPS5 (Macma4_04_g37480)	C4 1DAI	-1.5950	0.0006	0.4047	24
putative Disease resistance protein RPS5 (Macma4_04_g37480)	C4 2DAI	-2.8971	<.0001	0.4047	24
putative Disease resistance protein RPS5 (Macma4_04_g37480)	C4 4DAI	-0.4050	0.3269	0.4047	24
putative Disease resistance protein RPS5 (Macma4_04_g37480)	PA 1DAI	-0.8543	0.0454	0.4047	24
putative Disease resistance protein RPS5 (Macma4_04_g37480)	PA 2DAI	-0.7701	0.0691	0.4047	24
putative Disease resistance protein RPS5 (Macma4_04_g37480)	PA 4DAI	-0.0204	0.9602	0.4047	24
Peroxidase 4 (Macma4_06_g16370)	C4 1DAI	-2.0261	0.0378	0.9076	19
Peroxidase 4 (Macma4_06_g16370)	C4 2DAI	-4.0639	0.0003	0.9076	19
Peroxidase 4 (Macma4_06_g16370)	C4 4DAI	0.7484	0.4198	0.9076	19
Peroxidase 4 (Macma4_06_g16370)	PA 1DAI	1.8085	0.0609	0.9076	19
Peroxidase 4 (Macma4_06_g16370)	PA 2DAI	0.1924	0.8431	0.9076	19
Peroxidase 4 (Macma4_06_g16370)	PA 4DAI	NA	NA	NA	NA
putative L-type lectin-domain containing receptor kinase IX.1 (Macma4_06_g30880)	C4 1DAI	-1.8941	0.0514	0.9240	24
putative L-type lectin-domain containing receptor kinase IX.1 (Macma4_06_g30880)	C4 2DAI	-1.6216	0.0920	0.9240	24
putative L-type lectin-domain containing receptor kinase IX.1 (Macma4_06_g30880)	C4 4DAI	0.7988	0.3959	0.9240	24
putative L-type lectin-domain containing receptor kinase IX.1 (Macma4_06_g30880)	PA 1DAI	0.5062	0.5889	0.9240	24
putative L-type lectin-domain containing receptor kinase IX.1 (Macma4_06_g30880)	PA 2DAI	1.6289	0.0906	0.9240	24
putative L-type lectin-domain containing receptor kinase IX.1 (Macma4_06_g30880)	PA 4DAI	-0.6900	0.4625	0.9240	24
Premnaspirodien oxygenase (Macma4_07_g21750)	C4 1DAI	-3.4524	0.0073	1.0714	12
Premnaspirodien oxygenase (Macma4_07_g21750)	C4 2DAI	-1.0712	0.3371	1.0714	12
Premnaspirodien oxygenase (Macma4_07_g21750)	C4 4DAI	1.8572	0.1086	1.0714	12
Premnaspirodien oxygenase (Macma4_07_g21750)	PA 1DAI	NA	NA	NA	NA
Premnaspirodien oxygenase (Macma4_07_g21750)	PA 2DAI	NA	NA	NA	NA
Premnaspirodien oxygenase (Macma4_07_g21750)	PA 4DAI	NA	NA	NA	NA
NDR1/HIN1-like protein 10 (Macma4_09_g31980)	C4 1DAI	-2.3838	0.0086	0.8329	24
NDR1/HIN1-like protein 10 (Macma4_09_g31980)	C4 2DAI	-4.5652	<.0001	0.8329	24
NDR1/HIN1-like protein 10 (Macma4_09_g31980)	C4 4DAI	-1.1301	0.1875	0.8329	24
NDR1/HIN1-like protein 10 (Macma4_09_g31980)	PA 1DAI	0.3558	0.6731	0.8329	24
NDR1/HIN1-like protein 10 (Macma4_09_g31980)	PA 2DAI	0.3580	0.6711	0.8329	24
NDR1/HIN1-like protein 10 (Macma4_09_g31980)	PA 4DAI	1.1372	0.1848	0.8329	24
Nematode resistance protein-like HSPRO2 (Macma4_10_g08320)	C4 1DAI	-3.9772	0.0001	0.8733	24
Nematode resistance protein-like HSPRO2 (Macma4_10_g08320)	C4 2DAI	-6.9753	<.0001	0.8733	24

Nematode resistance protein-like HSPRO2 (Macma4_10_g08320)	C4 4DAI	-0.6214	0.4836	0.8733	24
Nematode resistance protein-like HSPRO2 (Macma4_10_g08320)	PA 1DAI	1.0109	0.2584	0.8733	24
Nematode resistance protein-like HSPRO2 (Macma4_10_g08320)	PA 2DAI	0.0589	0.9468	0.8733	24
Nematode resistance protein-like HSPRO2 (Macma4_10_g08320)	PA 4DAI	-0.6498	0.4641	0.8733	24
Cysteine-rich repeat secretory protein 55 (Macma4_10_g17470)	C4 1DAI	-2.3427	0.1519	1.5829	24
Cysteine-rich repeat secretory protein 55 (Macma4_10_g17470)	C4 2DAI	-2.1273	0.1915	1.5829	24
Cysteine-rich repeat secretory protein 55 (Macma4_10_g17470)	C4 4DAI	-1.5934	0.3242	1.5829	24
Cysteine-rich repeat secretory protein 55 (Macma4_10_g17470)	PA 1DAI	2.2201	0.1735	1.5829	24
Cysteine-rich repeat secretory protein 55 (Macma4_10_g17470)	PA 2DAI	-1.6347	0.3120	1.5829	24
Cysteine-rich repeat secretory protein 55 (Macma4_10_g17470)	PA 4DAI	-0.5457	0.7333	1.5829	24
Stilbene synthase 1 (Macma4_10_g24620)	C4 1DAI	-1.9008	0.0086	0.5498	8
Stilbene synthase 1 (Macma4_10_g24620)	C4 2DAI	-3.6496	0.0002	0.5498	8
Stilbene synthase 1 (Macma4_10_g24620)	C4 4DAI	NA	NA	NA	NA
Stilbene synthase 1 (Macma4_10_g24620)	PA 1DAI	NA	NA	NA	NA
Stilbene synthase 1 (Macma4_10_g24620)	PA 2DAI	NA	NA	NA	NA
Stilbene synthase 1 (Macma4_10_g24620)	PA 4DAI	NA	NA	NA	NA
Protein SAR DEFICIENT 1 (Macma4_08_g16850)	C4 1DAI	-2.7027	0.0016	0.7613	24
Protein SAR DEFICIENT 1 (Macma4_08_g16850)	C4 2DAI	-4.1904	<.0001	0.7613	24
Protein SAR DEFICIENT 1 (Macma4_08_g16850)	C4 4DAI	-0.8587	0.2705	0.7613	24
Protein SAR DEFICIENT 1 (Macma4_08_g16850)	PA 1DAI	-0.3113	0.6862	0.7613	24
Protein SAR DEFICIENT 1 (Macma4_08_g16850)	PA 2DAI	-0.3778	0.6242	0.7613	24
Protein SAR DEFICIENT 1 (Macma4_08_g16850)	PA 4DAI	1.7596	0.0297	0.7613	24
Pathogenesis-related protein 1 (Macma4_03_g08420)	C4 1DAI	-3.0373	0.0483	1.4597	24
Pathogenesis-related protein 1 (Macma4_03_g08420)	C4 2DAI	-1.1180	0.4512	1.4597	24
Pathogenesis-related protein 1 (Macma4_03_g08420)	C4 4DAI	-1.0386	0.4836	1.4597	24
Pathogenesis-related protein 1 (Macma4_03_g08420)	PA 1DAI	-0.7857	0.5954	1.4597	24
Pathogenesis-related protein 1 (Macma4_03_g08420)	PA 2DAI	-1.6211	0.2778	1.4597	24
Pathogenesis-related protein 1 (Macma4_03_g08420)	PA 4DAI	0.9065	0.5405	1.4597	24
Thaumatococcus-like protein 1 (Macma4_05_g17130)	C4 1DAI	-4.7951	<.0001	0.5971	24
Thaumatococcus-like protein 1 (Macma4_05_g17130)	C4 2DAI	-4.2196	<.0001	0.5971	24
Thaumatococcus-like protein 1 (Macma4_05_g17130)	C4 4DAI	-1.3750	0.0303	0.5971	24
Thaumatococcus-like protein 1 (Macma4_05_g17130)	PA 1DAI	0.8645	0.1606	0.5971	24
Thaumatococcus-like protein 1 (Macma4_05_g17130)	PA 2DAI	-0.1127	0.8519	0.5971	24

Supplementary table 3. Differentially expressed genes (DEGs) selected from *Musa acuminata* subsp. *burmannica* accession ‘Calcutta 4’ during infection with *Fusarium oxysporum* f. sp. *cubense* STR4, categorized by their functions in defence pathways against biotic stresses. Each gene group is described according to the role in the plant immune response to pathogens.

PRR membrane receptors						
Gene	LogFC	Treatment	Product			
Macma4_01_g23280	2.6554813	1DAI	SNF1-related protein kinase regulatory subunit beta-2			
Macma4_05_g06390	2.331157	1DAI	Protein kinase domain-containing protein			
Macma4_01_g23280	2.0359087	2DAI	SNF1-related protein kinase regulatory subunit beta-2			
Macma4_02_g09450	3.9492313	2DAI	Leucine-rich repeat receptor-like serine/threonine/tyrosine-protein kinase SOBIR1			
Macma4_02_g20630	3.0494334	2DAI	putative LRR receptor-like serine/threonine-protein kinase At2g16250			
Macma4_03_g15650	2.4562566	2DAI	Receptor-like cytoplasmic kinase 176			
Macma4_04_g01380	2.0343969	2DAI	putative LysM domain receptor-like kinase 4			
Macma4_04_g09660	2.1517576	2DAI	Protein LYK5			
Macma4_06_g36370	3.5230592	2DAI	Mitogen-activated protein kinase 5			
Macma4_07_g02340	2.2084521	2DAI	Serine/threonine-protein kinase RIPK			
Macma4_07_g13490	2.7838592	2DAI	Protein kinase domain-containing protein			
Macma4_07_g13510	2.1845243	2DAI	Protein kinase domain-containing protein			
Macma4_07_g25020	2.326438	2DAI	CBL-interacting protein kinase 2			
Macma4_08_g19310	3.50357	2DAI	Mitogen-activated protein kinase kinase 7			
Macma4_08_g29160	2.5986138	2DAI	putative Receptor-like proteL. in kinase HSL1			
Macma4_09_g07690	2.236141	2DAI	Serine/threonine-protein kinase OX11			
Macma4_10_g11450	2.4979967	2DAI	putative Chitin elicitor receptor kinase 1			
PR proteins						
Gene	LogFC	Treatment	Product			
Macma4_02_g16620	2.3984057	1DAI	Pathogenesis-related protein 1C			
Macma4_05_g17130	3.8620141	1DAI	Thaumatin-like protein 1			
Macma4_05_g17130	3.4699425	2DAI	Thaumatin-like protein 1			
Macma4_02_g16620	2.456543	4DAI	Pathogenesis-related protein 1C			
Macma4_03_g08400	2.1305036	4DAI	Pathogenesis-related protein 1			
Macma4_03_g08410	2.8131865	4DAI	Pathogenesis-related protein 1			
Macma4_03_g08420	4.1450963	4DAI	Pathogenesis-related protein 1			
Macma4_10_g08320	2.7534003	1DAI	Nematode resistance protein-like HSPRO2			
Macma4_05_g32600	4.5281875	2DAI	Nematode resistance protein-like HSPRO2			
Macma4_10_g08320	6.2958242	2DAI	Nematode resistance protein-like HSPRO2			
Macma4_11_g23440	4.5714334	2DAI	Nematode resistance protein-like HSPRO2			
Ion binding						
Gene	LogFC	Treatment	Product			
Macma4_04_g11230	4.9274949	2DAI	Calcium-transporting membrane-type	ATPase	7%2C	plasma
Macma4_07_g28680	4.4333735	2DAI	Calcium-transporting membrane-type	ATPase	7%2C	plasma
Macma4_06_g37980	3.9236182	2DAI	putative Zinc finger protein ZAT8			
Macma4_06_g11520	2.4535559	2DAI	Calcium-binding protein CML24			
ROS and SAR activation						

Gene	LogFC	Treatment	Product
Macma4_02_g06440	3.6752019	1DAI	Peroxidase 1
Macma4_02_g25270	2.6420187	1DAI	Peroxidase 59
Macma4_03_g02750	2.7743013	1DAI	Peroxidase
Macma4_07_g09370	3.0961857	1DAI	Peroxidase 52
Macma4_02_g25270	2.1632778	2DAI	Peroxidase 59
Macma4_05_g23620	2.1857948	2DAI	Peroxidase 5
Macma4_06_g16330	4.3739818	2DAI	Peroxidase 4
Macma4_06_g16370	4.7767745	2DAI	Peroxidase 4
Macma4_10_g31460	3.5071063	2DAI	Cationic peroxidase 1
Macma4_09_g31980	3.5742491	2DAI	NDR1/HIN1-like protein 10
Macma4_08_g16850	3.5223906	2DAI	Protein SAR DEFICIENT 1 (SARD1)
Macma4_05_g23620	2.291539	4DAI	Peroxidase 5
Macma4_10_g01630	4.8714811	4DAI	Cationic peroxidase 1
Macma4_10_g10810	4.0093618	4DAI	Peroxidase 4

Chitinases

Gene	LogFC	Treatment	Product
Macma4_08_g34260	2.1167916	1DAI	Hevamine-A
Macma4_01_g21300	3.0692668	2DAI	Acidic endochitinase
Macma4_03_g29600	3.1021082	2DAI	Chitinase
Macma4_03_g29630	4.2183551	2DAI	Basic endochitinase C
Macma4_05_g18860	2.9754202	2DAI	Chitinase 6
Macma4_09_g12390	4.0919451	2DAI	Chitin-inducible gibberellin-responsive protein 1
Macma4_03_g29600	2.6756914	4DAI	Chitinase
Macma4_05_g18860	3.2708696	4DAI	Chitinase 6
Macma4_05_g18850	3.1710397	4DAI	Chitinase 6
Macma4_06_g23390	2.2505533	4DAI	Endochitinase EP3

Transcription factors

Gene	LogFC	Treatment	Product
Macma4_02_g16390	3.4702747	1DAI	putative protein TIFY 5A
Macma4_01_g00810	5.4612341	2DAI	WRKY domain-containing protein
Macma4_02_g16390	7.2581061	2DAI	putative protein TIFY 5A
Macma4_02_g23700	2.5371601	2DAI	WRKY transcription factor 6
Macma4_03_g01960	4.063008	2DAI	Protein TIFY 10b
Macma4_04_g28760	3.7710131	2DAI	Transcription factor MYB44
Macma4_06_g34540	6.2655799	2DAI	putative protein TIFY 5A
Macma4_07_g04870	2.664359	2DAI	Transcription factor MYC2
Macma4_08_g27870	3.2876526	2DAI	Transcription factor MTB1
Macma4_09_g10730	7.3035838	2DAI	putative protein TIFY 5A
Macma4_09_g16730	2.2762969	2DAI	Transcription factor JAMYB
Macma4_09_g24850	3.1234421	2DAI	WRKY transcription factor WRKY51
Macma4_09_g27000	2.4607728	2DAI	Dof-type domain-containing protein
Macma4_09_g30650	2.7082652	2DAI	Transcription factor AS1
Macma4_11_g08360	5.8966374	2DAI	putative WRKY transcription factor WRKY28
Macma4_02_g16390	3.0227137	4DAI	putative protein TIFY 5A

Plant hormones

Gene	LogFC	Treatment	Product
Macma4_06_g02820	4.8070978	1DAI	Abscisic acid receptor PYL2

Macma4_05_g23430	2.2630154	1DAI	Protein DETOXIFICATION 51
Macma4_08_g11580	2.5729141	1DAI	Protein DETOXIFICATION 51
Macma4_11_g03200	3.2884419	1DAI	Protein DETOXIFICATION 51
Macma4_04_g05760	2.7925433	1DAI	Ethylene-response factor C3
Macma4_05_g22840	3.315146	1DAI	putative Ethylene-responsive transcription factor 1B
Macma4_05_g30420	3.0206545	1DAI	putative Ethylene-responsive transcription factor 9
Macma4_11_g21380	3.6475217	1DAI	Ethylene-responsive transcription factor 1B
Macma4_04_g03250	2.2516855	1DAI	Salicylic acid-binding protein 2
Macma4_06_g02820	3.0645495	2DAI	Abscisic acid receptor PYL2
Macma4_02_g13270	5.0577883	2DAI	Protein DETOXIFICATION 51
Macma4_05_g16180	4.7952644	2DAI	Brassinosteroid-responsive RING protein 1
Macma4_03_g13220	2.4207851	2DAI	Ethylene-responsive transcription factor 3
Macma4_04_g04680	6.7688855	2DAI	Ethylene-responsive transcription factor ERF098
Macma4_04_g05760	2.7560745	2DAI	Ethylene-response factor C3
Macma4_05_g22840	3.2835999	2DAI	putative Ethylene-responsive transcription factor 1B
Macma4_05_g24740	3.9110988	2DAI	Ethylene-responsive transcription factor ERF071
Macma4_05_g30420	7.1619321	2DAI	putative Ethylene-responsive transcription factor 9
Macma4_07_g05580	4.5939776	2DAI	Ethylene-responsive transcription factor ERF010
Macma4_07_g07090	3.9008527	2DAI	Ethylene-responsive transcription factor ERF105
Macma4_08_g19660	3.8883521	2DAI	Ethylene-responsive transcription factor ERF021
Macma4_08_g22960	2.1158082	2DAI	Protein ETHYLENE-INSENSITIVE 3-like 1a
Macma4_09_g11860	4.0023363	2DAI	Ethylene-responsive transcription factor 4
Macma4_10_g09800	4.9283062	2DAI	putative Ethylene-responsive transcription factor ERF019
Macma4_10_g29480	4.6762398	2DAI	putative Ethylene-responsive transcription factor ERF017
Macma4_10_g30580	2.1102614	2DAI	EIN3-binding F-box protein 1
Macma4_11_g23270	4.4809041	2DAI	putative Zinc finger protein ZAT18
Macma4_06_g17460	2.9976014	2DAI	Jasmonate-induced oxygenase 1
Macma4_04_g41220	2.5003622	2DAI	Allene oxide synthase 1. chloroplastic
Macma4_04_g03250	2.0525105	2DAI	Salicylic acid-binding protein 2
Macma4_09_g31980	3.5742491	2DAI	NDR1/HIN1-like protein 10
Macma4_11_g21380	2.1756112	4DAI	Ethylene-responsive transcription factor 1B
Macma4_04_g04680	7.3069688	4DAI	Ethylene-responsive transcription factor ERF098
Macma4_04_g05760	2.6007519	4DAI	Ethylene-response factor C3
Macma4_05_g22840	3.2218542	4DAI	putative Ethylene-responsive transcription factor 1B
Macma4_05_g24740	2.1419974	4DAI	Ethylene-responsive transcription factor ERF071

ETI

Gene	LogFC	Treatment	Product
Macma4_01_g04340	4.0346392	2DAI	putative NB-ARC domain-containing protein
Macma4_01_g04350	4.9313868	2DAI	NB-ARC domain-containing protein
Macma4_01_g04360	3.3235121	2DAI	NB-ARC domain-containing protein
Macma4_01_g04380	3.9665522	2DAI	NB-ARC domain-containing protein
Macma4_04_g37480	2.3797411	2DAI	putative Disease resistance protein RPS5

Secondary metabolites

Gene	LogFC	Treatment	Product
Macma4_07_g21750	5.1190997	1DAI	Premnaspirodiene oxygenase
Macma4_06_g34090	3.8430513	1DAI	Cytochrome P450 81Q32
Macma4_06_g34090	3.8430513	1DAI	Cytochrome P450 81Q32

Macma4_05_g09040	2.9055502	1DAI	Cytochrome P450 78A5
Macma4_06_g34080	2.7432176	1DAI	Cytochrome P450 81Q32
Macma4_10_g22190	2.3835385	1DAI	Cytochrome P450 81Q32
Macma4_07_g21750	5.2249605	2DAI	Premnaspirodien oxygenase
Macma4_10_g24620	3.2427373	2DAI	Stilbene synthase 1
Macma4_10_g10790	2.0512354	2DAI	Exocyst complex component EXO70B1
Macma4_03_g21730	7.409999	2DAI	Cytochrome P450 94B3
Macma4_08_g31110	6.1032483	2DAI	Cytochrome P450 94C1
Macma4_05_g19410	5.3958947	2DAI	Cytochrome P450 94C1
Macma4_10_g22190	3.8704946	2DAI	Cytochrome P450 81Q32
Macma4_10_g22160	3.2172508	2DAI	Cytochrome P450 81Q32
Macma4_06_g34090	2.7874358	2DAI	Cytochrome P450 81Q32
Macma4_05_g09040	2.5611441	2DAI	Cytochrome P450 78A5
Macma4_06_g34080	2.1712447	2DAI	Cytochrome P450 81Q32
Macma4_09_g21760	2.5332706	4DAI	Tricetin 3'-O-trimethyltransferase
Macma4_09_g14290	2.5809809	4DAI	Cytochrome P450 CYP73A100
Macma4_01_g15210	2.1110233	4DAI	Cytochrome P450 71A1

CONCLUSÕES GERAIS E PERSPECTIVAS

Esta tese de doutorado teve como objetivo investigar as respostas de defesa de *Musa acuminata* subsp. *burmannica* ‘Calcutta 4’ contra *Fusarium oxysporum* f. sp. *cubense* raça STR4, pela primeira vez, analisando a expressão diferencial de genes e os mecanismos estruturais de defesa, em comparação com o genótipo suscetível ‘Prata-anã’. Os resultados destacam a eficácia de ‘Calcutta 4’ responder precocemente e de forma robusta o ataque de *Foc* STR4, impedindo a colonização do patógeno e evolução da doença, conforme evidenciado por análises histológicas, sintomatológicas e transcritômicas.

Mostramos que é a identificação robusta de raças dentro de isolados de *Foc* só é possível por meio de análises genômicas, envolvendo, por exemplo, a comparação de ortologia de genes. Assim, após a identificação de ortogrupos do isolado de *Foc* 218A, obtido de plantas sintomáticas de ‘Nanica’ (subgrupo Cavendish) oriundas do estado de São Paulo em 2014, fornecido pela Embrapa Mandioca e Fruticultura (CNPMPF), com demais representantes de raças de *Foc*, foi possível concluir que o isolado 218A possui maior similaridade de genes com um isolado de *Foc* STR4 (VCG 0120), classificando-o como *Foc* STR4.

A ausência de sintomas visíveis em ‘Calcutta 4’ e a presença de amarelecimento foliar e necrose do rizoma em ‘Prata-Anã’ confirmam as respostas contrastantes desses dois genótipos à *Foc* STR4. Assim como nas análises histológicas que revelaram mecanismos estruturais de defesa em ‘Calcutta 4’, como a deposição de calose e compostos fenólicos, que estavam ausentes no genótipo suscetível ‘Prata-Anã’.

A análise de transcriptômica identificou um total de 1.416 genes diferencialmente expressos (DEGs), com a maioria regulados positivamente detectados em 2 DAI, reforçando a rápida ativação da defesa em ‘Calcutta 4’ ao processo de infecção de *Foc* STR4. O enriquecimento de genes envolvidos no reconhecimento do patógeno, sinalização e respostas de defesa destaca a natureza dinâmica da resposta da planta ao ataque do patógeno.

Importantes famílias gênicas relacionadas ao mecanismo de defesa de plantas ao ataque de patógenos foram encontrados durante a interação de *Foc* STR4 e ‘Calcutta 4’, bem como a ativação das vias PTI e ETI, fitormônios, fatores de transcrição, MAPK, proteínas de reconhecimento de membrana, quitinases e fitoalexinas. A forte regulação positiva de vias de metabolismo secundário, incluindo aquelas responsáveis pela produção de fitoalexinas e outros compostos de defesa, ressalta a estratégia de defesa química da planta. Esses achados são

consistentes com estudos anteriores entre outras espécies de *Musa* e raças de *Foc*, demonstrando a importância das respostas de defesa precoce e da sinalização hormonal na resistência de *Musa spp.* à *Foc*.

A identificação de genes-chave de resistência em 'Calcutta 4' oferece oportunidades promissoras para seu uso em programas de melhoramento, com foco na introgressão de genes alvo por meio de biobalística ou agroinoculação, além de edição por CRISPR. Essas técnicas visam aumentar a resistência em cultivares suscetíveis de *Musa* de forma concisa, sem que haja perda de características agronômicas desejadas, e com foco na resistência a diversos patógenos. Aqui, demonstramos importantes genes de resistência candidatos de 'Calcutta 4' a *Foc* STR4, como os genes R pertencentes à família de NLRs, que podem ser utilizados na engenharia genética para criar cultivares de bananas comerciais resistentes à doença. Contudo, pesquisas adicionais focadas na validação funcional desses genes *in planta* serão essenciais para sua incorporação em programas de melhoramento de bananas comerciais e no desenvolvimento de variedades resistentes a Murcha de Fusarium, contribuindo para a sustentabilidade global da produção de bananas.