## BREEDING FOR COFFEE QUALITY

#### Christophe Montagnon, Pierre Marraccini & Benoit Bertrand

In line with the focus on specialty coffee, we will concentrate on the breeding of Coffea arabica. Contrary to what many may believe, breeding for coffee quality is a relatively new initiative. The challenges and opportunities that breeders face when selecting varieties for high quality are discussed.

In the coffee production sector, when talking about varieties, most people will mention Robusta and Arabica. This confusion between species, Coffea canephora and C. Arabica respectively, and varieties is typical of a pure commodity sector where only the largest and most obvious differences are recognized. According to the International Union for the Protection of New Varieties of Plants, a variety is "a plant grouping within a single botanical taxon of the lowest known rank, which grouping" can be: "i) defined by the expression of the characteristics resulting from a given genotype or combination of genotypes, ii) distinguished from any other plant grouping by the expression of at least one of the said characteristics and iii) considered as a unit with regard to its suitability for being propagated unchanged". One would argue that Bourbon or Tipica are Arabica coffee varieties and it is true that most coffee people would be able to roughly describe the phenotype of each. However, referring to the definition of a variety: exactly what are the characteristics expressed by Bourbon or Tipica? Are they distinguishable from other Arabica plants? Further adding to the complexity is the recognition by the International Coffee Organization (ICO) of three Arabica coffee categories: Colombian Milds, Other Milds and Brazilian Natural. Historically, the mainstream coffee market has been based largely on this classification and the reputation of quality coffee tends to be associated with geographic origin irrespective of the variety or botanical type.

In this chapter, the concept of coffee variety and its relation to market demand are discussed. The current knowledge of coffee genetics and quality are also discussed, along with the gaps in knowledge that still remain. Breeding strategies are then proposed, stressing the need for new high throughput phenotyping of selection criteria linked to quality. Finally, some thoughts are offered about how producers might take advantage of new advances in genetics and selection methodologies.

#### INTRODUCTION

Surprisingly, it is only recently that breeders have begun to breed for improved coffee quality. In this chapter, the challenges and opportunities for breeding *Coffea arabica* varieties with improved quality traits are discussed. In line with the scope of this book, which focuses on specialty coffee, this chapter is limited to improving quality of *Coffea arabica*. Readers interested in coffee breeding in general are invited to read Lashermes *et al* (2009) for Arabica, Montagnon *et al* (1998 a&b; to be published) for *C. canephora* (Robusta) and the recent review of Lashermes *et al*, (2008).

The Coffea genus is composed of more than 100 species (Bridson and Verdcourt, 1988). Arabica and Robusta coffees represent 65 % and 35 % respectively of the coffee produced worldwide. *C. arabica* is a self compatible amphidiploid (2n=4x=44), whereas all other Coffea species are diploid (2n=2x=22) and strictly allogamous. Sometime within the past 100,000 years, *C. arabica* was formed from the two closely related species *C.canephora* and *C. eugenioides* (Lashermes *et al*, 1999).

According to FAO (database : FAOSTAT: http://faostat.fao.org/site/567/default. aspx#ancor), coffee yield has stagnated since 1960 in all coffee producing countries except Brazil, Colombia and Vietnam (Figure 1). Vietnam focused on Robusta in response to government stimuli at a time when the agricultural



**Figure 1:** Evolution of Yield (Hg green coffee / Ha) since 1961: Brazil, Colombia, Vietnam vs others (FAOSTAT database)

sector was liberalizing and when there were favorable prices in the world market. Brazil and Colombia both invested consistently and continuously in coffee R&D for many decades, with breeding of improved varieties as a major part of their development programs. Plant breeding requires a large long term investment, but over the long term the returns on investment are extremely high.

Most of the main crops in the world benefit from a strong seed/breeding private sector, but coffee and several other tropical crops do not. Coffee and several other tropical crops do not. The main reason is that private breeding companies do not see the coffee sector as an interesting market for improved varieties. Nevertheless, several studies indicate that lack of credit is the major bottleneck that prevents producers purchasing improved coffee varieties. This point of view is supported by our experience in Central America where producers buy new F1 coffee hybrids when credit is available (Killian, unpublished data; Haggar, unpublished data). In the light of the demand for improved coffee varieties and the high rates of return on investment in plant breeding, we suggest that coffee breeding and especially breeding for coffee quality should be high on the agenda of all those who are involved in the quality coffee business.

## VARIETIES AND THEIR MARKET-DEMANDED CHARACTERISTICS

Coffee growers have consciously or unconsciously selected plants with specific traits for well over one thousand years. As background information the reader is referred to the genealogy of the main varieties of coffee (Figure 2) and their characteristics (Table 1). The main characteristics described in Table 1 should be treated with caution as (i) there is no well-established, internationally acknowledged description of "coffee" varieties; (ii) the name of the variety is not always a guarantee that it really is the variety it is stated to be; and (iii) the expression of varietal traits varies according to environmental conditions such as altitude.

#### Varieties in the coffee sector

In the coffee production sector, most people use the terms Robusta and Arabica to distinguish between varieties, when in reality the term "Robusta" refers to the species *C. canephora* and "Arabica" to *C. arabica*. This confusion between varieties and species occurs when coffee is treated as a commodity with only the major and most obvious differences being recognized. The International Union for the Protection of New Varieties of Plants (UPOV) defines a variety as "a plant grouping within a single botanical taxon of the lowest known rank, which grouping...... can be: (i) defined by the expression of the characteristics resulting from a given genotype or combination of genotypes, (ii) distinguished from any other plant grouping by the expression of at least one of the said characteristics and (iii) considered as a unit with regard to its suitability for being



**Figure 2:** Schematic representation of the genealogy of the main varieties of *Coffea* arabica

Variety type	Examples	Advantages	Limitations	Market
Varieties with special mutations related to quality	Maragogype (Maracaturra, Pacamara), Laurina / Bourbon Pointu	Special Quality	Low productivity, disease susceptibility	Niche markets
Classic tall varieties	Tipica, Bourbon	Reputation for quality is standard to good. Robust varieties	Rather low productivity, disease susceptibility. More adapted to agroforestry systems.	Upper to niche markets
	Java	Good quality. Robustness, partial resistance to CBD and rust, well-adapted for use by small producers.	Has not yet developed a reputation for quality. Reputation for quality to be constructed. Rather low productivity.	Upper markets
	Geisha	Excellent reputation for quality.	Highly unstable variety. Needs further breeding.Only in Agroforestry systems.	Upper markets
	Ethiopian landraces	Quality is standard to excellent. Rust or CBD resistance in some accessions.	Productivity low to regular. Only in Agroforestry systems.	Upper markets
Improved tall varieties	Mundo Novo (tall)	Standard quality and standard productivity. Adapted to mechanization.	Disease susceptibility	Mainstream
	Ethiopian F1 hybrids	Good to very good productivity. Partial resistance to CBD and rust in some hybrids.	Reduced the diversity of Ethiopian quality. Difficulties of diffusion. Only in Ethiopia	Mainstream
Classic dwarf varieties	Caturra, Catuai	Good productivity. Quality standard. Well-adapted to intensification	Disease susceptibility	Mainstream to upper markets

#### Table 1: General description of the main C. arabica varieties

Introgressed dwarf varieties	Catimors, Sarchimors or derived	Standard to good productivity. Partial resistance to CBD or nematodes in some accessions rust resistance.	Questionable quality. Needs further breeding for quality	Mainstream
Improved dwarf F1 hybrids	Centro America (Central America), Diamond Coffee	Excellent productivity. Partial resistance to CBD and rust resistance in some hybrids. Good to excellent quality.	Quality reputation to be constructed. Production capacities to be increased to meet producers demand	Mainstream to upper / niche markets, depending on the F1 hybrid variety

Table 1: General description of the main C. arabica varieties (continued)

propagated unchanged". In other words, a variety can be precisely described, distinguished from any other variety and be reproduced in such manner that the varietal identity is unchanged. In the same way that a trade mark protects the inventors intellectual property rights, the name of a variety, when in accordance with the UPOV regulations and used by a well-organized seed sector, ensures that the purchaser does not get an unpleasant surprise. Some would argue that Bourbon and Tipica are Arabica coffee varieties and it is true that many coffee producers will be able to describe the phenotype of each. However, if we ask:

- Can the precise characteristics of Bourbon or Tipica be described? The answer is "yes". A solid botanical description was performed in the 40's.
- Are they distinguishable from any other Arabica plants? The answer is "yes" based on this solid description
- Are they propagated unchanged? Here is the real difficulty. With some exceptions, the lack of control along the chain of dissemination of these varieties, often by producers themselves, makes it difficult to be sure what the real genetic status of a cultivated variety is.

More generally, beyond the Typica and Bourbon situation, most coffee "varieties" have duly been described during the process of breeding. Nevertheless, it is questionable whether this description holds true as these "varieties" are often propagated and passed from one producer or country to another, or from one country to another with no guarantee of true-to-type replication of the original variety.

Since the 1980's breeding efforts have emphasized disease resistance due to the threat of coffee leaf rust and coffee berry disease. Resistant selections are based on the introgression of resistant *C. canephora* genes, using the natural interspecific (*C. arabica* x *C. canephora*) Timor Hybrid as the source for introgression. These new coffees are named Catimors or Sarchimors. The best known of these are CR95 in Costa Rica, Ruiru 11 in Kenya, IAPAR59 and other derivative cultivars in Brazil, Colombia in Colombia and Oro Azteca in Mexico. These populations are the closest to what might be called a variety, with Oro Azteca being the only variety, to our knowledge, that has been registered with UPOV. Nevertheless, when the seed production system gets away from the control of the National Coffee Institute, plots that are supposedly producing these varieties are, in reality, highly heterogeneous and lacking in acceptable genetic quality (Montagnon and Bertrand, personal observation in Meso-America).

Thus, we conclude that in the coffee sector, even if "varieties" are generally welldescribed as part of the breeding process, seed production and dissemination systems do not always maintain the varietal purity of the populations distributed to growers. Hence, referring to coffee types as Bourbon, Tipica, Mundo Novo and Caturra frequently does not always provide the grower with credible information on the type of coffee they possess. It is worth mentioning that some countries like Brazil, Colombia and Costa Rica do have a seed production chain that ensures genetic conformity, but a lot more effort is required in other countries.

Vagueness concerning what can be described as a variety in no way invalidates the empirical knowledge of producers and roasters concerning characteristics of coffee types. Thus, for example, planting Bourbon or Tipica will generally ensure a good cup quality. At the same time, we note that the coffee sectors in many countries do not have the capacity and organization required to ensure dissemination of true-to-type varieties. However, the situation is changing with the new generation of F1 hybrids becoming more popular as the sector moves towards an era of true coffee varieties.

### Characteristics demanded by an evolving market

The International Coffee Organization (ICO) recognizes three Arabica coffee categories: Colombian Milds, Other Milds and Brazilian Natural. The mainstream coffee market is largely based on this classification and the reputation for coffee quality depends more on the geographic origin than on the variety or botanical type. The geographic source may be associated with a particular type of coffee grown in that country or region; however, in the market these associations are not clear. Thus, roughly speaking, in the mainstream markets the demand for specific varieties is extremely limited. The main concern of the market is for a consistent quality that is expected from the particular country or region: the roaster expects specific quality traits when he purchases coffee from a particular origin. The roasters are suspicious of any modification of the quality that they have come to expect from a particular producer (Bertrand *et al.*, 2006a).

Nevertheless, the situation has changed rapidly with double digit growth in the specialty coffee market over the past ten years (see Giovannucci *et al*, 2008 for a detailed review). The specialty market is often described as a "decommoditization" of the coffee market (Daviron and Ponte, 2005) with growing consumer demand for differentiated and high quality coffees. The rise of the specialty high quality market has led to opposing perceptions of the quality of coffee "varieties". On the one hand, several producer countries have argued that the Catimor types do not have a negative impact on quality (see for example, Van der Vossen, 2009), while, on the other, roasters have been virulent critics of the coffee produced by the Catimor types. The roasters and the market seem to have the final say, and there is renewed interest in the variety as a key factor in determining coffee quality. This perception has stimulated a renewed awareness of the importance of coffee varieties as key factors for quality.

In parallel with the increased market share for specialty coffee, there is a growing fear among roasters of a shortage of good quality coffee in the mainstream volume market. This fear is compounded by likely effects of climate change on coffee production and quality (see Chapter 1.3 of this book). The coffee sector is becoming increasingly aware of the need to increase both productivity and quality. Low productivity of coffee is associated with high production costs and is increasingly seen as the main reason for shortages of high quality coffee. Low productivity levels lead directly to low overall production and shortages of coffee as growers switch to more profitable options. Hence, after many years of indifference, the coffee sector now realizes that appropriate varieties are required so as to produce sufficient volumes of high quality coffee to meet market demand. Several research institutions, including CIRAD and its partners, have identified this need for a new generation of varieties that combine quality and productivity (Bertrand et al, 2011) as part of an overall "Ecologically Intensive Agriculture" axis of research (www.cirad.fr). This has been further conceptualized by Nespresso with the neologism: qualitivity<sup>™</sup>.

Compound (% dry matter)	C. arabica	C. canephora
Total lipids	13 - 17	7.2 - 11
Caffein	0.7 - 2.2	1.5 - 2.8
Chlorogenic acids	4.80 - 6.14	5.34 - 6.41
Trigonelline	1 - 1.2	0.6 - 1.7
Oligosaccharide	6 - 8	5 - 7
Total Polysaccharides	50 - 55	37 - 47

**Table 2:** Composition of *C. arabica* and *C. canephora* for the main precursors of aroma (after Leroy *et al*, 2006)

### The breeder's perspective

During the years of what we have called "indifference" to quality, breeders made productivity the main criteria for selection, with disease resistance a major means of maintaining productivity. Leaf rust was emphasized on a worldwide basis and Coffee Berry Disease in East and Central Africa. Investment in breeding was greatest in countries like Brazil, which adopted intensive coffee production systems with full exposure to the sun rather than shade. These breeding programs produced Catimor-type varieties, which are productive, resistant or tolerant to the main diseases and well-adapted to intensive full sun systems. However, their quality has been repeatedly questioned by the market, as well as their poor adaptation to agro-forestry systems, which favor good quality and are frequently associated with ecological sustainability. Finally, this generation of Catimor-like varieties did not widely replace the traditional varieties.

The biological cycle of coffee is 5-10 years, and once a grower plants a tree he may wish to keep it for a further twenty years or more. Breeders may therefore face uncertainty about what the market will be demanding when they finally release a variety. This partly explains the disillusion with the Catimor-type varieties: quality was not such a major concern when breeding programs started in the 80's and it was only some years later that their deficiencies in terms of quality were perceived to be a problem.

Some breeders anticipated the demand for new varieties that combine quality and productivity by some 10-15 years. Their varieties are now becoming available to meet today's market demand. However, the market demand still lacks clarity (see section 3.4). Breeders have two strategic options (Table 3). The first is to improve productivity while maintaining commonly recognized high quality standards. We call this strategy Standard Quality Conservative Selection. The second option, which we call Quality Innovative Selection, focuses on simultaneously improving both productivity and quality. Quality improvement may emphasize innovative quality characteristics that are a distinctive feature of a particular variety that might qualify it for trade as an exclusive product.

It is still not clear to breeders which strategy to choose or how much weight to give to each strategy. Standard Quality Conservative Selection would likely aim at large volumes in a mainstream quality market, whereas Quality Innovative Selection would target roasters looking for exclusivity in what we might call a hyper-specialty market. The strategy options have implications for breeding and selection methodologies which are highlighted in the following sections.

# CURRENT KNOWLEDGE ON COFFEE GENETICS AND QUALITY

The creation of new varieties requires knowledge about the factors (and their genetics) that define coffee quality.

Breeding strategy	Objective	Market	Type of material	Methodology	Urgent need
Standard Quality Conservation Selection	New high yielding varieties with no loss of quality	All quality coffee markets	Introgressed varieties	Screening introgressed varieties	Sound, valid, cheap and rapid prediction / phenotyping of quality
				Genome-wide Selection	
			F1 hybrids	Screen unused Arabica diversity for parent selection	
Quality Innovative Selection	New varieties with a special /specific quality	Need to be integrated in a well- defined value chain	Pure Arabica lines (likely Ethiopian)	Screen unused Arabica diversity for specific qualities / mutations	
				Mutagenesis	

**Table 3:** Synthetic view and description of possible coffee breeding strategies for quality

#### Selection criteria for quality and phenotyping

The prerequisite for evaluating the genetic basis, and hence the opportunity of breeding, for Coffee Cup Quality (CCQ) is to be able to measure/evaluate it. CCQ is determined by sensory evaluation known as cupping. Each player in the industry has his own way of cupping. There has been an attempt to standardize cupping, especially for the specialty coffee market (see for example SCAA, 2009). However, rules of standardization have not been applied or tested in a research context to establish statistical differences or similarities between varieties or other factors. Researchers have tried to establish some quantitative methodologies that allow statistical analysis (Moschetto et al, 1996; Puerta, 2000; Muschler et al, 2001; Bertrand et al, 2006b; Perriot et al, 2006; Vaast et al, 2006; Leroy et al, 2010). However, to our knowledge, the evaluation methods were never tested for repeatability and statistical power, as is the case for several other products, including wine (O'Mahony and Odbert, 1985; O'Mahony and Goldstein, 1986; Murray et al, 2001). Furthermore, we have not found any specific comparison of CCQ evaluations carried out by the coffee industry and by researchers: there is no well-established and validated cupping method available for research. The SCAA cupping method may be suitable for research and statistical differentiation between varieties, but this remains to be tested. There is a need to integrate the activities of the breeders with those of the coffee industry so as to develop a standardized means of evaluating cupping quality. We are quite optimistic that new initiatives, like the Global Coffee Quality Research Initiative (GCQRI), will provide a good framework to achieve this integration.

Sensory evaluation is often a time consuming and costly process. For this reason, chemical predictors or indicators of CCQ quality have been sought. The predictor (or "bio-marker") is often a chemical storage (SC) or volatile compound (VOC) (see Wishart, 2008 for review). Ribeiro *et al* (2009) provide an excellent synthesis of the known precursors of coffee aroma and the corresponding volatile compounds (Figure 3).

The major storage compounds of mature C. arabica beans are cell wall polysaccharides (CWP, 48-60% Dry Matter (DM)), mainly galactomannans and arabinogalactan-proteins, lipids (13-17% DM), proteins (11-15% DM), sucrose (7-11% DM) and chlorogenic acids (CGA, 5-8% DM) (for a review see De Castro and Marraccini, 2006). Recently the metabolic processes that occur in the course of coffee bean development were described in detail (Joët et al, 2009). Each of these major storage compounds plays several crucial roles in the complex chemistry of roasting (Flament, 2002; Ribeiro et al, 2009). For example, proteins and amino acids are essential for the conversion of reducing sugars into aroma precursors through Maillard reactions. Reducing sugars themselves results from the degradation of sucrose and CWP. In addition, triacylglycerols are the major carriers of aroma in the roasted bean. Their fatty acid (FA) composition determines the generation of thermally-induced oxidation products, in particular aldehydes, which react readily with Maillard intermediates, giving rise to additional aroma compounds. CGA and caffeine are responsible for bitterness. However, caffeine has never been clearly related to coffee cup quality (Montagnon et al, 1998; Joët et al, 2010).

Numerous factors influence the basic chemical composition of the coffee bean: soil-climatic conditions (Bertrand *et al*, 2006b), agricultural practices (Vaast *et al*, 2006; Geromel *et al*, 2008) and post-harvest processes (Selmar *et al*, 2006). It is generally accepted that altitude and shade improve coffee quality (Avelino *et al*, 2005; Decazy *et al*, 2003; Guyot *et al*, 1996), although at very high altitudes shade can lower quality (Bosselman *et al*, 2009). Low temperatures have been suggested to slow down the ripening process, which in turn leads to greater accumulation of aroma precursors (Vaast *et al*, 2006). Joët *et al* (2010) proved that air temperature (linked to altitude and shade) has a major impact on the chemical composition of coffee beans.

*C. arabica* produces a better CCQ than *C. canephora*. The species differ in the content of the main precursors of aroma (Table 2., Ky *et al*, 2001a; Leroy *et al*, 2006). Differences between the species led to the hypothesis that a higher content of lipids, trigonelline and sugars coupled with a lower content of GCA might be linked to better CCQ. During the last decades, much research has been devoted to the confirmation of this hypothesis.

Total lipid content was reported to either decrease (Guyot *et al*, 1996), be stable (Avelino *et al*, 2005; Joët *et al*, 2010) or increase (Decazy *et al*, 2003; Bertrand



Figure 3: Main precursors of aroma in the coffee bean (Ribeiro et al, 2009)

*et al*, 2006b) with increasing altitude. Bertrand *et al* (2006b) recently showed that the composition of fatty acids rather than total lipid content might be related to CCQ. The composition of fatty acids was shown to be highly influenced by environment (Villarreal *et al*, 2008), suggesting the need for further studies on the relationship between fatty acids and CCQ.

GCAs were reported in one study to accumulate with an increase in altitude (Avelino et al, 2005), but to remain stable in another (Guyot *et al*, 1996). 5-CQA, rather than total GCA, was shown to increase with altitude (Bertrand *et al*, 2006b). Farah *et al* (2006) found a significant positive correlation between 3,4-DCQA levels in green beans and cup tasting results with Brazilian natural coffees. Here again, the fine composition of GCA rather than its total content seems to be of interest.

Farah *et al* (2006) found a significant positive correlation between trigonelline levels in green beans and cup tasting results for Brazilian natural coffees.

Sugars were reported to increase (Guyot *et al*, 1996) or be stable (Bertrand *et al*, 2006b; Joët *et al*, 2009) with higher altitude. Sugars also increased (Vaast *et al*, 2006) or decreased (Geromel *et al*, 2008) with increasing shade. Joët *et al* (2010) showed that different classes of sugars react differently to decreasing air temperature.

The chemical composition of wet- and dry-processed beans may differ significantly, as observed for free amino acids, organic acids, and non-structural carbohydrates (Bytof *et al*, 2005; Knopp *et al*, 2006). Dry-processed coffees are generally characterized as having more body, whereas in most terroirs, wet-processed coffees have a better aroma, generally resulting in higher acceptance (Selmar *et al*, 2002). In both treatments, the freshly processed coffee beans remain viable and exhibit active metabolic processes (Bytof *et al*, 2007).

Green coffee and roasted coffee display 300 and 850 volatile compounds respectively (Grosch, 2001; Flament, 2002). Studies on the relation between CCQ and volatile compounds are often specifically concerned with the identification of off-notes in roasted coffee or brew (see for example, Mateus *et al*, 2007; Tranchida *et al*, 2009; Lindinger *et al*, 2009). Studying 58 Arabica varieties, Ribeiro *et al* (2009) could predict CCQ from the volatile composition of roasted coffee.

Studies on volatile compounds of green coffee related to cup quality also addressed strong cup defects such as stinking beans (Guyot *et al*, 1982) and mouldy/earthy defect (Cantergiani *et al*, 1999). However, through aroma extract dilution analysis (AEDA) and gas chromatography/olfactometry (GCO), up to 28 potent odorants were identified in green coffee (Grosch, 2001). However, it is only recently that studies have determined a relationship between the volatile compound composition of green coffee and the evolution of CCQ during storage (Scheidig *et al*, 2007) or to specifically low CCQ (Mancha Agresti *et al*, 2008; Toci and Farah, 2008). Furthermore, Gonzalez-Rios *et al* (2007) were able to characterize and observe the difference in volatile compositions of green coffee according to four different post-harvest techniques.

In conclusion, even if some trends relate CCQ to aroma precursors, a reliable methodology would, by definition, be based on solid data that can be used to predict CQQ from aroma precursor profiles. There are some indications that CCQ may be related to the fine composition of lipids or GCA and the volatile composition of green coffee. Several other precursors like proteins and carotenoids have never been studied. The evolving technologies enabling fast and wide cost-effective screening of both SCs and VOs open the way to the identification of solid predictors of CCQ that might serve for high-throughput germplasm phenotyping.

## Coffee genetics and quality

There are several approaches to studying the genetic basis of CCQ, including the examination of statistical differences between varieties, determination of genetic correlations between quality and molecular markers linked to Quantitative Trait Loci (QTLs) and identification of specific genes associated with quality.

#### Introgression, new F1 crosses and quality

In the 1980s, the Timor Hybrid (TH) was crossed with traditional Arabica coffees. TH is a natural hybrid of Arabica and Robusta found in Timor Island (see also Chapter 2.5). The amount of introgressed genome from Robusta represents 8-27 % of the TH genome (Lashermes et al 2000a, Mahé et a., 2007). Several cultivar lines (i.e. cv. 'Costa Rica 95', cv. 'Obatã', cv. 'IAPAR59') have been fixed after several generations of pedigree selection. However, inevitably, the introgression process has not been restricted to resistance traits and Robusta genes implicated in quality are present in the genome of the introgressed lines. Though most breeders' were positive about the quality of the fixed introgressed lines (Fazuoli et al, 1977; Owuor, 1988; Moreno et al, 1995; Puerta, 2000; Van der Vossen, 2009), most coffee buyers claimed that the quality of the new introgressed varieties was below standard. Bertrand et al (2003) confirmed that, on average, introgressed lines were disappointing in terms of beverage acidity and preference. However, the same study indicated that some introgressed lines were similar to the control and that furthermore, cup quality was not related to the amount of introgressed Robusta genome. This strongly suggests that it is possible to obtain good guality traits in introgressed lines, and also that Marker Assisted Selection (MAS) can be an effective means to avoid undesirable introgressed fragments suspected of having a negative effect on CCQ (Lashermes et al, 2000b).

Since the late 90's, CIRAD and partners have developed a new generation of varieties called F1. These varieties are the result of crosses between traditional varieties (both introgressed and non-introgressed) and Ethiopian genotypes. They exhibit hybrid vigor or heterosis with up to 50% superiority for yield as compared to the best parent (Bertrand *et al*, 2005). Furthermore, the cup quality of several of these F1 varieties was equal or superior to the traditional varieties (Bertrand *et al*, 2006b). These varieties also perform well in coffee agroforestry systems (Bertrand *et al.*, 2011)

To our knowledge, no study of quantitative genetic parameters has been made on *C. arabica* quality. *C. canephora* (Montagnon *et al*, 1998) and interspecific crosses (Barre *et al*, 1998; Ky *et al*, 1999; Ky *et al*, 2001b) have been studied, but the findings are not necessarily applicable to *C. arabica*.

#### Quantitative Trait Loci and candidate genes

*C. arabica* is an amphipolyploid species, which makes it difficult to use standard methodologies to identify Quantitative Trait Loci (*QTLs*<sup>1</sup>). There is no published information on QTLs linked to quality in *C. arabica*. The first work identifying QTLs linked to quality (cup quality and chemical composition) dealt with *C. canephor* (Leroy *et al*, submitted). However, as the species in the *Coffea* genus are closely related (Lashermes *et al*, 1997; Cubry *et al*, 2008), it may be possible to identify quality genes in any of the diploid species and look for the homologous genes in *C. arabica*. This approach may explain the existence of studies that identify genes associated with quality in several of the diploid species (for a detailed review, see Joët *et al*, 2011).

Two genes involved in the fruiting time (Akaffou *et al*, 2003) and maturation (Bustamente-Porras *et al*, 2006) of the coffee fruit were identified in interspecific crosses. More recently, genes of bean expansion that could be implicated in the control of bean size were reported (Budzinski et al, 2011). Finally, genes directly involved in the metabolism of sucrose (Geromel *et al.*, 2006; Privat *et al.*, 2008; Joët *et al.*, 2009) and polysaccharides, (mainly the galactomannans) were identified (Marraccini *et al.*, 2005, 2011; Pré *et al.*, 2008; Joët *et al.*, 2009; Figueiredo *et al.*, 2011).



Figure 4: The "Omics" cascade (Dettmers *et al*, 2007)

<sup>1</sup> QTL stands for Quantitative Trait Loci: a QTL is a region of the genome associated with the expression of a quantitative trait.

Genes involved in the metabolism of caffeine have been widely studied and described (Ogawa *et al.*, 2001; Ogita *et al.*, 2003 and 2004; Ashihara *et al.*, 2006; Uefuji *et al.*, 2003; Salmona *et al*, 2008). A natural caffeine-free mutation (called "caffeine-free") in a *C. arabica* plant (Silvarolla *et al*, 2004) has a nucleotide mutation in the CADXMT1 gene (Maluf *et al*, 2009) coding for an N-méthyltransferase enzyme.

The chlorogenic acids form a family of esters from trans-cinnamic acids and quinic acids. The 5-O-caffeoylquinic acid (5-CQA) accounts for the major part (70%) of GCAs (Hanson, 1965). The identification and expression of several genes involved in 5-CQA metabolism have been described (Mahesh *et al.*, 2007; Lepelley *et al.*, 2007; Koshiro *et al.*, 2007; Joët *et al.*, 2009 an 2010).

Other genes have been described for oleosin (Simkin *et al*, 2006) and carotenoid (Simkin *et al*, 2008) pathways.

Gene discovery is of great interest as it may help to decipher the metabolism of compounds involved in quality. However, the use of this knowledge in classical breeding is still not clear. The discovery of genes usually opens the way to genetically modified varieties through genetic engineering. Today, GM coffees are not formally considered for commercial purposes<sup>2</sup>.

### BREEDING STRATEGIES FOR COFFEE QUALITY

One of the most important features of any breeding progam is the development of screening techniques.

## High throughput phenotyping of coffee quality

An efficient breeding strategy relies on sound selection criteria. The only inviolate selection criterion for cup quality is cup quality evaluation *per se*: there are no reliable indirect selection techniques that serve as cheap and rapid predictors of coffee quality (see Chapter 2.1). More research is needed to establish clear and robust sensory evaluations that can be statistically analyzed. However, even if such techniques were available they would likely be too costly and time consuming to be routinely applied in a breeding program to screen a large number of genotypes.

The objective of screening is to identify traits or components of the coffee bean that are associated or correlated with particular quality traits. In the following paragraphs we describe some of the promising new techniques that could be

<sup>2</sup> This is a formal decision of the Common Code for the Coffee Community

developed to assist breeders in the selection process. Some, like transcriptomics, are probably not useful for coffee quality as a large portion of the transcripts is not translated, but stored as mRNA until germination (Joët *et al*, 2009).

Major non-protein storage compounds (lipids, sugars, alkaloids, chlorogenic acids and carotenoids and the different forms of these families) can be analysed with high pressure liquid chromatography (HPLC) combined with mass spectrometry (MS) (Gelpi, 2002). This setup allows the separation, identification and quantification of hundreds of metabolites in a single analysis.

Simultaneous chemical and sensory analyses (mdGC-MS-Olfactometry = mdGC-MS-O) can be used to rank and identify specific volatile and semi-volatile compounds responsible for specific aromas and defects. Specific volatiles can be associated with both specific aroma defects and desired aromas. The method starts with solid-phase microextraction (SPME) with extracts passing through mdGC-MS-O. There are several advantages of combining SPME and mdGC-MS-O, especially lower detection thresholds and increased chromatographic separations (see for example Koziel et al, 2001a&b; Koziel et al, 2006).

Near Infrared Spectrophotometry (NIRS) is a rapid low cost technique for characterizing samples after the calibration curves or equations have been developed. NIRS is already used to predict the total content of the main family of precursors: lipids, chlorogenic acids, sugars, trigonelline, and caffeine (Montagnon et al, 1998; Bertrand et al, 2006; Kathurima et al, 2010). Ribeiro et al (2010) suggest that integrated use of the NIRS technology is effective for quality prediction in roasted coffee. However, further work is needed to develop the technique and develop calibration curves for the precursors of quality in green coffee.

While coffee breeders anxiously await the development of these techniques and their incorporation into selection schemes, they are limited to only the technology that is currently available.

## Standard Quality Conservative Selection: productivity and disease resistance without loss of quality

Standard Quality Conservative Selection aims to produce highly productive and disease resistant varieties without losing quality. The selection schemes are based on incorporation of previously unused genetic variation in introgressed and F1 hybrid varieties.

There are two main sources of genetic variability for coffee quality: Ethiopian germplasm and introgressed genotypes. The traditional cultivated lines of C. arabica have a very narrow genetic base because of the small number of trees from which they all originated in the 18th century (Berthaud and Charrier, 1998). This narrow genetic base limits their potential use in breeding programs. On the other hand, the Ethiopian germplasm is much more diverse and has a much broader genetic variability (Anthony et al, 2002; Alemayehu et al, 2010). Selections made directly from the wild Ethiopian germplasm have not been widely cultivated as their agronomic performance is poor when compared to traditional varieties. However, in light of their contribution to the new generation of F1 hybrids (Bertrand et al, 2005) as well as the growing need for high quality coffees, breeders are showing renewed interest in incorporating them into selective breeding programs as they undoubtedly present a great opportunity for breeding improved quality coffee varieties. The second source of genetic diversity is the wide range of introgressed genotypes. As already stated, most introgressed selected varieties did not satisfy the market' criteria for high quality coffee, but they were not selected for their quality. Bertrand et al, (2003) reevaluated the pool of introgressed varieties and discovered much genetic variability for guality. Thus, with adequate evaluation methods (including molecular markers), an efficient breeding process could lead to the identification of introgressed, disease-resistant varieties with excellent quality (Lashermes et al, 2000b). It is worth noting that well-chosen introgressed lines might be used as one parent of F1 hybrids.

### Quality specific selection: Specific quality

Quality specific selection aims at selecting a particular exceptional cup quality. This breeding strategy must be integrated into a well-defined coffee value chain. Indeed, breeding for a particular quality only makes sense if a coffee roaster is able to take advantage of the added value obtained from a distinct quality profile. Selection for an extraordinary quality profile will almost certainly carry a tradeoff in terms of lower productivity or the need for more intensive management. Hence, a premium price is required for the particular quality trait in order to balance higher costs of production. The Geisha variety in Panama illustrates how a price premium can compensate for low natural productivity and specialized crop management.

We suggest that Quality Specific Selection should only be embarked upon when there is a close partnership between one or several producers and a limited number of coffee roasters: the coffee roasters will almost certainly look for exclusivity as part of their marketing strategy.

Quality Specific Selection will depend on the exploitation of currently unused genetic diversity. It is unlikely that selection from within populations of currently available introgressed varieties will produce a distinctive and exceptional high quality coffee. Initial screening should focus on the pure Arabicas, mainly the Ethiopian lines, to identify genotypes that are sources of exceptional quality, but

also unlikely to have high levels of productivity. Crosses between these exceptional quality lines and materials with better agronomic characteristics and disease resistance should then provide the basis for selection of exceptional quality materials with acceptable levels of productivity and agronomic characteristics.

#### Breeding and climate change?

Any guality coffee selection program has to take into account the fact that varieties selected from crosses made today will still be in commercial production thirty years hence. Consequently, breeders have to face the challenge of climate change and its likely impact on quality (Baker and Haggar, 2007; Laderach et al, 2009; Vinecky et al, 2010). When breeders address climate change they must integrate their efforts with those of the whole sector. There are various possible responses to climate change by coffee growers. One is to continue growing coffee at the current location, modify production practices and select varieties well-adapted to the new climatic conditions. An alternative is to move crops to regions or sites with climatic conditions similar to those where high quality coffee is currently produced. This latter option may be quite limited as increased temperatures would mean moving to higher altitudes in most regions, where potential areas for coffee production is reduced (see Chapter 1.3 climate change). Furthermore, temperatures are certain to rise in areas where coffee is currently grown and drought is also more likely. Consequently, it is likely that growers will increasingly require varieties of coffee that produce high quality coffee under warmer and dryer/wetter conditions. There is little doubt that climate change will alter rainfall patterns, but it is not clear whether the high quality coffee growing areas will be drier, wetter or the same in twenty or thirty years from now. This suggests that breeders will need to provide the coffee industry with varieties that are capable of producing high quality coffee (i) under conditions that may be up to 4°C warmer than the current conditions and (ii) that are well-adapted to a wider range of rainfall conditions. Breeders may assist the coffee industry in addressing these problems principally by (i) selecting varieties adapted to coffee growing systems designed to buffer variation in rainfall and warmer temperatures and (ii) selecting stress tolerant varieties that per se that are well-adapted to both drought and excess rainfall and warmer temperatures. The selection of varieties adapted to Coffee Agroforestry Systems, such as F1 hybrids, will likely lead to varieties adapted to systems that buffer the effects of climate change (Bertrand et al, 2011). The selection of abiotic stress tolerant varieties will rely on identifying abiotic stress tolerant germplasm and selection in extensive multilocation trials.

While basic Genotype x Environment interaction (GxE) studies have been neglected in the era of molecular biology, practical varietal improvement for specific conditions is likely to be most effectively achieved by selection under conditions that are as similar as possible to those encountered in commercial plantations. This means selecting a series of sites for trials that encompass the range of conditions to be experienced in the future by high quality coffee

arowers. This suggests that GxE trials are an efficient tool to address issues related to climate change (Braun et al, 2010). The few studies that explicitly explore GxE for coffee quality conclude that the best variety in one environment remains the best in others even if the overall quality varies with environments (Moschetto et al, 1996; Bertrand et al, 2006b), and that although there may be some statistically significant interactions for chemical compounds, they are small and probably of little commercial significance (Villarreal et al, 2008). This mirrors common empirical knowledge in the coffee production sector. Air temperature (correlated with altitude) is one of the main factors influencing green coffee composition: an increase of temperature due to climate change would damage quality if no action is taken to mitigate the effect (Joët et al, 2010). A direct consequence of the reduction in quality at higher temperatures is the need to carefully interpret the results of GxE trials. Lack of a G x E interaction does not necessarily mean that all is well. The fact that there is no G x E interaction for temperature and coffee quality may simply indicate that all varieties react in a similar manner to higher temperatures and that at higher temperatures quality is not as good as at lower temperatures. If this is the case, then the lack of a G x E interaction indicates that none of the varieties are well-adapted (in terms of quality) to higher temperatures. However, those varieties that produce better quality coffee under today's conditions will also produce better quality under the conditions of the future. Thus, just to maintain quality under higher temperature conditions in the future, in the absence of G x E, new improved quality varieties will be needed. Thus, to combat rising temperature and its negative effects on coffee quality breeding for quality under today's climate may provide varieties with better quality under the likely conditions of the future.

To develop improved quality varieties adapted to warmer conditions and varied rainfall patterns, early greenhouse screening of different varieties submitted to varying water regimes and temperatures may provide data similar to that obtained from multilocation trials at a much lower cost. Similarly, the use of controlled water regimes through sophisticated irrigation systems in one site in an arid region may provide variation in water regimes similar to that obtained from a large number of multilocation trials. Marraccini *et al* (2011) used both types of experiments (controlled stress in greenhouse and in irrigated fields) to evaluate drought resistant *C. canephora* and *C. arabica* accessions. Single site trials with controlled variation in stress should discriminate between tolerant and susceptible varieties: this view is supported by single site trials that revealed massive variation in drought resistance of *C. canephora* (Montagnon and Leroy, 1993).

Identifying abiotic stress tolerant germplasm might be achieved through a genetic background that is less sensitive to environmental variation. Heterosis in plants is generally associated with a more stable, homeostatic response to changes in the environment (Gallais 2009). Bertrand *et al* (2006b) confirmed that the lipid content of F1 hybrid varieties is less influenced by environmental changes than that of traditional varieties. Furthemore, Bertrand *et al* (2011) showed

that the superiority of F1 hybrids over traditional varieties was even greater at lower altitudes with higher temperatures; thus, the higher the temperatures, the greater the advantage of F1 hybrids. On the other hand, Marraccini *et al* (2011) suggested that introgressed Arabica varieties are more tolerant to drought.

In their efforts to produce varieties well-adapted to the changing environment, breeders will also draw on basic physiological and molecular studies that identify markers for tolerance of variable rainfall patterns and warmer conditions. Identifying molecular mechanisms involved in abiotic stress tolerance will shed light on the possible metabolic connections with quality pathways. Recent pioneering work is paving the way for this new research area (Bardil *et al*, 2011) at the *C. arabica* genome level and at the Rubisco level (Marranccini *et al*, 2011).

## THE ROLE OF THE PRIVATE SECTOR IN COFFEE BREEDING

## The coffee industry as an active player in coffee breeding

The coffee industry should play an important role in coffee breeding. This does not mean that every roasting company should have a breeding department, but the coffee industry should provide guidelines to the breeding sector on what types of varieties are needed and also provide financial support, either bilaterally or multilaterally<sup>3</sup>. This might take the form of either a common industry platform fostering breeding projects or bilateral agreements whereby a given company specifically requests that a breeding company provides a variety with a given set of characteristics. Knowing the characteristics of the desired end product (the ideotype) is essential to breeders. If they are not provided with guidelines, they are likely to be either very conservative or follow their own whims, which may not be in accordance with the needs of the growers and roasters. The risk of not producing varieties with the desired traits is particularly serious in perennial crops like coffee, which take a long time to improve and then are maintained for long periods in the field.

## The specific issue of mass production and diffusion of varieties

Once new varieties have been selected, a system must exist to mass produce and distribute them to producers. This is a very important issue in the case of *C. arabica*, an autogamous plant for which hand-pollination for massive seed production is not economically viable. Until recently, only homozygous lines were produced in isolated seed gardens. With this system it not possible to

<sup>3</sup> It is worth mentioning here the Global Coffee Quality Research Initiative (www.gcqri.org) which is a coffee industry R&D initiative that, inter alia, fosters breeding and provides guidance to breeders

distribute F1 hybrid varieties or unfixed, but interesting, introgressed lines. Cirad and partners have developed a somatic embryogenesis method of clonal propagation adapted to coffee (Etienne, 2005) that was recently scaled up to an industrial level through a partnership between Cirad and the Ecom Group with a potential production of 5-10 millions embryos/year (Etienne *et al*, 2010). This has established the first system that offers heterozygous varieties of *C. arabica* on a worldwide basis, and provides growers with the chance to fully benefit from high quality genetically improved plants (Menendez-Yuffa *et al*, 2010a&b).

It is unlikely that such activities need to be replicated. The main private players will not necessarily be from the coffee sector, they may also be seed companies that extend their activities to coffee.

## CONCLUSION

Although growers have selected for quality for more quality for more than one thousand years, modern selective breeding methods have only been applied to coffee in the past two decades. Breeders still face many challenges in their efforts to improve quality. A major impediment to their efforts is the lack of sound well-established selection criteria for high quality coffee varieties. The coffee community simply cannot ignore this knowledge gap and should invest time and money for an ambitious research program to develop criteria and methodologies for selection. The Specialty Coffee Industry, aware of the risk of doing nothing, should support the efforts through private-public partnerships. Furthermore, the coffee industry must face the challenge of meeting the need for an increased volume of high quality coffee not only under present conditions but also under the varied scenarios presented by the very real threat of climate change.

The challenges are great but the opportunities are also immense: through the exploration of key metabolic pathways, unused genetic diversity, powerful techniques of multiplication, and involvement of the private sector in breeding and varietial dissemination. At the launch of the Global Coffee Quality Research Initiative, it was clearly stated that "the cost of doing nothing in R&D is just too high".

The future of coffee breeding for productivity and quality in coffee producing countries depends on the emergence of public-private partnerships where private companies invest in innovation that is likely to be based on findings from public research (see Etienne *et al.*, 2010; Bertrand *et al.*, 2011). Indeed, without investment by the private sector, progress in breeding new coffee varieties is unlikely to proceed rapidly. Coffee producers desperately need a professional private-public sector partnership dedicated to the development of a true catalog of new varieties.

#### REFERENCES

- Akaffou DS, Ky CL, Barre P, Hamon P, Louarn J, Noirot M (2003) Identification and mapping of a major gene (Ft1) involved in fructification time in the interspecific cross *Coffea pseudozanguebariae* × *C. liberica* var. Dewevrei: impact on caffeine content and seed weight. Theoretical and Applied Genetics 106:1486–1490.
- Alemayehu T. N., Pétiard V., Broun P. and Crouzillat, 2010. Molecular genetic diversity of Arabica coffee (*Coffea arabica* L.) Using SSR Markers. ASIC conference, Bali (IND) Oct 2010, to be published.
- Anthony F., Combes M. C., Astorga C., Bertrand B., Graziosi G. and P. Lashermes, 2002. The origin of cultivated *Coffea arabica* L. varieties revealed by AFLP and SSR markers. Theor Appl Genet, 104:894–900.
- **Ashihara H,** Zheng XQ, Katahira R, Morimoto M, Ogita S, Sano H., 2006. Caffeine biosynthesis and adenine metabolism in transgenic *Coffea canephora* plants with reduced expression of N-methyltransferase genes. Phytochem; 67: 882-886.
- **Avelino, J.,** Barboza, B., Araya, J. C., Fonseca, C., Davrieux, F., Guyot, B., *et al*, 2005. Effects of slope exposure, altitude and yield on coffee quality in two altitude terroirs of Costa Rica, Orosi and Santa Maria de Dota. Journal of the Science of Food and Agriculture, 85: 1869–1876.
- **Baker, P.** and J. Haggar, 2007. Global warming: The impact on Global Coffee. SCAA conference, Long beach: http://web.catie.ac.cr/congreso/jeremy/Global\_Warming.pdf
- Bardil A, Dantas de Almeida J, Combes MC, Lashermes P, and B Bertrand, 2011. Genomic expression dominance in the natural allopolyploid Coffea arabica is massively affected by growth temperature. New Phytologist, in press." [20100720]. http://www.intl-pag.org/18/abstracts/W20\_PAGXVIII\_157.html
- Barre P, Akaffou S, Louarn J, Charrier A, Hamon S, Noirot M (1998) Inheritance of caffeine and heteroside contents in an interspecific cross between a cultivated coffee species *Coffea liberica* var *dewevrei* and a wild species caffeine-free *C. pseudozanguebariae*. Theor Appl Genet 96:306–311.
- **Berthaud**, J and Charrier A., 1998. Genetic Resources of Coffea. pp. 1-42. In: R. J. Clarke and R. MacRae (eds). Coffee Vol. 4 Agronomy, Elsevier Appl. Sci., London and New York.
- **Bertrand B.,** Guyot B., Anthony F., Lashermes P.2003. Impact of *Coffea canephora* gene introgression on beverage quality of *C. arabica*. Theor. Appl. Genet. 107:387-394.
- **Bertrand B,** Etienne H, Cilas C, Charrier A, Baradat P (2005). *Coffea arabica* hybrid performance for yield, fertility and bean weight. Euphytica 141:255-262.
- **Bertrand B.,** Etienne H., Davrieux F. and B. Guyot, 2006a. Central America and the Caribbeans: varietal improvement for quality. In : Montagnon Christophe (ed.). Coffee: terroirs and qualities. Versailles : Ed. Quae, p. 147-162.
- **Bertrand B.**, Vaast P., Alpizar E., Etienne H., Davrieux F., Charmetant P., 2006b Comparison of bean biochemical composition and beverage quality of arabica hybrids involving Sudanese-Ethiopian origins with traditional varieties at various elevations in Central America. *Tree physiology* 26 : 1239-1248.
- Bertrand B., Alpizar E., Llara L., SantaCreo R., Hidalgo M., Quijano J.M., Charmetant P., Montagnon C., Georget F. and H. Etienne, 2011. Performance of *Coffea arabica* F1 hybrids in comparison with American pure line varieties. Euphytica: DOI 10.1007/ s10681-011-0372-7
- **Bosselmann,** A.S., Dons, K., Oberthür, T., Smith Olsen, C., Ræbild, A.and H. Usma. 2009. The influence of shade trees on coffee quality in small holder coffee agroforestry systems in Southern Colombia. *Agriculture, Ecosystems & Environment*, Vol. 129: 253-260,

- Braun, H.J., G. Atlin and T. Payne, 2010. Multi-location testing as a tool to identify plant response to global climate change. In: Reynolds M.P. (Ed), Climate Change & Crop Production. CABI, Cambridge (MA, USA): 263-284.
- Bridson D, Verdcourt B. 1988. Coffea. In: Polhill RM, ed.Flora of tropical East Africa (part 2). Rotterdam: A.A.Balkema, 703-723.
- **Budzinski IGF,** Santos TB, Será T, Pot D, Vieira LGE, Pereira LFP (2011). Expression patterns of three a-expansin isoforms in *Coffea arabica* during fruit development. Plant Biology 13: 462-471.
- **Bustamante-Porras J.,** C. Campa, V. Poncet, M. Noirot, T. Leroy, S. Hamon and A. de Kochko, 2006. Molecular characterization of an ethylene receptor gene (CcETR1) in coffee trees, its relationship with fruit development and caffeine content. molecular genetics and genomics, 277, : 701-712.
- **Bytof,** G., Knopp, S. E., Schieberle, P., Teutsch, I., and D. Selmar, D., 2005. Influence of processing on the generation of gamma-aminobutyric acid in green coffee beans. European Food Research and Technology, 220: 245–250.
- **Bytof,** G., Knopp, S. E., Kramer, D., Breitenstein, B., Bergervoet, J. H. W., Groot, S. P. C., *et al.*, 2007. Transient occurrence of seed germination processes during coffee postharvest treatment. Annals of Botany, 100: 61–66.
- **Cantergiani,** E., Brevard, H., Amado, R., Krebs, Y., Feria-Morales, A., & Yeretzian, C. (1999). Characterisation of mouldy/earthy defect in green mexican coffee. In proceedings of the 18th ASIC Conference, ASIC :43–49.
- **Cubry P.,** Musoli C.P., Legnaté H., Pot D., De Bellis F., Poncet V., Anthony F., Dufour M., Leroy T.. 2008. Diversity in coffee assessed with SSR markers: Structure of the genus Coffea and perspectives for breeding. Genome, 51 : 50-63.
- **Daviron B.** and Ponte S., 2005. The Coffee Paradox: Global Markets, Commodity Trade and the Elusive Promise of Development. Londres, Zed Books. 288 p.
- **De Castro,** R. D. and Marraccini, P. (2006). Cytology, biochemistry and molecular changes during coffee fruit development. Brazilian Journal of Plant Physiology, 18:175–199.
- **Decazy, F.,** Avelino, J., Guyot, B., Perriot, J. J., Pineda, C., and C. Cilas, 2003. Quality of different Honduran coffees in relation to several environments. Journal of Food Science, 68: 2356–2361.
- **Dettmer K,** Aronov PA, and B.D. Hammock, 2007. Mass spectrometry-based metabolomics. Mass Spectrometry Reviews, 26:51–78.
- Etienne H., 2005. Protocol of somatic embryogenesis: Coffee (*Coffea arabica* L. and *C. canephora* P.). In: Protocols for somatic embryogenesis in woody plants. Series: Forestry Sci Vol. 77, Jain SM, Gupta PK (Eds). Springer, the Netherlands. ISBN: 1-4020-2984-5, pp. 167-179.
- Etienne H., Bertrand B., Ribas A., Lashermes P., Malo E., Montagnon C. Alpizar E., Bobadilla R., Simpson J., Dechamp E., Jourdan I., Georget F. 2010. Current application of coffee (*Coffea arabica*) somatic embryogenesis for industrial propagation of elite heterozygous materials in Central America and Mexico and for routine functional genomics : [Abstract]. In : IUFRO Working Party 2.09.02 : Somatic Embbryogenesis of Forest Trees Conferences, August 19-21, 2010, Suwon, Korea. Advances in somatic embryogenesis of trees and its application for the future forests and plantations . s.l. : s.n., p. 54-55. IUFRO Working Party 2.09.02 : Somatic Embryogenesis of Forest Tree Conference, 2010-08-19/2010-08-21, Suwon, Corée.
- **Farah A.,** Monteiro M.C., Calado V., Franca A.S. and L.C. Trugo, 2006. Correlation between cup quality and chemical attributes of Brazilian coffee. Food Chemistry 98 : 373–380.
- Fazuoli L.C., Carvalho A., Monaco L.C., Texeira A.A., 1977. Qualidade da bebida do café ICATU. Bragantia 36:165-172.

- **Fernandez-Cornejo J.**, 2004. The Seed Industry in U.S. Agriculture: An Exploration of Data and Information on Crop Seed Markets, Regulation, Industry Structure, and Research and Development. Resource Economics Division, Economic Research Service, U.S. Department of Agriculture. Agriculture Information Bulletin Number 786.
- **Figueiredo SA, Lashermes P,** Aragão FJL (2011). Molecular characterization and functional analysis of the  $\beta$ -galactosidase gene during *Coffea arabica* (L.) fruit development. Journal of Experimental Botany, DOI:10.1093/jxb/erq440
- Flament, I. (2002). Coffee flavour chemistry. Chichester, UK: John Wiley and Sons.
- **Gallais,** A., 2009. Hétérosis et varieties hybrids en amelioration des plantes. Quae Edition (Versailles), 356 pp.
- **Gelpi, E.,** 2002. Interfaces for coupled liquid-phase separation/mass spectrometry techniques. An update on recent developments. Journal of Mass Spectrometry, 37 :241–253.
- Geromel C., Ferreira L.P., Cavalari A.A., Pereira L.F.P., Guerreiro S.M.C., Vieira L.G.E., Leroy T., Pot D., Mazzafera P., Marraccini P. (2006). Biochemical and genomic analysis of sucrose metabolism during coffee (*Coffea arabica*) fruit development. J Exp Bot 57: 3243-3258.
- Geromel C., Ferreira L.P., Davrieux F., Guyot B., Ribeyre F., dos Santos Scholz M.B., Pereira L.F.P., Vaast P., Pot D., Leroy T., Androcioli Filho A., Vieira L.G.E., Mazzafera P., Marraccini P. (2008). Effects of shade on the development and sugar metabolism of coffee (*Coffea arabica* L.) fruits. *Plant Physiol. Biochem.* 46: 569-579.
- **Giovannucci,** D., Liu, P. and Byers, A., 2008. Adding Value: Certified Coffee Trade in North America. In Pascal Liu (Ed.) Value-adding Standards in the North American Food Market – Trade Opportunities in Certified Products for Developing Countries. FAO. Rome.
- **González-González M.,** Mayolo-Deloisa K., Rito-Palomares M. and R. Winkler, 2010. Colorimetric protein quantification in aqueous two-phase systems. Process Biochemistry, in press.
- **Gonzalez-Rios,** O., Suarez-Quiroz, M.L., Boulanger, R., Barel, M., Guyot, B., Guiraud, J.P. and S. Schorr-Galindo, 2007. Impact of "ecological" post-harvest processing on the volatile fraction of coffee beans: I. Green coffee. Journal of Food Composition and Analysis, 20: 289-296.
- **Grosch W.**, 2001. Chemistry III: Volatile compounds. In: Clarke R.J. and O.G. Vitzhum (Eds), Coffee: Recent developments, Blackwell Science, London: 68-89.
- Guyot, B., Cros, E., and J.C. Vincent, 1982. Caracterisation et identification des composes de la fraction volatile d'un cafe vert arabica sain et d'un cafe vert arabica puant. Café Cacao The, XXVI : 279–289.
- **Guyot, B.,** Gueule, D., Manez, J. C., Perriot, J. J., Giron, J., Villain L (1996). Influence de l'altitude et de l'ombrage sur la qualité des cafés Arabica. Plantations, Recherche, Développement, 3 : 272–280.
- **Hanson K.R.,** 1965. Chlorogenic acid biosynthesis. Chemical synthesis and properties of the mono-O-cinnamoylquinic acids. Biochemistry 4 : 2719-2730.
- Joët, T., Laffargue, A., Salmona, J., Doulbeau, S., Descroix, F., Bertrand, B., et al., 2009. Metabolic pathways in tropical dicotyledonous albuminous seeds: Coffea arabica as a case study. New Phytologist, 182: 146–162.
- Joët T, Laffargue A, Descroix F, Doulbeau S., Bertrand B., de kochko A. and S. Dussert, 2010. Influence of environmental factors, wet processing and their interactions on the biochemical composition of green Arabica coffee beans. Food Chem, 118 : 693-701.
- Joët T., Pot D., Ferreira L.P., Dussert S., Marraccini P. 2011. Génomique fonctionnelle du développement de la graine: une approche essentielle pour l'identification des déterminants moléculaires de la qualité du café. To be published in « Les Cahiers de l'Agriculture ».

- Kathurima C.W., Kenji G.M., Muhoho S.M., Boulanger R. and F. Davrieux F.. 2010. Discrimination of *Coffea arabica* hybrids of the composite cultivar ruiru 11 by sensorial evaluation and biochemical characterization. Advance journal of food science and technology, 2 (3): 148-154.
- **Knopp,** S., Bytof, G., and D. Selmar, 2006. Influence of processing on the content of sugars in green Arabica coffee beans. European Food Research and Technology, 223: 195–201.
- **Ky C.L.,** J. Louarn, B. Guyot, A. Charrier, S. Hamon and M. Noirot , 1999. Relations between and inheritance of chlorogenic acid content in an interspecific cross between Coffea pseudozanguebariae and Coffea liberica var 'dewevrei'. Theoretical and Applied Genetics 98 : 628–637.
- **Ky C.L.,** J. Louarn, S. Dussert, B. Guyot, S. Hamon and M. Noirot, 2001a. Caffeine, trigonelline, chlorogenic acids and sucrose diversity in wild *Coffea arabica* L. and *C. canephora* P. accessions. Food Chemistry, 75: 223-230.
- **Ky C.L.,** B. Guyot, J. Louarn, S. Hamon and M. Noirot, 2001b. Trigonelline inheritance in the interspecific cross between Coffea pseudozanguebariae × C. liberica var. 'dewevrei'. Theoretical and Applied Genetics 102 : 630–634.
- **Koshiro Y**, Jackson MC, Katahira R, Wang ML, Nagai C, Ashihara H. Biosynthesis of chlorogenic acids in growing and ripening fruits of *Coffea arabica* and *Coffea canephora* plants. Z Naturforsch C 2007; 62 : 731-742.
- **Koziel,** J.A., J. Noah, and J. Pawliszyn. 2001a. Field sampling and determination of formaldehyde in indoor air with solid phase microextraction and on-fiber derivatization. Environmental Science & Technology. 35, 1481-1486.
- Koziel, J.A., M. Odziemkowski, and J. Pawliszyn. 2001b. Sampling and analysis of airborne particulate matter and aerosols using in-needle trap and SPME fiber devices. Analytical Chemistry. 73, 47-53.
- Koziel, J.A., L. Cai, D. Wright, S. Hoff. 2006. Solid phase microextraction as a novel air sampling technology for improved, GC-Olfactometry-based, assessment of livestock odors. Journal of Chromatographic Science, 44(7), 451-457.
- Laderach, P., M. Lundy, A. Jarvis, R. Julián, E. Pérez Portilla and K. Schepp.2009. Predicted impact of climate change on coffee-supply chains. Climate change CIAT conference : 20 pp. http://www.uci.ac.cr/descargas/conferencias/Predicted-impact-of-climatechange-on-coffee-supply-chains.pdf
- Lashermes, P., Combes, M.C., Trouslot, P., and Charrier, A., 1997 Phylogenetic relationships of coffee tree species (*Coffea* L.) as inferred from ITS sequences of nuclear ribosomal DNA. Theor. Appl. Genet. 94, 947–955.
- Lashermes P., Combes M.C., Robert J., Trouslot P., D'Hont A. *et al*, 1999. Molecular characterization and origin of the allotetraploid *Coffea arabica* L. genome. Mol Gen Genet, 261:259-266.
- Lashermes, P., S. Andrzejewski, B. Bertrand, M. C. Combes, S. Dussert, G. Graziosi, P. Trouslot, and F. Anthony, 2000a. Molecular analysis of introgressive breeding in coffee (*Coffea arabica* L.). Theor. Appl. Genet. 100, 139–146.
- Lashermes P., Combes M.C., Topart P., Graziosi G., Bertrand B., Anthony F., 2000b. Molecular breeding in coffee (Coffee arabica L.). In: Sera T, Soccol CR, Pandey A, Roussos S (eds), Coffee biotechnology and quality, pp.101-112. Kluwer Academic, Dordrecht, The Netherlands.
- Lashermes P., Carvalho Andrade A., Etienne H.. 2008. Genomics of coffee, one of the world's largest traded commoditiesIn : Moore Paul H. (ed.), Ming Ray (ed.). Genomics of tropical crop plants. New York : Springer [Etats-Unis], p. 203-226.
- Lashermes P., Bertrand B., Etienne H.. 2009. Breeding coffee (*Coffea arabica*) for sustainable production. In : Jain Shri Mohan (ed.), Priyadarshan P.M. (ed.). Breeding plantation tree crops : tropical species. New York : Springer [Etats-Unis], p. 525-543.

- **Lepelley M**, Cheminade G, Tremillon N, *et al*. Chlorogenic acid synthesis in coffee: an analysis of CGA content and real-time RT-PCR expression of HCT, HQT, C3H1, and CCoAOMT1 genes during grain development in *C. canephora*. Plant Sci 2007; 172 : 978-996.
- **Leroy, T.,** Ribeyre, F., Bertrand, B., Charmetant, P., Dufour, M., Montagnon, C., *et al*, 2006. Genetics of coffee quality. Brazilian Journal of Plant Physiology, 18 :229-242.
- Leroy T., De Bellis B., Legnate H., Kanamura E., Gonzales G., Pereira L.F., Carvalho Andrade A., Charmetant P., Montagnon C., Cubry P., Marraccini P., Pot D., de Kochko. A. (2010). Improving quality of African Robustas: QTL for agronomic and quality related traits in *Coffea canephora*. Tree Genetics and Genomes. DOI 10.1007/s11295-011-0374-6
- Lindinger, C. Pollien, P., de Vos, R. C. H., Tikunov, Y., Hageman, J. A., Lambot, C., Fumeaux, R., Voirol-Baliguet, E. and I. Blank, 2009. Identification of Ethyl Formate as a Quality Marker of the Fermented Off-note in Coffee by a Nontargeted Chemometric Approach. Journal of Agricultural and Food Chemistry 57: 9972-9978.
- Mahé, L., V. M. P. Várzea, D. Le Pierrès, M.-C. Combes and P. Lashermes, 2007. A new source of resistance against coffee leaf rust from New-Caledonian natural interspecific hybrids between *Coffea arabica* and *Coffea canephora*. Plant Breeding, 126: 638-641.
- Mahesh V, Million-Rousseau R, Ullmann P, et al. 2007. Functional characterization of two p-coumaroyl ester 3'-hydroxylase genes from coffee tree: evidence of a candidate for chlorogenic acid biosynthesis. Plant Mol Biol, 64:145-159.
- Maluf MP, da Silva CC, de Oliveira MDA, Tavares AG, Silvarolla MB, Guerreiro O. Altered expression of the caffeine synthase gene in a naturally caffeine-free mutant of *Coffea arabica*. Genet Mol Biol 2009; 32 : 802-810.
- Mancha Agresti, P.M.C, Franca, A.S, Oliveira, L.S. and R.Augusti, 2008. Discrimination between defective and non-defective Brazilian coffee beans by their volatile profil. *Food Chemistry*, 106:787-796.
- **Marraccini P.,** Rogers W.J., Allard C., André M.-L., Caillet V., Lacoste N., Lausanne F., Michaux S. (2001). Molecular and biochemical characterization of endo-*b*-mannanases from germinating coffee (*Coffea arabica*) grains. *Planta* 213: 296-308.
- Marraccini P., Rogers W.J., Caillet V., Deshayes A., Granato D., Lausanne F., Lechat S., Pridmore D., Pétiard V. (2005). Biochemical and molecular characterization of *a*-Dgalactosidase from coffee beans. *Plant Physiol, Biochem.* 43: 909-920.
- Marraccini, P., Freire, L.P., Alves, G.S.C, Vieira, N.G., Vinecky, F., Elbelt, S., Ramos, H.J.O., Montagnon, C., Vieira, L.G.E., Leroy, T., Pot, D., Silva, V.A., Rodrigues, G.C. and A. C. Andrade, 2011. RBCS1 expression in coffee: *Coffea* orthologs, *Coffea arabica* homeologs, expression variability under drought stress and between genotypes. *BMC Plant Biology*, accepted for publication.
- Mateus M.L., Lindinger C., Gumy J.C.and R. Liardon, 2007. Release Kinetics of Volatile Organic Compounds from Roasted and Ground Coffee: Online Measurements by PTR-MS and Mathematical Modeling. J. Agric. Food Chem., 55 :10117–10128.
- Menendez-Yuffa A., Barry-Etienne D., Bertrand B., Georget F., Etienne H. 2010a. A comparative analysis of the development and quality of nursery plants derived from somatic embryogenesis and from seedlings for large-scale propagation of coffee (Coffea arabica L.). Plant cell, tissue and organ culture, 102 (3) : 297-307.
- Menendez-Yuffa A., Bertrand B., Georget F., Jourdan I., Lashermes P., Malo E., Montagnon C., Santoni S., Tollon C., Etienne H. 2010b. Microsatellite polymorphism in hybrids of Coffea arabica (L.) produced industrially by somatic embryogenesis : [Abstract]. In : IAPB. 12th IAPB Congress. Sustainability through agricultural biotechnology : food, biomatrerials, energy and environment, June 6-11, 2010, Saint Louis, Missouri, USA . s.l. : s.n., 1 p. IAPB Congress. 12, 2010-06-06/2010-06-11, Saint Louis, Etats-Unis.
- **Montagnon C.,** Leroy T. 1993. Response to drought of young *Coffea canephora* coffee trees from different genetic groups in the Côte d'Ivoire. *Café Cacao Thé*, 37 (3) : 179-190.

- **Montagnon C.,** Leroy T., Eskes A. 1998a. Varietal improvement of *Coffea canephora*. 1: criteria and breeding methods. Plantations, Recherche, Développement, 5 (1) : 18-33.
- Montagnon C., Leroy T., Eskes A. 1998b. Varietal improvement of *Coffea canephora*. 2: breeding programmes and their results Plantations, Recherche, Développement, 5 (2) : 89-98.
- **Montagnon C.,** Cubry P. and T. Leroy. Breeding and varietal creation of *C. canephora*. Les cahiers de l'Agriculture. To be published in 2011.
- Moreno G, Moreno E and G. Cadena, (1995) Bean characteristics and cup quality of the Colombia variety (*Coffea arabica*) as judged by international tasting panels. In: 16th International Scientific Colloquium on Coffee. Kyoto, pp.574-583.
- **Moschetto D.,** Montagnon C., Guyot B., Perriot J.J., Leroy T., Eskes A. 1996. Studies on the effect of genotype on cup quality of *Coffea canephora*.Tropical Science, 36 : 18-31.
- **Murray,** J.M., C.M. Delahunty and I.A. Baxter, 2001. Descriptive sensory analysis: past, present and future. Food Research International, 34:461-471.
- **Muschler,** R.G., 2001. Shade improves coffee quality in a sub-optimal coffee-zone of Costa Rica. Agroforestry Systems 85: 131–139.
- **Ogawa M,** Herai Y, Koizumi N, Kusano T, and H. Sano, 2001. 7-Methylxanthine methyltransferase of coffee plants. Gene isolation and enzymatic properties. J Biol Chem; 276 : 8213-8218.
- **Ogita S,** Uefuji H, Yamaguchi Y, Koizumi N, Sano H. Producing decaffeinated coffee plants. Nature 2003; 423 : 823.
- **Ogita S,** Uefuji H, Morimoto M, Sano H. Application of RNAi to confirm theobromine as the major intermediate for caffeine biosynthesis in coffee plants with potential for construction of decaffeinated varieties. Plant Mol Biol 2004; 54 : 931-941.
- O'Mahony, M. and Odbert, N., 1985. A Comparison of Sensory Difference Testing Procedures: Sequential Sensitivity Analysis and Aspects of Taste Adaptation. Food Science 50:1055-1058.
- **O'Mahony,** M. and L.R. Goldstein, 1986. Effectiveness of Sensory Difference Tests: Sequential Sensitivity Analysis for Liquid Food Stimuli. Food Science 51:1550-1553.
- **Owuor J.B.**, 1988. An assessment of the cup quality of the new disease resistant *Coffea arabica* cultivar RUIRU 11 in Kenya. Kenya Coffee , 53:333-336.
- **Perriot J.J.**, Ribeyre F. and Montagnon C. 2006. The qualities of a coffee. In : Montagnon Christophe (ed.). Coffee: terroirs and qualities. Versailles : Ed. Quae, p. 11-20.
- Pré M, Caillet V, Sobilo J, McCarthy J., 2008. Characterization and expression analysis of genes directing galactomannan synthesis in coffee. Ann Bot 102 : 207-220.
- Privat I, Foucrier S, Prins A, et al. Differential regulation of grain sucrose accumulation and metabolism in Coffea arabica (Arabica) and Coffea canephora (Robusta) revealed through gene expression and enzyme activity analysis. New Phytol 2008; 178 : 781-797.
- **Puerta G.I.,** 2000. Calidad en taza de algunas mezclas de variedades de café de la especie *Coffea arabica L. Cenicafé* 51:5-19.
- **Ribeiro,** J.S., Augusto F., Salva T.J., Thomaziello G R.A and M.M.C. Ferreira, 2009. Prediction of sensory properties of Brazilian Arabica roasted coffees by headspace solid phase microextraction-gas chromatography and partial least squares. Analytica Chimica Acta, 634 : 172–179.
- **Ribeiro J.S.,** M.M.C. Ferreira and T.J.G. Salva, 2010. Chemometric models for the quantitative descriptive sensory analysis of Arabica coffee beverages using near infrared spectroscopy. Talanta, in press.

- **Salmona J,** Dussert S, Descroix F, de Kochko A, Bertrand B, Joët T. Deciphering transcriptional networks that govern *Coffea arabica* seed development using combined cDNA array and real-time RT-PCR approaches. Plant Mol Biol 2008; 66: 105-124.
- SCAA Specialty Coffee Association of America, 2009. SCAA Protocols | Cupping Specialty Coffee.http://www.scaa.org/PDF/PR%20-%20CUPPING%20PROTOCOLS%20 V.21NOV2009A.pdf.
- Scheidig,C. Czerny,M. and P. Schieberle, 2007. Changes in Key Odorants of Raw Coffee Beans during Storage under Defined Conditions. J. Agric. Food Chem., 55: 5768–5775
- **Selmar,** D., Bytof, G.,and S.Knopp, 2002. New aspects of coffee processing: The relation between seed germination and coffee quality. In Proceedings of the international congress of ASIC, p. 19.
- Selmar, D., Bytof, G., Knopp, S. E. and B. Breitenstein, 2006. Germination of coffee seeds and its significance for coffee quality. Plant Biology, 8: 260–264.
- Silvarolla MB, Mazzafera P; Fazuoli LC. A naturally decaffeinated arabica coffee. Nature 2004; 429 : 826.
- Simkin A.J., T. Qian, V. Caillet, F. Michoux, M. Ben Amor, C. Lin, S. Tanksley and J. McCarthy, 2006. Oleosin gene family of *Coffea canephora*: Quantitative expression analysis of five oleosin genes in developing and germinating coffee grain. Journal of Plant Physiology, 163: 691-708.
- Simkin A.J., M. Kuntz, H. Moreau and J. McCarthy, 2008. Carotenoid profiling and the expression of carotenoid biosynthetic genes in developing coffee grain. Journal of Plant Physiology 165: 1087-1106
- Toci, A.T and A. Farah, 2008. Volatile compounds as potential defective coffee beans' markers. Food Chemistry, 108 : 1133–1141
- **Tranchida P.Q.,** Purcaro G., Conte P., Dugo G. and L. Mondello, 2009. Enhanced resolution comprehensive two-dimensional gas chromatography applied to the analysis of roasted coffee volatiles. Journal of Chromatography A, 43 :7301-7306.
- **Uefuji H**, Ogita S, Yamaguchi Y, Koizumi N, Sano H., 2003. Molecular cloning and functional characterization of three distinct N-methyltransferases involved in the caffeine biosynthetic pathway in coffee plants. Plant Physiol; 132 : 372-380.
- Vaast P., B. Bertrand, J.J. Perriot, B. Guyot and M. Génard, 2006. Fruit thinning and shade improve bean characteristics and beverage quality of coffee (*Coffea arabica* L.) under optimal conditions. J Sci Food Agric 86:197–204.
- **Van der Vossen,** H.A.M, 2009. The cup-quality of disease-resistant cultivars of Arabica coffee (*Coffea arabica*). Experimental Agriculture. 45: 323-332.
- Villarreal D., Laffargue A., Posada H., Bertrand B., Lashermes P., Dussert S. 2009. Genotypic and environmental effects on coffee (*Coffea arabica* L.) bean fatty acid profile: Impact on variety and origin chemometric determination. *Journal of agricultural and food chemistry*, 57 (23) : 11321-11327.
- Vinecky, F., Davrieux, F., Alves, G.C.S., Mera, A.C., Leroy, T., Bonnot, F., Pot, T., Rocha, O.C., Guerra, A.F., Rodrigues, G.C., Marraccini, P. and A.C. Andrade. Effects of water stress on bean biochemical composition of Coffea arabica cv. Rubi. In: The 23rd International Conference on Coffee Science. Bali - Indonesia. October 3rd - 8th, 2010, to be published.
- Wishart, D.S., 2008. Metabolomics: applications to food science and nutrition research. Trends in Food Science & Technology, 19 : 482-493.