https://doi.org/10.1590/1678-4499.20240146

CROP BREEDING
Article

Large-scale gene expression analysis reveals the role of primary metabolism regulation in resistance to Brazilian *Pseudomonas coronafaciens* pv. *garcae* in coffee

Juliana Camargo Martinati Schenk[1], Lucas Mateus Rivero Rodrigues[1,*], Natália Arruda[1], Paula de Souza Guimarães[1], Leandro Cardamone Diniz[2], Antonio Mauro Rezende[3], Suzete Aparecida Lanza Destéfano[4], Lilian Padilha[5], Mirian Perez Maluf[5], Oliveiro Guerreiro Filho[1]

1. Instituto Agronômico de Campinas – Centro de Café Alcides Carvalho – Campinas (SP), Brazil.
2. Embrapa Soja – Londrina (PR), Brazil.
3. Fundação Oswaldo Cruz – Instituto René Rachou – Belo Horizonte (MG), Brazil.
4. Instituto Biológico – Laboratório de Bacteriologia Vegetal – Campinas (SP), Brazil.
5. Embrapa Café – Brasília (DF), Brazil.

Received: Jul. 3, 2024 | **Accepted:** Oct. 1, 2024

Section Editor: Carlos Alberto Scapim

***Corresponding author:** lucasmriverorodrigues@gmail.com

How to cite: Schenk, J. C. M., Rodrigues, L. M. R., Arruda, N., Guimarães, P. S., Diniz, L. C., Rezende, A. M., Destéfano, S. A. L., Padilha, L., Maluf, M. P. and Guerreiro Filho, O. (2025). Large-scale gene expression analysis reveals the role of primary metabolism regulation in resistance to Brazilian *Pseudomonas coronafaciens* pv. *garcae* in coffee. Bragantia, 84, e20240146. https://doi.org/10.1590/1678-4499.20240146

ABSTRACT: This study investigated the response of arabica coffee plants to the pathogen *Pseudomonas coronafaciens* pv. *garcae* using RNA-seq technology. Susceptible and resistant coffee plants were inoculated with the bacteria, and leaf samples were collected at different time points for RNA sequencing. Seven genes related to different defense pathways were chosen for expression quantification in time-course experiments using infected leaves from resistant and susceptible plants, as well as non-infiltrated and water-infiltrated leaves as controls. The results obtained revealed that response mechanisms differ between genotypes and provide insights into the genetic basis of early defense in coffee plants against *P. coronafaciens* pv. *garcae*, offering potential strategies for genetic breeding.

Key words: bacterial halo blight, *Coffea arabica*, RNASeq, defense mechanism.

INTRODUCTION

Bacterial halo blight (BHB), which is caused by *Pseudomonas coronafaciens* pv. *garcae* (PCG), is an emergent and uncontrollable disease in nurseries and plantations. The mainly cultivated *Coffea arabica* and *C. canephora* cultivars are highly susceptible to the disease (Rodrigues et al. 2019).

The disease symptoms usually begin on young leaves as irregular, translucent lesions surrounded by a yellowish halo. This pattern reflects the colonization mode of tissues characterized by movement via vessels and growth near or around stomata, wounds, and other structures (Rodrigues et al. 2015).

Given the recent expansion in the geographic distribution of the disease compared to others, research on PCG remains limited and relatively recent. Rodrigues et al. (2019) identified variability in the resistance response to BHB among 13 *Coffea* species. Immunity to PCG has been observed in some species, including *C. arabica*; however, to date, only one cultivar, IPR 102 (Sera et al. 2017), has been identified as resistant to the pathogen.

The salicylic acid (SA)-mediated defense pathway in plants plays a crucial role in the resistance against pathogens, and recent findings by Ariyoshi et al. (2022) suggest a significant involvement of serine/threonine kinase in this process in coffee plants. This connection between SA signaling and serine/threonine kinase not only reinforces existing hypotheses, but also hints at deeper, unexplored mechanisms in the coffee-PCG resistance interaction, paving the way for further investigation.

High-throughput RNA sequencing (RNA-seq) has been previously utilized to identify differentially expressed genes in coffee plants challenged by various pathogens (Vieira et al. 2016, Castro et al. 2022). In our study, we employed RNA-seq technology to assemble the transcriptome of two *C. arabica* cultivars — one resistant and one susceptible to PCG — to identify genes potentially associated with defense mechanisms. These findings could prove valuable for the selection of markers in breeding programs aimed at enhancing resistance in arabica coffee plants.

MATERIAL AND METHODS

Plant material and inoculation

All experiments were conducted with *C. arabica* cultivars IAC 125 RN (Fazuoli et al. 2018) characterized as susceptible to the BHB and IPR 102 (Sera et al. 2017), classified as resistant to the BHB.

The PCG IBSBF 1197 strain was selected due to its well-known high pathogenicity to coffee plants (Rodrigues et al. 2017). Cryopreserved strain from the Phytobacteria Culture Collection of the Instituto Biológico was used as inoculum (108 CFU·mL^{-1}).

The inoculation experiment was carried out with 6-month-old seedlings. Young and fully expanded coffee leaves from the first internode were inoculated by abrasion with PCG bacterial suspension on the foliar limb, with a total of four plants per genotype. After inoculation, the plants were kept in a laboratory environment, and leaves were collected at 6 and 24 hours post inoculation (HPI) and immediately frozen in liquid nitrogen for subsequent RNA extraction.

RNA sequencing, library construction and data analysis

Total RNA was individually extracted using the RNeasy Plant Mini Kit (QIAGEN), following the manufacturer's protocol. Equal amounts of RNA from susceptible plants collected after 6 and 24 HPI were pooled in a single sample (SS.6-24), as well as resistant plant (SR.6-24), and diluted to the concentration of 50 ng/μL. After pooling, aliquots from each sample were sent in duplicate (SS.6-24.1; SS.6-24.2; SR.6-24.1; SR.6-24.2) for RNA sequencing. The steps of RNA to cDNA conversion, cDNA precipitation and purification, cDNA quantification by spectrophotometry, library construction, and sequencing using Illumina HiSeq2500 technology were performed by Biodome laboratory. A total of four libraries (two replicates of each treatment SS6_24 and SR6_24) was subjected to paired-end sequencing (2×125 pb).

Quality of reads was verified using the FASTQC tool. Subsequently, all reads were mapped against a reference genome of *C. canephora*, (https://coffee-genome.org/download), using the STAR tool with the quantMode, GeneCounts, and twpassMode, Basic parameters. After mapping, gene read counts for individual samples were loaded into R (https://www.R-project.org/), and the DESeq2 package (Love et al. 2014) was utilized for differential gene expression analysis. Functional annotation and categorization based on Gene Ontology, as well as mapping to the Kyoto Encyclopedia of Genes and Genomes, and the metabolic pathways were performed using the Blast2GO program (Conesa et al. 2005).

Differentially expressed genes (DEG), in susceptible (control) and resistant (test) coffee plants with log2 foldchange values > 3 were identified and annotated respectively. From these, seven genes were selected for validation analyses.

Validation of selected genes

Six-month-old coffee seedlings were used for infiltration with PCG suspension, prepared as previously described. Inoculations were performed according to an original procedure from Harborg (1970) modified by Mazzafera and Eskes (1977)[1] by infiltrating the bacterial suspension into the foliar limb, with a total of 12 plants per genotype.

[1] Mazzafera and Eskes, A. B. (1987). Comparação de dois métodos para infiltração de substâncias no mesofilo de folhas de cafeeiro (p. 113-114). *In* 14 Congresso Brasileiro de Pesquisas Cafeeiras and I Congresso Latino-Americano de Tecnologia Cafeeira. Campinas (SP), Brazil.

Control groups consisted of plants infiltrated with distilled water (n = 12) and healthy coffee plants without contact with the pathogen or injuries from inoculations (n = 6). After infiltration, the plants were kept in a laboratory environment, and leaves were collected at 8 and 24 HPI (n = 6 for infiltrated plants and n = 3 for mock plants), and immediately frozen in liquid nitrogen for subsequent RNA extraction. For gene validation, the total RNA of each time sampling was extracted in duplicate by cetyltrimethylammonium bromide (CTAB) method (Chang et al. 1993). Samples used for validations were not pooled to detect expression along bacterial infection. Quantification of the expression of seven selected genes was conducted by quantitative polymerase chain reation (qPCR) using an ABI7300 instrument. The qPCR reactions were carried out in a final volume of 15 µL, containing 400 ng of cDNA, 200 mM of each primer, and 1X SYBR Green master mix (Promega). All samples were amplified in triplicates, and the quantification was performed in three independent analyses. Negative controls without cDNA were included to detect any signs of contamination.

To confirm the presence of single amplicons, polymerase chain reaction (PCR) products were subjected to a dissociation curve with a temperature ranging between 60 and 95°C. The constitutive gene GAPDH was used as an endogenous control for normalization. The relative expression of all treatments was calculated using the values obtained from samples of cultivar IAC 125 RN used as a calibrator.

RESULTS AND DISCUSSION

RNAseq data and differentially expressed genes

To gain a comprehensive understanding of genes expressed during PCG infection in coffee leaves, this study employed an RNASeq approach to compare the responses in resistant (IPR 102) and susceptible (IAC 125 RN) cultivars of *C. arabica* at two-time points during bacterial infection.

The generated reads obtained by transcriptomic analysis were mapped to the reference genome of *C. canephora* (https://coffee-genome.org/download), and the percentage of mapping was approximately 80%, indicating high quality sequencing data (Table 1).

Table 1. Summary of reads extraction and mapping.

Sample	Total Reads	Mapped reads	Mapping percentage
IPR 102 pool sample 1 (SS.6-24.1)	28,220,384	22,387,445	79.33
IPR 102 pool sample 2 (SS.6-24.2)	23,353,905	18,822,888	80.60
IAC 125 RN pool sample 1 (SR.6-24.1)	28,606,102	24,307,643	84.97
IAC 125 RN pool sample 2 (SR.6-24.2)	31,564,698	24,938,418	79.01

Functional categorization of these DEG revealed the presence of 91 genes differentially expressed that were up regulated in the resistant plants compared to 28 genes up regulated in the susceptible plants. Most activated genes (26%) in resistant plants belong to the category of integral membrane component genes, with a wide range of functions such as signal recognition particle receptor complex, zinc ion binding, potassium ion transmembrane transporter activity, and lipid metabolic process. Following these are the genes responsible for catalytic activity, cellular wall modification, and inositol catabolic process, each accounting for 5% of the expression (Fig. 1). In susceptible plants, most activated genes belong to the disease resistance NB-LRR family and uncharacterized protein unknown function class, each representing 21% of the expressions (Fig. 2).

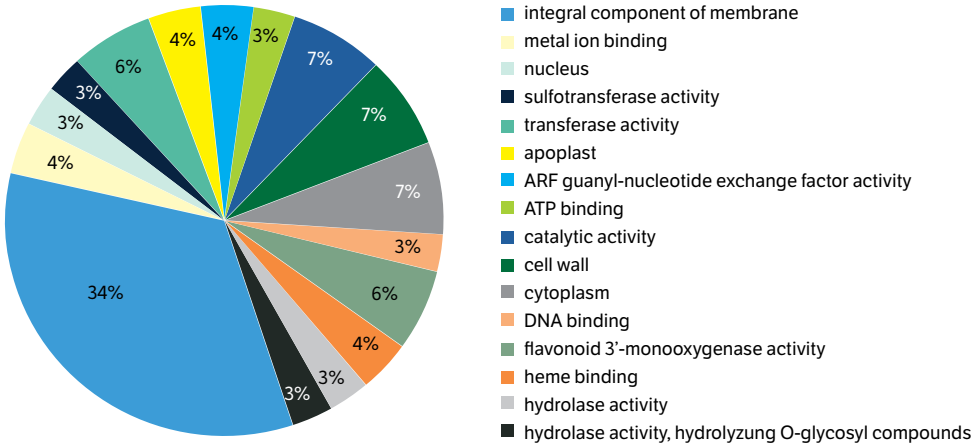


Figure 1. Relative amount of differentially expressed genes in resistant coffee genotype, IPR 102, inoculated with *Pseudomonas coronafaciens* pv. *garcae* at 6–24 hours post inoculation. Each fragment represents a biological process, or a molecular function of those genes identified as upregulated in resistant coffee plants, according to Kyoto Encyclopedia of Genes and Genomes pathways.

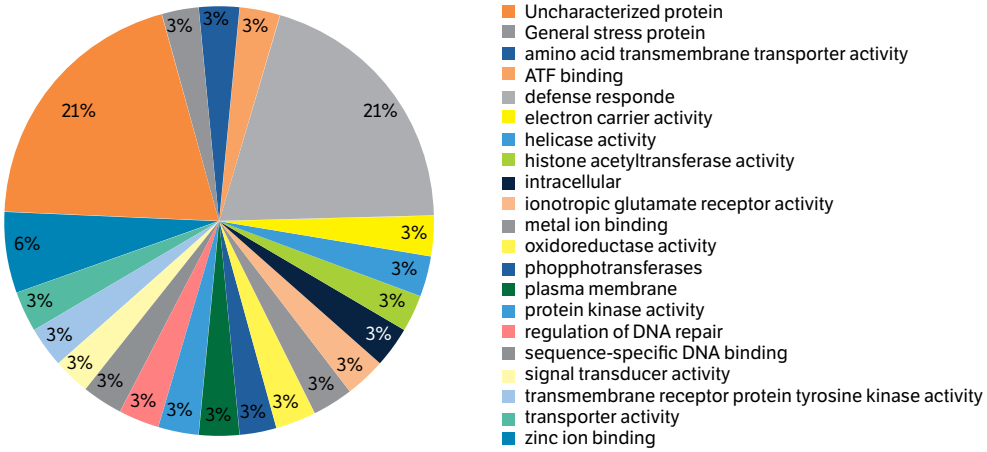


Figure 2. Relative amount of differentially expressed genes in susceptible coffee genotype, IAC 125 RN, inoculated with *Pseudomonas coronafaciens* pv. *garcae* at 6–24 hours post inoculation. Each fragment represents a biological process, or a molecular function of those genes identified as upregulated in susceptible coffee plants, according to Kyoto Encyclopedia of Genes and Genomes pathways.

The well described mechanism in the first line of response to bacterial attack is the pathogen/microbe-associated molecular patterns (PAMP/MAMP), recognized by the transmembrane pattern recognition receptors (PRR). After MAMP recognition, the PRR initiates signal transduction and activates pattern-triggered immunity (PTI), which includes a burst of signaling molecules, such as reactive oxygen/nitrogen species (ROS/RNS) and Ca2+ and calcium-dependent protein kinases (Newman et al. 2013).

In the second phase, the pathogen successfully deploys effectors that results in effector-triggered susceptibility (ETS). In the third phase, these effectors are recognized by the NB-LRR proteins that intercept pathogen effectors and an effector-triggered immunity (ETI) is stablished (Jones and Dangl 2006). The NB-LRR proteins are a family of proteins (nucleotide-binding and leucine-rich repeat) classified as receptor-like kinases (RLKs) and receptor-like proteins (RLPs) (Tang et al. 2017), in which the most R genes belong (Cesari et al. 2014). The third phase is only effective for resistance, when bacteria isolates carry the recognized effector called an avirulence protein (AVR).

The transcriptome analysis was taken in the first 24 HPI and displayed the primary defense responses in coffee plants infected with PCG. The activation of major membrane and related genes in resistant coffee plants could explain the intense

modulation in the cell membrane at the early stages against PCG attack and could help to stablish a linking between gene expression and the physiology of defense mechanism.

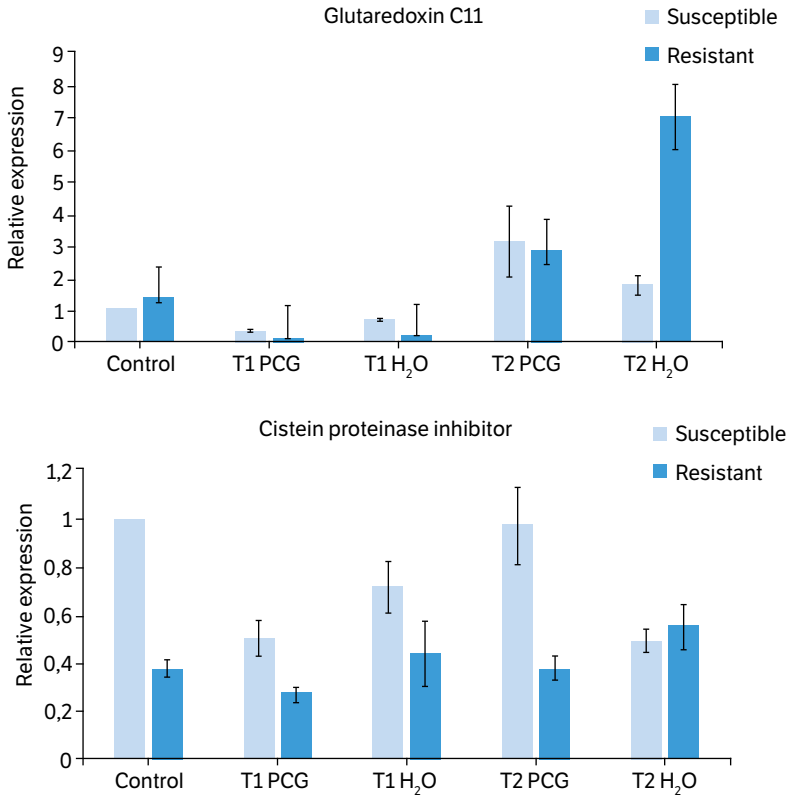
Resistant plants present an activation mostly in cell membrane genes including an ADP-ribosylation factor (ARF) exchange factor activity. This factor is essential to activate the ARF proteins. Likewise, the ARF proteins family is a part of a group of proteins responsible for the modulation of the lipid membrane composition by regulating membrane traffic and organelle structure (Singh and Jürgens 2018).

Furthermore, shikimate dehydrogenase protein was also found in resistant plants indicating that shikimate synthesis at the first 24 HPI. It is known that the shikimate pathway is an essential metabolic path in plants (Herrmann and Weaver 1999), being a precursor for the biosynthesis of aromatic amino acids such as phenylalanine, tyrosine, and tryptophan (Almeida et al. 2024).

Although susceptible coffee plants also activated defense response genes, most of them are related to an "uncharacterized" ADP-binding protein. This mode of action in susceptible coffee plants exhibits an attempt to inhibit the disease spread, but it does not seem to be an effective defense mechanism. The difference in the expression of these genes in resistant and susceptible coffee plants could be explained by the agility of the immune system in responding to PCG attack and an orchestrated expression in resistant plants, mostly in genes related to membranes.

Based on the results of the differential expression analyses, we established a cutoff of log2 foldchange values > 3 in both susceptible and resistant plants, to select genes for validation based on their potential involvement in defense response and use in assisted selection for plant breeding strategies. The selected genes are associated with different defense response pathways, including redox (Glutharedoxin C11), hormone signaling (Snakin-2 and Win-1), pathogen inhibition (CY-proteinase inhibitor), and ion transport (TP1, TP2, and PAP). The results of these analyses are shown in Figs. 3, 4 and 5.

The qPCR profile of the gene glutaredoxin C11 shows similar expression patterns to the RNASeq profile, with activation observed in both susceptible and resistant control plants (Fig. 3). However, upon infection, this expression profile shifts, with the gene being repressed at 8 HPI in both bacterial and mock-inoculated plants, and then activated again at 24 HPI.



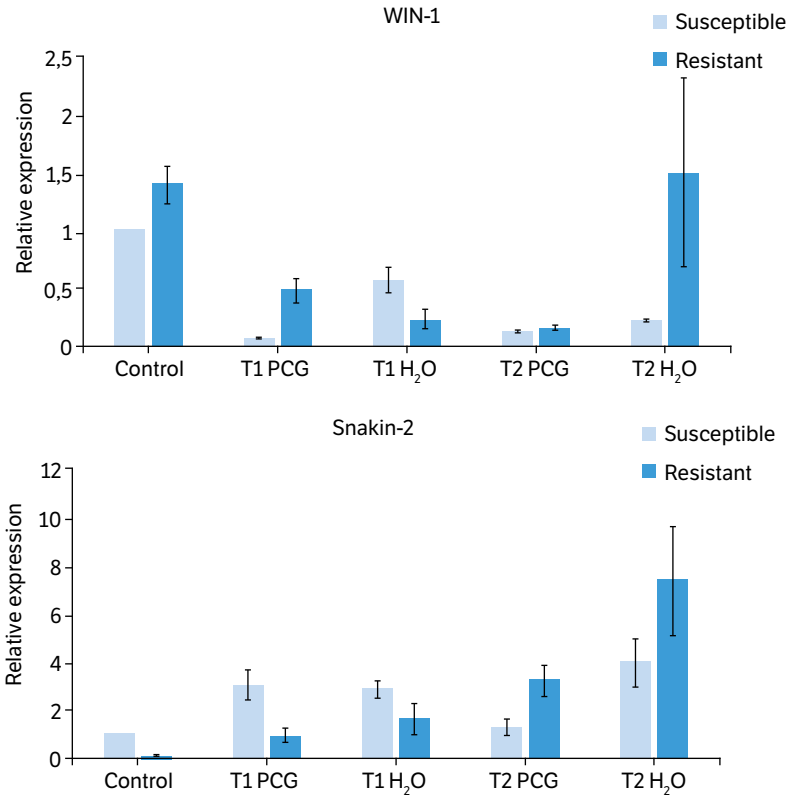
T1: 8 hours post inoculation; T2: 24 hours post inoculation; bars represent the standard deviation.

Figure 3. Relative expression of defense related genes in resistant (IPR 102) and susceptible (IAC 125 RN) plants inoculated with *Pseudomonas coronafaciens* pv. *garcae* (PCG) or water (H_2O).

The oxidative burst, an important step in plant defense, requires the activation of several genes, including members of the glutaredoxin gene family (Meyer et al. 2012), which are important components of the redox control system. The expression pattern of a glutaredoxin family member observed here suggests an adjustment to oxidative stress response in both resistant and susceptible plants, but it is not specific to bacterial infection. Another hypothesis for the observed expression levels of C11 may be the control exerted by the bacterium via virulence genes on the jasmonic acid-mediated defense pathways (Gupta et al. 2020). In this case, the expression levels of C11 are maintained at low levels in both genotypes at 24 HPI when compared with water infiltration, indicating that the resistance is not affected by this strategy of PCG.

The profile of CY-proteinase inhibitor, as observed in both RNASeq and qPCR analyses, indicates higher expression in control susceptible plants, followed by a decrease upon infection (Fig. 3). This repression is also observed in resistant plants, although to a lesser extent, suggesting that this gene may not be directly involved in the defense and resistance responses.

Another gene with a repression profile upon bacterial infection is WIN-1, as evidenced by a decline in transcript accumulation in both susceptible and resistant plants (Fig. 4). However, mock-inoculated plants exhibit a different expression pattern, with a recovery of transcript accumulation observed in resistant plants at 24 HPI.



T1: 8 hours post inoculation; T2: 24 hours post inoculation; bars represent the standard deviation.

Figure 4. Relative expression of differentially expressed genes related to signaling and activation of defense routes in resistant (IPR 102) and susceptible (IAC 125 RN) plants inoculated with *Pseudomonas coronafaciens* pv. *garcae* (PCG) or water (H_2O).

The results obtained for WIN-1, a key element in the ethylene signaling pathway, imply that the defensive response orchestrated by ethylene might be suppressed during the initial phases of infection. However, it is important to acknowledge that the outcomes derived from the gene WIN analysis do not provide a robust foundation for drawing a definitively conclusive assertion.

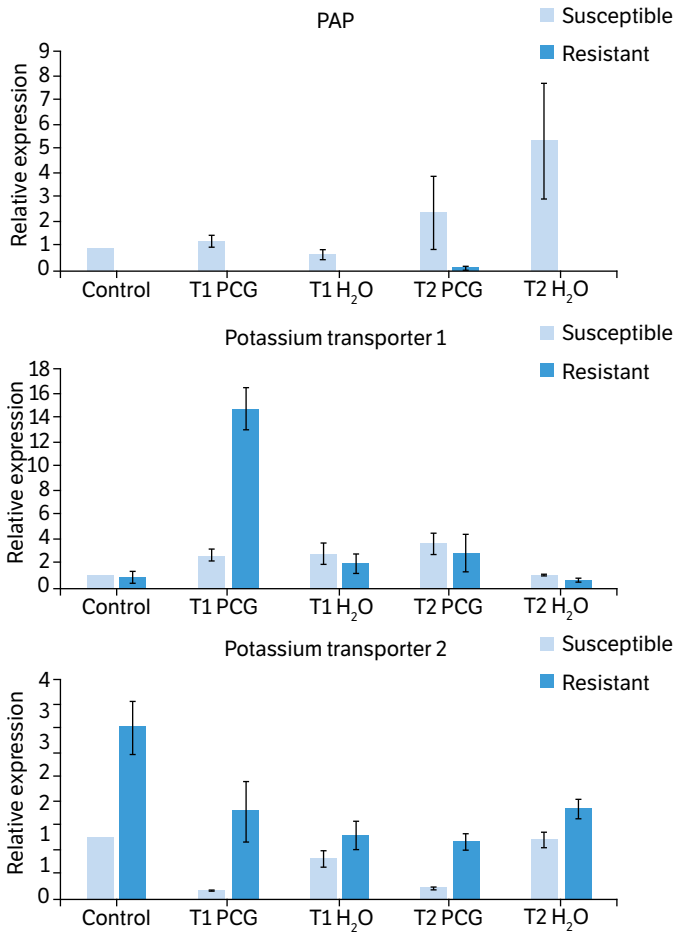
Snakin-2, which is associated with the SA signaling pathway (Fig. 4), is another defense-related gene evaluated in this study. It is activated in both susceptible and resistant plants upon infection, but with different timing. Susceptible plants activate the gene during the first 8 HPI and then repress it at 24 HPI, while resistant plants display a gradual accumulation of Snakin-2 transcripts upon infection, peaking at 24 HPI.

On the other hand, the expression profile of Snakin-2 showed that gene activation is more pronounced upon water infection in both genotypes, indicating the activation of a non-specific defense response. Ariyoshi et al. (2022) demonstrated the probable involvement of serine/threonine kinase in the defense against PCG in coffee plants, reinforcing the hypothesis that the SA-associated pathway is closely related to the resistance interaction between coffee and PCG.

The expression of this gene in susceptible control plants suggests that the Snakin gene is constitutively expressed (Herbel et al. 2017). This gene is also expressed in various tissues during development (Berrocal-Lobo et al. 2002) and is locally induced by wounds (Herbel et al. 2017). Therefore, its function is primarily associated with both constitutive and inducible defense barriers (Berrocal-Lobo et al. 2002) and is also linked to ROS balance. Leaves of transgenic potato plants with silenced Snakin-1 exhibited increased levels of ROS and significantly reduced ascorbic acid content (Nahirñak et al. 2019). It is also known that SA signaling is hindered in plants with Snakin overexpression (Zhang and Wang 2011).

Perhaps, this higher level of expression in susceptible plants during the initial stages blocks the entire SA signaling cascade, thereby influencing the resistance process. The "late" expression of the Snakin gene in resistant plants suggests a strategic contribution to sustained defense responses. Further research is crucial to elucidate the precise mechanisms underlying these intricate functions.

Other category of genes evaluated in this study includes those related to phosphorus foraging and recycling, the purple acid phosphatases (PAP), and potassium transporters (PT1 and PT2). The expression of PAP dramatically increases upon infection in susceptible plants, but it is practically absent in resistant plants at all evaluated time points. At 24 HPI, there is a slight increase in expression observed in resistant plants, but it is not as pronounced as in susceptible plants (Fig. 5).



T1: 8 hours post inoculation; T2: 24 hours post inoculation; bars represent the standard deviation; PAP: purple acid phosphatases.

Figure 5. Relative expression of differentially expressed genes from primary metabolism pathways in resistant (IPR 102) and susceptible (IAC 125 RN) plants inoculated with *Pseudomonas coronafaciens* pv. *garcae* (PCG) or water (H_2O).

Previous studies have already demonstrated the association between PAP gene expression and the SA signaling pathway (Ravichandran et al. 2015, Zhang et al. 2007). The expression of genes involved in the SA pathway signaling, such as PDF and PR genes, was significantly suppressed in PAP knockout plants at 24 HPI, leading to an enlargement in lesion size. This inference may indicate that the preferentially employed signaling pathway by PAP genes derives from the phenylalanine ammonia lyase (PAL) route rather than the isochorismate synthase (ICS) route in the SA metabolic pathway (Ravichandran et al. 2015).

The expression levels of PAP genes also seem to have a direct impact on the modulation of defense genes. Ravichandran et al. (2015), examining the expression of the PAP5 gene in *Pseudomonas syringae* pathovar *tomato* strain, DC3000 (Pst DC3000)-infected Arabidopsis, reported that an optimal level of PAP5 expression is necessary for complete basal resistance. Overexpression of PAP5 in *Arabidopsis* impaired the expression of ICS1 and PR1 genes, as well as the accumulation of SA, similarly observed in mutant pap5 knockout plants. Furthermore, PAP5 overexpression in plants also hindered the accumulation of H_2O_2 in response to Pst DC3000. Although this is not this study focus, the higher expression levels in susceptible coffee plants could potentially be explained. The same authors suggest that, since PAP5 is induced only during the initial stages (6 HPI) of Pst DC3000 infection and its localization within the peroxisome, PAP5 might also function as a component in reactive oxygen intermediate (ROI) generation.

Two different potassium transporter genes were validated in this study. Interestingly, while no differential expression was detected by RNASeq, qPCR analysis revealed distinct expression profiles for these genes (Fig. 5). Despite their similar function, PT2 is activated in control resistant plants and its expression decreases over time during the treatment. A similar expression pattern is observed in susceptible plants, but the transcript levels decline more significantly in bacterial-infected plants. Finally, 8 HPI the PT1 gene shows an extremely high level of expression in resistant plants inoculated with PCG, in relation to the other treatments.

The validation of potassium transport indicates that coffee plants may utilize different routes for potassium mobilization during defense, and one of these routes may be triggered by pathogen recognition. The expression profile of PT1 indicates a specific response to bacterial infection in resistant plants, as transcript levels increase at 8 HPI and then decrease thereafter. In susceptible plants, PT1 expression is slightly activated compared to control samples, but it does not vary along treatments. On the other hand, expression of PT2 is repressed by bacteria infection on susceptible plants, confirming that potassium transport plays an important role on resistance mechanisms.

Studies indicate that bacteria benefit from the absence or deficiency of K+ within the plant (Medzhitov and Janeway Jr. 2002). During the recognition of PAMPs, the transport of K+ across the membrane is essential to initiate plant defense signaling processes. The loss of K+ promotes PAMP activation and aids in bacterial growth. Upon PAMP activation, a substantial influx of K+ occurs in the cell, signaling a downstream immune response (Medzhitov and Janeway Jr. 2002).

In animal cells, TLR4 binds to bacterial PAMP lipopolysaccharide (LPS) and induces efflux of K+ through the MaxiK K+ channel, activating signaling cascades. This leads to the release of pro-inflammatory tumor necrosis factor-alpha and potassium transporters of the HAK5 type, along with other kinases, contributing to the intracellular immune response (Brauer et al. 2016).

Resistant coffee plants to *Pseudomonas* display higher expression of K+ transporter genes, indicating a heightened mobilization of this element in their metabolism. Beyond the primary response to bacterial infection, potassium levels also positively influence K+ regulation, resulting in reduced ROS production in plants (Hasanuzzaman et al. 2020). This reduction is accompanied by a decrease in NADPH oxidases and the maintenance of photosynthetic electron transport activity, which assists in lowering ROS levels (Foyer 2018).

Among the seven genes evaluated, only PT1 and WIN-1 were biologically validated, as they exhibit compatible expression profiles between RNAseq and real-time qPCR techniques in the evaluation of the transcriptome. These genes likely act as key elicitors in the resistance response pathway to PCG.

The validated genes from this study will be further analyzed to assess their utility in selecting resistant coffee plants and eliminating undesirable individuals. By targeting genes involved in defense mechanisms such as redox processes, hormone signaling, pathogen inhibition, and ion transport, this research aimed to refine breeding strategies. Successful validation

of these genes will enhance our understanding of plant resistance and support the development of coffee varieties with improved resistance to bacterial infections.

These findings highlight the complexity of the interaction between PCG-coffee plants, emphasizing the need for further studies to better understand the mechanisms involved. Understanding the genetic basis of resistance and susceptibility to pathogens in plants is fundamental for the development of strategies for genetic improvement and disease management, aiming to guarantee the sustainability and productivity of coffee crops.

CONCLUSION

Our study provided data for *C. arabica* infected with the bacterial pathogen *P. coronafaciens* pv. *garcae* and revealed a transcriptomic profile response to this pathogen that involves multiple responses including a basal non-host defense strategy, a balance in ROS producing/scavenging, and a pathogen response associated with potassium transport. The differences in time-expression genes between resistant and susceptible coffee plants were revealed and supported that an early defense state is crucial for triggering a rapid resistance response in coffee plants.

CONFLICT OF INTEREST

Nothing to declare.

AUTHORS' CONTRIBUTION

Conceptualization: Rodrigues, L. M. R. and Guerreiro Filho, O.; **Methodology:** Schenk, J. C. M., Arruda, N., Guimarães, P. S. and Destéfano, S. A. L.; **Formal analysis:** Diniz, L. C. and Rezende, A. M. **Writing – original draft:** Rodrigues, L. M. R., Padilha, L., Maluf, M. P. and Guerreiro Filho, O. **Writing – review and editing:** Schenk, J. C. M., Rodrigues, L. M. R., Padilha, L., Maluf, M. P. and Guerreiro Filho, O. **Project administration:** Rodrigues, L. M. R. and Guerreiro Filho, O.

DATA AVAILABILITY STATEMENT

Not applicable.

FUNDING

Coffee Research Consortium
Grants Nos.: 2 13 02 001 00 03; 02 13 02 001 00 07

Conselho Nacional de Desenvolvimento Científico e Tecnológico
Grant No.: DT 307.610/2020-9

Instituto Nacional de Ciência e Tecnologia – Café
Grant No.: 465.580/2014-9

ACKNOWLEDGMENTS

The authors are grateful to the National Council for Scientific and Technological Development (CNPq) for a research fellowship (OGF CNPq DT 307.610/2020-9), the Coordination of Superior Level Staff Improvement, the Foundation for Research Support of the State of Minas Gerais, the Nacional Institute of Coffee Science and Technology, the Secretariat of Agriculture and Supply of the State of São Paulo, and the Coffee Research Consortium for financial support.

REFERENCES

Almeida, A. M., Marchiosi, R., Abrahão, J., Constantin, R. P., Santos, W. D. and Ferrarese-Filho, O. (2024). Revisiting the shikimate pathway and highlighting their enzyme inhibitors. Phytochemistry Reviews, 23, 421-457. https://doi.org/10.1007/s11101-023-09889-6

Ariyoshi, C., Sant'ana, G. C., Felicio, M. S., Sera, G. H., Nogueira, L. M., Rodrigues, L. M. R., Ferreira, R. V., da Silva, B. S. R., Resende, M. L. V., Destéfano, S. A. L., Domingues, D. S. and Pereira, L. F. P. (2022). Genome-wide association study for resistance to *Pseudomonas syringae* pv. *garcae* in *Coffea arabica*. Frontiers in Plant Science, 13, 989847. https://doi.org/10.3389/fpls.2022.989847

Berrocal-Lobo, M., Segura, A., Moreno, M., López, G., García-Olmedo, F. and Molina A. (2002). Snakin-2, an antimicrobial peptide from potato whose gene is locally induced by wounding and responds to pathogen infection. Plant Physiology, 128, 951-961. https://doi.org/10.1104/pp.010685

Brauer, E. K., Ahsan, N. D. R., Kato, N., Coluccio, A. E., Piñeros, M. A., Kochian, L. V., Thelen, J. J. and Popescu, S. C. (2016). The raf-like kinase ILK1 and the high affinity K+ transporter HAK5 Are required for innate immunity and abiotic stress response. Plant Physiology, 171, 1470-1484. https://doi.org/10.1104/pp.16.00035

Castro, I. S. L., Freitas-Lopes, R. L., Ferreira, S. S., Maciel, T. E. F., Florez, J. C., Zambolim, E. M., Zambolim, L. and Caixeta, E. T. (2022). Transcriptome Analysis Uncovers the Gene Expression Profile of *Hemileia vastatrix* (Race XXXIII) during the Interactions with Resistant and Susceptible Coffee. Agronomy, 12, 444. https://doi.org/10.3390/agronomy12020444

Cesari, S., Kanzaki, H., Fujiwara, T., Bernoux, M., Chalvon, V., Kawano, Y., Shimamoto, K., Dodds, P., Terauchi, R. and Kroj, T. (2014). The NB-LRR proteins RGA4 and RGA5 interact functionally and physically to confer disease resistance. EMBO Journal, 33, 1941-1959. https://doi.org/10.15252/embj.201487923

Conesa, A., Götz, S., García-Gómez, J. M., Terol, J., Talón, M. and Robles, M. (2005). Blast2GO, a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatic, 15, 3674-3676. https://doi.org/10.1093/bioinformatics/bti610

Chang, S., Puryear, J. and Cairney, J. (1993). A simple and efficient method for isolating RNA from pine trees. Plant Molecular Biology Reporter, 11, 113-116. https://doi.org/10.1007/BF02670468

Fazuoli, L. C., Braghini, M. T., Silvarolla, M. B., Gonçalves, W., Mistro, J. C., Gallo, P. B. and Guerreiro Filho, O. (2018). IAC 125 RN - A dwarf coffee cultivar resistant to leaf rust and root-knot nematode. Crop Breeding and Applied Biotechnology, 18, 237-240. https://doi.org/10.1590/1984-70332018v18n2c35

Foyer, C. H. (2018). Reactive oxygen species, oxidative signaling and the regulation of photosynthesis. Environmental and Experimental Botany, 154,134-142. https://doi.org/10.1016/j.envexpbot.2018.05.003

Gupta, A., Bhardwaj, M. and Tran, L. S. P. (2020). Jasmonic acid at the crossroads of plant immunity and *Pseudomonas syringae* virulence. International Journal of Molecular Sciences, 21, 7482. https://doi.org/10.3390/ijms21207482

Harborg, W. A. F. (1970). A device for injecting solution and suspension into thin leaves of plants. Canadian Journal of Botany, 48, 1135-1136. https://doi.org/10.1139/b70-165

Hasanuzzaman, M., Bhuyan, M. H. M. B., Zulfiqar, F., Raza, A., Mohsin, S. M., Mahmud, J. A., Fujita, M. and Fotopoulos, V. (2020). Reactive oxygen species and antioxidant defense in plants under abiotic stress, Revisiting the crucial role of a universal defense regulator. Antioxidants, 9, 681. https://doi.org/10.3390/antiox9080681

Herbel, V., Frank, J. S. and Wink, M. (2017). The antimicrobial peptide snakin-2 is upregulated in the defense response of tomatoes (*Solanum lycopersicum*) as part of the jasmonate-dependent signaling pathway. Journal of Plant Physiology, 208, 1-6. https://doi.org/10.1016/j.jplph.2016.10.006

Herrmann, K. M., & Weaver, L. M. (1999). The shikimate pathway. Annual Review of Plant Biology, 50, 473-503. https://doi.org/10.1146/annurev.arplant.50.1.473

Jones, J. D. G. and Dangl, J. L. (2006). The plant immune system. Nature, 444, 323-329. https://doi.org/10.1038/nature05286

Love, M. I., Huber, W. and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology, 15, 550. https://doi.org/10.1186/s13059-014-0550-8

Medzhitov, R. and Janeway Jr., C. A. (2002). Decoding the patterns of self and nonself by the innate immune system. Science, 296, 298-300. https://doi.org/10.1126/science.1068883

Meyer, Y., Belin, C., Delorme-Hinoux, V., Reichheld, J. P. and Riondet, C. (2012). Thioredoxin and glutaredoxin systems in plants, molecular mechanisms, crosstalks, and functional significance. Antioxidants & Redox Signaling, 17, 1124-1160. https://doi.org/10.1089/ars.2011.4327

Nahirñak, V., Rivarola, M., Almasia, N. I., Barón, M. P. B., Hopp, H. E., Vile, D., Paniego, N. and Rovere, C. V. (2019). Snakin-1 affects reactive oxygen species and ascorbic acid levels and hormone balance in potato. PLoS One, 14, e0214165. https://doi.org/10.1371/journal.pone.0214165

Newman, M. A., Sundelin, T., Nielsen, J. T. and Erbs, G. (2013). MAMP (microbe-associated molecular pattern) triggered immunity in plants. Frontiers in Plant Science, 4, 139. https://doi.org/10.3389/fpls.2013.00139

Ravichandran, S., Stone, S. L., Benkel, B., Zhang, J., Berrue, F. and Prithiviraj, B. (2015). Optimal level of purple acid phosphatase5 is required for maintaining complete resistance to *Pseudomonas syringae*. Frontiers in Plant Science, 6, 568. https://doi.org/10.3389/fpls.2015.00568

Rodrigues, L. M. R., Almeida, I. M. G., Patrício, F. R. A., Beriam, L. O. S., Maciel, K. W., Braghini, M. T. and Guerreiro Filho, O. (2017). Aggressiveness of strains and inoculation methods for resistance assessment to bacterial halo blight on coffee seedlings. Journal of Phytopathology, 165, 105-114. https://doi.org/10.1111/jph.12543

Rodrigues, L. M. R., Destéfano, S. A. L., Almeida, I. M. G., Beriam, L. O. S., Braghini, M. T. and Guerreiro Filho, O. (2019). Multiple resistance to bacterial halo blight and bacterial leaf sport in *Coffea* spp. Arquivos do Instituto Biológico, 86, 1-9. https://doi.org/10.1590/1808-1657000632018

Rodrigues, L. M. R., Queiroz-Voltan, R. B. and Guerreiro Filho, O. (2015). Anatomical changes on coffee leaves infected by *Pseudomonas syringae* pv. *garcae*. Summa Phytopathologica, 41, 256-261. https://doi.org/10.1590/0100-5405/2049

Sera, G. H., Sera, T. and Fazuoli, L. C. (2017). IPR 102 - Dwarf Arabica coffee cultivar with resistance to bacterial halo blight. Crop Breeding and Applied Biotechnology, 17, 403-407. https://doi.org/10.1590/1984-70332017v17n4c60

Singh, M. K. and Jürgens, G. (2018). Specificity of plant membrane trafficking – ARFs, regulators and coat proteins. Seminars in Cell and Developmental Biology, 80, 85-93. https://doi.org/10.1016/j.semcdb.2017.10.005

Tang, D., Wang, G., & Zhou, J. M. (2017). Receptor kinases in plant-pathogen interactions: more than pattern recognition. The Plant Cell, 29, 618-637. https://doi.org/10.1105/tpc.16.00891

Vieira, A., Cabral, A., Fino, J., Azinheira, H. G., Loureiro, A., Pedro Talhinhas, P., Pires, A. S., Vitor Varzea, V., Moncada, P., Oliveira, H., Silva, M. C., Paulo, O. S. and Batista, D. (2016). Comparative validation of conventional and RNA-Seq data-derived reference genes for qPCR expression studies of Colletotrichum kahawae. PLoS One, 11, e0150651. https://doi.org/10.1371/journal.pone.0150651

Zhang, S. and Wang, X. (2011). Overexpression of GASA5 increases the sensitivity of Arabidopsis to heat stress. Journal of Plant Physiology, 168, 2093-2101. https://doi.org/10.1016/j.jplph.2011.06.010

Zhang, W., Gruszewski, H. A., Chevone, B. I. and Nessler, C. L. (2007). An Arabidopsis purple acid phosphatase with phytase activity increases foliar ascorbate. Plant Physiology, 146, 431-440. https://doi.org/10.1104/pp.107.109934