The Drop of Beverage Quality Caused by *Coffea canephora* Gene Introgression Can be Avoided by Selection

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SUMMARY

The introgression of *Coffea canephora* genes in *C. arabica* is suspected of causing a drop in beverage quality. Coffee samples from lines introgressed by *C. canephora* via the Timor Hybrid, and well-known for their resistance to coffee leaf rust (*Hemileia vastatrix*) and to the nematode *Meloidogyne exigua* were studied for beverage quality, chemical composition and amount of introgressed genetic material. Chemical analyses (caffeine, chlorogenic acids, fat, trigonelline, sucrose) were carried out. The amount of AFLP markers introgressed from the Timor Hybrid varied from 1 to 37 for the studied lines. In a first study the introgressed cv. CR95, was compared to the traditional non-introgressed cv. Caturra in 13 locations. In a second study, the cv. 'Veranero', was compared to Caturra in 14 contrasted locations. Finally, 15 introgressed pure lines were compared with the tradional cv. Caturra, Villa-Sarchi and Catuai. The cv. CR95 revealed a lower beverage quality to Caturra and a lower content of fat. In the second study, there were significant differences between lines for all the biochemical compounds analysed and for the acidity and the overall standard of the beverage. Two lines (T17927, T17924) were significantly poorer than the controls for sucrose and beverage acidity. T17924 also had more chlorogenic acids and was poorer for the overall standard. However, two highly introgressed lines, T17934 and T17931, (25 and 30 AFLP markers respectively) did not differ from the non-introgressed controls. There were no correlations between the amount of AFLP markers and the chemical contents or beverage attributes. It was concluded that it should be possible to find lines with resistance genes and good beverage quality. Selection can avoid accompanying the introgression of resistance genes with a drop in beverage quality.

INTRODUCTION

Two types of coffee are consumed worldwide, Robusta (*Coffea canephora* Pierre) and Arabica (*C. arabica* L.). Robusta coffee has been characterized as a neutral coffee, weak-flavoured, and occasionally strong and pronounced bitterness (Charrier and Berthaud, 1985). Arabica fetches a higher price as it makes a milder, fruitier and acidulous beverage. The species *C. arabica* ($2n = 4x = 44$) is an allotetraploid containing two genomes that originated from two different diploid wild ancestors, *C. canephora* and *C. eugenioides* Moore (Lashermes et al., 1999). The species is characterized by low genetic diversity (Lashermes et al., 1996), which is attributable to its reproductive biology and evolution. Among other things, the low variability is reflected in its susceptibility to most diseases (Bertrand et al., 1999). In contrast, *C. canephora* is a diploid species ($2n = 2x = 22$) with considerable variability (Charrier and Berthaud, 1985; Lashermes et al., 1999). Since the second half of the 20th century, most breeding programmes implemented throughout the world (Brazil, Colombia, Kenya, Ethiopia, Costa Rica, Honduras) have transferred resistance to rust (*Hemileia vastatrix* Berk. and Br.), root-knot nematodes *(Meloidogyne* sp.) and Coffee Berry Disease (*Colletotrichum kahawae* sensu Hindorf) from the Timor Hybrid to cultivars of *C. arabica*.

The original Timor Hybrid from the island of Timor (Bettencourt, 1973) is derived from a wild interspecific cross between *C. arabica* and *C. canephora*. The Timor Hybrid has been crossed with commercial varieties such as 'Caturra' or 'Villa-Sarchi'. The F_1 hybrid has been selfed and a plant breeding programme, based on pedigree selection (Carvalho et al. 1989), has been carried out for five to eight generations. Based on this strategy, several cultivars generally known as 'Catimors' or 'Sarchimors' have been released in Brazil ('IAPAR 59', 'TUPI', 'OBATA'), Colombia ('Colombia') or Central America ('IHCAFE 90', 'Costa Rica 95' or 'T5175'). These varieties are resistant to most known races of rust and therefore produce around 20% more than traditional varieties. It has been estimated that several hundred thousand hectares have been planted with these new varieties. Given this success, it can be expected that breeding of the Arabica species for resistance to pests and diseases will be based for some time on crosses derived from the Timor Hybrid. Lashermes et al. (2000a), using AFLP (Amplified Fragment Length Polymorphism) markers recently estimated that the approximate amounts of introgressed materials in many introgressed Arabica lines ranged from 8% to 27% of the *C. canephora* genome. The amount of alien genetic material is therefore substantial. It thus seems likely that the introgression process has not been restricted to resistance traits but could also involve undesirable genes. For example, Herrington et al. (1983) discovered that introgression can be a source of bitterness in watermelon (*Citrullus lanatus*). As Robusta does not have such a good beverage quality (BQ) as Arabica, it is reasonable to wonder whether introgression might have a negative impact on BQ. In addition, the defence exhibited by plants against pathogens depends to a large extent on chemical compounds (Agrios 1997), which might interfere with end-use quality. Ky et al. (1999) suggested that coffee species like Robusta, producing more chlorogenic acids (12-13% *vs* 7-8% for Arabica), are well protected against many pathogens but of poor BQ. Guerrero et al. (2001) were able to discriminate the two *Coffea* species, using quantitative and qualitative differences of chlorogenics acids. The caffeine content is higher in the *C. canephora* beans (2-4%) than in the *C. arabica* beans (0.8-1.7%), and fat, sucrose and trigonelline are lower (Clifford, 1985). Based on organoleptic evaluation and using scientific procedures, introgressed lines of Arabica were found to produce good BQ, that was similar to the non-introgressed standard (Fazuoli et al., 1977; Castillo, 1990; Moreno et al., 1995; Puerta, 1998; Puerta, 2000; Owuor, 1988). However, most coffee buyers claim that new introgressed varieties have a poorer BQ than the 'Caturra' standard. In this study, by linking the amount of alien genetic materiel as estimated by AFLP analysis in Timor Hybrid-derived lines with beverage quality and the chemical compositions of beans, we attempted to adress this question, which has crucial implications for genetic improvement of the species.

MATERIALS AND METHODS

Plant material

Experiment 1

The cv 'CR95' (see Table 1), was compared to cv 'Caturra'. Samples of the two cultivars coming from 13 contrasted locations were compared.

Experiment 2

The cv 'Veranero' (see Table 1), was compared to cv 'Caturra'. Samples of the two cultivars coming from 14 contrasted locations were compared. The cv 'Veranero' is an introgressed cultivar derived from the cross between Typica and *C. canephora* (Anthony et al., 2002).

Table 1. Description of plant material. Numbers of AFLP markers attributable to introgression detected in 22 introgression lines and a non-introgressed cultivar, cv Caturra. Resistance (R) and susceptibility (S) to leaf rust *Hemileia vastatrix* (race II) and to root-knot nematode *Meloidogyne exigua* (population of Costa Rica) are from Bertrand et al. (1997) and Bertrand et al. (2001), respectively.

Line	Description	Origin	Introgression markers	Reaction to Leaf rust	Reaction to nematode
T17924	Introgressed cultivar « Colombia » (CIFC1343)	Colombia	32	R	R
T17925	Introgressed cultivar « Colombia » (CIFC1343)	Colombia	14	R	R
T17926	Introgressed cultivar « Colombia » (CIFC1343)	Colombia	28	R	R
T17927	Introgressed cultivar « Colombia » (CIFC1343)	Colombia	30	R	R
T17928	Introgressed cultivar « Colombia » (CIFC1343)	Colombia	26	R	S
T17929	Introgressed cultivar « Colombia » (CIFC1343)	Colombia	1	S	S
T17930	Introgressed cultivar « Colombia » (CIFC1343)	Colombia	14	R	R
T17931	Introgressed cultivar « Colombia » (CIFC1343)	Colombia	30	R	R
T17933	Introgressed cultivar « Colombia » (CIFC1343)	Colombia	16	R	R
T17934	Introgressed cultivar « Colombia » (CIFC1343)	Colombia	25	R	R
T17935	Introgressed cultivar « Colombia » (CIFC1343)	Colombia	16	R	R
T17936	Introgressed cultivar « Colombia » (CIFC1343)	Colombia	37	R	R
T17937	Introgressed cultivar « Colombia » (CIFC1343)	Colombia	33	R	R
T17938	Introgressed cultivar « Colombia » (CIFC1343)	Colombia	10	R	R
T17940	Introgressed cultivar « Colombia » (CIFC1343)	Colombia	19	R	R
CV 'Veranero'	Introgressed cultivar	Costa Rica	?	S	S
cv CR95	Introgressed cultivar (CIFC 832/1)	Costa Rica	12	R	S
cv Caturra	Non-introgressed cultivar	Costa Rica	0	S	S
cv Catuai	Non-introgressed cultivar	Costa Rica	0	S	S
cv Villa-Sarchi	Non-introgressed cultivar	Costa Rica	0	S	S

Experiment 3

15 introgressed Arabica lines (from generation F_4 and onwards) derived from different progenies of the Timor Hybrid (i.e. CIFC 832/1, CIFC 832/2, CIFC 1343) and three non-introgressed commercial cultivars, cv Caturra, cv Catuai, cv Villa-Sarchi (Table 1) formed the plant material. The samples came from a trial set up at the ICAFE research centre in Costa Rica in 1998, located in Heredia at 1,200 m above sea level on an andosol type soil. In each of the four replicates of the trial, the plots consisted of 10 trees (i.e. 40 trees per line).

Samples taken for organoleptic and chemical analysis

Composite samples were taken from plots, using ripe, healthy cherries harvested from the upper branches of the trees during the harvest peak. Two kg of coffee cherries were subjected

to the wet process (pulping, fermentation and drying) to obtain 1 kg of green coffee beans. The samples of green coffee were screened through a size 17 sieve and the most defective beans were eliminated. In experiment 1 and 2, samples were submitted for organoletpic analysis in 2000. In experiment 3, samples harvested in 1998 (Y1) and 1999 (Y2) were submitted for organoleptic analysis (see below). The samples harvested in Y1 and Y3 (2000) were submitted for chemical analysis (see below). For the Y3 harvest, 15 out of 18 lines were harvested, the three other lines (T17936, T17937 and T17938) did not produce a sufficient quantity for harvest.

Organoleptic analysis

After roasting for 6-7 min, BQ tests were carried out on an infusion (120 ml) prepared from 12 g of roasted coffee. In experiment 1 and 2 a panel od 16 evaluators compared the two cultivars for each location (repsctiveley CR95 vs Caturra and Veranero vs Caturra) following a triangular test. In experiment 3, a panel of eight evaluators tasted 120 ml of infusion following a quantitative test. The major taste and flavour attributes, aroma, body (i.e. strength), acidity were scored using scales ranging from 0 to 5 where 0 = nil, 1 = very light, 2 = light, 3 = medium, 4 = strong and 5 = very strong. There was also an overall standard for liquor quality based on the above attributes and ranging from 0 to 5 where 0 = unacceptable, 1 = bad, 2 = regular, 3 = good, 4 = very good, 5 = excellent.

Chemical analysis

The analyses were performed by near infrared spectrometry by reflectance (Williams and Norris 1990) of green coffee (50 g) after grinding (ground to < 0.5 mm) using a NIR spectrometer system (model 6500, by NIRSystem, Inc. 1201 Tech Road Silver Spring, Md 20904) driven by NIRS2 (4.0) software (Intrasoft Intl., LLC, RD109, Sellers Lane, Port Matilda, Pa 16870). For the Y1 and Y3 samples from Trial 1 (experiment 2) and for all samples from experiment 1, a NIR spectrum (NIRS) was acquired in reflectance (R) mode in the 1104-2456 nm range (Downey et al., 1994; Downey and Boussion, 1996; Scanlon et al., 1999). Using specific calibrations (Guyot et al., 1988; Guyot, 1993), it is possible to determine the caffeine, trigonelline, fat and sucrose contents. These contents were determined for all samples (experiment 1) and for Y1 samples (experiment 2). For the Y3 samples, a NIR spectrum was acquired in reflectance (R) mode at intervals of 26 nm for a total of 52 data points. The reflectance, expressed as log (1/R) values, gave a characteristic signature for each sample.

AFLP protocol

Leaf samples were taken from 20 trees representing 17 introgressed lines and three non-introgressed lines as controls (cv Caturra, cv Catuai, cv Villa Sarchi) (Table 1). The AFLP protocol described by Vos et al. (1995) was basically followed with minor modifications to suit coffee DNA as reported by Lashermes et al. (2000a). For each sample, 500 ng of genomic DNA were digested using two restriction enzymes, EcoRI and MseI. Restricted DNA fragments were ligated with EcoRI and MseI adapters using T4 DNA ligase (Gibco BRL). In pre-selective amplification, the ligation mixture was amplified using primers complementary to the adapters with one additional selective 3'-nucleotide. Two sets of primers with three selective nucleotides were used for amplification. The EcoRI primers were end labelled with γ -[^{33}p]-ATP using T 4 polynucleotide kinase. PCR amplifications were carried out using a total of 42 AFLP primer combinations as described in Lashermes et al. (2000a). Amplification products were electrophoresed on 6% denaturing polyacrylamide gel. The gels were dried and exposed to Kodak Bio Max X-ray film.

Data analysis

Experiment 1 and 2 were tested using a triangular test design. The normal dsitribution was used like an approximation of the binomial distribution to test the significance of the differences (P = 0.05). For the experiment 3, for each introgressed line, the number of introgressed AFLP marker bands was determined as previously described (Lashermes et al., 2000a). Number of introgressed AFLP markers was calculated for each line, and subsequently analysed for possible association, using a Pearson correlation test, with mean values for each line for chemical contents (Y1 samples) and organoleptic scoring (Y1 and Y2 samples). Results obtained in experiment 3 were used to analyse the amount of variation between lines for chemical contents determined on the Y1 samples as well as for the organoleptic scoring performed on the Y1 and Y2 samples. Data were therefore subjected to analyses of variance (ANOVA) using lines as the grouping variable (each lines represented by 3-4 values for the different plots), and followed by a comparison of the means for the different lines using a Newman-Keuls multiple range test. Based on the NIR wavelength data from the Y3 samples, a discriminant function analysis was performed (Statistica 4.3, Statsoft Inc. 1993). The Squared Mahalanobis distances between lines were calculated and significance of the variation among distances was determined by a Wilks' lambda test.

RESULTS

Experiment 1

The jury was able to discriminate the 2 cultivars in 73% of the tests ($P < 0.001$). Caturra was preferred to CR95 in 65% of the tests ($P < 0.01$).

Experiment 2

The jury was able to discriminate the 2 cultivars in 84% of the tests ($P < 0.000$). Caturra was preferred in 75% of the tests ($P < 0.000$).

Experiment 3

The mean number of markers introgressed per line was 21.1 among the 22 introgressed Arabica lines, with extreme values ranging from 1 (T17929) to 37 (T17936) (Table 1). The previously determined resistances to race II of coffee rust as well as to *M. exigua* are indicated in Table 1. The variations in chemical compound contents were low (Table 2) and within the range determined for a set of more than 300 Arabica coffee samples of 'Caturra' and 'Catuai' produced in Central America (Guyot, unpublished data). There were significant differences between lines for all the biochemical compounds analysed. For caffeine, five introgressed lines had significantly higher values than the non-introgressed controls (1.26 to 1.27%). Lowest and highest values were noted in lines T17925 (1.23%) and T17933 (1.45%). For chlorogenic acids, a single line (T17924) had a content (8.34%) that was significantly higher than in the controls (7.43 to 7.66%). However, this extreme content was not beyond the range found for the chlorogenic acid content of Arabica coffee produced in Central America (7.5% to 8.3%; Guyot, unpublished data). Two lines (T17933 and T17937) had a lower chlorogenic acid content than the controls. For the fat content, significant differences existed between the lines. One line (T17928) was characterized by a significantly lower content (13.78%) than the three controls (14.42, 14.46 and 14.50%). Again, this value was within the range for this compound in Central America (13.0% to 15.0%). The trigonelline content varied between 0.95 (T17930) and 1.14% (T17926). Seven lines had higher contents than the controls (1.02 to 1.03%) and two had lower values. Sucrose revealed more marked differences between lines

than the other compounds studied. The non-introgressed controls had the highest values (7.14 to 7.23%). Four lines had significantly lower values than the controls. However, deviations were slight (6.41 to 7.10%) and still within the range for Arabica from this region (6.2% to 9%).

Table 2. Comparison of chemical components (% of dry weight) of coffee produced by the Timor Hybrid-derived lines and cv Caturra, cv Catuai and cv Villa-Sarchi as non-introgressed controls. Means followed by the same suffix in the same column are not significantly different at $p \leq 0.05$.

Line	Caffeine	Chlorogenic acids	Fat	Trigonelline	Sucrose
T17924	1.37 bcd	8.34 a	14.47 abc	1.127 a	6.41 c
T17925	1.23 d	7.75 b	14.31 abc	1.125 a	6.78 abc
T17926	1.30 cd	7.77 b	14.20 abc	1.143 a	6.66 bc
T17927	1.31 bcd	7.73 b	14.30 abc	1.127 a	6.71 bc
T17928	1.37 abc	7.45 bc	13.87 c	1.017 d	7.01 ab
T17929	1.32 bcd	7.41 bc	14.03 bc	1.022 bc	6.92 ab
T17930	1.38 ab	7.39 bc	14.43 abc	0.952 e	6.83 abc
T17931	1.30 bcd	7.28 cd	14.36 abc	1.032 bc	6.88 abc
T17933	1.45 a	7.06 d	14.61 ab	0.962 e	7.02 ab
T17934	1.31 bcd	7.55 bc	14.83 a	1.072 bc	6.89 abc
T17935	1.37 bc	7.75 b	14.26 abc	1.097 ab	6.86 abc
T17936	1.28 cd	7.26 cd	14.29 abc	1.016 d	7.10 ab
T17937	1.28 cd	7.22 d	14.21 abc	1.022 cd	6.96 ab
T17938	1.33 bcd	7.59 bc	14.21 abc	1.095 ab	6.64 c
T17940	1.37 abc	7.73 b	14.16 abc	1.095 ab	6.79 abc
cv Caturra	1.26 d	7.43 bc	14.42 abc	1.030 cd	7.23 a
cv Catuai	1.27 d	7.54 bc	14.46 abc	1.042 cd	7.16 a
cv Villa-Sarchi	1.26 d	7.66 bc	14.50 abc	1.017 d	7.14 a

There were no significant differences between the introgressed lines and the non-introgressed controls for body attributes (Table 3). For the flavour attribute, the differences found in Y1 were barely significant and there were no significant differences between lines in Y2. However, significant differences were found for acidity and the overall standard in Y1 and Y2. For example, it was found that line T17926 was poorer than the non-introgressed controls for the overall standard and for acidity in Y1. Nevertheless, that result was not confirmed in Y2 since T17926 was not significantly different from the controls. Only two lines were significantly poorer than the controls for two years running. They were line T17927, which was poorer than the controls for acidity in Y1 and in Y2, and line T17924, which was significantly poorer than the controls for acidity and for the overall standard in Y1 and in Y2.

Discrimination between lines based on their NIRS

Based on the NIR wavelength data from the Y3 samples, squared Mahalanobis distances between lines were calculated (Table 4). It was not possible to distinguish between the non-introgressed controls (probabilities indicated in the upper matrix of Table 4) based on their NIR wavelength. Three introgressed lines could not be distinguished from one or two of the non-introgressed controls. They were T17929, T17935 and T17940. There was significant discrimination between the other lines and the three non-introgressed controls based on their NIRS. The 15 lines are represented on the two principal components in Figure 1.

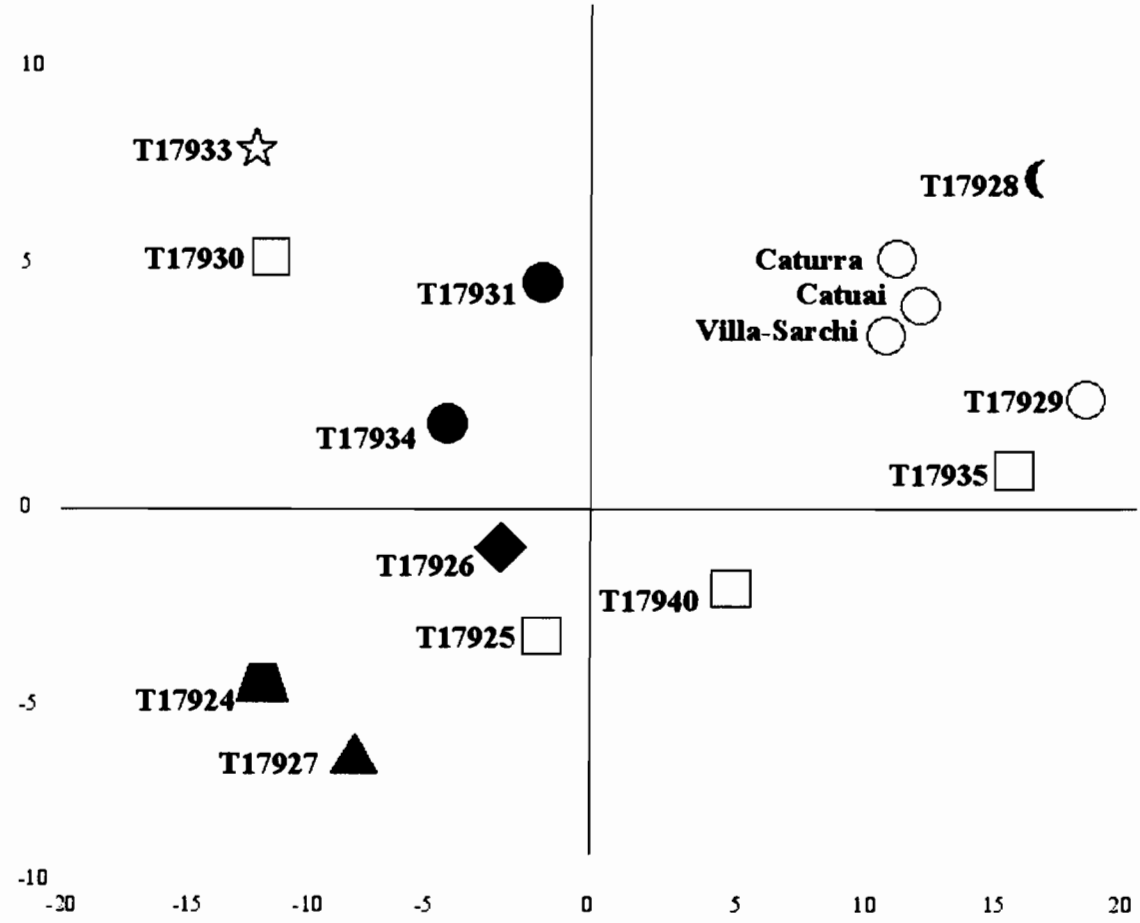

Figure 1. Representation of 15 *Coffea arabica* accessions based on the Mahalanobis distance calculated on the near infrared spectroscopy spectrum from the Y3 samples. For each accession, the line number (centroid) represents the means for 3-4 samples on the two principal components. Differences, for chemical contents from Y1 samples and the organoleptical attributes from Y1 and Y2, between the introgressed lines and the non-introgressed controls are indicated by a symbol (shape and colour). *Circle*: non-introgressed controls and introgressed lines identical for chemical contents and BQ; *square*: lines significantly higher than the controls, for trigonelline and/or caffeine contents; *moon*: line significantly higher than the controls for caffeine contents and significantly lower for fat contents; *star*: lines significantly lower than the controls for chlorogenic acids and trigonelline contents; *lozenge*: line significantly higher than the controls for trigonelline and lower for sucrose contents; *triangle*: line significantly lower than the non-introgressed controls for sucrose content and for beverage acidity; *trapeze*: line significantly lower than the controls for sucrose and chlorogenic acid contents and for beverage acidity and overall standard. The *black symbols* represented lines with 21-32 introgressed markers; the *grey symbols* represented lines with 5-20 introgressed markers; the *white symbols* represented lines with 0-1 introgressed markers.

Relations between introgression levels and line characteristics

There were no significant correlations between the BQ attributes of the lines or their chemical contents and their amount of introgressed AFLP markers. It can be seen in Figure 1 that line T17929 (1 introgression marker) and two highly introgressed lines (T17934 and T17931, 25 and 30 markers respectively) did not differ from the non-introgressed controls for either the chemical contents or the organoleptic analysis attributes. The other introgressed lines differed

from the non-introgressed controls to varying degrees. Lines T17935, T17940, T17930, and T17925, which displayed fewer than 20 introgression markers, differed from the controls for caffeine or trigonelline content. Line T17928 had a higher caffeine content than the controls, but a lower fat content. Line 17933 (16 markers) had less trigonelline and sucrose than the non-introgressed controls. Line T17926 (26 markers) had significantly more sucrose and trigonelline than the control. Line T17927 (30 introgression markers) differed significantly from the controls for trigonelline and sucrose and the beverage of this line was judged to be less acidic in Y1 and Y2. Lastly, line T17924 (32 introgression markers), differed from the controls for trigonelline, sucrose, chlorogenic acids, beverage acidity and the overall standard in Y1 and Y2.

Table 3. Beverage characteristics of Timor Hybrid-derived lines and cv Caturra, cv Catuai and cv Villa-Sarchi as non-introgressed controls. Scores for acidity, body, flavour estimated using scales ranging from 0 to 5, 0 = nil, 5 = very strong. Overall standard, 0 to 5, 0 = unacceptable, 5 = excellent. Data obtained from a panel of eight evaluators. Means followed by the same suffix in the same column are not significantly different at $p \leq 0.05$.

	Year 1				Year 2			
	Overall Standard	Acidity	Body	Flavour	Overall Standard	Acidity	Body	Flavour
T17924	2.18 bc	2.21 bc	2.50 a	2.50 ab	1.86 b	1.64 c	2.57 a	2.93 a
T17925	2.39 ab	2.18 bc	2.50 a	2.82 ab	2.36 ab	2.36 abc	2.57 a	2.93 a
T17926	1.75 c	1.75 c	2.40 a	2.40 ab	2.36 ab	2.64 ab	3.14 a	3.50 a
T17927	2.11 c	2.00 bc	2.54 a	2.75 ab	2.00 ab	1.86 c	2.71 a	3.14 a
T17928	2.18 bc	2.11 bc	2.46 a	2.75 ab	2.64 a	2.64 ab	3.07 a	3.07 a
T17929	2.47 ab	2.33 ab	2.80 a	2.91 ab	2.28 ab	2.14 abc	2.86 a	2.71 a
T17930	2.47 ab	2.50 ab	2 .61a	2.75 ab	2.14 ab	2.14 abc	2.57 a	3.21 a
T17931	2.24 abc	2.14 bc	2.57 a	2.76 ab	2.79 a	2.57 ab	2.86 a	3.35 a
T17933	2.19 bc	2.14 bc	2.47 a	2.62 ab	2.69 a	2.39 ab	3.15 a	3.07 a
T17934	2.76 a	2.71 ab	2.86 a	2.91 ab	2.36 ab	2.14 abc	2.57 a	2.85 a
T17935	2.53 ab	2.57 ab	2.75 a	2.82 ab	2.79 a	2.86 ab	3.14 a	3.14 a
T17936	2.19 bc	2.09 bc	2.71 a	2.62 ab	2.36 ab	2.57 ab	3.00 a	3.21 a
T17937	2.53 ab	2.46 a	2.64 a	2.96 ab	2.29 ab	2.07 abc	2.71 a	3.50 a
T17938	2.04 bc	1.93 c	2.43 a	2.29 b	2.57 ab	3.07 a	3.14 a	3.07 a
T17940	2.36 ab	2.25 bc	2.53 a	2.57 ab	2.71 a	3.07 a	2.71 a	3.43 a
cv Caturra	2.79 a	2.81a	2.81 a	3.13 a	2.33 ab	2.33 ab	2.90 a	3.04 a
cv Catuai	2.73 a	2.99 a	2.67 a	2.60 ab	2.97 a	2.87 ab	2.90 a	2.84 a
cv Villa-Sarchi	2.60 a	3.05 a	2.86 a	2.36 ab	3.04 a	3.05 a	2.81 a	2.82 a

Table 4. Discriminant analysis performed to classify 20 lines using Mahalanobis distances on the basis of NIRS wavelengths. Each sample was characterized by 52 wavelengths in the 1104-2448 nm interval. Lower matrix, Mahalanobis distance between the lines, upper matrix, probability of Wilks' lambda tests between the lines.

Lines	T17924	T17925	T17926	T17927	T17928	T17929	T17930	T17931	T17933	T17934	T17935	T17940	Villa-Sarchi	Catuai	Caturra
T17924		0.15	0.02	0.50	0.00	0.00	0.01	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00
T17925	191.28		0.92	0.38	0.00	0.01	0.02	0.32	0.00	0.27	0.01	0.28	0.01	0.02	0.00
T17926	218.49	62.40		0.36	0.00	0.00	0.01	0.17	0.00	0.12	0.00	0.35	0.01	0.00	0.00
T17927	109.56	194.33	149.72		0.00	0.00	0.04	0.07	0.01	0.05	0.00	0.07	0.00	0.00	0.00
T17928	1512.76	785.81	755.71	1203.07		0.42	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00
T17929	1270.40	561.27	537.36	1014.76	121.71		0.00	0.00	0.00	0.00	0.22	0.03	0.04	0.07	0.04
T17930	209.57	337.91	280.69	276.13	1374.67	1209.44		0.05	0.10	0.01	0.00	0.00	0.00	0.00	0.00
T17931	266.61	158.34	134.43	267.41	750.66	658.07	164.99		0.01	0.43	0.00	0.08	0.00	0.00	0.00
T17933	398.59	495.76	370.52	399.12	1249.32	1139.57	107.97	228.23		0.00	0.00	0.00	0.00	0.00	0.00
T17934	234.06	150.19	126.37	261.09	820.45	671.72	197.71	74.92	334.14		0.00	0.02	0.00	0.00	0.00
T17935	1157.21	652.51	599.42	952.27	278.67	245.81	1183.39	713.04	961.59	702.92		0.05	0.00	0.16	0.16
T17940	392.64	167.80	100.48	271.19	503.67	360.37	412.90	173.07	353.95	204.09	306.29		0.00	0.10	0.03
Villa-Sarchi	916.01	451.88	350.58	695.34	426.96	324.15	753.59	424.20	782.57	428.56	621.28	366.63		0.08	0.11
Catuai	737.73	347.46	295.87	597.31	303.54	236.92	617.66	300.02	481.47	343.07	183.30	134.18	194.35		0.18
Caturra	970.56	546.37	423.35	790.93	353.32	310.29	843.23	425.17	616.21	486.46	210.28	234.32	169.28	127.15	

DISCUSSION

The variation in number of markers introgressed per line reflected a level of variability similar to that detected with another set of samples of introgressed lines by Lashermes et al. (2000a). All but two of the lines (T17929, cv 'Veranero') were resistant to leaf rust (race II). That resistance was introgressed from *C. canephora* via the Timor Hybrid (Kushalappa and Eskes, 1989; Gonçalvez and Pereira, 1998). Resistance to *M. exigua*, which also came from *C. canephora* (Bertrand et al., 2001) was found in 13 of the 17 lines. Line T17929, which only had a single AFLP attributable to introgression was susceptible to both parasites. The Timor Hybrid-derived lines therefore had great variability for the number of introgression markers. The presence of large amounts of introgressed genetic materials from *C. canephora* in many introgressed Arabica lines indicates that plant breeding has resulted in contrasting situations between lines. These lines are choice germplasm for studying the effect of introgression on BQ.

For many crops, undesirable effects are often associated with introgressed segments (Grandillo et al., 1999). For Arabica, most work published on the quality of introgressed lines concludes that BQ has not been modified by the introgression of genes from *C. canephora* (Fazuoli, 1977; Moreno et al., 1995; Puerta, 1998). Nevertheless, our results seem to show that these conclusions need to be moderated. For the two cultivars CR95 and 'Veranero' and for some lines in selection there would seem to be a drop in quality attributable to introgression. That was the case with line T17924 which displayed significant differences from the non-introgressed controls for most of the chemical contents (trigonelline, sucrose and chlorogenic acids), and for beverage acidity and the overall standard. However, there were also highly introgressed lines that revealed no difference from the non-introgressed controls. Such was the case with lines T17934 and T17931, which did not differ for either the chemical content or the BQ. As the latter reveal genetic resistances to coffee leaf rust and *M. exigua*, it can be concluded that the presence of resistance genes has no pleiotropic effects on beverage quality. This is an encouraging result for the future of genetic improvement programmes based on the introgression of resistance genes from *C. canephora* via the Timor Hybrid. However, if it is to be more effective and, in particular, if it is to avoid maintaining undesirable introgressed fragments suspected of having a negative effect on BQ, selection could be assisted by specific markers of resistance to pests/diseases (Lashermes et al., 2000b). This programme would be much more efficient if it were possible to detect chemical compounds with variations that are highly correlated to quality defects attributable to introgression. In our study, the lowest sucrose contents and the highest chlorogenic acid contents seemed to be linked to a poor BQ.

Genetic improvement of Arabica, based on the introgression of genes from the species *C. canephora* in order to create varieties resistant to the main parasites of the crop, has resulted in lines that have a variable amount of introgression markers, thereby illustrating the problems involved in reducing introgression to only those genes of agronomic interest via traditional selection. Nevertheless, it would seem that selection can avoid accompanying the introgression of resistance genes with a drop in BQ.

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