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Coffea arabica L. genes from isoprenoid metabolic pathways are more expressed in full sun cultivation systems than in agroforestry systems

Fernanda Freitas de Oliveira [a,b], Juarez Pires Tomaz [b], Bruna Silvestre Rodrigues da Silva [a,b], Tiago Benedito dos Santos [b,c], Suzana Tiemi Ivamoto-Suzuki [b,d], Maria Brígida dos Santos Scholz [e], Luiz Filipe Protasio Pereira [a,b,f,*]

[a] Department of Biology, Graduate Program in Genetics and Molecular Biology, Universidade Estadual de Londrina, Londrina, PR, Brazil
[b] Genetic Improvement Area, Plant Biotechnology Laboratory, Instituto de Desenvolvimento Rural do Paraná - Iapar-Emater (IDR-Paraná), Londrina, PR, Brazil
[c] Graduate Program in Plant Production, Universidade do Oeste Paulista, Presidente Prudente, SP, Brazil
[d] Departament of Agronomy, Universidade Estadual de Londrina, Londrina, PR, Brazil
[e] Ecophysiology Area, Plant Physiology Laboratory, Instituto de Desenvolvimento Rural do Paraná - Iapar-Emater (IDR-Paraná), Londrina, PR, Brazil
[f] Empresa Brasileira de Pesquisa Agropecuária (Embrapa Café), Brasília, DF, Brazil

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ABSTRACT

Coffee synthesizes two unique isoprenoid diterpenes, cafestol ($C_{20}H_{28}O_3$) and kahweol ($C_{20}H_{26}O_3$), ent-kaurene compounds produced through the mevalonate or mevalonate-independent pathway. These compounds are relevant to beverage quality, plant defense, human health and nutraceutical properties. We investigate the expression profile of genes in the early (*CaHMGR*, *CaMVD*, *CaDXR* and *CaIDS*) and final steps of isoprenoid diterpenes biosynthesis (*Coffea arabica* L. cytochrome P450s: *CaCYP82C2*, *CaCYP71A25*, *CaCYP74A1* and *CaCYP701A3*) in two fruits development stages (90 and 210 days after flowering). We also observed total lipid, cafestol and kahweol accumulation in *C. arabica* grown using two crop systems (full sun and agroforestry) at IDR-Parana Londrina, PR, Brazil. Plants in full sun presented higher gene expression than plants grown in agroforestry systems, except for *CaHMGR*. Fruits showed higher levels of transcript activity 210 days after flowering, about 2.8 times more in the genes expressed during the first steps and 3.6 times in those in the later steps. Although lipid, cafestol and kahweol were higher in the full sun than the agroforestry system, only increased lipid content was statistically significant. Cultivation system plays an important role in the expression of the genes, particularly as regards light incidence.

1. Introduction

World coffee consumption is increasing (USDA, 2019) and cup quality, which has attracted the attention of consumers and markets, is correlated with bean chemical composition (Ky et al., 2001; Butt and Sultan, 2011), which can be affected by genotype background, environment and growth conditions.

Coffee bean quality is the result of a combination of several compounds including sugars, soluble solids, chlorogenic acids, caffeine, trigonelline and lipids (Ky et al., 2001; Cavin et al., 2002). Interestingly, coffee produces two unique isoprenoid diterpenes detected in the unsaponifiable lipid fraction: cafestol (CAF - $C_{20}H_{28}O_3$) and kahweol (KAH - $C_{20}H_{26}O_3$) (Speer and Kölling-Speer, 2006; Dias et al., 2010), which have good antioxidant (Lee and Jeong, 2007), anticarcinogenic (Cavin et al., 2002), anti-inflammatory and anti-angiogenic properties (Cárdenas et al., 2011; Wang et al., 2012). However, one report relates CAF to increases in serum cholesterol (Orsetti and Fitzgerald, 1999). Added to their pharmaceutical potential, diterpenes have attracted

Abbreviations: DAF, days after flowering; FSS, full sun system; AFS, agroforestry system; MVA, mevalonate pathway; MEP, mevalonate-independent pathway; HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase; MVD, mevalonate diphosphate decarboxylase; MVPP, 5-pyrophosphate; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; DXR, 1-Deoxy-5-xylulose phosphate reductase; IDS, isopentenyl diphosphate synthase; NIRS, Near-infrared spectroscopy; CYP450, Cytochromes P450; CAF, Cafestol; KAH, Kahweol.

* Corresponding author at: Scientist at Embrapa Café, Plant Biotechnology Laboratory IDR-Paraná, Rodovia Celso Garcia Cid, 375 Km, CP: 481, CEP:86001-970, Londrina, Paraná, Brazil.
E-mail address: filipe.pereira@embrapa.br (L.F.P. Pereira).

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interest for use as nutraceuticals, flavors and fragrances, and for their biopesticide and anti-herbivory potential (Pateraki et al., 2015).

The biosynthesis of these isoprenoid diterpenes occurs either through the mevalonate pathway (MVA) in the cytosol or through the mevalonate-independent pathway (MEP) in the plastids (Tholl and Lee, 2011). In the MVA pathway, 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR; EC 1.1.1.34, Bach, 1986) is the key enzyme that controls the metabolic flux in the early steps (Tiski et al., 2011; Hemmerlin et al., 2012). Later in the biosynthesis, mevalonate diphosphate decarboxylase (MVD; EC 4.1.1.33) converts mevalonate 5-pyrophosphate into isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), basic units in isoprenoid biosynthesis (Pereira and Ivamoto, 2015). On the other hand, in the MEP pathway, 1-deoxy-5-xylulose phosphate reductase (DXR; EC 1.1.1.267) is the key enzyme (Takahashi et al., 1998). In this pathway, isopentenyl diphosphate synthase (IDS) plays a similar role to the enzyme MVD, producing IPP and DMAPP (Eisenreich et al., 2001; Rodríguez-Concepción, 2004).

The final stages of coffee diterpene biosynthesis, after the formation of IPP, have not been well elucidated. The few studies that have been conducted indicate the involvement of P450 cytochromes (P450) in the biosynthesis of diterpenes (Ivamoto et al., 2017), which are the third largest gene family in plants (Nelson and Werck-Reichhart, 2011). P450s cytochromes participates in the biosynthesis and metabolism of sterols and steroid hormones as well in the metabolism of various lipids (Omura, 1999). In *C. arabica*, 87 genes encoding P450 have already been identified *in silico* using expressed sequencing tags (Ivamoto et al., 2015).

Originally, coffee was discovered growing naturally under trees (Perfecto and Vandermeer, 1996) and the first coffee plantations in Brazil were developed under canopy trees to reproduce their natural environment (agroforestry system, AFS) (DaMatta, 2004). Over the years, coffee crops were changed to full sun system (FSS) in order to increase production (DaMatta, 2004). This domestication process required adaptation, which might involve changes in several metabolic pathways, such as for isoprenoids and lipids. Gene characterization related to isoprenoid pathways and to the biosynthesis of specific diterpenes has been performed on plants under FSS cultivation (Tiski et al., 2011; Ivamoto et al., 2017), but there is no such study under AFS.

Coffee beans from AFS have been characterized as larger, heavier and with a better cup taste (Muschler, 2001; Bote and Struik, 2011; Coltri et al., 2015; Ivamoto et al., 2017) than FSS coffee beans. Shade can also protect coffee trees from extreme weather events, producing lower hydric stress than FSS cultivation (Bote and Struik, 2011; Coltri et al., 2015), and can decrease the infestation and population density of the coffee berry borer *Hypothenemus hampei* F. (Mariño et al., 2016).

In order to better understand how coffee cultivation systems can affect quality, we started to analyze the lipids and coffee diterpenes produced in FSS and AFS cultivation. We analyzed the transcriptional profile of isoprenoid-pathway genes (*CaHMGR*, *CaMVD*, *CaDXR* and *CaIDS*), as well as the P450 genes involved in diterpenes production (*CaCYP82C2*, *CaCYP71A25*, *CaCYP74A1* and *CaCYP701A3*) at two coffee fruit development stages (90 and 210 days after flowering, DAF).

2. Material and methods

2.1. Plant material and experimental conditions

Fruits of *C. arabica* L. cv. IAPAR59 from two different cultivation systems were used: AFS, with coffee plants growing under *Hevea brasiliensis* L. trees, and FSS, cultivated under field conditions, at the Experimental Station of he Instituto de Desenvolvimento Rural do Parana IAPAR-EMATER (IDR-Paranã), at latitute 23° 18′ S, longitude 51° 09′ W, average altitude 585 m, on a a dystrophic red latosol (oxisol), in Londrina, Paraná State, Brazil. The rubber trees (clone PB 235) were planted during the first half of 1999 and *C. arabica* L. cv. IAPAR59 was planted in the first half of 2000. Coffee plants, in both cultivation system, were arranged with spacings of 2.5 m between rows and 1 m between plants in the row. Global solar radiation was measured year around usin pyranometers, with LI-COR - Model LI200X. The sensors were positioned in the coffee line, between plants in both AFS and FSS conditions. Solar radiation interception index in AFS was 72% compared with FSS. Fruits were collected at 90 (December 2014) and 210 DAF (April 2015), green and ripe fruits, respectively. These two periods were chosen due to their importance in bean and fruit development. 90 DAF is when perisperm tissue is almost replace by the endosperm tissue which is fully mature at 210 DAF (De Castro and Marraccini, 2006). For each treatment, three biological samples were collected (fruit of individual plants for each repetition). Fruits were immediately frozen in liquid nitrogen and kept at −80 °C for RNA isolation. Fruits at 210 DAF were also sun dried for lipid and diterpene analysis.

2.2. RNA isolation and cDNA synthesis

Endosperm tissues from the frozen fruits (Fig. 1) were separated and ground into a powder in liquid nitrogen. Total RNA was isolated as described by Chang et al. (1993). To eliminate contaminant genomic DNA, samples of total RNA were treated with DNase I (RNAse-free, Invitrogen, Carlsbad, CA, USA). The quality and integrity of RNA was verified by 1% agarose gel electrophoresis, stained with ethidium bromide, and its concentration and purity were determined using a NanoDrop ND-100 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The absence of genomic DNA contamination was confirmed by PCR using *glyceraldehyde-3-phosphate-dehydrogenase* gene (*GAPDH*) primers (Cruz et al., 2009) with 100 ng of RNA (data not shown). Complementary DNA (cDNA) was synthesized using SuperScript III reverse transcriptase (Invitrogen) according to the manufacturer's recommendations, with 5 μg of total RNA. Three independent cDNA synthesis reactions were performed for each sample and used as technical replicates in quantitative real-time PCR (RT-qPCR) analyses. The final cDNA products were diluted ten-fold prior to use in RT-qPCR.

2.3. Primer design and amplification efficiency

The gene sequences used in this study were obtained from NCBI (ncbi.nlm.nih.gov/). We designed primers for MVA and MEP pathway enzyme genes: *CaIDS*, *CaDXR*, *CaHMGR*, *CaMVD* (Table 1) using Primer Express software (Applied Biosystems™). *CaCYP82C2*, *CaCYP71 A25*, *CaCYP74A1* and *CaCYP701A3* primers have been described previously (Ivamoto et al., 2017). Primer specificity was verified using dissociation curve analysis, the amplicon length was verified by 2% agarose gel electrophoresis and its efficiency was calculated using the software LinRegPCR (Ramakers et al., 2003).

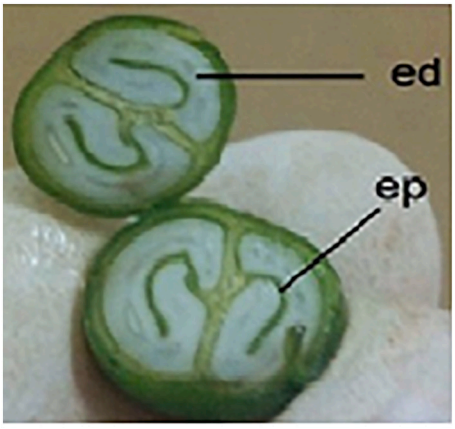

Fig. 1. Coffee fruit: endosperm (ed) and perisperm (ep) tissues at 90 DAF.

Table 1
Primer sequences for RT-qPCR of genes coding for enzymes of MEP and MVA pathways.

Primer	Forward sequence (5′-3′)	Reverse sequence (5′-3′)	Efficiency (100%)	Amplicon size (bp)
CaHMGR	CCACTACGGAGGGCTGTTTG	CATCCCATCTCTCAACAGGATACC	100	100
CaMVD	CCCAGACGCCCACCAATATA	CAAGGGTGACGCTGATGCT	100	100
CaDXR	CCACGCCTTGATCTTTGCA	CCCAGCAGCATATGCGAGTT	97	100
CaIDS	ATTGGACAATGGCGGTGCTA	AACTTTACCCCCAGCATTCACA	100	100

2.4. Quantitative RT-qPCR

The transcriptional levels of eight genes were evaluated by RT-qPCR in a 7500 Fast Real-Time PCR System (Applied Biosystems™) using SYBR Green PCR Master Mix (Applied Biosystems™). The reaction mixture contained 5 μL of SYBR Green Master Mix, 0.5 μL of each primer (10 μM), 1 μL of cDNA diluted 1:10, and Milli-Q water to a total volume of 10 μL. The reaction mixture was incubated at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 60 s. Dissociation curves were analyzed to verify the presence of a single product, including a negative control. All reactions were performed with three biological samples and three technical replicates. The *GAPDH* gene was used as internal control, as recommended by Barsalobres-Cavallari et al. (2009) and Cruz et al. (2009). Relative expression was calculated as $(1 + E)^{-\Delta Cq}$, where $\Delta Cq_{target} = Cq_{target\ gene} - Cq_{GAPDH}$ (Livak and Schmittgen, 2001) and $\Delta\Delta Ct = \Delta Ct_{target} - \Delta Ct_{reference\ sample}$. The Ct values for both target and integral control gene were the means of triplicate independent PCRs. The sample with the lowest transcription level for each gene was used as reference.

2.5. Lipid and diterpene analyses

Coffee fruits at 210 DAF were analyzed by Near-Infrared Spectroscopy (NIRS), according to Scholz et al. (2014), to determine CAF, KAH and lipid contents in g.100 g^{-1} of fruit. Coffee fruits were manually selected and sun-dried. For chemical determinations and spectral collections, coffee beans were ground in a laboratory disk mill (Perten 3600, Kungens Kurva, Sweden) to particles of 0.5 mm. Samples were analyzed in a NIRS 6500 instrument (Foss NIRSystems, Silver Spring, MD, USA).

2.6. Statistical analyses

Experimental results were analyzed statistically in triplicate and the data were expressed as the mean and standard deviation (±SD). Statistical analysis was performed using Assistat Software (de Silva and Azevedo, 2009). The difference between treatments was determined by variance analysis, ANOVA, followed by Student's *t*-test ($p < 0.05$).

3. Results

3.1. Expression pattern of genes related to MEP and MVA pathways

Genes encoding enzymes of the MVA pathway (*CaHMGR* and *CaMVD*) showed different expression patterns for both cropping system and fruit stage (Fig. 2). The *CaHMGR* gene expression was twice as high in fruits at AFS 210 DAF than in AFS 90 DAF, FSS at 90 DAF and 210 DAF. For *CaMVD*, the highest expression was observed in FSS 90 DAF fruits, which was about twice that of FSS fruits at 210 DAF and AFS at 210 DAF. Also, at AFS 90 DAF expression of *CaMVD* was 5.5 times lower than FSS at 90 DAF.

On the other hand, MEP-pathway genes *CaDXR* and *CaIDS* showed the same expression pattern, being expressed at higher levels in FSS fruits 210 DAF, which was about 3.5 times higher than both FSS and AFS at 90 DAF, and about 5.5 times higher than 210 DAF AFS fruits.

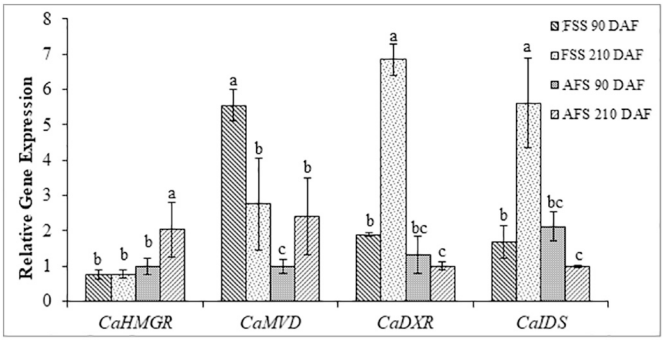

Fig. 2. Relative expression of genes related to mevalonate-independent and mevalonate pathways. Transcript abundances were analyzed by RT-qPCR in fruits of *C. arabica* grown under FSS and AFS at 90 and 210 DAF. Abundance of specific transcripts was normalized using *GAPDH* as endogenous control. Data are represented by the mean of three technical repetitions. Equal letters means no significant difference. $p < 0,05$.

3.2. Relative expression of P450 genes

The genes *CaCYP82C2* and *CaCYP74A1* showed the same expression pattern: higher expression in FSS 210 DAF fruits, followed by FSS 90 DAF and AFS 210 DAF (Fig. 3). Moreover, both genes were expressed at lower levels in AFS fruits 90 DAF. The *CaCYP82C2* gene was expressed at levels about 1.4 times higher in fruits in FSS at 210 DAF than in those from FSS at 90 DAF, 2.7 times higher than AFS at 210 DAF and 4.8 times higher than AFS at 90 DAF.

With regard to *CaCYP71A25*, in FSS fruits at 210 DAF it was 3.5 times more highly expressed than in FSS fruits at 90 DAF, 3.6 times than AFS at 210 DAF and 15.7 times than AFS fruits at 90 DAF. In the same way, *CaCYP74A1* was 1.7 times more highly expressed in fruits from FSS at 210 DAF than those from FSS at 90 DAF, 2.8 times more than AFS 210 DAF and 15.2 times more than AFS 90 DAF.

However, the *CaCYP701A3* gene showed a different pattern from the other three *P450* genes, with higher expression observed in FSS fruits at 90 DAF, which did not statistically differ from FSS at 210 DAF, but it was

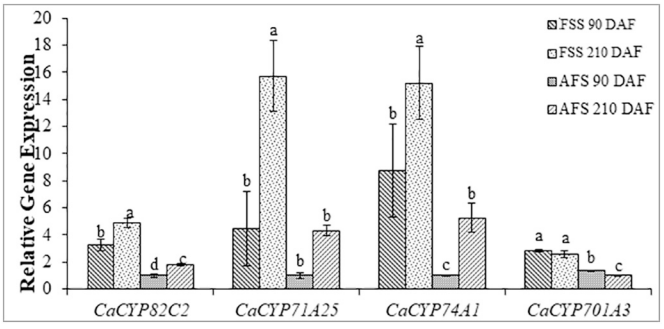

Fig. 3. Relative expression of *P450* genes. Transcript abundances of *C. arabica* fruit grown under FSS and AFS, 90 DAF and 210 DAF, were analyzed by RT-qPCR. The abundance of specific transcripts was normalized using *GAPDH* as endogenous control. The data are represented by the mean of three technical replicates. Equal letters means no significant difference. $p < 0,05$.

two times higher than AFS at 90 DAF and 2.8 times higher than AFS at 210 DAF.

3.3. Lipid and diterpene contents

Levels of lipids, CAF and KAH, assayed in 210 DAF fruits, showed higher content in FSS (Fig. 4). However, only lipid content presented significant differences (ANOVA, $p < 0.05$).

4. Discussion

4.1. Genes in isoprenoid metabolic pathways are more highly expressed in FSS

The results of the RT-qPCR demonstrated that the genes from the MVA pathway had a different expression pattern related to both fruit development and cultivation system. Expression of *CaHMGR* was the lowest of all the genes studied, mainly at FSS. There was an increase in *CaHMGR* only at 210 DAF in AFS. *HMGR* expression is regulated by both biotic and abiotic factors, including light intensity (Stermer et al., 1994; Venkatachalam et al., 2009; Li et al., 2014; Tian et al., 2015), which downregulate its expression (Learned, 1996); *HMGR* was down-regulated by blue light in *Salvia miltiorrhiza* hairy roots (Chen et al., 2018). Furthermore, the dark treatment induced *PgHMGR* promoter activity in an etiolated hypocotyl (*Panax ginseng*) (Kim et al., 2014). In further support, several photoreceptors are involved in *HMGR* repression in Arabidopsis seedlings (Rodríguez-Concepción et al., 2004), which means the lower the incidence of light over these genes the more expressed they would be. Similarly, in *Azadirachta indica* it was observed that *AiHMGR1* did not show difference in expression between fruit stages, while *AiHMGR2* had late expression in fruits, being four times higher in the fourth stage of fruit formation compared to the first three stages (Bhambhani et al., 2017). Likewise, in our study, *CaHMGR*, at 210 DAF in AFS presented increased expression when compared with the 90 DAF AFS fruit stage.

CaMVD was more highly expressed in FSS fruits 90 DAF, however in AFS the highest expression was in 210 DAF fruits. Interestingly, it seems that *MVD* expression is upregulated by light intensity. In *H. brasiliensis HbMVD* was 1.3 times higher in the first leaf from the top compared to the second, third and fourth leaf, suggesting that this difference is due to incitation by incidence of light (Wu et al., 2017). In transgenic tobacco, light application significantly increased *GbMVD* (*Ginkgo biloba* MVD) pro-driven *GUS* activity in two times (Liao et al., 2015). In the same way, the results presented here indicate *CaMVD* expression increases under light intensity (FSS), therefore this is the first evidence of *CaMVD* expression regulation in coffee due to cultivation system (AFS or FSS) and fruit developmental stage.

Interestingly, *CaDXR* and *CaIDS* showed the same expression pattern, suggesting common regulation mechanisms. In FSS, these genes showed high expression at the late stages of fruit development (210 DAF). In *Vitis vinífera*, *VvDXR* tends to be highly expressed in ripe fruits that present higher carotenoid and chlorophyll content (Leng et al., 2017), which can be related to both chlorophyll presence in fruit ripening, light incidence and the increase of carotenoids content due to the ripening process. Meanwhile, in AFS the highest expression was observed in fruits at 90 DAF, which can be related to the delay in fruit maturation in AFS (Geromel et al., 2008), whereas in *S. miltiorrhiza*, Chen et al. (2018) demonstrates that DXR expression was 1.2-fold higher with red light than with dark treatment. Both genes *CaDXR* and *CaIDS* are from the MEP pathway found in plastids, therefore they are probably highly responsive to light and may be important to the formation of photosynthetic pigments such as chlorophyll and carotenoids.

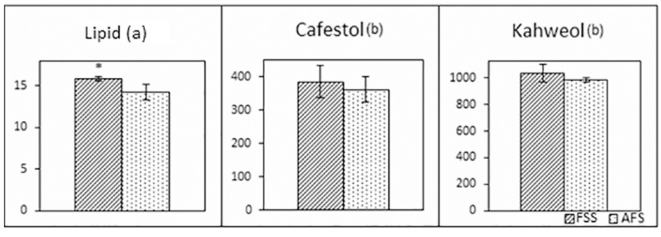

Fig. 4. Lipid, cafestol and kahweol content of fruits of plants grown under FSS and AFS at 210 DAF, expressed in a: g.100 g^{-1}; b: mg.100 g^{-1}. * $p < 0.05$.

Gene expression in coffee beans during early endosperm development is affected by light exposure (Geromel et al., 2008). Moreover, light is one of the major environmental signals for the differential accumulation of isoprenoid-derived metabolites during plant growth and development (Vranová et al., 2013). Martins et al. (2014) also suggest that for species such as coffee, which evolved in shady habitats but can develop successfully under full light, energy expenditure through metabolic reprogramming can be crucial for increasing growth, photosynthetic capacity and suitability when light energy is abundant.

Ivamoto et al. (2017) characterized transcriptional activity of seven P450 genes, and four of these, *CaCYP82C2*, *CaCYP71A25*, *CaCYP74A1* and *CaCYP701A3*, showed expression patterns that are possibly related to diterpene accumulation in coffee perisperm. Our results with endosperm showed higher activity in fruits at 210 days. This is interesting since at 210 DAF endosperm tissue is the preeminent tissue, while the perisperm is limited to a thin layer involving the endosperm. Therefore, we have probably observed continuity in the expression of those genes, already present at the beginning of fruit development.

Despite the fact that Ivamoto et al. (2017) did not find a statistically significant difference associated with perisperm tissue at different maturation stages, here in our study, an increase in *CaCYP82C2* transcriptional level during fruit maturation was observed for FSS and for AFS. The higher gene expression in fruits of plants grown in FSS than in those from AFS also indicates the influence of light on the regulation of this gene. For the same gene, Zhang et al. (2008) observed low expression in dark treatments in Arabidopsis. This difference may also be due to a higher incidence of pests in this cropping system (Mariño et al., 2016), since this gene was related to novel pathogen defense-related secondary metabolites (Rajniak et al., 2015; de Simone et al., 2017).

While we observed higher *CaCYP71A25* expression at 210 DAF in the FSS condition, Ivamoto et al. (2017) reported higher expression in the perisperm at 90 DAF, and that *CaCYP71A25* can be involved in CAF and KAH formation rather than monoterpenes, as observed in tomato (Li et al., 2010). It has also been reported that in *Sorghum bicolor, CYP71A25* is upregulated under drought conditions (Johnson et al., 2014) and in nitrogen-stress sensitive genotypes (Gelli et al., 2014). In apple (*Malus* x *domestica* Borkh.) *CYP71A25* is also involved in stress response (Kumar et al., 2016). Interestingly, in coffee plants, *CaCYP71A25* expression could be explained by the response to the FSS environment regarding to AFS. Coffee is a plant that grows naturally under the forest canopy and it would be interesting to investigate possible roles of this gene in plant adaptation to full sun cultivation systems.

Whereas Ivamoto et al. (2017) did not find a difference in the transcriptional profile of *CYP74A1* between DAF, we observed higher expression at 210 DAF in both cultivation systems. *CYP74A1* was described in both Arabidopsis and soybeans, and is associated in plant primary metabolism and allene oxide synthase (AOS). AOS is one of the key enzymes of jasmonic acid (JA) biosynthesis (Haga and Iino, 2004; Svyatyna and Riemann, 2012), which is involved in plant defense against herbivores, pathogens and abiotic stress (Koeduka et al., 2015). Furthermore, light plays a central role in regulating JA biosynthesis and signaling pathways, and has implications in stress response pathways (Carvalho et al., 2010), which might explain why this gene was more highly expressed in FSS fruits, mainly at 210 DAF.

CaCYP701A3 exhibit higher expression in FSS compared to AFS. Also a decrease in expression was observed in AFS 210 DAF compared to AFS 90 DAF, while there was no difference between 90 and 210 DAF in FSS.

This result corroborates with Ivamoto et al. (2017), who also found a decrease on expression of *CaCYP701A3* among fruit ripening in coffee perisperm, what shows that this gene has the same pattern in coffee endosperm and perisperm. *CYP701A3* belongs to the CYP701 family, whose members are described as ent-kaurene oxidase, which performs three catalytic reactions in the initial stages of the biosynthesis of gibberellin and brassinosteroids, and its last product is considered to be the precursor of the diterpenes CAF and KAH (Morrone et al., 2010).

4.2. Lipid, cafestol and kahweol contents in fruits of plants in FSS

Despite the differences in gene expression related to diterpenes biosynthesis between plants grown in FSS and AFS, there is no difference in the concentration of CAF and KAH in the fruits grown in these systems of evaluation. Scholz et al. (2016) analyzed CAF and KAH concentration in Ethiopian coffee plants growing in FSS during three years and did not observe variation in the content of these compounds (Scholz et al., 2016). Together, results suggest that coffee diterpenes are not heavily influenced by environmental conditions. Interestingly, similar results were observed by Geromel et al. (2008) in relation to sucrose content of the bean in coffee plants growing under shade and in full sun conditions. Despite differences in sucrose synthase and sucrose-phosphate synthase expression in both gene and enzymatic level, the bean sucrose content between both systems was similar at the end of fruit ripening.

With regard to lipid content, it was more highly accumulated in fruits grown in FSS (about 11% more), which corroborates with Geromel et al. (2008), who observed that light conditions had a significant effect on lipids content, where higher values were observed in FSS relative to the AFS condition. In the same way, Geromel et al. (2008) and Delaroza et al. (2017) also observed higher lipid content in coffee plants under higher light intensity, while Izquierdo et al. (2009) suggests that increased light intensity stimulates lipid accumulation in maize and soybean.

In summary, this study showed that plants in FSS present different isoprenoid and diterpene CYP450 gene transcription patterns from plants growing in AFS. This is the first time that gene expression and accumulation of lipid, CAF and KAH have been described taking into consideration growth conditions and fruit age. Moreover, it is also the first time that coffee endosperm tissue was used in expression analysis of isoprenoids genes. We have demonstrated that cultivation system plays an important role in gene expression, particularly as regards light incidence. The higher incidence of light in the cultivation system increases the expression of the majority of the genes studied. Most of the genes analyzed were more highly expressed in fruits at 210 DAF, which can also be related to fruit ripening. Additionally, no differences in CAF and KAH concentration between the growth systems were observed, reinforcing that the ambient has little influence on diterpene accumulation. Comprehension of these pathways is relevant for better understanding of coffee bean composition, which is related to quality, as well as for the development of genetic engineering technologies available for coffee breeding.

Declaration of competing interest

The authors declare that there is no conflict of interest that could be perceived as prejudicial to the impartiality of the reported research.

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