



Transcriptome Analyses of Leaves Reveal That Hexanoic Acid Priming Differentially Regulate Gene Expression in Contrasting *Coffea arabica* Cultivars

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INTRODUCTION

Coffee (*Coffea* spp.) is one of the most important traded commodities in the international market [International Coffee Organization (ICO), 2018]. *C. arabica*, the only allotetraploid species in the genus, is the most planted one. Despite its economic importance, evaluation of physiological changes taking account molecular responses to biostimulants are still scarce. When plants recognize potential biotic/abiotic challenges, they often switch to a primed state of enhanced defense. This mechanism enables plants to respond robustly after exposure to stress (Aranega-Bou et al., 2014). The response of *C. arabica* plants to priming, in terms of transcriptional profiles, is a big gap in this area. Hexanoic acid (Hx) is a natural priming agent with proven efficiency in a wide range of host plants and pathogens (Llorens et al., 2016), including coffee pathogens. In this study we aimed to investigate the effect of Hx priming in *C. arabica* leaves transcriptome. We hypothesize if Hx application could modulate genes related to defense responses, being a potential eliciting agent in *C. arabica*. To test this effect, we applied Hx in roots of two Brazilian *C. arabica* cultivars with distinct breeding histories and contrasting resistance to the major disease in Arabica coffee, coffee leaf rust. While Catuai Vermelho is among the most used cultivars in Brazil, but it is susceptible to leaf rust, Obatã is a moderately resistant cultivar (Del Grossi et al., 2013). We performed transcriptome analysis of leaves. Reads were mapped to the *C. arabica* public genome and up to 94% of reads were mapped. Transcript expression level was quantified and differentially expressed genes (DEGs) were identified based on FPKM ratio and statistical analyses. A total of 57 and 63 DEGs were found in Catuai Vermelho and Obatã, respectively. Most DEGs correspond to upregulated genes in response to Hx, in both cultivars (86% Catuai Vermelho and 73% Obatã). Eight DEGs were found modulated in both cultivars, including ferredoxin-NADP reductase and phenylalanine ammonia-lyase. DEGs were functionally annotated through Blast2GO. Biological process and molecular function categorizations revealed that DEGs related to cellular, oxidation-reduction, organic substance and primary metabolic process, as well as transferase and ion binding activity might play a dominant role in the leaf response to priming. These data contribute to identify key genes differentially expressed in response to Hx as well as indicate pathways modulated by this eliciting agent.

VALUE OF DATA

- The species *C. arabica* is responsible for 60% of the world coffee production due to its fine flavor and aroma. Brazil is the largest producer and exporter of this commodity.
- Hexanoic acid (Hx) is a priming agent which leads plant to a physiological state that enables them to respond more rapidly and/or robustly to biotic or abiotic stress scenario.
- To date, there is no transcriptome analysis for *C. arabica* cultivars Catuai Vermelho and Obatã in response to the priming agent hexanoic acid (Hx) application.
- These data will contribute to identify key genes differentially expressed in response to Hx application and it can also indicate which pathways are modulated by Hx.

MATERIALS AND METHODS

Plant Material

Five-month-old plants of *C. arabica* cv. Catuai Vermelho IAC 144 and Obatã IAC 1669-20 (4–5 leaf pairs) were used in this study. Plants were selected based on size uniformity and were transferred to pots containing 3 L of aerated nutrient solution (ANS), adapted from Clark (1975) by de Carvalho et al. (2013). The hexanoic acid experiment was carried out as described in Silva et al. (2020), under controlled temperature ($23 \pm 2^\circ\text{C}$) and light/dark cycle (12/12 h, photosynthetically active photon flux density of $\sim 400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). The following treatments were assessed: (a) ANS (control); (b) ANS + hexanoic acid (Merck, final concentration 0.55 mM) for 48 h. Plants were grown in 3 to 6 plastic pots in which three pots received each treatment. The experiments were repeated 3 times to obtain biological replicates. The potted plants were grouped in “pools” (made of 9–18 plants), which were considered a biological replicate. Three biological replicates were used. The mature leaves of the middle third of the plants were collected within the 3rd hour of the light period and stored at -80°C to further analyses.

Total RNA Extraction and Quality Control, Library Preparation, and RNA-Seq

Total RNA was extracted from leaves pools using the RNeasy Plant kit (Qiagen, Hilden, North Rhine-Westphalia, Germany). Total RNA samples were purified using the RNeasy Minielute Cleanup kit (Qiagen, Hilden, North Rhine-Westphalia, Germany). The purity of RNA was determined using a NanoDrop ND-100 spectrophotometer (Thermo Scientific, San Jose, CA, USA) and concentrations were obtained using Qubit fluorimeter (Thermo Fisher Scientific, Wilmington, DE, USA). Poly(A) RNA sequencing library was prepared following Illumina's TruSeq-stranded-mRNA sample preparation protocol (Illumina Technologies, San Diego, CA). Paired-ended sequencing (2 X 150 bp) was performed on Illumina's NovaSeq 6000 sequencing system at LC Sciences (Houston, TX, USA). Data were deposited into the European Nucleotide Archive (ENA), submission ERA6282544 and in Zenodo (doi: 10.5281/zenodo.5517785).

TABLE 1 | Statistics of *C. arabica* cv. Catuai Vermelho and Obatã RNA-seq analysis.

Cultivar/Treatment	Raw reads	Valid reads		
Pre-Assembly				
<i>C. arabica</i> cv. Catuai Control	151,526,548	116,851,828		
<i>C. arabica</i> cv. Catuai Hx	159,026,378	119,089,560		
<i>C. arabica</i> cv. Obatã Control	144,857,610	123,342,262		
<i>C. arabica</i> cv. Obatã Hx	142,817,402	124,659,366		
Cultivar/Treatment	Mapped reads	Uniquely mapped reads	Spliced reads	
Post-Assembly				
<i>C. arabica</i> cv. Catuai Control	109,447,784	68,090,059	30,207,564	
<i>C. arabica</i> cv. Catuai Hx	111,351,338	70,544,331	31,056,743	
<i>C. arabica</i> cv. Obatã Control	114,541,061	64,917,974	32,597,596	
<i>C. arabica</i> cv. Obatã Hx	113,645,733	62,705,577	31,283,461	

RNA-Seq Analysis and Differential Transcript Abundance

Adaptor contamination, low quality bases and undetermined bases were removed by using Cutadapt (Martin, 2011) and in house PERL scripts. Sequence quality was verified using FastQC (Andrews, 2010). HISAT2 (Kim et al., 2015) was used to map reads to the *C. arabica* genome (ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/003/713/225/GCF_003713225.1_Cara_1.0/Mapped) reads were assembled using StringTie (Pertea et al., 2015). **Table 1** shows the statistics of the transcriptome analysis. StringTie was also used to detect expression level for mRNAs by calculating FPKM. The differentially expressed genes (DEGs) were selected with \log_2 (fold change) >1 or \log_2 (fold change) <-1 and with statistical significance (p value < 0.05) by R package edgeR (Robinson et al., 2010). A second analysis was done on the differentially expressed mRNAs and only the ones with FPKM (ratio) ≥ 2 or FPKM (ratio) ≤ -2 ; coefficient of variation $\leq 30\%$ and average FPKM ≥ 5 . These genes are reported in **Supplementary Table 1**.

DEGs Data Annotation and Gene Ontology (GO) Analysis

Sequence annotation and functional analysis were done for the DEGs by using *Blast2GO* (Conesa et al., 2005), at the *OmicBox* (Götz et al., 2008) platform. Sequences were annotated by blasting nucleotide sequences against the NCBI

NR database (BLASTX, $e\text{-value} \leq 1.10^{-5}$). A total of 57 and 63 DEGs were found in Catuai Vermelho and Obatã, respectively. Most DEGs correspond to upregulated genes in response to Hx, in both cultivars (86% Catuai Vermelho and 73% Obatã). Eight DEGs were commonly found in the Catuai Vermelho and Obatã cultivars (AAA-ATPase, auxin-induced protein (two transcripts), ferredoxin-NADP reductase, premnaspirodiene oxygenase-like, glutathionyl-hydroquinone reductase, flavonol 3-O-glucosyltransferase and phenylalanine ammonia-lyase). DEGs were analyzed by Gene Ontology and were functionally assigned to the relevant terms. Biological process and molecular function categorizations revealed that DEGs related to cellular, oxidation-reduction, organic substance, and primary metabolic process, as well as transferase and ion binding activity might play a dominant role in the leaf response to priming. Most of the DEGs have a role in plant defense, corroborating to our hypothesis. These data contribute to identify key genes differentially expressed in response to Hx as well as indicate pathways modulated by this eliciting agent.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://www.ebi.ac.uk/ena, PRJEB39901, https://doi.org/10.5281/zenodo.5517786>.

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AUTHOR CONTRIBUTIONS

DD conceived the idea and acquired funding. PC, RR, NC, and SI-S conducted the experiment. IB performed analysis on the data. IB and DD wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2021.735893/full#supplementary-material>

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