

Introduction

Colombian cacao has a growing demand due to its appreciated flavor and aroma; this crop is affected by pests and diseases that mitigate its production. Different species of the oomycete *Phytophthora* produced the Black pod disease worldwide. *Phytophthora palmivora* Butler is present in all cacao-producer countries and causes losses of 20 to 30% of the production and 10% of the death of cacao trees (Bailey y Meinhardt, 2016).

In Colombia, pod production and the generation of seedlings in nurseries are severely affected by this disease. This study's purpose was to understand better the defense mechanisms related to the plant response during the pathogen infection. We used a transcriptomic approach to obtain information about the genes involved in the resistance to diseases. Dual RNA-sequencing permitted the simultaneous capture of pathogen-specific transcripts and the plant defense-expressed genes during infection, providing a complete view of the interaction. Our goal was to identify the pathogen and plant genes expressed during the early stages of black pod infection (0h, 24h, 48h, and 96h), comparing the expression patterns in two cacao genotypes with contrasting responses.

Methodology

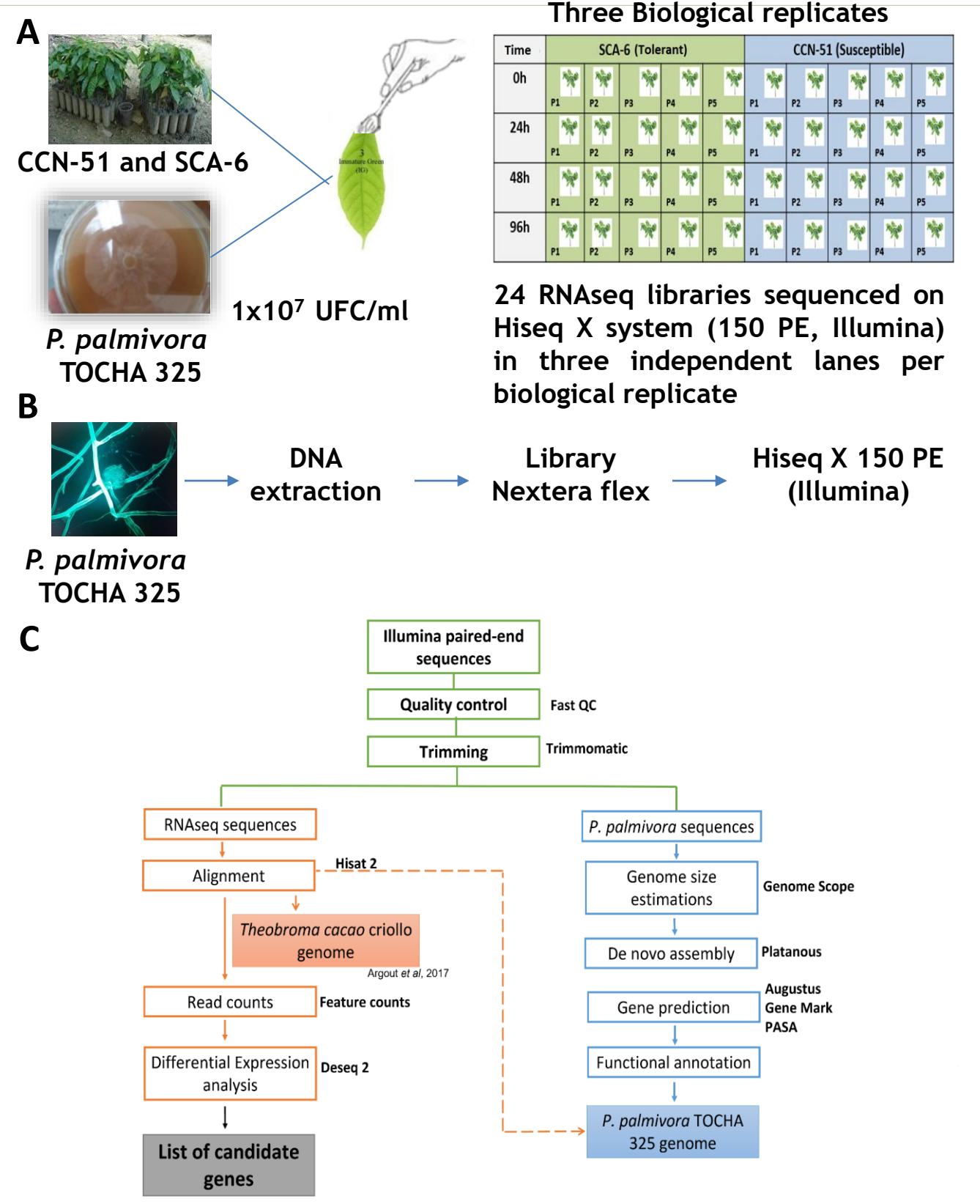


Figure 1: Methodology for conducting dual transcriptomics in *P. palmivora* *T. cacao*. A. Experimental design for the RNAseq assay. B. Workflow for obtaining *P. palmivora* genome C. Bioinformatic workflow to conduct *P. palmivora* assembly and dual RNAseq assembly.

Preliminary results

Table 1. Main statistics of *P. palmivora* Tocha 325 genome assembly and annotation.

<i>P. palmivora</i> Tocha325	
Total length	129.459.292
# Scaffolds	35.910
Largest Scaffold	101.544
N50	8.039
L50	4.006
% GC	49,42
BUSCO Complete Stramenopiles	89%(89/100)
BUSCO Complete Alveolata	86,6%(148/171)
Genes	41.032
Transcripts	40.793

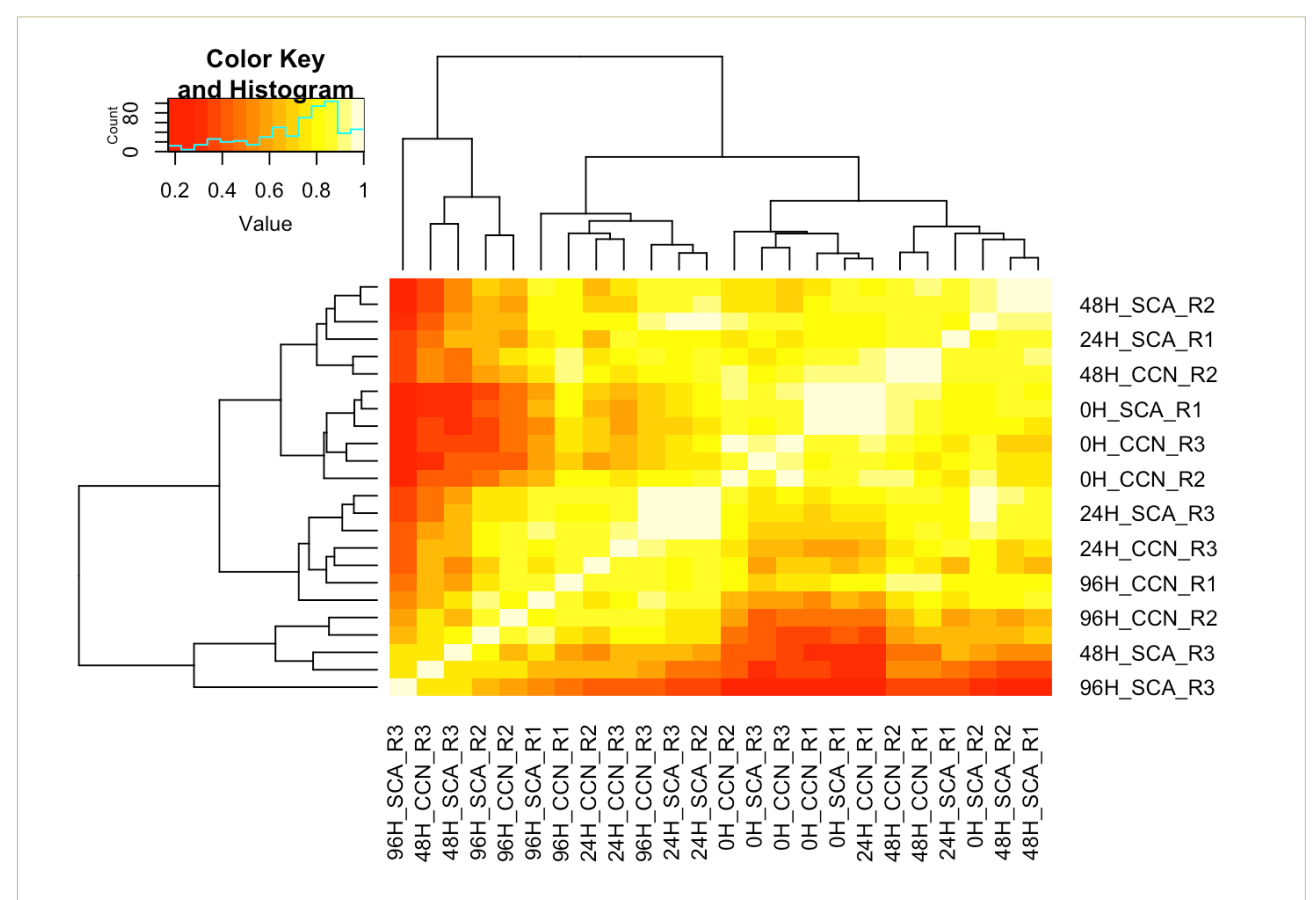


Figure 2: Heatmap of RNA-Seq transcriptome analysis showing differential gene expression in susceptible and tolerant *T. cacao* genotypes during *P. palmivora* infection

Table 2. List of possible candidate genes involved in *Theobroma cacao*'s response to *P. palmivora* infection

Gene in <i>T. cacao</i> genome	Putative Gene	Function based on UNIPROT
Tc02v2_t027470_1	Putative_Ribonuclease_1	Wound response
Tc07v2_t005420_4	Putative_Protein_synthesis_inhibitor_II	Antifungal constitutive agent in plants
Tc09v2_t014860_2	VIN3-like_protein_2	Involved in the way of vernalization and photoperiod regulating gene expression
Tc10v2_t005600_1	Protein_IQ-DOMAIN_1,	Required for the correct "splicing" of specific resistance genes R
Tc06v2_t002510_2	Formamidopyrimidine-DNA glycosylase	Involved in the repair of DNA damaged by oxidation or by mutagenic agents
Tc05v2_t022680_2	Probable_low-specificity_L-threonine_aldolase_1	Component of the MAC (Membrane Attack Complex) complex that probably regulates the defense response through transcriptional control and is then essential for plant immunity.
Tc01v2_t030600_2	Neomenthol dehydrogenase	Involved in basal resistance against pathogens

Conclusions

We present preliminary results in which some candidate defense response genes were identified. A new genome for *P. palmivora* was assembled and annotated. The next step will be to use this genome to identify pathogen effectors and continue to explore the transcriptomic data to identify the associations between the plant defense response and the pathogen effectors. These results are an essential tool to identify genes responsible for the resistance and generate improved varieties with resistance to black pod disease to reduce losses due to diseases caused by *Phytophthora* sp.

References

- Argout, X., Salse, J., Aury, J.M., Guiltinan, M.J., Droc, G., Gouzy, J., Allegre, M. et al. (2011). The genome of *Theobroma cacao*. Nat. Genet. 43, 101-108.
 Bailey, B. A., y Meinhardt, L. W. Cacao diseases: A history of old enemies and new encounters. Cacao Diseases: A History of Old Enemies and New Encounters. Springer. 2016

Acknowledgements

This study was funded by the Ministerio de Agricultura of Colombia. We thanks Leonora Rodríguez, Martha Carrera, Mauricio Soto, Esperanza Torres and Bayardo Parra for their guidance and help in the experimental work.