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Genetic control of the sensory quality of coffee beverage

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ABSTRACT

The sensory quality of coffee drink is a quantitative characteristic, that is, polygenic, being directly associated with compounds such as: caffeine, trigonellin, lipids, proteins, sucrose and chlorogenic acids that are precursors of aromas and flavors in coffee. Thus, the objective of this review was to identify the main genes related to the synthesis of these compounds and to understand how the expression of these genes occurs throughout the development of coffee fruits. The studies studied in the review demonstrate that the use of molecular biology has allowed greater advances not only in the identification of the main compounds related to the sensory quality of coffee drink, but also in the identification of enzymes involved in its biosynthesis and the main genes that control the synthesis of these enzymes. The deepening of these studies generates great expectations, because it may enable the development of strategies that can control the expression of these genes and thus interfere in the chemical constitution of the grains in a favorable way for the production of quality precursor compounds. Another important aspect raised in the review was the study of genes associated with fruit development and maturation, and it is possible to verify how the expression of these genes occurs throughout the period of fruit development and how they are responding to environmental stimuli and controlling fruit maturation.

Keywords: Specialty coffees, Coffee genome, Gene expression, Coffee chemistry.

1 INTRODUCTION

In the current context of coffee growing, the differentiation of quality is a priority for greater value addition to the final product, being increasingly the demand of the world market in relation to the excellence of aromas and flavors of coffees. Thus, it becomes a great challenge for farmers to seek the best combinations of factors associated with quality such as genetics, environment, processing and

management method, which promotes greater efficiency in the production of coffees with differential of aromas, flavors and even nutraceutical characteristics (MALTA et al., 2020).

The sensory quality of coffee beverage is mainly determined by the flavor and aroma formed during roasting, from precursors present in the raw bean. Therefore, it is a polygenic characteristic, so it is highly influenced by factors such as environmental conditions, harvest and post-harvest processes and genetic (LEROY et al., 2006; MINTESNOT et al., 2018).

Thus, it can be considered that variations in the chemical composition and sensory quality of coffee are due to events involving the interaction genotype, environment and processing method and may be interconnected, thus allowing to establish associations (WORKU et al., 2018).

The chemical constitution of coffee beans is directly associated with the sensory quality of the beverage, compounds such as caffeine, trigonelline, lipids, proteins, sucrose and chlorogenic acids are precursors of aromas and flavors in coffee (FREITAS et al., 2020).

Advances in molecular biology have contributed to help understand where the proteins that encode the synthesis of these compounds are formed in cells, which genes control the synthesis of these proteins, how cells communicate with each other, and how this whole process of communication, protein synthesis, gene expression can influence the development of characteristics related to the aroma and taste of coffee (QUINTERO et al., 2018).

Thus, the objective of this review was to identify the main genes related to the synthesis of precursor chemical compounds of the sensory quality of beverage in Arabica coffee, as well as to understand how the expression of these genes occurs throughout the development of coffee fruits.

2 GENES INVOLVED IN CAFFEINE BIOSYNTHESIS IN COFFEE BEANS

Caffeine is a purinic alkaloid that accumulates in various tissues of the coffee tree because of its low catabolism. The substance is synthesized from xanthosine, an intermediate in the catabolic pathways of purines. The caffeine contents in coffee beans are found variedly, and in the Arabica Coffee species the contents vary from 0.6 to 1.8%, while in the Coffee canephora species the contents vary from 1.2 to 4.0% (GUIMARÃES et al., 2021).

The caffeine contents in the coffee bean have been the target of several studies, mainly due to its pharmacological effects and also on the direct impact on the sensory quality of the beverage, being responsible for the characteristic bitterness that contributes to the final quality of the drink (RIBEIRO et al., 2016).

Regarding the genes, which code for N-methyltransferases, are involved in the biosynthesis of caffeine: 7-methylxanthosine synthase (MS), theobromine synthase (TS) and caffeine synthase (CS). Methylation of substrates occurs in the first, third and fourth steps of the biosynthetic pathway. In the second step occurs the removal of ribose by a nucleosidase (OGITA et al., 2005; PINTO et al., 2020).

The table below presents the genes identified in studies conducted from silica analysis in NCBI gene banks and in the coffee genome project database.

Table 1 – Silica analysis corresponding to the genes involved in the synthesis of caffeine in the fruits of the species Coffee arabica.

Gene	Homology			Size of (pb)	
	Organism	Access	EST No. Coffee	Expected	Observed
Caffeine Synthase - CS	Arabica Coffee	AB 086414	66	292	300
Methylxanthosine Synthase - MS	Arabica Coffee	FROM 048793	36	299	300
Theobromine Synthase - TS	Arabica Coffee	AB 048794	60	332	320

Source: Author
Legend: pb – Base pairs

The polymorphisms of genes involved in the biosynthetic pathway of caffeine have an outstanding importance in the improvement of the coffee tree. MIZUNO et al., (2003) identified, in fruits of Coffea arabica, genes with a certain homology to the caffeine synthase and theobromine synthase genes present in young tea leaves and present in genomic databases, suggesting the occurrence of gene polymorphism. These researchers obtained, through the use of the RT-PCR technique, clones of the caffeine synthase gene and verified that they had 80% similarity with theobromine synthase and 40% similarity with caffeine synthase tea, concluding that if cloned they can assist in the development of transgenic C. arabica with low caffeine content by antisense mRNA technology or by gene silencing.

Several scientific reports demonstrate that the reduced caffeine content in the seeds of the cultivar Laurina is conditioned by the expression of a recessive gene, and the species has low caffeine accumulation due to the slow synthesis of the alkaloid (MALUF et al., 2009; PINTO et al., 2020).

Studies carried out by LIN et al., (2005), related to the genes of the biosynthetic pathway of caffeine, evidenced their participation in different plant tissues such as endosperm and young leaves. The genes of theobromine synthase and caffeine synthase were expressed in immature fruits and the expression of the theobromine synthase gene was higher, not only in small green fruits (chumbinho phase), but also in pericarp and in immature endosperm of larger green fruits (green phase). The researchers observed through RT-PCR technology that there was no expression of methylxanthosine, theobromine and caffeine synthase in ripe fruits, which could explain the absence of caffeine accumulation during the maturation process in the fruits evaluated.

Studies have verified that the expression of the genes methylxanthosin (CmXRS1), theobromine (CTS2) and caffeine synthase (CCS1), occurs in different ways throughout the stages of development of fruits of C. arabica cultivar Mokka and in C. canephora. The results showed regular expression of CmXRS1 and CCS1, and irregular expression of CTS2, during fruit development. Peaks of accumulation of CTS2 transcripts were observed in the expansion stage of the fruits, while the

accumulation of transcripts of the CmXRS1 and CCS1 genes declined during maturation. These authors concluded that caffeine synthesis occurs in unripe fruits in the green stage. In the later stages, sugarcane green and cherry, the enzyme caffeine synthase has no activity, although the fruits have stable levels of caffeine (PERROIS et al., 2014).

In addition to the genotype, the accumulation and concentration of caffeine in coffee beans can be influenced by environmental factors. Several studies have found significant variation in caffeine content as a result of the effect of altitude. In relation to this factor, it is observed that caffeine increases its accumulation in coffee beans as the altitude increased, (CHENG et al. 2016; WORKU et al. 2018).

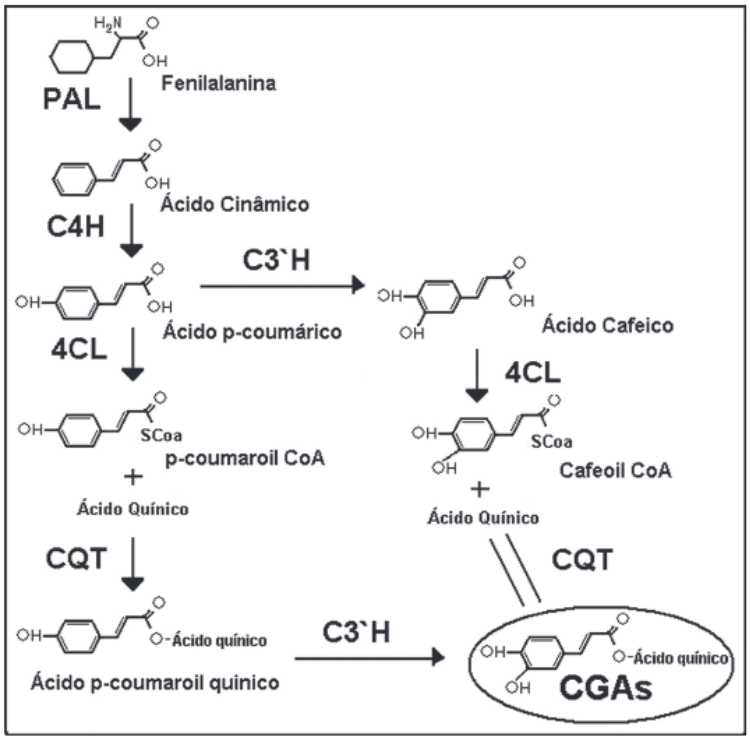
This increase in caffeine contents with the elevation of altitude, may be associated with lower stresses suffered by the plant due to high temperatures, because high temperatures in addition to reducing the production and translocation of photoassimilates to the grain due to malfunction or destruction of photosystems, reduces the quality of the same which causes the reduction of the quality and quantity of the various compounds biosynthesized during the filling and maturation of the coffee beans (VAAST et al., 2006; CHENG et al., 2016).

3 GENES INVOLVED IN THE BIOSYNTHESIS OF CHLOROGENIC ACIDS IN COFFEE BEANS

The Chlorogenic Acids (CGAs), are phenolic compounds present in the fruits of the coffee tree, with contents ranging from 5 to 8% in Coffea arabica and 7% to 11% in Coffea canephora, these compounds are present in all stages of fruit development, considering a great precursor of coffee quality (RIBEIRO et al., 2016).

Chlorogenic acids are composed of secondary metabolisms and derive from the biosynthesis of phenylalanine by the phenylpropanoid pathway. There are two pathways of synthesis of CGAs, first the enzyme phenylalanine ammonialiase (PAL) catalyzes phenylalanine in cinnamic acid, cinnamate 4 hydroxylase (C4H) catalyzes the second hydroxylation of cinnamic acid to coumaric acid, this is catalyzed by the enzyme 4 couramate CoA ligase (4CL) in p-coumaril CoA. From this point two different routes can be taken. In the first, p-coumaric acid can be catalyzed into caffeic acid by 4-couramate 3-hydroxylase (C3'H), which is then catalyzed by the enzyme 4CL in Cafeoyl CoA, in which the quinic acid molecule is added by the enzyme hydroxycinnamoyl transferase quinate (CQT), giving rise to the molecules of CGAs. In the second, p-coumaric acid is added to the quinic acid molecule by the CQT enzyme that originates p-coumaroyl quinic acid, which is catalyzed by the C3'H enzyme in CGAs (KNEVITT et al., 2017).

FIGURE I - Biosynthetic Route of Chlorogenic Acids



Source: Adapted from Ivamoto et al. (2015).

Work by Ivamoto et al., (2017), identified 45 candidate genes for the biosynthesis pathway of chlorogenic acids. Results include the genes: phenylalanine ammonialiase (PAL), cinnamate 4-hydroxylase (C4H), 4 couramate coa ligase (4CL), 4-couramate 3-hydroxylase (C3'H), hydroxycinnamoyl quinate transferase (CQT) and caffeoyl-CoA O-methyltransferase (CCoAMOT). Table 2 below presents the candidate genes for biosynthesis of Chlorogenic Acids in Coffee.

Table 2 - Candidate genes for biosynthesis of Chlorogenic Acids

Gene Name	Number of Genes	Description Protein
CaPAL	3	Phenylalanine 4 - hydroxylase
CaC4H	3	Cinamato4-hidroxilase
Ca4CL	5	4 couramato CoA ligase
CaCQT	14	Hidroxicinamoil quinato transferase
Ca3'H	17	4-couramato 3-hidroxilase
CaCCOAMOT	3	Caffeoyl-CoA O-methyltransferase

Source: Adapted from Ivamoto et al 2017.

Studies of expression analysis by RT-PCR have shown that the expression of genes related to the production of chlorogenic acids decreases at the end of the maturation of coffee fruits. Results of PCR amplification analysis of the PAL gene, evidences the difference of transcripts between the phases of fruit development, and the highest intensity of the fragment was observed in the ovary and green sugarcane phase and the lowest intensities occurred in the ripe fruits (IVAMOTO et al., 2013).

Recent studies have demonstrated variation in the intensity of expression of the PAL gene in the ripe fruits of different Arabica coffee cultivars, and the cultivars Mundo Novo and Icatu precoce presented lower expressions followed by Catuaí Vermelho, Icatu and Obatã (SOBREIRA et al., 2016).

In addition to the genotype the environment can also influence the chlorogenic acid contents of the beans, studies have shown that the amount of chlorogenic acids in coffee beans have a positive correlation with the elevation of altitude. This being one of the factors that can explain the higher quality of coffees at higher altitudes (JÖET et al., 2010; PEPPER, 2020).

4 GENES INVOLVED IN SUCROSE SYNTHESIS IN COFFEE BEANS

The predominant sugars in coffee are non-reducing ones, particularly sucrose. Sucrose is one of the compounds of the coffee bean that was considered as an important precursor of the flavor and aroma of the beverage, as it degrades rapidly during roasting, forming several compounds (GEROMEL et al., 2006). During the coffee roasting process, the reducing sugars mainly react with amino acids (Maillard reaction), giving rise to aliphatic acids, hydroxymethyl furfural and other furans in addition to pyrazines. These compounds are considered essential for the constitution of the taste and aroma of the beverage, either as volatile or non-volatile components.

The species Coffee arabica presents contents of 5.1% to 9.4% of sucrose, while the Coffee canephora species these values are always lower, usually ranging from 4% to 7% (CAMPA et al., 2004).

Sucrose synthesis is usually catalyzed by SPS sucrose phosphate synthase, along with a specific phosphatase: SPP (EC 3.1.3.24), which hydrolyzes sucrose-6P into sucrose and SUS phosphate synthase which is more closely related to sucrose degradation. In addition to SUS, sucrose degradation can be catalyzed by invertases, being an irreversible reaction (QUINTERO et al., 2018).

Geromel et al., 2006, identified two isoforms of SUS that perform different metabolic functions within the cell, being the genes CaSUS1 and CaSUS2. These genes are not expressed at the same time in the tissues, and it seems clear that, in C. arabica, the CaSUS2 gene is related to sucrose synthesis, because its strong expression overlaps with SUS peaks and sucrose accumulation in certain stages of the pericarp and endosperm, while the CaSUS1 gene seems to be related to sucrose degradation.

Reports in the literature demonstrate that the higher activity of the enzyme of the SUS enzyme occurs in the final stages of maturation, being accompanied by the detection of the expression of the CaSUS2 gene, and also by the accumulation of sucrose in this tissue. Thus, relating the data of enzymatic activities, expressions of the CaSUS1 and CaSUS2 genes and the accumulation of sucrose in the different tissues of coffee fruits, it is likely that these genes encode isoforms of SUS that perform different functions within the cells (VAAST et al., 2006; RIBEIRO et al., 2016).

5 GENES INVOLVED IN THE BIOSYNTHESIS OF LIPIDS IN COFFEE BEANS

Lipids are found predominantly in the endosperm of the coffee bean, with contents around 15% of the total chemical compounds of the species Coffea *arabica* and 10% in *Coffea canephora* (SCHOLZ et al., 2016).

The lipids have a beneficial effect on the quality of the coffee drink both in aroma and flavor, and during roasting they are concentrated in the external areas forming a protective layer on the seed, thus avoiding any losses during this process. However, part of the lipids is lost in the milling process, a fact that explains why the best quality coffees have the highest lipid contents (PIMENTA et al., 2003; RIBEIRO et al., 2016).

The diterpenes cafestol (CAF) and kahweol (KAH) are the main components of the unsaponifiable lipid fraction in green and roasted coffee beans, and the quantitative analysis of these diterpenes in C. arabica genotypes showed significant intraspecies variability in their concentrations, suggesting genetic control of the trait (MAFU et al., 2016).

Cytochromes P450 (P450s) can recognize and modify the caurano skeleton leading to the production of diterpenes, and are also responsible for several secondary metabolites, phytohormones, and plant defense compounds (Pateraki et al., 2015).

Recent studies with seven candidate genes for P 450 allowed the identification of five genes possibly related to the production of these diterpenes: CaCYP71A25, CaCYP701A3, CaCYP76C4, CaCYP82C2 and CaCYP74A1. Since these genes showed transcriptional patterns similar to the accumulation of CAF and KAH in coffee organs and tissues, and the concentrations the highest levels of CAF accumulation, were verified in flower buds and flowers, while the highest levels of KAH accumulation were observed during fruit development reaching the peak at 120 days after flowering (IVAMOTO et al., 2017). Table 3 below presents the seven candidate genes for P 450 identified by in silic approach.

Table 3 - P 450 candidate genes identified by in silic approach

Gene	EST Coffee	C. canephora genome	Putative function P450
CapCYP72A15	Contig16992	Cc05_g08890	Biosynthesis of triterpene
CapCYP94B1	GW461079	Cc01_g18610	jasmonic acid catabolism
CapCYP76C4	Contig146	Cc02_g36410	Biosynthesis of monoterpenoids
CapCYP74A1	Contig7490	Cc10_g03570	lipoxygenase biosynthesis
CapCYP82C2	Contig17481	Cc04_g10600	Homoterpeno ent-caureno oxidase
CapCYP701A3	Contig11456	Cc10_g03710	hydroxylation of monoterpenes
CapCYP71A325	Contig14459	Cc04_g11300	-

Source: Adaptation Ivamoto et al., 2017

6 GENES INVOLVED IN THE FRUIT MATURATION PROCESS

The stage of ripening of the fruit is a determining factor for the sensory quality of coffee drink, and the ripe fruit is a determining factor for the production of specialty coffees. In this context, the study of the main genes involved in the physiological processes of maturation as well as their expressions is of paramount importance for understanding the genetic mechanisms of quality control (PIMENTA et al., 2020). Table 4 presents the main genes involved in the synthesis of enzymes related to the maturation process of the coffee fruit.

Table 4 - Genes involved in enzyme synthesis associated with coffee fruit maturation

		Homology			Size of (pb)	
Gene	Product	Organism	Access	Nº EST Café	Expected	Observed
ACO	ACC oxidase	*L. esculentum*	ABO 13101	32	317	300
ACS	ACC synthase	*L. esculentum*	ABO 13101	02	324	310
CAT	Catalase	*L. esculentum*	OF 112368	155	329	300
Csp1	11S protein	*C. arabica*	Y16976	43	350	350
DH	Dehydrin	*C. canephora*	DQ333960	22	235	235
EMB3	LEA Protein	*Picea glauca*	L47601	20	337	330
ER5	LEA Protein	*L. esculentum*	U77719	09	345	330
YARD	Ethylene response	*L. esculentum*	AY192367	15	320	300
ETR	*Ethylene receptor*	*L. esculentum*	38666	1	371	370
GR	*Glutathione reductase*	*Pisum sativum*	X98274	9	363	350
ICL	*Isocitrate liase*	*L. esculentum*	U18678	4	310	300

Reports found in the literature demonstrate that the genes related to ethylene synthesis (ACO, ACS, ERF and ETR), as well as the GR, DH, ERS and ICL genes increased the intensity of fragments in the final stages of fruit development. The DH and EMB3 genes maintained constant expression throughout all stages of fruit development (GASPARI et al., 2012).

Several studies have demonstrated the possibility of identifying candidate genes for genetic markers of the development phases of the fruits of the Arabica Coffee, and these genes can be used associated with agronomic and physiological attributes as a molecular parameter to define the ideal phase of the harvest, which can ensure a more balanced chemical composition of the fruits and higher beverage quality (DE CASTRO et al., 2006; SAINTS, 2020).

7 FINAL CONSIDERATIONS

The reflections addressed in the review allow us to understand that the sensory quality of the coffee drink, translated through aromas and flavors is an extremely complex characteristic, being determined by precursors of flavors and wires originated during the development of the fruits and developed in the roasting process.

In this context it is possible to understand that the sensory quality of the beverage is a quantitative characteristic, that is, polygenic, being directly associated with compounds such as:

caffeine, trigonellin, lipids, proteins, sucrose and chlorogenic acids that are precursors of aromas and flavors in coffee. Thus, it is possible to verify that it is a characteristic that suffers high influence of environmental factors, crop management and processing.

Thus, it is possible to verify that the advancement of molecular biology allows not only to identify the main compounds related to the sensory quality of coffee drink, but also the enzymes involved in its biosynthesis and the main genes that control the synthesis of these enzymes. The deepening of these studies generates great expectations, because it may enable the development of strategies that can control the expression of these genes and thus interfere d in the chemical constitution of the grains.

Another important aspect raised in the review was the study of genes associated with fruit development and maturation, and it is possible to verify how the expression of these genes occurs throughout the period of fruit development and how they are responding to environmental stimuli and controlling fruit maturation.

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