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Review

Influence of genotype and environment on coffee quality

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Background: Coffee is one of the most valuable commodities exported worldwide. Greater understanding of the molecular basis of coffee quality is required to meet the increasing demands of consumers. Genotype and environment (G and E) have been shown to influence coffee quality. Analysis of coffee metabolism, the genes governing the accumulation of key components and the influence of environment on their expression during seed development supports the identification of the molecular determinants of coffee quality.

Scope and approach: The metabolism of important biochemical components of the coffee bean: caffeine, trigonelline, chlorogenic acids sucrose and lipids in coffee was reviewed. Analysis focused on how coffee metabolism was regulated by G and E throughout seed development and evaluation of transcriptome studies as an effective tool for use in understanding this system.

Key findings and conclusions: An overview of metabolism of the key components of coffee identified critical metabolic steps regulating the final concentration of metabolites that determine coffee quality. Coffee metabolism is influenced by both G and E and explains the higher quality of Arabica when compared to Robusta as well as the improvement of coffee quality by shade. Interaction of G and E (G $\times$ E) also contributes to quality. However, coffee metabolism is still not fully understood and there is scope for further studies to explain the contributions of G, E and G X E.

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1. Introduction

Coffee is one of the most valuable commodities traded (Fridell, 2014; Osorio, 2002). Increasing awareness of quality, taste and health among consumers is increasing demand for high quality and speciality coffees (Ashihara & Crozier, 2001; Upadhyay & Mohan Rao, 2013). Assessment of coffee quality is usually focused on factors that influence utilization of the final product with consumer preferences being assessed in three primary ways: physical (e.g. bean size), sensorial (cup quality) and chemical analysis (key compounds attributed to quality) (Fridell, 2014) (see Table 1). However, coffee quality results from interaction among many different factors including genotype (G) and environment (E) (Muschler, 2001; Sunarharum, Williams, & Smyth, 2014). Consumers of high quality coffee may exercise preference for genotype with labelling of species (e.g. arabica) or environment of production (usually country).

Coffee quality varies in different genotypes. Arabica coffee, which contributes around 70% of the world coffee production (ICO 2013), is higher quality with lower caffeine and produces a more aromatic brew when compared to Robusta coffee (C. L Ky et al., 2001; Silvarolla, Mazzafera, & Fazuoli, 2004). Environment factors, such as shade and high altitude have been observed to improve coffee quality (Joët, Salmona, Laffargue, Descroix, & Dussert, 2010). Diversity of coffee quality due to G and E, result from influences on the biochemical components of the coffee bean accumulated during seed development (Joët et al., 2010).

To improve coffee quality, it is essential to understand coffee metabolism and the genes governing the accumulation of the molecular determinants of coffee flavor during bean development. Numerous studies have been conducted in this field, especially in relation to biochemical constituents such as caffeine, trigonelline, chlorogenic acids (CGAs), sucrose and lipids, considered to influence commercially important sensory traits. The metabolism of these compounds has been studied for decades. However, significant knowledge gaps still exist and more studies are required to more fully define G and E influences on coffee quality.

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Table 1
Key components in coffee and changes after roasting.

Component	Flavour attribute	Influence of roasting
Caffeine	Perceived strength, body and bitterness	stable (Oestreich-Janzen, 2010)
Trigonelline	Overall aromatic perception, bitterness	60–90% degraded (Clarke & Macrae, 1985)
Chlorogenic acids	Acidity, astringency and bitterness	59.7–98% degraded (Trugo & Macrae, 1984)
Sucrose	Flavour precursor	disappear (Grosch, 2001, pp. 68–89)
Lipids	Flavour carriers, texture and mouthfeel	stable (Oestreich-Janzen, 2010)

2. Overview of coffee quality traits influenced by genotype and environment

2.1. Physical attributes

2.1.1. Bean size

Price is related to bean size and small beans of the same variety bring lower prices; However, larger beans do not necessarily taste better; Ideally, roasting should be processed with uniform beans (Wintgens, 2012). When roasting with uneven beans, the smallest tend to burn or over roasted while the largest tend to be under-roasted, which affects both the visual appearance of coffee beans and cup quality (Barel & Jacquet, 1994; Muschler, 2001). Arabica coffee beans are larger than Robusta coffee beans, ranging between 18-22 g and 12–15 g per 100 beans respectively (Wintgens, 2012). Bean size also changes with different environments (Dessalegn, Labuschagne, Osthoff, & Herselman, 2008; Muschler, 2001). As a positive factor, shade increases and unifies bean size by reducing the solar radiance in the coffee canopy and results in a lower air temperature and slowing down of coffee maturation. In addition, as floral initiation is light dependent, fewer flowers developed under lower solar radiance resulting in lower fruit productivity. Both these factors enable more bean filling due to longer assimilation into fewer beans (Michael N Clifford, 2012; Muschler, 2001; Vaast, Bertrand, Perriot, Guyot, & Genard, 2006). Interestingly, even when grown in the same shade environment provided by shade trees, the adaption to shade varies in different genotypes, for example, a relatively greater increase in bean size was found in *C.arabica var. Catimor* than in *C. arabica var. Caturra* (29% and 20% increase in large bean size, respectively). This suggests that Catimor may prefer or be more adapted to shade than Caturra (Muschler, 2001). This interaction is a genotype by environment interaction (G $\times$ E) which is common in many plants (Des Marais, Hernandez, & Juenger, 2013).

2.1.2. Bean colour

The colour of green beans is a sign of freshness, moisture content, the level of defective beans and homogeneity (Mendonça, Franca, & Oliveira, 2009). The green-bluish colour of washed Arabica beans is preferred relative to the browner beans of Robusta (Wintgens, 2012). Bean colour changes with different environments, for example, coffee grown at high altitude is often greenly-blue and if grown in soil lacking zinc, coffee beans may become light-grey in colour (Wintgens, 2012).

2.1.3. Sensory evaluation

Flavour, namely cup quality, is the primary standard in worldwide coffee trade (A Farah, Monteiro, Calado, Franca, & Trugo, 2006). Having an even bean size and good appearance without defective beans does not always result in good coffee flavour (Wintgens, 2012). For this reason, it is important to judge the flavour quality in relation to the final utilization, such as roasted, liquid canned coffee, etc. Cup quality analysis aims to evaluate coffee flavour with a group of trained people in an objective and reproducible way to create a profile using established terminology, such as aroma, flavour, body and acidity, which has been established by the International Coffee Organization (ICO).

Coffee flavour is very sensitive to G and E changes. Acidity, for example, ranges dramatically in different washed Arabica, while Robusta has been described as low or no acidity at all with coarse liquor, harsh and cereal notes and thick body (Van der Vossen & Walyaro, 1981). Ultimately, Arabica coffee is sold as blends with varying proportions of Robusta coffee, but Robusta coffees are seldom used alone (Wintgens, 2012). The same genotype planted in different environment may vary greatly in quality. For example, increasing positive attributes (appearance and preference) together with decreasing negative attributes (bitterness and astringency) was found in shade grown coffee (see Table 2) (Geromel et al., 2008; Muschler, 2001; Vaast et al., 2006). This improvement may come from a balance of filled and uniform ripening coffee berries from the shade. A positive interaction of genotype and a particular environment results in premium coffee. Similarly to bean size, Catimor flavour was found to be improved more by shade than Caturra flavour, which further suggests that Catimor is more adapted to shade (Ashihara & Crozier, 2001). Another factor positively influencing quality is high altitude, which was shown to increase beverage quality of coffee (Avelino et al., 2005). Genotypes, such as Blue Mountain, SL-28, Pluma Hidalgo are famous worldwide due to their premium flavour, however, if grown in places other than their preferred environments do not always have a good flavour (Jean, Jacques, Alejandra, & Christophe, 2006). Nevertheless, little is known about how G and E combinations generate high quality coffee.

2.1.4. Chemical attributes

The chemistry of coffee quality is highly complex with a wide range of compounds that change during fruit development. A few key components, such as caffeine, trigonelline, lipids, sucrose and chlorogenic acids (CGAs), are regarded as significant in influencing coffee quality. These components either stay stable and act as flavour attributes reaching the coffee brew or are degraded during roasting accounting for flavour precursors (see Table 1) (Wintgens, 2012).

2.1.5. Caffeine

Caffeine is one of the most important bitterness attributes contributing to coffee quality. When caffeine is consumed moderately by humans, increased energy availability, alertness and concentration, decreased fatigue and boosted physical performance have been reported, however, too much caffeine may result in undesired effects such as cardiovascular disease, depression, and even addiction (Jiang, Ding, Jiang, Li, & Mo, 2014). Nowadays, caffeine is the world's most famous behaviourally active drug and is consumed primarily from coffee (Davis, Govaerts, Bridson, & Stoffelen, 2006; Oestreich-Janzen, 2010). The recent sequencing of *C. canephora* genome revealed that caffeine evolved separately in coffee and in other plants such as tea suggesting a biologically important role for caffeine (Denoeud et al., 2014).

Arabica coffee is popular for its lower caffeine content compared to Robusta, with 0.6–1.8% and 1.2–4.0% respectively (Bicho, Leitão, Ramalho, de Alvarenga, & Lidon, 2013b; Hečimović, Belščak-

Table 2
Shade and altitude influences on key coffee components.

Phenotypic trait	Shade	Altitude	References
Bean size/fruit weight	↑	–	(Avelino et al., 2005; Muschler, 2001; Odeny et al., 2014; V. & Giridhar, 2013; Vaast et al., 2006)
Preference	↑	↑	
Caffeine	↑	↑	
Trigonelline	↓	↑	
Chlorogenic acids	↓	↑	
Sucrose	↓	↓	
Lipids	↑	↑(fat)	

Cvitanović, Horžić, & Komes, 2011; Mazzafera & Carvalho, 1991; Viani, 1993); In some coffee species there is no caffeine at all (Mazzafera & Carvalho, 1991). As a typical purine alkaloid, caffeine (1, 3, 7-trimethylxanthine) is synthesized mainly through three methylation steps (S-adenosyl-methionine- (SAM) - dependent methylation steps) and a nucleosidase step (ribose removal step) catalyzed by specific genes encoding enzymes (see Fig. 1). The caffeine biosynthesis pathway has been thoroughly reviewed by Hiroshi (Ashihara & Crozier, 2001; Ashihara, Sano, & Crozier, 2008). Caffeine is formed in immature coffee fruits and gradually accumulates during seed development. Meanwhile, transcription and enzyme activity is high in immature fruits and decreases drastically in the last stages of bean development (Koshiro, Zheng, Wang, Nagai, & Ashihara, 2006; Maluf et al., 2009; Perrois et al., 2015). Caffeine accumulation is dependent on highly expressed *CcDXMT* transcripts while less correlated with *CcMXMT1*; Expression of *CaXMT1*, *CaMXMT1* and *CaDXMT2* (Cc and Ca relate to paralogue genes of *C. canephora* and *C. arabica*) were found to be lower in *C. arabica* compared to *C. canephora* (parental sub-genome in *C. arabica*) and this explains why lower caffeine is found in Arabica. Additionally, a combination of reduced *C. eugenioides* sub-genome (maternal genome to *C. arabica*) expression in Arabica further explains the lower amount of caffeine (Perrois et al., 2015). The low caffeine content of *C. eugenioides* is due to reduced caffeine biosynthesis together with a rapid catabolism that is regulated by genes such as those encoding (7-N) demethylase activity (Ashihara & Crozier, 1999).

Other than genotype, caffeine accumulation and the final concentration are also influenced by environment. Earlier research showed caffeine was increased when shade levels were increased from 0 to 45% in *C. arabica cv* K7 beans and the same result was observed in a second year (Vaast et al., 2006). When shade was increased to 30%, 50%, 70% and 80%, a consistent improvement of caffeine content was shown in *C.arabica cv* Costa Rica 95 beans compared with the full sun treatment (Odeny, Chemining'wa, & Shibairo, 2014). In 55% shade, research showed that caffeine in *C.arabica cv* IAPAR 59 was increased when harvest 214 day after flowering (DAF) but decreased by 281 DAF compared with full sun grown coffee beans (Geromel et al., 2008). Shade delays coffee maturity about a month and biochemical composition varies significantly at different bean development stages, therefore it is difficult to draw a conclusion about shade influences on caffeine content when harvesting coffee beans of two treatment at the same time. In transcript studies, a short term of complete darkness in Robusta coffee seedlings resulted in a dramatically decrease in both chemical (0.094% caffeine) and transcript levels (almost no *CcDXMT* as well as *CcXMT and CcMXMT* expressed) in young leaves but increased after light for 6 or 12 h (Kumar, Simmi, Naik, & Giridhar, 2015). Research measuring transcript profiles in Robusta leaves and fruits gave the same pattern (Perrois et al., 2015). This apparent contradiction may be due to light being required for caffeine synthesis but the optimal level of light required being very low. Many metabolites and structures at the coffee leaf and whole-plant levels, fruits included, have been shown to adjust to particular light conditions (Lusk, Reich, Montgomery, Ackerly, & Cavender-Bares, 2008; Walters, 2005). However, long-term influence of light on caffeine accumulation is not known. High altitude is another factor increasing caffeine content (see Table 2) (Avelino et al., 2005). However, research to date has not investigated the influence on transcription to better understand the impact of high altitudes.

The role of *N*-methylnucleosidase (NMT) in catalysing the ribose removal step has been unclear. NMT was initially found to be a nonspecific purine-nucleoside phosphorylase (PNP) and a crude preparation of XMT from coffee was shown to be not involved in this reaction (Mizuno et al., 2003; H.; Uefuji, Ogita, Yamaguchi, & Koizumi, 2003). However, more recentlyXMT was purified and crystallized and shown to catalyse the ribose remove step as well (McCarthy & McCarthy, 2007). This was shown by detecting 7-methylxanthine (7mX) in the xanthosine (XR) methylation (McCarthy & McCarthy, 2007). This result was not obtained when S-adenosyl-L-Cys (SAH) was added to the crystallization trials (McCarthy & McCarthy, 2007). Additionally, xanthosine, with its free N7 atom, was assumed to be a better substrate for PNP (McCarthy & McCarthy, 2007). This phenomenon gives a clue as to why transgenic tobacco can produce caffeine while only XMT, MXMT, DXMT are expressed (Hirotaka Uefuji et al., 2005). XMT has no activity in the last two steps, which are catalyzed by MXMT and DXMT (McCarthy & McCarthy, 2007). All these indirect studies show that further research is required in this field.

2.1.6. Trigonelline

Particularly high levels of trigonelline are present in green coffee beans and the content varies from 0.3 to 1.3% (Stennert & Maier, 1994). Next to caffeine, trigonelline is the second most abundant

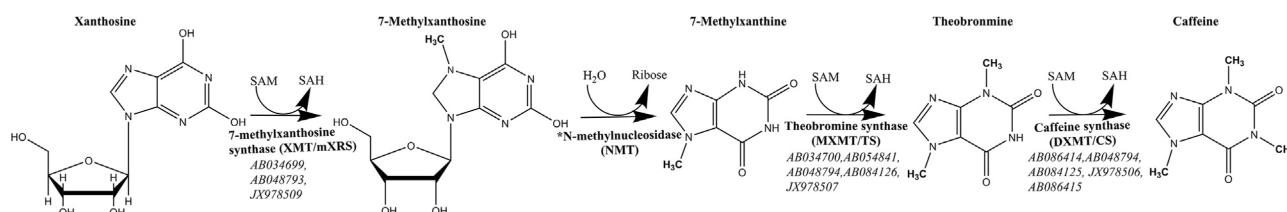

Fig. 1. *Major caffeine biosynthesis pathway in coffee seeds*. Gene accession numbers are marked below the enzyme encoded in italics. * Genes not unidentified.

alkaloid in coffee beans. As a pyridine derivative (see Fig. 2), trigonelline is an aroma precursor that contributes to the desirable flavour products formed during coffee roasting, including pyrazine, furans, alkyl-pyridines and pyrroles (M. N. Clifford, 1985; Dart & Nursten, 1985; De Maria, Trugo, Neto, Moreira, & Alviano, 1996). Trigonelline decomposes rapidly depending on the roasting temperature. During the degradation process, a de-methylation process generates a water-soluble B vitamin, nicotinic acid, which is bioavailable in coffee beverages compared with other natural sources in bound forms (Trugo, 2003). Coffee consumption of 3.5 standard cups per day accounts for one-third of the minimum dietary nicotinic acid requirement for an adult (Teply & Prier, 1957). Therefore, coffee is a significant dietary source of nicotinic acid (Perrone, Donangelo, & Farah, 2008).

Unlike caffeine, Arabica contains higher levels of trigonelline than Robusta with 0.80–1.82% and 0.7–1.24%, respectively (Bicho, Leitão, Ramalho, de Alvarenga, & Lidon, 2013a; Campa et al., 2004; de Oliveira Fassio et al., 2016; C. L; Ky et al., 2001; MacRae, Hill, Henning, & Mehuys, 1989). Lower trigonelline was found in shade grown coffee beans (Vaast et al., 2006) (see Table 2). However, this trend is not consistent with increased shade levels. Recent research, for example, revealed a decrease of trigonelline when increasing shade levels of 0–30%, 50% and then a rise at 70% and continued reduction in 80% (Odeny et al., 2014). Opposite to the impact of shade, high altitude increases trigonelline content during all stages of Robusta cherry development, however, in Arabica coffee, there is no difference detected (Sridevi & Giridhar, 2013).

Trigonelline was shown to follow the same biosynthetic pattern to caffeine, accumulating rapidly in young coffee fruits and synthesis decreasing markedly before the mature stage (Koshiro et al., 2006). Trigonelline is probably formed through nicotinic acid as shown in Fig. 2. Although limited studies have explored the trigonelline biosynthesis pathway, Arabica trigonelline synthase (nicotine *N*-methyltransferase) was shown to belong to the motif B' methyltransferase family (Mizuno et al., 2014). Two highly identical genes (CTgS1 and CTgS2) encoding this *N*-methyltransferase were found to be homologous to those in caffeine synthesis (Mizuno et al., 2014). There have been no reports of the influence of G and E on trigonelline during different development stages.

2.1.7. Chlorogenic acids

Chlorogenic acids (CGAs) are a group of phenolic compounds that show multiple attributes. During roasting, a large percentage of the CGAs degrade to form caffeic acid, lactones and other phenol derivatives through Maillard and Strecker's reactions, which result in increased bitterness, astringency and aroma (Upadhyay & Mohan Rao, 2013). CGAs are thermally unstable and in Arabica coffee the loss of CGAs after light roasting and after very dark roasting of beans corresponds to 60.9% and 96.5% respectively while in Robusta this loss corresponds to 59.7% to almost 98% respectively (Trugo & Macrae, 1984). Although most CGAs are lost by roasting, a sharp increase in total antioxidant activity was reported in the coffee beverage which suggested that the breakdown products of CGAs are antioxidants (Upadhyay & Mohan Rao, 2013). For the coffee plant itself, CGAs are significant plant metabolites that are associated with the protection of plant cells against stress, for example, oxidative stress, UV irradiation and pathogen infection (Matsuda, Morino, Miyashita, & Miyagawa, 2003; Niggeweg, Michael, & Martin, 2004; Peterson, Harrison, Snook, & Jackson, 2005).

The three main subgroups of CGAs, caffeoylquinic acids (CQA; 5CQA included), di-caffeoylquinic acids (3diCQA) and feruloylquinic acids (FQA), represent approximately 67%, 20% and 13% of total CGAs in Robusta, and, 80%, 15% and 5% in Arabica (Adriana Farah, Monteiro, Donangelo, & Lafay, 2008; C. L Ky et al., 2001). The total amount of CGAs is relatively lower in Arabica (4.0–8.4%) compared to Robusta (7.0–14.4%) (Bicho et al., 2013b; C.-L.; Ky, Noirot, & Hamon, 1997; Upadhyay & Mohan Rao, 2013). The major components of CGAs, both 5CQA and diCQA are lower in Arabica (Bicho et al., 2013a; M.; Clifford, 1997). When the three diCQA isomers are mixed equally, a bitter, lingering aftertaste and a subsequent metabolic bitter taste can be detected down each side of the tongue (Upadhyay & Mohan Rao, 2013). Research has found more bitterness was associated with the taste of diCQA rather than 5CQA, however, the astringency of diCQA is reduced when present with 5CQA (Clarke & Macrae, 1985; Ohiokpehai, Brumen, & Clifford, 1982). In water and beverages, both 5CQA and the three isomers of diCQA have a threshold ranging between 0.05 and 0.1 mg/mL while the caffeine threshold is 0.094 mg/mL (Drewnowski, 2001; Upadhyay & Mohan Rao, 2013). Compared to caffeine, larger amounts of CQA (5.2–6.5% in Arabica and 5.5–8.0% in Robusta) and smaller amounts of diCQA (0.7–1.0% in Arabica and 1.4–2.5% in Robusta) are present in coffee (M. Clifford, 1997). Together after roasting, (light or medium roasted with 67.7% and 76.4% lost) there is still an amount of CGAs left in the coffee brew which contributes significant bitterness compared with caffeine further explaining why Robusta is more bitter than Arabica.

The major CGAs isomers, 5-CQA, di3, 5-CQA, accumulate primarily in immature coffee beans and decrease dramatically by maturity. For example, diCQA (mainly di3, 5-CQA) reduces from 8.4% in green coffee beans to 2.3% in red coffee beans; in contrast, minor components, such as 3-CQA and 4-CQA, accumulate progressively throughout bean maturation (Joët et al., 2010; Lepelley et al., 2007). Corresponding to this flux, different genes were reported at particular development stages. Upstream enzymes encoded by *PAL*, *C4H*, *4CL*, *C3Hc* and *HCT* were activated at the early bean development at 90–120 DAF (day after flowering) to produce the major components and this accumulation reached a plateau at 120–150 DAF, followed by activation of *CCoAOMT* and other genes regulating remobilization of minor compounds (1/8 of 5-CQA was converted to di3, 5-diCQA and 1/10 to 5-FQA) and lignin biosynthesis, which is responsible for cell wall hardening, from 150 to 210 DAF. Later on at the mature stage, a very dense network involving co-expression of almost all phenylpropanoid genes in this

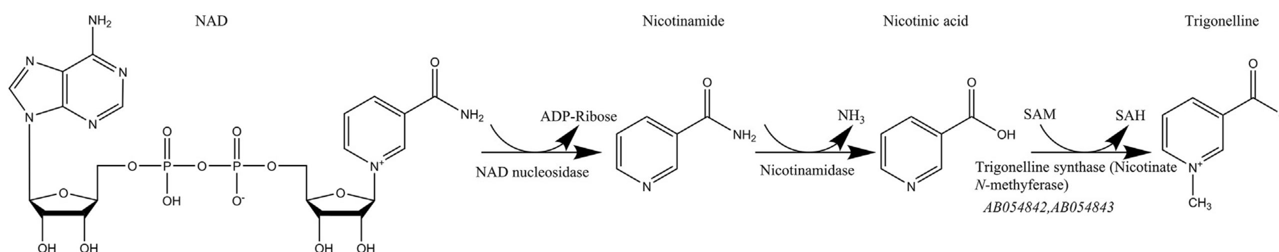

Fig. 2. *Possible trigonelline biosynthesis pathway in coffee seeds.* Gene accession numbers are marked below the enzyme encoded in italic.

biosynthesis pathway (Fig. 3) was expressed (Joët et al., 2010). At 120–150 DAF, the final 5-CQA content increased with HQT and 4CL8 expression (Joët et al., 2010). The lower content of CGAs in *C.canephora* FRT64 compared to *C.canephora* FRT05 was probably a result of higher HCT gene expression, as HCT has proved to be an S and L lignin formation donor (Lepelley et al., 2007). Differential expression of gene homologues activated at different stages of plant development may have different functions. *PAL1* and *PAL3* in *C.canephora* were expressed in immature coffee fruit and were highly correlated with CGAs content, however, PAL2 was activated and expressed at very low levels at the mature stage which corresponds to the pattern of flavonoid accumulation (Lepelley et al., 2012). All this complexity of expression contributes to the final CGA content in coffee. Therefore, it is very important to understand these biosynthetic pathways and the important developmental stages and regulatory steps.

No significant relationship was found between CGA content and rainfall or evapotranspiration, and a minor negative correlation observed with solar irradiance. Temperature is not to be ignored as a highly significant difference was detected (Joët et al., 2010). A warm climate provided by the low altitude coffee zone activated early accumulation of the major components and favoured subsequent remobilization of minor compounds. A delay of 5-CQA accumulation was found in a cooler climate and in 150–210 DAF of seed development 5-CQA accumulation was found to be positively correlated with temperature. Maximal CGA content was influenced by temperature because the CGA peaks at different stages in different climates and the variation in minor components remains until maturity (Joët et al., 2010). This suggests that transcriptional expression and co-regulation of genes of CGAs biosynthesis were influenced by bean developmental stage and temperature. For instance, *PAL2* and *C4H* expression were positively correlated with temperature at 90–120 DAF, however, the reverse correlation was observed at 120–150 DAF. Temperature was not found to be positively correlated with final the CGA content. This is probably because all the coffee from 16 different locations (270–1032 m above sea level) was harvested at the same time. There are three reasons that might account for this outcome. The first is that high altitude with low temperature delays coffee seeds maturity resulting in a different maturity for each plot. The second

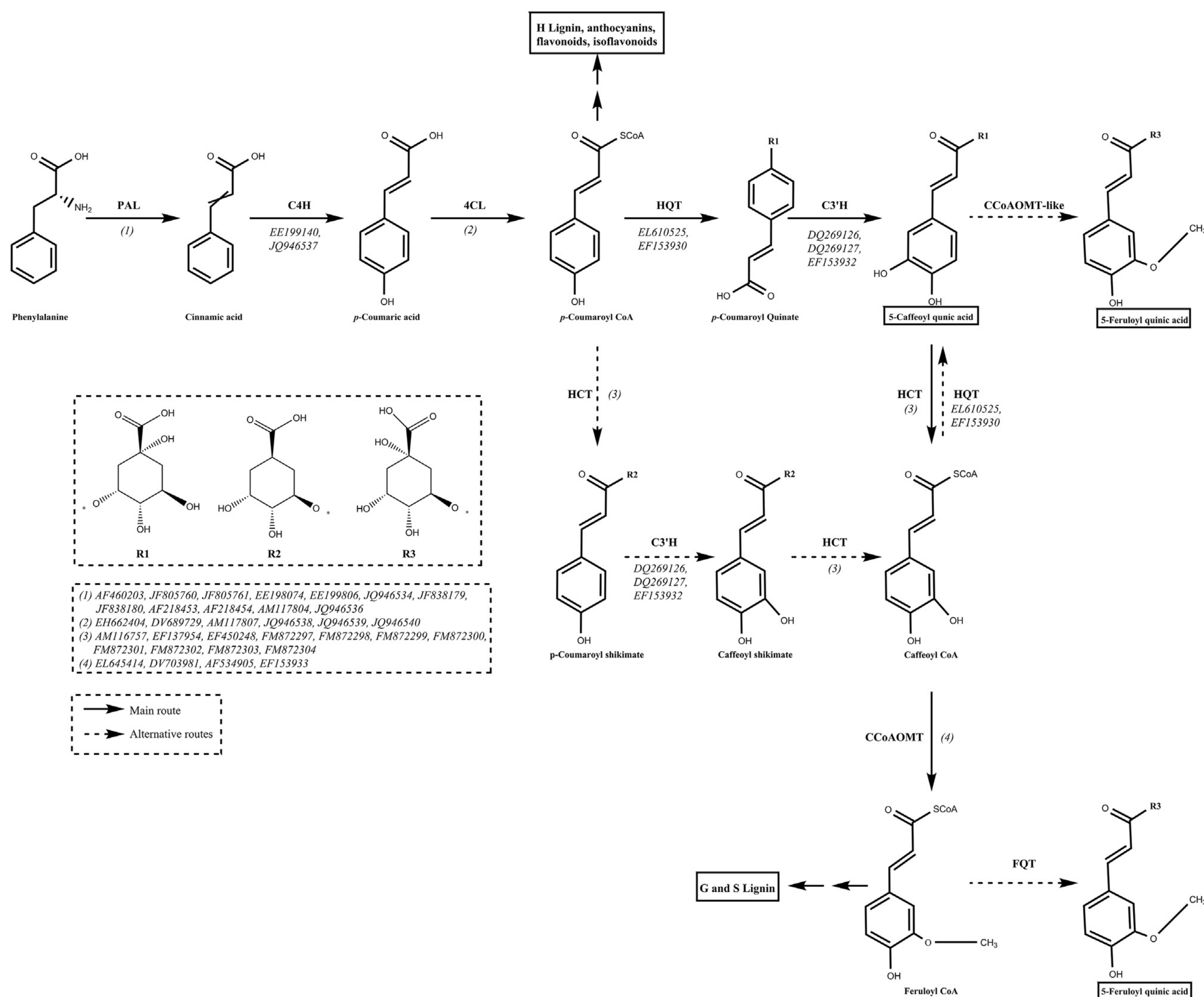

Fig. 3. *Chlorogenic acids biosynthetic pathway in coffee seeds.* PAL, phenylalanine ammonia lyase; C4H, *trans*-cinnamate 4-hydroxylase; 4CL, 4-coumarate: CoA ligase; HCT, hydroxycinnamoyl-CoA:shikimate/quinate hydroxycinnamoyl transferase; HQT, hydroxycinnamoyl-CoA quinate hydroxycinnamoyl; C3′H, *p*-coumaroyl CoA 3-hydroxylase; CCoAOMT, caffeoyl-CoA 3-*O*-methyltransferase; FQT, feruloyl-CoA quinate feruloyl transferase. Gene accession numbers are marked in italics and additional accession numbers are labelled (1)–(4) and are given on the left.

possible case is that CGA accumulation may be favoured by specific altitudes. Last but not least, this study was not designed specially for altitude and the environment was complicated by a combination of different climate factors.

Temperature is positively correlated with CGA content (Joët et al., 2010). Shade was found to be another influencing factor as 45% shade (by slope inclination) slightly reduced CGA content in *C.arabica* Costa Rica 95 and increased shade from 0 to 80% (by shade trunk) caused a significant drop in CGA levels in *C.arabica* K7 (Odeny et al., 2014; Vaast et al., 2006). Additionally, this difference may also come from genotypic differences in shade adaption. It is difficult to make a conclusion from these results as a weak correlation between solar irradiance or rainfall and CGA content is reported (Joët et al., 2010). Shade influences may arise from a different microclimate produced by shade or different genotype adaptations to shade. Transcriptional evidence is needed to determine the influence of shade on CGA accumulation. Moreover, steps involved in 5FQA biosynthesis remain to be discovered, although a CCoAOMT or CCoAOMT-like enzyme was reported to be involved. Numerous reports on environment influence on CGA in coffee relate to the role of CGA in plant resistance to disease or pests. The genes, *PAL* and *C4H* regulating CGAs biosynthesis, were found to be subject to a changing environment, including biotic or abiotic stress (Benveniste, Salaün, & Durst, 1978; Tovar, Romero, Girona, & Motilva, 2002). However, there is no research on their role in environmental influence on coffee quality rather than coffee plant adaption.

2.1.8. Sucrose

As an aroma precursor, sucrose degrades rapidly during roasting and forms volatile and non-volatile compounds, such as furans, pyrazine, aliphatic acids and hydroxymethyl furfural through Millard reactions (Grosch, 2001, pp. 68–89). Sucrose contributes more than 90% of the total low molecular carbohydrates in green coffee beans (Knopp, Bytof, & Selmar, 2006). Other low molecular sugars, for instance, glucose and fructose may also degrade or react with amino acids, but in green beans they represent only about 0.5% of the total (C-L Ky et al., 2000).

The extensively studied sucrose metabolism of tomato (*Solanum lycopersicum*) is an appropriate model for coffee as both belong to the asteroid I class of flowering plants and share a large number of common genes. Sucrose metabolism is a fairly complex process that involves two main steps (Fig. 4): 1) breakdown of sucrose by sucrose synthase (SUS) and invertase (including three isomers in cell wall, cytosol and vacuole); 2) biosynthesis of sucrose by sucrose phosphate synthase (SPS) and sucrose phosphate phosphatase (SPP) encoding genes as well as interaction with transported hexose or sucrose and starch metabolism (Nguyen-Quoc & Foyer, 2001).

Sucrose content depends on the coffee genotype, with 7.4–11.1% in Arabica and 4.05–7.05% in Robusta (Tran, Slade Lee, Furtado, Smyth, & Henry, 2016). Sucrose remained constant in early growing stages of the perisperm-endosperm transition, then increased during endosperm development and drastically increased from middle stage (large green to yellow fruit) and this increase was slowed down until maturity while Robusta accumulates gradually throughout seed development (Joët et al., 2009; Privat et al., 2008). Genes regulating sucrose metabolism are expressed differently during bean development. Higher sucrose synthase (CcSUS1) and acid invertase activities (CcINV2) were shown to be expressed in early development stages and lower *CcSPS1* (sucrose phosphate synthase) activity to re-synthesis sucrose at the end of development (Privat et al., 2008).

Candidate genes of sucrose metabolism expressed differently through coffee seeds development (including paralogous genes). Genes encoding invertase and sucrose phosphate phosphatase (SPP) peak at the first stage (Joët et al., 2009). This suggests a biosynthetic and catabolic balance of sucrose in young coffee seeds which responses to a constant concentration of sucrose in young coffee fruits. The highest expression of *SPS* was detected at 60–90 DAF. This is the key enzyme encoding sucrose biosynthesis in coffee seeds. Meanwhile, genes encoding cell wall invertase (*CWIN*) reaches a plateau which parallels the highest hexose transporter 1 (*HT1)* expression at the same stage (Joët et al., 2009). This implies higher sucrose containing tissues (source) transport sucrose to the cell wall. The sucrose is degraded to hexose and transported by *HT1* to the developing seeds (sink) cytosol. Degradation of sucrose to hexose and transport contributes to the increase in sucrose content of coffee seeds after the stage of constant sucrose. Consistent with this increase, sucrose transporter 1 (*ST1*) expression peaks at 90–120 DAF (Joët et al., 2009). This explains the sucrose increase by remobilization of sucrose from source and transport by *ST1* through the cell wall to the sink. *ST1* expression remained at a plateau until 120–150 DAF, when the *ST2* expression level reached the highest level. However, *SUS1* was found to be the most highly expressed at 120–150 DAF (Joët et al., 2009). This explains why sucrose was drastically increased during the period 90–150 DAF and then slowed down. All gene expression drops at the following stage. Subsequently, *INV* reached another maximum at 210–240 DAF while *SUS2* and *HT2* increases and were the most highly expressed in the final stage. Another peak was found at 210–240 DAF in *SPS* expression. This did not result in a rise in *SPP* expression, which regulated the downstream reaction towards sucrose, however, sucrose increased until maturity (Joët et al., 2009). This implies *SPS* is the predominant enzyme controlling sucrose synthesis. Finally, all genes expression levels went down except *HT2* and *SUS2*. From 120 to 150 DAF until >240 DAF, the gene expression pattern followed in parallel with the gradual increase of sucrose in the coffee seeds (Joët et al., 2009). Other research provided evidence that *CWIN* was expressed in early seed development to regulate endosperm and embryo cell proliferation, while SUS tends to be expressed in the middle to late stage controlling biosynthesis of lipids, starch, cellulose and proteins (Ruan, 2014). Shade was found to reduce sucrose significantly by delaying the peak of SUS and SPS enzyme activity and resulting in higher *SUS2* transcripts in coffee endosperm. The same SS and SPS activities were found in full sun and shade at 214 DAF, however, increases of these two enzyme activities until 260 DAF were reported in coffee grown under a shade treatment (Geromel et al., 2008). However, this research only studied three enzymes, including acid invertase, which did not shown much difference in transcripts. Many reports have shown that plant adaption to low temperature involves an increase in soluble sugars, especially sucrose (Winter & Huber, 2000). Maximum SPS protein activity was found in respond to cold exposure in spinach leaves (Guy, Huber, & Huber, 1992). In potato tubers, a new form of *SPS* was even observed at low temperature (Reimholz et al., 1997). It is not known whether this happens in coffee seeds. As sucrose metabolism is highly diverse and complex and involved with multigenes, further work is also needed to prove the negative influence of shade. Additionally, more work is required to determine how genotype, environment or the interaction of these two factors influences sucrose accumulation in coffee seeds during ripening.

2.1.9. Lipids

Coffee lipids, ranged between 7 and 17% in green coffee beans, which includes coffee oil in the endosperm: triacylglycerols (70–80%), esters of diterpene alcohols and fatty acids (15–18.5%), and other low concentration compounds contributing 0.1–3.2% of total lipids, and coffee wax (0.1–0.3% of total bean weight) outside the beans, which is sometimes removed by technological treatment

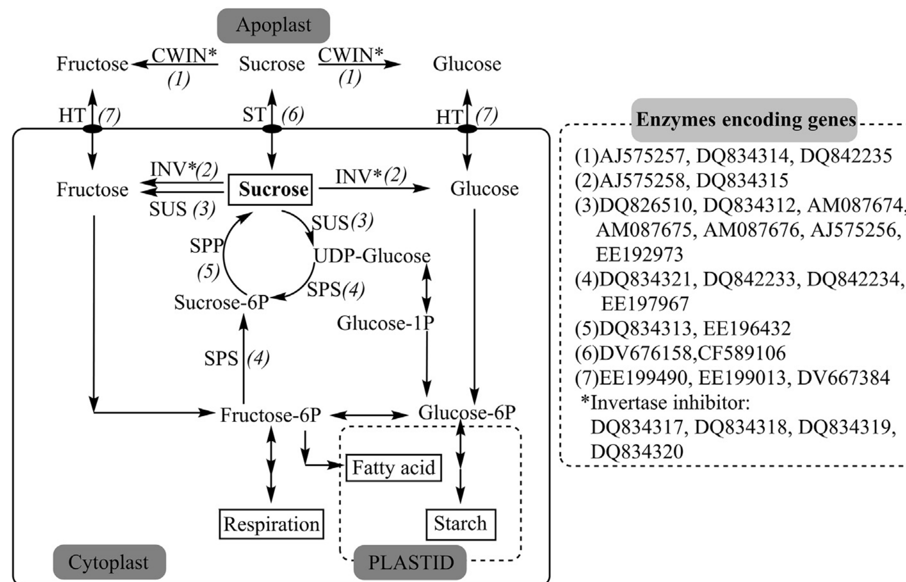

Fig. 4. *Sucrose biosynthesis in coffee seeds*. CWIN, Cell wall invertase; HT, Hexose transporter; ST, Sucrose transporter; INV, invertase; SUS, Sucrose synthase; SPP, Sucrose phosphate; SPS, Sucrose phosphate synthase.

such as polishing, dewaxing or decaffeinating the beans which makes the coffee beverage more attractive (Kurzrock & Speer, 2001; Nikolova-Damyanova, Velikova, & Jham, 1998; Oestreich-Janzen, 2010). Coffee lipids contribute to the texture and mouth feel of the beverage as they carries flavours and fat-soluble vitamins (Oestreich-Janzen, 2010). Kahweol and cafestol in the class of diterpene fatty acids attract much attention due to their paradoxical physiological effects that may increase serum cholesterol but potentially protect against carcinogenesis. However, tocopherols, another important group of lipids in green coffee beans that are present in small amounts, show antioxidant activities to humans (Cavin, Holzhäuser, Constable, Huggett, & Schilter, 1998; Lam, Sparnins, & Wattenberg, 1987; Speer & Kölling-Speer, 2006). Roasting does not change most of the coffee lipids, however, they are difficult to retain in the final beverage. In normal filtered preparations, for example, there are less than 0.2% lipids in the brew; for strongly roasted expresso, lipids accounts for 1–2% (Ranheim & Halvorsen, 2005; Speer & Kölling-Speer, 2006). Some lipids are identified with the species, for example, kahweol is detected mainly in Arabica while 16-*O*-methylcafestol (16-OMC) is only observed in Robusta and a higher content of lipid is found in Arabica relative to Robusta coffee (with average lipids of 15% and 10%, respectively). However, tocopherols are higher in Robusta than Arabica (Oestreich-Janzen, 2010).

To explain these differences, gene profiling has been conducted for five oleosin genes (*OLE1-5*), which encode the main seed oil storage proteins, in *Coffea arabica* and *Coffea canephora* from different ripening stages. Two predominant genes, *OLE-1* and *OLE-2*, together with *OLE-4* were significantly more highly expressed in every development stage in Arabica. However, *OLE-3* and *OLE-5* were expressed in Robusta at all development stages except the small green coffee seed stages, where all the five *OLE* genes were observed to have almost no expression (Simkin et al., 2006). This implies that Arabica starts to store oil earlier and in higher concentrations than Robusta. The mid-stage is an important changing point for lipid metabolism. For instance (see Fig. 5), a concomitance of FATb, KASII, ASD and FaTa, and the highest lipid synthesis and a sharp drop of linolenic acid (18:3) all occurred at 90–150 DAF. Fatty acids synthase acyl-CoA was shown to be at 90–120 DAF. Compared to homologous genes in other species, a strikingly high palmitic (16:0) and linoleic acid (18:2) (40%) is correlated with *FATb* transcription during lipid synthesis (Joët et al., 2009). Overexpression of *FATb* in transgenic plant supports this conclusion with high palmitic (16:0) accumulation (Dörmann, Voelker, & Ohlrogge, 2000).

Lipid content is also subject to environmental factors, such as shade, which results in higher levels (Odeny et al., 2014; Vaast et al., 2006). High altitude is another factor in favour of fat accumulation (Avelino et al., 2005). Moreover, a study working with short time extreme low temperature treatments found that five coffee genotypes responded differently to cold temperature; lipids increased in some coffee leaves to maintain cellular integrity but this was difficult for some genotypes which were observed to have lipid degradation together with damaged membranes (Campos, nia Quartin, chicho Ramalho, & Nunes, 2003). Therefore, it would be interesting to see whether these differences were regulated by genes controlling lipid accumulation and whether paralog genes in coffee endosperm (beans) have the same pattern of expression and response to cold. Whether shade and high altitude have the same influence on genes expression as low temperature remains to be determined. However, lipid metabolism is complex and needs further work to be fully understood. To date, no genes encoding malonyl-CoA ACP transacylase and ketoacyl-ACP synthase III have been found in the *Coffea* EST databases (Joët et al., 2009). Therefore, more work is needed to understand how these important components accumulate and what factors influences their accumulation.

3. Methods for analysis of coffee quality influenced by G and E

Physical and chemical traits of coffee are influenced by G and E as discussed above and interactions between G and E are observed that can be expressed as G × E. Together with physical and chemical analysis (metabolites), candidate genes expression profiling enables identification and quantification of transcripts and shows significant success in detecting molecular differences under different environment (Cullum, Alder, & Hoodless, 2011). Currently, an increasing number of reports are being published using transcriptome analysis to investigate G and E influences on the coffee plant. A comparison of *C. arabica* and its parents (*C. canephora* and *C. euginoides*) seedlings showed that the Arabica transcriptome was

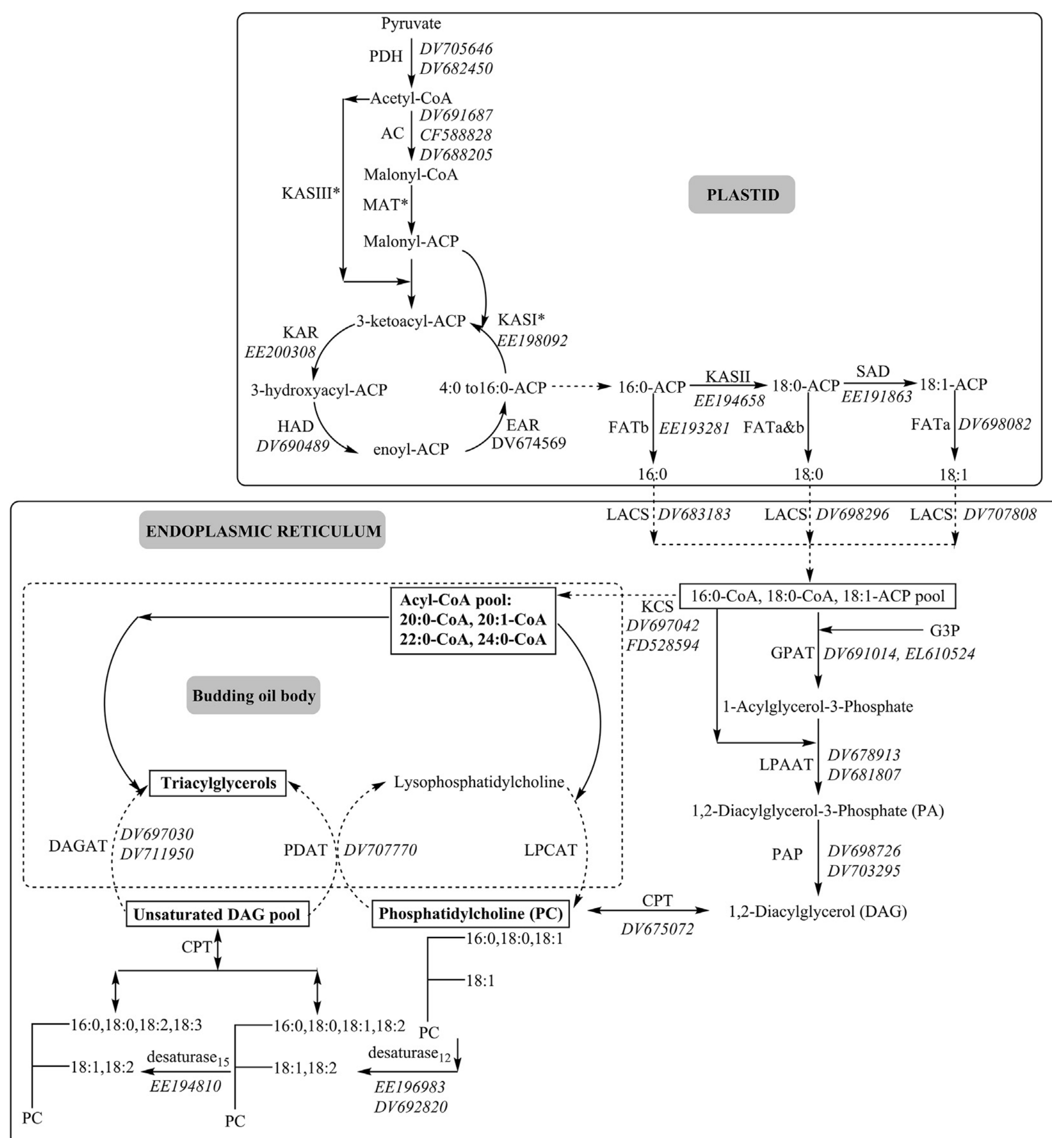

Fig. 5. *Lipid biosynthesis in coffee seeds.* The genes encoding these enzymes are in italics. *indicates genes not found in the Coffea expressed sequence tag (EST) database. PDH, pyruvate dehydrogenase; AC, acetyl-CoA carboxylase; MAT, malonyl-CoA ACP transacylase; KAS, ketoacyl-ACP synthase; KAR, ketoacyl-ACP reductase; HAD, hydroxyacyl-acyltransferase; EAR, enoyl-ACP reductase; SAD, stearoyl-ACP desaturase; j FAT, acyl-ACP thioesterase; LACS, long-chain acyl-CoA synthetase; GPAT, glycerol-phosphate acyl-transferase; KCS, beta ketoacyl-CoA synthase; LPAAT, acylglycerol-phosphate acyltranferase; PAP, phosphatidate phosphatase; CPT, diacylglycerol cholinephosphotransferase; DAGAT, diacylglycerol acyltransferase; PDAT, phospholipid diacylglycerol acyltransferase; LPCAT, lysophosphatidylcholine.

more stable to temperature variation compared to its progenitors (Bardil, de Almeida, Combes, Lashermes, & Bertrand, 2011). Plants, including fruits, have the plasticity to adapt to different environment. However, in practical, whether Arabica coffee fruits have the same pattern as seedlings being stable when treated with the same environment is yet to be confirmed. Recent transcriptome analysis in *C. eugenioides*, provides a global view of highly transcriptional expressed genes with various function in fruits and leaves: biological process related genes were significantly highly expressed in fruits while molecular function is lower compared to leaves, indicating tissues specific functions (Yuyama et al., 2015). Importantly, this study improves our understanding of the *C. arabica* background and future studies can benefit from this resource from *C. eugenioides* (Yuyama et al., 2015).

Coffee quality is influenced by metabolism of key compounds in coffee and the composition may be cross-influenced. Sucrose, for example, is a precursor for lipid and protein biosynthesis (Ruan, 2014). Transcriptome analysis, an overview of all the possible influences, aims to complete the whole set of transcripts in a cell and reveal the changing expression levels across different genotypes and environments. Transcriptome analysis using next genome sequencing technologies (NGS) have the advantage of high throughput and efficiency compared to previous technologies (Reuter, Spacek, & Snyder, 2015; Tran et al., 2016).

4. Limitations of current analysis of coffee quality as influenced by G and E

Although plenty of studies have been conducted on G and E influences on coffee quality, it is a highly complex process. A huge gap in knowledge remains. Firstly, coffee metabolism of key components is yet to be fully understood. The main metabolism pathways, including enzymes and their encoding genes need to be identified. In caffeine metabolism, for example, a highly purified 7-

methylxanthosine synthase, encoding caffeine biosynthesis, native to coffee is yet to be isolated (Ogawa, Herai, Koizumi, Kusano, & Sano, 2001). Secondly, transcriptional profiling using emerging next generation sequencing techniques is required to further understand coffee metabolism. Cost effective sequencing yielding more depth and coverage of sequencing coupled with improved data analyzing software will all support wide ranging experiments and gene expression analysis. For example, the Pacbio sequencing platform provides longer reads (average length >10 k) as compared to the Illumina platform, allowing the use of this platform to generate a reference transcript database especially for species like *C. arabica* without a reference genome. Even though there is a higher error rate (11%) for Pacbio, higher depth and coverage will circumvent this issue (Denoeud et al., 2014). Thirdly, transcriptome studies are mainly analyzed with coffee plants instead of fruits, however, for coffee quality, fruit analysis is essential. Moreover, there is limited work combining metabolic analysis together with transcriptional analysis to define G and E influences on coffee quality.

5. Future prospects

Plants have a striking ability to evolve and adapt to different environment conditions with a phenotypic plasticity associated with physiology and metabolism changes. Genotype or environment influences can be captured by phenotypic or transcriptional profiling during coffee bean development. The release of the *C. canephora* genome recently and more transcriptional work published on *C. engenioids* and *C. arabica* have increased the potential for the study of G and E influences on Arabica or Robusta quality using transcriptome analysis. However, to take advantage of this opportunity, further studies will be required. Firstly, the main coffee metabolic pathways need to be fully understood during different development stages with key metabolites, enzymes and encoding genes identified. Secondly, different genotypes or environment impacts on coffee metabolism needs to be analyzed to determine how to use this knowledge to improve coffee quality. Furthermore, it is essential to understand how G × E affects coffee quality and how to utilize this knowledge to improve coffee quality. This can be used as a guide to RNA interference and transgenic studies to control key genes and modify the final accumulation of key quality components to improve coffee. However, one needs to make a balance between coffee quality and yields. For example, some environment factors, like shade, have been shown to positively improve coffee quality, but they were also observed to decrease coffee yield. A reduction in caffeine or CGAs may reduce bitterness in the brew, however, this might be a problem for the coffee plant if caffeine or CGAs levels are too low to allow the plants resist to pests and diseases.

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