


Transcriptional profiling by RNA sequencing of black pepper (*Piper nigrum* L.) roots infected by *Fusarium solani* f. sp. *piperis*

Edith C. O. Moreira¹ · Daniel G. Pinheiro^{2,3} · Sheila M. C. Gordo¹ · Simone M. Rodrigues⁴ · Elaine Pessoa^{5,7} · Hubert Schaller^{6,7} · Oriel F. de Lemos⁴ · Artur Silva⁵ · Horacio Schneider¹ · Wilson A. Silva Jr.² · Iracilda Sampaio¹ · Sylvain Darnet^{5,7} 

Received: 12 April 2017/Revised: 9 July 2017/Accepted: 12 September 2017/Published online: 21 September 2017
© Franciszek Górski Institute of Plant Physiology, Polish Academy of Sciences, Kraków 2017

Abstract Black pepper (*Piper nigrum* L.) is the most traded spice worldwide and therefore has great economic value. One of the major limitations of its production is yield losses in fields due to root rot, a disease caused by *Fusarium solani* f. sp. *piperis*. This soil-borne pathogen disseminates rapidly in tropical countries. Biotechnological breeding is often presented as an efficient tool to produce resistant pepper cultivars. An RNA-sequencing experiment was used to take a snapshot of the root transcriptome 60 days after infection by the pathogen. The mapping of 67 million SOLiD single-end reads to the *Fusarium* genome identified many fungal transcripts coding constitutive proteins and two proteins involved in virulence and conidial formation. The annotation of black pepper transcripts obtained by de novo assembly predicted three proteins restricted to this species. While these transcripts were upregulated in infected roots, the corresponding predicted

proteins had no hit in databases. A global analysis of differentially expressed black pepper genes, using terms of gene ontology, has demonstrated an enrichment of genes involved in proteolysis, plastid degradation, cell-wall remodeling and secondary metabolism, consistent with toxicity and necrotrophic fungal interactions that force plants to collaborate at the metabolic level. Detailed descriptions of up- or downregulated genes associated with plant defense suggested several genes implied in the biosynthesis of isoprenoids, especially for an upregulated terpene synthase, a putative geranylgeranyl diphosphate synthase, and a downregulated brassinosteroid signaling component (*BAK1*). These results provide new clues for investigating further target genes and biotechnological approaches for breeding black pepper resistance to *Fusarium*.

Keywords Differential expression · Root rot · *Piper nigrum* · RNA-seq · *Fusarium*

Communicated by E. Kuzniak-Gebarowska.

Electronic supplementary material The online version of this article (doi:10.1007/s11738-017-2538-y) contains supplementary material, which is available to authorized users.

✉ Sylvain Darnet
shd@laposte.net

- 1 Genetics and Molecular Biology Laboratory, Coastal Studies Institute, Bragança Campus, Universidade Federal do Pará, Bragança, Pará, Brazil
- 2 Department of Genetics, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil
- 3 School of Agricultural and Veterinary Sciences, São Paulo State University (Unesp), Jaboticabal, São Paulo, SP, Brazil
- 4 Brazilian Agricultural Research Corporation - EMBRAPA Amazônia Oriental, Belém, Pará, Brazil

- 5 Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, PA, Brazil
- 6 Plant Isoprenoid Biology (PIB) Team, Institut de Biologie Moléculaire des Plantes du CNRS, Université de Strasbourg, Strasbourg, France
- 7 Laboratoire International Associé PALMHEAT CNRS/UFPA, Direction Europe de la Recherche et Coopération Internationale (DERCI)- Campus, Gérard-Mégie 3, rue Michel-Ange, 75794 Paris Cedex 16, France

Introduction

The genus *Piper* is highly diverse, containing about 2000 species, and is distributed at tropical latitudes. Many of these species are of biotechnological interest due to their high contents of active compounds such as the lysine-derived piperidine, quinolizidine, indolizidine and lycopodium alkaloids (Jaramillo and Manos 2001; Menezes et al. 2009). Black pepper (*Piper nigrum* L.) is the most traded spice in the world, widely used in cooking, food preservation and perfume production. Some black pepper chemicals also have medicinal properties, such as antioxidant, anti-inflammatory and anticancer activities (Liu et al. 2010; Meghwal and Goswami 2013; Nishimura et al. 2011). The global production of black pepper from tropical countries is about 334,000 tonnes, totaling 1.8 billion US dollars. Up to 83% of this production is imported to Europe and North America (FAO 2011). This culture consequently has great economic and social importance in developing countries.

Phytopathological problems such as foot rot and root rot caused by fungi from the genera *Phytophthora* and *Fusarium*, respectively, represent a major threat to global *Piper* production (Duarte and Albuquerque 2001; Joy et al. 2007). *Fusarium solani* f. sp. *piperis* (Fsp) and its teleomorphic form known as *Nectria haematococca* f. sp. *piperis*, is a biotrophic soil fungus belonging to the class Ascomycetes that is endemic to the Brazilian Amazon region. The temperature and humidity in this region are constantly high and so offer optimal conditions for pathogen sporulation, which increases pathogen dissemination. Many black pepper plantations have thus been contaminated, affecting yield production and drupe quality (Albuquerque 1961; Albuquerque et al. 2001; Hamada et al. 1988). The pathogenic infection impairs the conducting vessels by increasing mycelial mass, leading to decreases in the supply of water and nutrients to the plant (Albuquerque et al. 2001; Fukutomi and Hirakata 1981). The physiology of the infection and disease is well known, but their underlying molecular mechanisms are poorly understood.

Evidence suggests that initial infections by root pathogens are generally established in the root elongation zone, as demonstrated for *Pisum sativum* (Cannesan et al. 2011; Gunawardena et al. 2005). The molecular plant-host interaction, however, is better known for foliar than root pathogens, which are poorly characterized and understood (Balmer and Mauch-Mani 2013; Okubara and Paulitz 2005). The elucidation at the molecular level of the defensive responses involved in the radicular pathosystem can consequently assist our understanding of these interactions and help us to design efficient biotechnological

approaches for plant breeding. Molecular interactions that characterize the interaction of *Fusarium* with its host plants have been successfully identified using transcriptomic profiles obtained by RNA sequencing (RNA-seq), in both model fungus/plant systems such as *Fusarium oxysporum*/*Arabidopsis thaliana* (L.) Heynh and in plants of agronomic interest (Berrocal-Lobo and Molina 2008). A previous experiment using black pepper suppression subtractive hybridization libraries has provided a partial view of the genes expressed in Fsp-plant interactions (de Souza et al. 2011).

We report an exhaustive analysis of the entire transcriptome of black pepper roots inoculated by Fsp, using RNA-seq to identify differentially expressed genes and to infer the global molecular mechanisms involved in plant defense against pathogenic fungi.

Materials and methods

Biological material

This study was approved by IBAMA under license number 02001.006478/2010-49 and special authorization 02/2008. Black pepper plants (cv. Bragantina) were inoculated with an isolate of *Fusarium solani* f. sp. *piperis*, provided by the Collection of Fungi and Phytopathogens of Amazon Biome at Embrapa Amazônia Oriental (de Castro et al. 2016). This isolate originated from black pepper plant roots with symptoms of fusariosis, collected in the Municipality of Baiao, Para, Brazil (de Castro et al. 2016). Two groups of thirty-five plants each were separately cultivated in a greenhouse: one group of control plants was inoculated with water, and the other group was inoculated with Fsp. The method of inoculation was based on the protocol described by Albuquerque et al. (2001). The pathogen was cultivated at 25 °C under a 12-h light/dark cycle on a water-agar culture medium containing potato dextrose. Disks of conidia and mycelia 0.7 cm in diameter were collected from these cultures and prepared as soil inocula. The black pepper plants were potted in vermiculite containing the *Fusarium* inoculum. The first symptoms of root rot were visible 60 days after inoculation, and plants from the above-mentioned two groups were then collected (Albuquerque 1961; de Castro et al. 2016).

cDNA preparation and SOLiD sequencing

Ten plants were randomly selected in each of the two groups of 35 plants inoculated with water or Fsp. Stem tissues and root-zone elongation tissues were sampled from

these pools of ten plants (Online resource 1). Total RNA was extracted from about 35 mg of fresh tissue. A non-inoculated root library (NIRL) and an infected root library (IRL) were prepared from these RNAs and sequenced on a SOLiD Next-Generation Sequencer (version 3.0), as described by Gordo et al. (2012) (Thermo Fisher Scientific, Waltham, USA). Library preparation and sequencing were not replicated for this experiment. The NIRL was previously published and registered as SRR1164727 in the NCBI-SRA database (Gordo et al. 2012). The accession number for the IRL reported in this study is SRR654187.

Bioinformatic analysis

A transcript sequence assembly (TSA) was obtained using the hybrid color-space read-additive method (Viegas et al. 2015), with a further step for UniVec removal of contaminated sequences (<https://www.ncbi.nlm.nih.gov/tools/vecscreen/univec/>), and deposited under accession GFIY00000000 in the NCBI database. IRL and NIRL read data sets were mapped on TSA contigs for transcript quantification using CLC Genomics Workbench (CLCg) with default parameters (<https://www.qiagenbioinformatics.com>). The transcripts were functionally annotated using Blast2GO (Conesa and Gotz 2008), with an initial comparison of the TSA data set with the Swiss-Prot/Uniprot database (<http://www.uniprot.org>) using BLASTX with an *E*-value of $1e-3$ (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The nucleotides of orphan gene sequences were compared using SRA-BLAST and NGS read data sets of leaf and drupe libraries, sequenced by HiSeq and MiSeq Illumina platforms and registered under NCBI accession SRX119532, SRX1716592, SRX2855187, SRX853366, SRX856632, SRX856639 and SRX890122 (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_PROGRAMS=megaBlast&PAGE_TYPE=BlastSearch&BLAST_SPEC=SRA&SHOW_DEFAULTS=on). For further characterization and modelling of molecular responses, black pepper transcripts were clustered by *A. thaliana* orthologs using the Araport 11 protein data set (<https://www.araport.org>) and BLASTX with an *E*-value of $1e-3$ as described for de novo transcriptomes in non-model species (Nogueira et al. 2016). Transcript expression was calculated for each *A. thaliana* homologous-gene cluster (AHGC) by summing the read counts for each black pepper transcript in the cluster. Identification of differentially expressed genes was based on Kal's Z-test in CLCg. The enrichment of terms of gene ontology (GO) between conditions was globally analyzed using the parametric analysis of gene set enrichment (PAGE) tool of the agriGO portal (Du et al. 2010). The AHGC expression data set was imported into MapMan for visualizing and interpreting the global molecular responses (Jung and An 2013). For the

digital expression of Fsp genes, IRL and NIRL read data sets were mapped with the CLCg mapper on the *N. haematococca* mpVI 77-13-4 genome (https://www.ncbi.nlm.nih.gov/assembly/GCF_000151355.1/), with a length fraction of 95% and a similarity fraction of 98% to ensure high-specificity mapping. Statistical analysis was performed using CLCg and Kal's Z-test.

Results and discussion

Library sequencing

We generated an IRL from RNAs extracted from a pool of ten plants to minimize individual variation. All plants displayed symptoms of *Fusarium* root rot such as blackened aspect of roots and collars and yellowish leaves (Albuquerque 1961) (Online resource 1). The blackened aspect of infected roots was most probably due to the production of toxins by the Fsp isolate (de Castro et al. 2016) that caused the symptoms of the disease on above-ground plant tissues, such as yellowish leaves and dried branches, and ultimately plant death (Albuquerque 1961; Albuquerque et al. 2001; de Castro et al. 2016; Duarte and Albuquerque 2001). All non-inoculated plants that were sampled to generate an NIRL had very clear roots (Online resource 1). NIRL and IRL were single-end sequenced using the SOLiD version 3 platform, and the raw sequence data sets contained about 72 million (3.57 Gbp) and 54 million (2.74 Gbp) reads, respectively.

Analysis of digital gene expression (DGE) of Fsp reads

Reads from Fsp were first identified by mapping the read data sets from NIRL and IRL on the *N. haematococca* genome, the teleomorph of *F. solani*. The mapping found only 180 mapped reads for NIRL and 31,186 reads for IRL. Transcripts should be considered non-expressed and under the limit of detection if they have <100 mapped reads or <1 transcript per million (TPM) reads. For NIRL, no genes were detected with values >100 mapped reads. For IRL, 60% of the reads (19,067 reads) were mapped in exons and 41.5% in introns, consistent with the use for library preparation of total RNAs depleted in rRNAs and containing ncRNAs. The low level of detected reads from pathogenic fungi, in this case 0.13% of the total reads, was previously reported for another pathosystem (Petre et al. 2012). The list of genes expressed with >100 reads is presented in Online Resource 2. Many of the detected genes were constitutive and should be considered fungal house-keeping genes, such as calmodulin, actin and tubulin, or genes involved in metabolism. Only a few candidate

genes were associated with plant-host interactions, such as the interferon-regulated resistance GTP-binding protein already isolated during the biosynthesis of zearalenone in the genus *Fusarium* (Lysøe et al. 2008). A molecular chaperone encoded by *HTPG*, which plays a crucial role in the vegetative growth, reproduction and virulence of *F. graminearum* (Bui et al. 2016), was expressed. Interestingly, the two most abundant transcripts were not well annotated, one without homology in the non-redundant (nr) NCBI database and the other with a low similarity to a histone-lysine N-methyltransferase and to the H3 lysine-9 specific dim-5 gene from *Neonectria ditissima*, a protein regulator of the circadian cycle and conidial formation (Ruesch et al. 2014). Only highly abundant genes were detected. These genes were annotated as constitutive genes, one uncharacterized gene and three putative central regulators of *Fusarium* reproduction and virulence.

DGE analysis of black pepper transcripts in roots infected by Fsp

Transcriptome assembly and annotation

The NIRL and IRL reads were mapped on the TSA data set as described by Viegas et al. (2015) for evaluating the abundance of black pepper transcripts. The TSA data set was obtained using a hybrid method to assemble the SOLiD single-end reads with Illumina NGS paired-end reads obtained from the same cultivar of black pepper by Joy et al. (2013). This reference transcriptome contained about 65.4 Mbp, with 59,863 predicted transcripts, an N50 of 1762 bp, 27,747 sequences longer than 1000 bp and a CEGMA score for complete genes of about 98%, indicating that the assembly was successful (Viegas et al. 2015). The annotation was performed by comparison with the Swiss-Prot database: 17,807 transcripts were fully annotated and 12,185 had homologies lower than 1e-100 with an orthologous sequence. All transcript annotations and information retrieved with Blast2Go as GO terms are listed in Online Resource 3. The total mapped reads were 15,484,438 for NIRL and 12,524,954 for IRL, representing 82.5 and 52.1% of the preprocessed read data sets, respectively. A statistical Z-test was performed between NIRL and IRL using the method described by Kal et al. (1999) for experiments with one sample per condition. The DGE analysis was interpreted in two steps: putative lineage-restricted genes upregulated in IRL and ncRNAs were first identified, then a global model of molecular responses was established based on the *A. thaliana* genome.

Prediction of orphan genes associated with Fsp infection

The orphan genes or genes restricted to *P. nigrum*, which were upregulated in IRL and associated with inoculation, were identified by filtering the transcripts based on the following criteria described by Nogueira et al. (2016): presence of a coding sequence, absence of homology in the UniProt/Viridiplantae or nr NCBI databases with *E*-values up to 1e-3, more than two-fold increases between NIRL and IRL ($FC_{IRL/NIRL}$), transcripts longer than 500 bp, false discovery rate less than 0.05, IRL read count over 100 and $P < 0.001$. This selection identified three transcripts. Transcript sequences were compared to seven different NGS libraries of *P. nigrum* leaves and drupes available in NCBI database using SRA-BLASTN to confirm the presence of the transcripts and to reject the hypothesis of putative contaminating microbial sequences. More than 250 NGS reads were mapped on each transcript, and the top-100 BLAST subset had very high sequence identities, about 99–100%, along the length of the three orphan gene transcripts, confirming the presence of these genes in the *P. nigrum* transcriptome (Online Resource 4). Two transcripts had full-length coding sequences (CDS): contig311778 with a predicted protein of 135 aa, and contig480387 with a predicted protein of 139 aa. A third transcript had a 5' partial CDS that corresponded to contig524182 with a predicted protein of 122 aa. All predicted protein sequences had no hit in the nr NCBI database, providing an opportunity to discover new proteins involved in defensive mechanisms in basal angiosperms.

ncRNAs and ncRNA precursors

IRL and NIRL were obtained from total RNAs that were depleted in rRNAs and consequently contained mRNAs but also ncRNA. Transcripts with no hit in the Viridiplantae/Uniprot database were submitted as queries to the RFAM server for predicting possible ncRNAs and ncRNA precursors (Nawrocki et al. 2015). About 350 transcripts were annotated as ncRNAs, 87% of which were identified as rRNAs (LSU_rRNA, SSU_rRNAs and 5S) of archaeobacteria, prokaryotes and eukaryotes (Online Resource 5). This result indicated that the depletion of rRNAs was not complete and the presence of microorganisms in the black pepper samples. These microorganisms most probably represented the background microbial population interacting with pepper plants grown in greenhouse conditions. Other classes of ncRNAs precursors were identified as miRNAs and snoRNAs. Among four microRNA

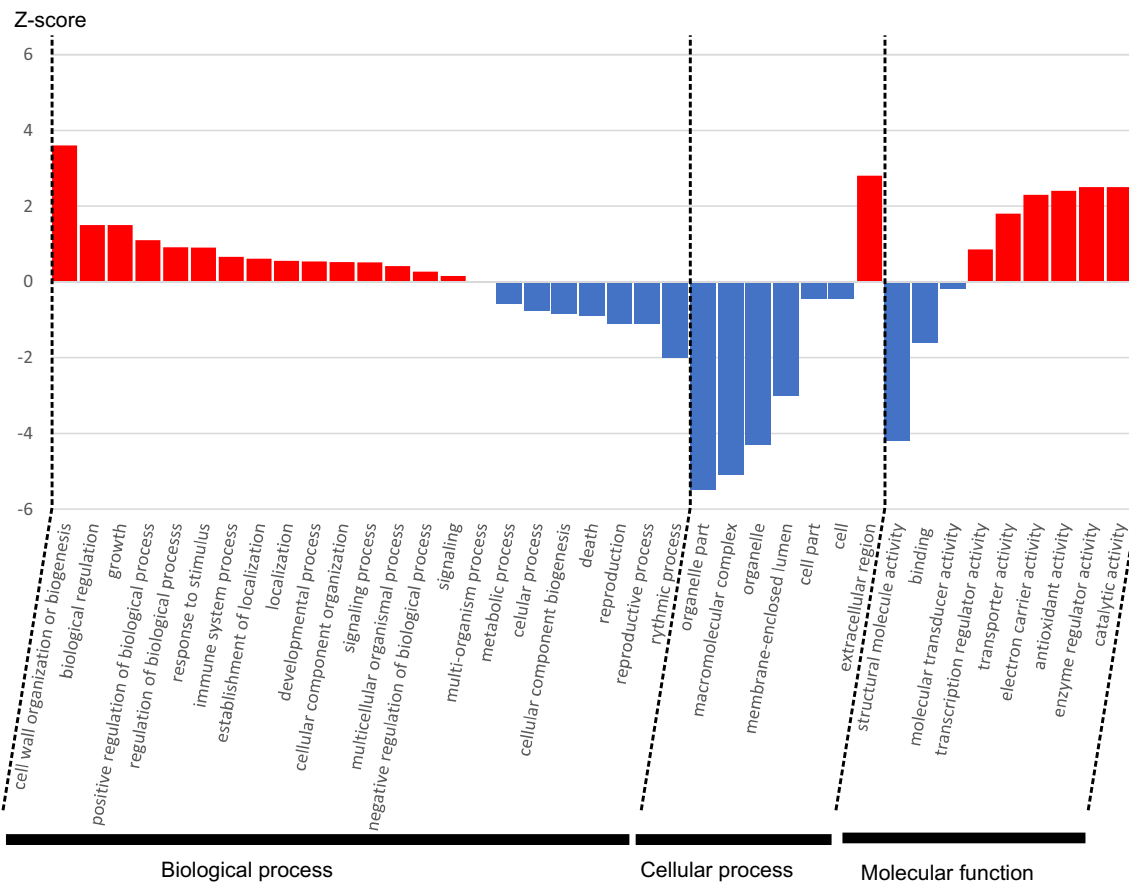


Fig. 1 Global enrichment and decrease in GO terms comparing black pepper transcripts, clustered by *Arabidopsis thaliana* orthologs, differentially expressed between roots uninfected and infected by

precursors, miR-171 and miR-166 had already been described in black pepper (Asha et al. 2012). All these miRNAs were expressed in root tissue, which did not substantially affect the size of IRL. Eleven snoRNA precursors were detected, with a sequencing coverage higher than 100 reads mapped at each transcript base position. Seven of these snoRNAs were statistically increased in IRL, up to 21-fold for snoZ161_228 and 22-fold for snoR117 (Online Resource 5). The functions of snoRNAs have not been well characterized in plants, but they may act as a central, dynamic, and highly versatile group of small regulatory RNAs, as do miRNAs and snRNAs (Dupuis-Sandoval et al. 2015; Zhu et al. 2016).

Modeling the molecular response

The black pepper transcripts were clustered by their *A. thaliana* orthologs for modeling the molecular response of the plant to the pathogen. The expression of each ortholog cluster was calculated based on its read counts (Online Resource 6), as described by Nogueira et al. (2016) for non-model transcriptomes. A Z-test was used for each

Fusarium solani f. sp. *piperis*. The Z-score was obtained using the parametric analysis of gene set enrichment tool of agriGO and indicates significant variation of the GO term categories

AHGC using the read count for each condition (Online Resource 6). A PAGE analysis for the GO terms was performed using AHGC and \log_2 of $FC_{IRL/NIRL}$ (Fig. 1). GO categories defined as biological processes, cellular processes and molecular functions with significant changes, enrichment or decreases are indicated in Fig. 1. Genes involved in cell-wall organization represented a highly variable category, with a Z-score near 4. For cellular processes, GO terms clearly decreased for genes associated with plastids and thylakoid lumens (GO:0031978, GO:0009543). In contrast, extracellular regions, especially plant-type cell walls (GO:0009505), were enriched. For molecular functions, many categories were enriched and were associated with antioxidant, transporter, regulatory and catalytic functions. Two subcategories of molecular function, pectinesterase activity (GO:0030599) and carboxylesterase activity (GO:0004091), were highly represented. The global view that was drawn from the analysis of GO enrichment (Fig. 1) indicated high activity in the apoplasmic compartment but also in the cell wall, because many genes were involved in dynamic cell-wall remodeling, which likely contributes to the deployment of plant

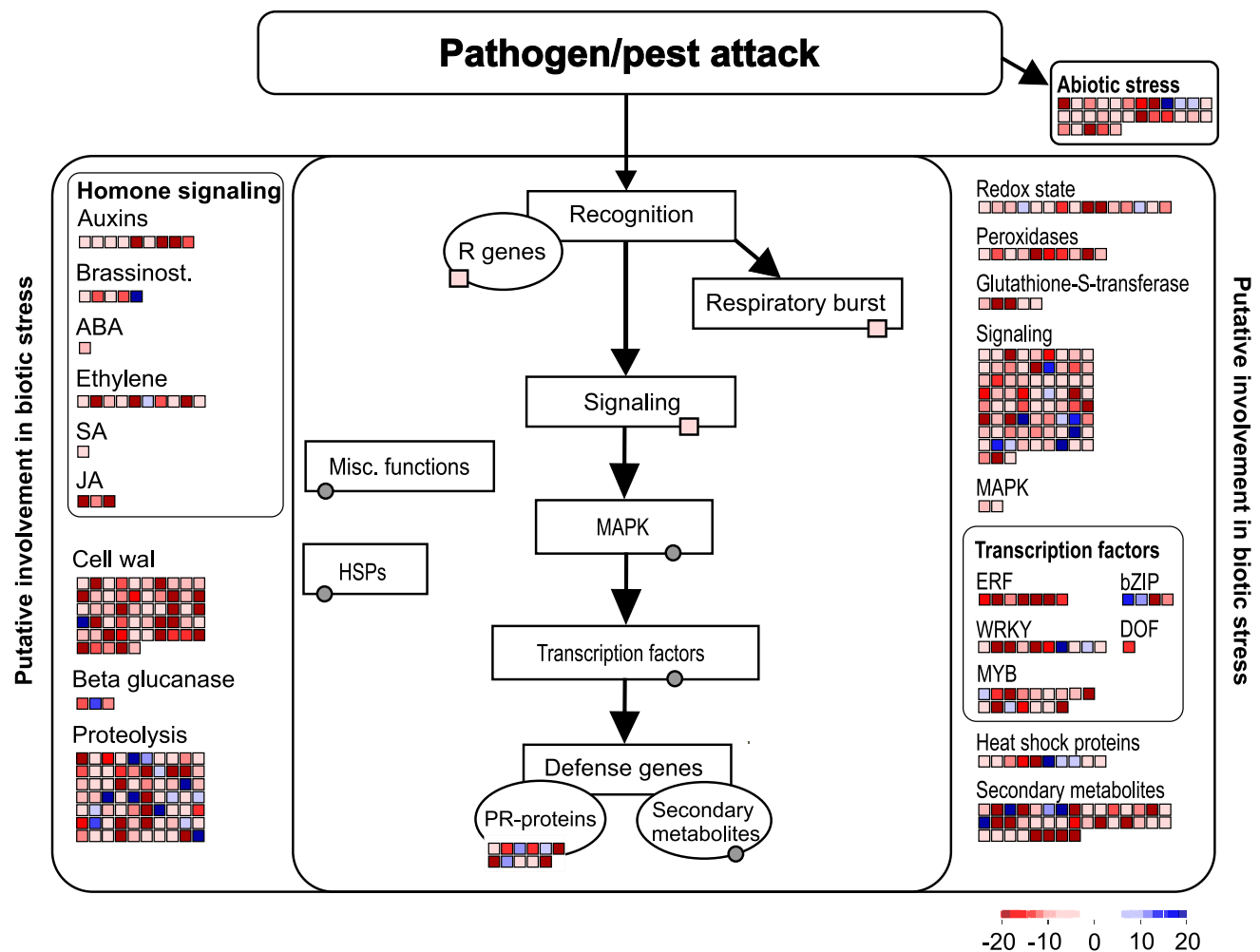


Fig. 2 Biotic-stress category of the MapMan overview showing black pepper transcripts, clustered by *Arabidopsis thaliana* orthologs, differentially expressed between roots uninfected and infected by *Fusarium solani* f. sp. *piperis*. Individual genes are represented by small squares, and their frequencies are indicated by color intensity.

Up- and downregulated genes are shown in red and blue, respectively. Scale bars display the changes in $FC_{\text{IRL/NIRL}}$. The gray circle indicates a category without mapped transcripts. The numeric values of each transcript represented in the figure are shown in Online Resource 6

responses against fungal disease (Bellincampi et al. 2014). The presence of plant and not fungal esterase activity was described by Bellincampi et al. (2014) as an ultimate stage of infection, when necrotrophic fungi force plants to collaborate in cell-wall degradation, thus favoring their own metabolism. An important decrease in GO categories for plastids and thylakoids is likely associated with the effect of fungal toxins, such as zearalenone, that can severely damage thylakoids (Chen et al. 2015; Lysøe et al. 2008). These results are in full agreement with our experiment describing plants with or without root-rot symptoms.

Transcripts with significant variation, i.e. $FC_{\text{IRL/NIRL}} > 5$ and $P < 0.001$, were mapped in the biotic-stress category using MapMan to obtain an overview of the black pepper molecular response (Fig. 2). Calculated values for each transcript classified into “biotic-stress related” functional plant category of MapMan are shown in Online Resource

7. Genes associated with biotic stress tended to be upregulated, with three categories highly represented: proteolysis, cell-wall components and secondary metabolism (Fig. 2). An intense proteolysis has been reported for other plant species infected by *Fusarium*, likely due to the action of the toxins deoxynivalenol and zearalenone (Matthaus et al. 2004; Schwarz et al. 2001).

Most importantly, the data collected in the present study indicated a large contribution of specialized metabolic pathways (or secondary metabolism) in the molecular response. Aspects of such a contribution have been reported (Campos-Bermudez et al. 2013). Many enzymes implicated in the biosynthesis of flavonoids, phenylpropanoids and isoprenoids were upregulated. Key genes controlling the level of isoprenoids through the so-called mevalonate (MVA) and methylerythritol 4-phosphate (MEP) pathways (Hemmerlin et al. 2012), coding for the

enzymes 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGR*) and 1-deoxy-D-xylulose-5-phosphate synthase (*DXS*), were overexpressed 5.1-fold and 8.7-fold ($FC_{\text{IRL}/\text{NIRL}}$), respectively. Interestingly, a putative gene encoding geranylgeranyl diphosphate synthase (*GGPPS*) was upregulated 110-fold in IRL. This *PnGGPPS* was apparently distant from other known *GGPPS*. The best hit was the *GGPPS* sequence of the Chinese peony that shared 34% aa identity. Many *GGPPS* have been identified from various species, and their expression has often been associated with plant defensive reactions, as for secologanin biosynthesis during the infection of wheat by *Fusarium* (Powell et al. 2016). A terpene synthase with 49% aa identity with a germacrene D synthase from *Citrus sinensis* was upregulated eightfold in IRL ($FC_{\text{IRL}/\text{NIRL}}$), suggesting a role as a defense-specific terpene synthase. A survey of plant-defense genes in our data sets identified many putative disease-resistance proteins: WRKY, bZIP and MYB transcription factors, a guanine nucleotide-binding protein and proteins involved in calcium signaling. The corresponding genes were expressed at levels similar to those observed in other studies of *Fusarium*-plant interactions, such as the *Fusarium*-wheat interaction (Powell et al. 2016). Many proteins were associated with drought and abiotic stress, such as ERD (early response to dehydration) and binding immunoglobulin protein. Such modified gene expression would likely account for vessel obstruction and general chlorosis (Carvalho et al. 2014; Rai et al. 2012). Molecular studies of *A. thaliana* inoculated with *Fusarium* have identified the hormonal response as an important component of plant defensive responses (Berrocal-Lobo and Molina 2008; Chen et al. 2014). Our results identified a few genes that were up- or downregulated in the abscisic acid and salicylic acid pathways. *BAK1* (*BRI1-associated receptor kinase*) was surprisingly downregulated, by 30-fold for $FC_{\text{IRL}/\text{NIRL}}$. *BAK1* is a central regulator of the brassinosteroid signaling pathway, which could indicate a reduced brassinosteroid response, although brassinosteroids can generally enhance disease tolerance. The *detiolated 2* (*DET2*) and *steroid 23-hydroxylase* (*CPD*) genes were upregulated by 5.3-fold and 12.3-fold, respectively. *DET2* and *CPD* are brassinosteroid biosynthetic enzymes. The genes encoding these enzymes were upregulated during biotic and abiotic stress in *Cucumis sativus*, suggesting a recruitment of the brassinosteroid biosynthetic machinery as part of the defensive response (Xia et al. 2011). These results indicate that the brassinosteroid pathway is involved in plant-pathogen interactions and thus represent a promising target for improving the resistance of black pepper to Fsp infection (Stegmann et al. 2017).

In summary, the transcriptional profiling reported in this study provides valuable information on the nature of the molecular response of black pepper during infection by

Fsp. It has identified previously unknown orphan genes involved in stress response, a strong increase of snoRNA expression, a plant carbohydrate metabolism reprogrammed by Fsp and a major role of isoprenoid biosynthesis and brassinosteroid signalling in black pepper defense. The expression of a gene encoding a terpene synthase that apparently had no functionally characterized counterpart in models such as *A. thaliana* was an important finding. Further functional characterization of this gene is needed. Our data surprisingly did not implicate pepper alkaloids in the defensive response, which reinforces the role of other metabolic pathways in the interaction with *Fusarium*. This study provides new insights on black pepper resistance to Fsp, because it addresses the global mobilization of the transcriptome rather than focusing only on specific resistance genes. This analysis also suggests new interesting studies for exhaustively characterizing the black pepper metabolic response for breeding Fsp-tolerant black pepper.

Author contribution statement ECOM, SMCG, SMR, ODdeL, AS, HS, WASJr and IS conceived and designed the study. DGP, EP, HS and SD analyzed and interpreted the data and SD and HS wrote the manuscript.

Acknowledgements The authors thank TR Rommel, G Silva and MSR Barbosa for their technical assistance. SD and HS are grateful to the Centre National de la Recherche Scientifique and Institut National des Sciences Biologiques (INSB) for funding the “Laboratoire International Associé PALMHEAT” CNRS/UFGA.

Compliance with ethical standards

Conflict of interest The authors of the manuscript have no conflict of interest to declare.

References

- Albuquerque F (1961) Podridão das raízes e do pé da pimenta-do-reino
- Albuquerque FC, Duarte ML, Benchimol RL, Endo T (2001) Resistência de Piperaceas nativas da Amazônia à infecção causada por *Nectria haematococca* f. sp. *piperis*. Acta Amaz 31:341–348
- Asha S, Nisha J, Soniya EV (2012) In silico characterisation and phylogenetic analysis of two evolutionarily conserved miRNAs (miR166 and miR171) from black pepper (*Piper nigrum* L.). Plant Mol Biol Rep 31:707–718. doi:10.1007/s11105-012-0532-5
- Balmer D, Mauch-Mani B (2013) More beneath the surface? Root versus shoot antifungal plant defenses. Front Plant Sci 4:256. doi:10.3389/fpls.2013.00256
- Bellincampi D, Cervone F, Lionetti V (2014) Plant cell wall dynamics and wall-related susceptibility in plant-pathogen interactions. Front Plant Sci 5:228. doi:10.3389/fpls.2014.00228
- Berrocal-Lobo M, Molina A (2008) *Arabidopsis* defense response against *Fusarium oxysporum*. Trends Plant Sci 13:145–150. doi:10.1016/j.tplants.2007.12.004

- Bui DC et al (2016) Heat shock protein 90 is required for sexual and asexual development, virulence, and heat shock response in *Fusarium graminearum*. *Sci Rep* 6:28154. doi:10.1038/srep28154
- Campos-Bermudez VA, Fauguel CM, Tronconi MA, Casati P, Presello DA, Andreo CS (2013) Transcriptional and metabolic changes associated to the infection by *Fusarium verticillioides* in maize inbreds with contrasting ear rot resistance. *PLoS ONE* 8:e61580. doi:10.1371/journal.pone.0061580
- Cannesan MA et al (2011) Association between border cell responses and localized root infection by pathogenic *Aphanomyces euteiches*. *Ann Bot* 108:459–469. doi:10.1093/aob/mcr177
- Carvalho HH et al (2014) The molecular chaperone binding protein BiP prevents leaf dehydration-induced cellular homeostasis disruption. *PLoS ONE* 9:e86661. doi:10.1371/journal.pone.0086661
- Chen YC, Wong CL, Muzzi F, Vlaardingerbroek I, Kidd BN, Schenk PM (2014) Root defense analysis against *Fusarium oxysporum* reveals new regulators to confer resistance. *Sci Rep* 4:5584. doi:10.1038/srep05584
- Chen YE, Cui JM, Su YQ, Yuan S, Yuan M, Zhang HY (2015) Influence of stripe rust infection on the photosynthetic characteristics and antioxidant system of susceptible and resistant wheat cultivars at the adult plant stage. *Front Plant Sci* 6:779. doi:10.3389/fpls.2015.00779
- Conesa A, Gotz S (2008) Blast2GO: a comprehensive suite for functional analysis in plant genomics. *Int J Plant Genomics* 2008:619832. doi:10.1155/2008/619832
- de Castro GLS, de Lemos OF, Tremacoldi CR, Moraes FKC, dos Santos LRR, Pinheiro HA (2016) Susceptibility of in vitro black pepper plant to the filtrate from a *Fusarium solani* f. sp. *piperis* culture. *Plant Cell Tissue Organ Cult* 127:263–268. doi:10.1007/s11240-016-1031-4
- de Souza CRB, Brígida ABS, Santos RCd, de Nazaré Monteiro Costa C, Darnet SH, Harada ML (2011) Identification of sequences expressed during compatible black pepper—*Fusarium solani* f. sp. *piperis* interaction. *Acta Physiol Plant* 33:2553–2560. doi:10.1007/s11738-011-0788-7
- Du Z, Zhou X, Ling Y, Zhang Z, Su Z (2010) agriGO: a GO analysis toolkit for the agricultural community. *Nucleic Acids Res* 38:W64–W70. doi:10.1093/nar/gkq310
- Duarte MdLR, Albuquerque F (2001) New diseases affecting black pepper crop in Brazil. *Int Pepper News Bull* 51–57
- Dupuis-Sandoval F, Poirier M, Scott MS (2015) The emerging landscape of small nucleolar RNAs in cell biology. *Wiley Interdiscip Rev RNA* 6:381–397. doi:10.1002/wrna.1284
- FAO (2011) Food and Agriculture Organization of the United Nations, statistics of agricultural production. FAO, Rome. <http://www.fao.org/faostat/>. Accessed 7 July 2017
- Fukutomi M, Hirakata KHM (1981) Studies on the stem rot and root rot diseases of black pepper. Anatomical observations on the distribution on the hyphae of the pathogenic fungus in invading tissues and the blockage of vessel cells. *Fitopatol Bras* 30(6):586
- Gordo SM et al (2012) High-throughput sequencing of black pepper root transcriptome. *BMC Plant Biol* 12:168. doi:10.1186/1471-2229-12-168
- Gunawardena U, Rodriguez M, Straney D, Romeo JT, VanEtten HD, Hawes MC (2005) Tissue-specific localization of pea root infection by *Nectria haematococca*. Mechanisms and consequences. *Plant Physiol* 137:1363–1374. doi:10.1104/pp.104.056366
- Hamada M, Uchida T, Tsuda M (1988) Ascospore dispersion of the causal agent of necrotic blight of *Piper nigrum*. *Japanese J Phytopathol* 54:303–308. doi:10.3186/jjphytopath.54.303
- Hemmerlin A, Harwood JL, Bach TJ (2012) A raison d'être for two distinct pathways in the early steps of plant isoprenoid biosynthesis? *Prog Lipid Res* 51:95–148. doi:10.1016/j.plipres.2011.12.001
- Jaramillo MA, Manos PS (2001) Phylogeny and patterns of floral diversity in the genus *Piper* (Piperaceae). *Am J Bot* 88:706–716
- Joy N, Abraham Z, Soniya EV (2007) A preliminary assessment of genetic relationships among agronomically important cultivars of black pepper. *BMC Genet* 8:42. doi:10.1186/1471-2156-8-42
- Joy N, Asha S, Mallika V, Soniya EV (2013) De novo transcriptome sequencing reveals a considerable bias in the incidence of simple sequence repeats towards the downstream of 'Pre-miRNAs' of black pepper. *PLoS ONE* 8:e56694. doi:10.1371/journal.pone.0056694
- Jung K-H, An G (2013) Application of MapMan and RiceNet drives systematic analyses of the early heat stress transcriptome in rice seedlings. *J Plant Biol* 55:436–449. doi:10.1007/s12374-012-0270-0
- Kal AJ et al (1999) Dynamics of gene expression revealed by comparison of serial analysis of gene expression transcript profiles from yeast grown on two different carbon sources. *Mol Biol Cell* 10:1859–1872
- Liu Y, Yadav VR, Aggarwal BB, Nair MG (2010) Inhibitory effects of black pepper (*Piper nigrum*) extracts and compounds on human tumor cell proliferation, cyclooxygenase enzymes, lipid peroxidation and nuclear transcription factor-kappa-B. *Nat Prod Commun* 5:1253–1257
- Lysøe E, Bone KR, Klemsdal SS (2008) Identification of up-regulated genes during zearalenone biosynthesis in *Fusarium*. *Eur J Plant Pathol* 122:505–516. doi:10.1007/s10658-008-9318-x
- Matthaus K et al (2004) Progression of mycotoxin and nutrient concentrations in wheat after inoculation with *Fusarium culmorum*. *Arch Anim Nutr* 58:19–35
- Meghwal M, Goswami TK (2013) *Piper nigrum* and piperine: an update. *Phytother Res* 27:1121–1130. doi:10.1002/ptr.4972
- Menezes IC, Cidade FW, Souza AP, Sampaio IC (2009) Isolation and characterization of microsatellite loci in the black pepper, *Piper nigrum* L. (piperaceae). *Conserv Genet Resour* 1:209–212. doi:10.1007/s12686-009-9051-6
- Nawrocki EP et al (2015) Rfam 12.0: updates to the RNA families database. *Nucleic Acids Res* 43:D130–D137. doi:10.1093/nar/gku1063
- Nishimura Y, Kitagishi Y, Yoshida H, Okumura N, Matsuda S (2011) Ethanol extracts of black pepper or turmeric down-regulated SIRT1 protein expression in Daudi culture cells. *Mol Med Report* 4:727–730. doi:10.3892/mmr.2011.487
- Nogueira AF et al (2016) Tetrapod limb and sarcopterygian fin regeneration share a core genetic programme. *Nat Commun* 7:13364. doi:10.1038/ncomms13364
- Okubara PA, Paulitz TC (2005) Root defense responses to fungal pathogens: a molecular perspective. *Plant Soil* 274:215–226. doi:10.1007/s11104-004-7328-9
- Petre B et al (2012) RNA-Seq of early-infected poplar leaves by the rust pathogen *Melampsora larici-populina* uncovers PtSultr3;5, a fungal-induced host sulfate transporter. *PLoS ONE* 7:e44408. doi:10.1371/journal.pone.0044408
- Powell JJ et al (2016) The *Fusarium* crown rot pathogen *Fusarium pseudograminearum* triggers a suite of transcriptional and metabolic changes in bread wheat (*Triticum aestivum* L.). *Ann Bot*. doi:10.1093/aob/mcw207
- Rai A, Suprasanna P, D'Souza SF, Kumar V (2012) Membrane topology and predicted RNA-binding function of the 'early responsive to dehydration (ERD4) plant protein. *PLoS ONE* 7:e32658. doi:10.1371/journal.pone.0032658
- Ruesch CE et al (2014) The histone H3 lysine 9 methyltransferase DIM-5 modifies chromatin at frequency and represses light-activated gene expression. *G3 (Bethesda)* 5:93–101. doi:10.1534/g3.114.015446

- Schwarz PB, Schwarz JG, Zhou A, Prom LK, Steffenson BJ (2001) Effect of *Fusarium graminearum* and *F. Poae* infection on barley and malt quality. *Monatsschrift Brauwiss* 54:55–63
- Stegmann M et al (2017) The receptor kinase FER is a RALF-regulated scaffold controlling plant immune signaling. *Science* 355:287–289. doi:[10.1126/science.aal2541](https://doi.org/10.1126/science.aal2541)
- Viegas A, Silva A, Darnet S (2015) HCRA: a hybrid colour-space read-additive method for de novo transcriptomic assembly integrating Illumina and SOLiD datasets. *Plant OMICS* 8:581–586
- Xia XJ, Zhou YH, Ding J, Shi K, Asami T, Chen Z, Yu JQ (2011) Induction of systemic stress tolerance by brassinosteroid in *Cucumis sativus*. *New Phytol* 191:706–720. doi:[10.1111/j.1469-8137.2011.03745.x](https://doi.org/10.1111/j.1469-8137.2011.03745.x)
- Zhu P, Wang Y, Qin N, Wang F, Wang J, Deng XW, Zhu D (2016) Arabidopsis small nucleolar RNA monitors the efficient pre-rRNA processing during ribosome biogenesis. *Proc Natl Acad Sci USA* 113:11967–11972. doi:[10.1073/pnas.1614852113](https://doi.org/10.1073/pnas.1614852113)