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B. Bertrand · B. Guyot · F. Anthony · P. Lashermes

Impact of the Coffea canephora gene introgression on beverage quality of *C. arabica*

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Abstract Lines of *Coffea arabica* derived from the Timor Hybrid (hybrid between *C. arabica* and *C. canephora*) are resistant to coffee leaf rust (*Hemileia vastatrix*) and to the nematode *Meloidogyne exigua*. The introgression of *C. canephora* resistance genes is suspected of causing a drop in beverage quality. Coffee samples from pure lines, compared in a Trial 1, and from F_1 hybrids and parental lines from a half-diallel trial in a Trial 2, were studied for beverage quality, chemical composition and amount of introgressed genetic material. Chemical analyses (caffeine, chlorogenic acids, fat, trigonelline, sucrose) were carried out with near-infrared spectrometry by reflectance of green coffee. The number of amplified fragment length polymorphic (AFLP) markers introgressed from the Timor Hybrid varied from 1 to 37 for the lines studied. There were significant differences between lines for all of 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 number of AFLP markers and the chemical contents or beverage attributes. Significant correlations were found between the performance of the parents and their general combining ability for beverage quality. It was concluded that it should be possible to find lines with both the desired resistance genes and good beverage quality. Selection can avoid accompanying the introgression of resistance genes with a drop in beverage quality.

Keywords Coffea arabica · Coffea canephora · Introgression · Chemical contents · AFLP · Near-infrared spectrometry

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B. Bertrand (✉) · B. Guyot · F. Anthony · P. Lashermes
IICA/PROMECAFE, Ap. Postal 55, 2200, Coronado, Costa Rica
e-mail: bertrand86@hotmail.com
Fax: +506-225-9894

Present address:
B. Guyot, CIRAD, BP 5035, Laboratoire d'analyses sensorielles, 34032 Montpellier, France

Present address:
F. Anthony · P. Lashermes, IRD (ex. ORSTOM), Genetrop, B.P. 5045, 34032, Montpellier Cedex, France

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 with a 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, and the F_1 hybrid 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 the 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, which produce more chlorogenic acids (8–13% versus 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 contents 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; Owuor 1988; Castillo 1990; Moreno et al. 1995; Puerta 1998; Puerta 2000). 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

Twenty-two 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, Caturra, Catuai and Villa-Sarchi (Table 1), formed the plant material. The samples came from two trials set up at the ICAFE research centre in Costa Rica, located in Heredia at 1,200 m above sea level on an andosol type soil. Trial 1, which was set up in 1988, comprised 15 lines derived from CIFC 1343, with cvs. Caturra, Catuai and Villa-Sarchi as controls. In each of the four replicates of the trial, the plots consisted of ten trees (i.e. 40 trees per line). Trial 2 was a half-diallel (10×10) including the selfed parental lines (Table 1) and a Caturra control. This trial was set up in 1996, using three replicates, and the plots in each replicate consisted of four trees. The trials were conducted without shade. The distance between plants was 0.84 m along the row and 1.68 m between rows – a density of 7,086 plants/ha. The plants received 1,000 kg/ha per year of N-P-K-Ca-Mg (18-3-10-8-0.5) in May and August, and 250 kg/ha/year of nitrogen in November, along with two applications of copper hydroxide per year against leaf diseases (coffee leaf rust and cercosporiosis).

Samples taken for organoleptic and chemical analysis

Composite samples from all trees in each plot were taken from plots in Trial 1 and Trial 2, using ripe, healthy cherries harvested from the upper branches of the trees during the harvest peak. Two kilograms of coffee cherries was 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 Trial 1, 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. In Trial 2, the samples collected in the 1998 harvest were submitted for organoleptic analysis.

Organoleptic analysis

After roasting the green coffee beans we carried out for 9–10 min, BQ tests on an infusion prepared from 12 g of the roasted coffee. A panel of eight evaluators tasted 120 ml of infusion. 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 that ranged from 0 to 5 where 0 = unacceptable, 1 = bad, 2 = regular, 3 = good, 4 = very good, 5 = excellent.

Chemical analysis

The analysis of green coffee beans was preferred to the analysis of roasted coffee beans since compositional changes occur during roasting (Clifford 1985). The analyses were performed by near-infrared spectrometry (NIRS) 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; NIRSystem, Silver Spring, Md.) driven by NIRS2 (4.0) software (Intrasoft, Port Matilda, Pa.). For the Y1 and Y3 samples from Trial 1, a NIR spectrum was acquired in reflectance (R) mode in the 1,104– to 2,456-nm range (Downey et al. 1994; Downey and Boussion 1996; Scanlon et al. 1999). Using specific calibrations (Guyot et al. 1988, 1993), it is possible to determine the caffeine, trigonelline, fat and sucrose contents. These contents were determined for the Y1

Table 1 Numbers of AFLP markers attributable to introgression detected in 22 introgression lines and a non-introgressed cultivar, 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[a]	Introgressed cultivar Colombia (CIFC1343)	Colombia	32	R	R
T17925[a]	Introgressed cultivar Colombia (CIFC1343)	Colombia	14	R	R
T17926[a]	Introgressed cultivar Colombia (CIFC1343)	Colombia	28	R	R
T17927[a]	Introgressed cultivar Colombia (CIFC1343)	Colombia	30	R	R
T17928[a]	Introgressed cultivar Colombia (CIFC1343)	Colombia	26	R	S
T17929[a]	Introgressed cultivar Colombia (CIFC1343)	Colombia	1	S	S
T17930[a,b]	Introgressed cultivar Colombia (CIFC1343)	Colombia	14	R	R
T17931[a,b]	Introgressed cultivar Colombia (CIFC1343)	Colombia	30	R	R
T17933[a,b]	Introgressed cultivar Colombia (CIFC1343)	Colombia	16	R	R
T17934[a]	Introgressed cultivar Colombia (CIFC1343)	Colombia	25	R	R
T17935[a]	Introgressed cultivar Colombia (CIFC1343)	Colombia	16	R	R
T17936[a]	Introgressed cultivar Colombia (CIFC1343)	Colombia	37	R	R
T17937[a]	Introgressed cultivar Colombia (CIFC1343)	Colombia	33	R	R
T17938[a]	Introgressed cultivar Colombia (CIFC1343)	Colombia	10	R	R
T17940[a]	Introgressed cultivar Colombia (CIFC1343)	Colombia	19	R	R
T18121[b]	Introgressed cultivar Catimor (CIFC832/1)	Brazil	8	R	S
T18130[b]	Introgressed cultivar Catimor (CIFC832/1)	Brazil	31	R	R
T8666[b]	Introgressed cultivar Catimor (CIFC832/1)	Brazil	11	R	S
T8667[b]	Introgressed cultivar Catimor (CIFC832/1)	Brazil	12	R	S
T18138[b]	Introgressed cultivar Sarchimor (CIFC832/2)	Brazil	32	R	R
T18140[b]	Introgressed cultivar Sarchimor (CIFC832/2)	Brazil	19	R	S
T18141[b]	Introgressed cultivar Sarchimor (CIFC832/2)	Brazil	20	R	R
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

[a] Line present in trial 1
[b] Parental line of the half-diallel (trial 2)

samples. 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 25 trees representing 22 introgressed lines and three non-introgressed lines as controls (cvs. Caturra, Catuai, 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 was digested using two restriction enzymes, *Eco*RI and *Mse*I. Restricted DNA fragments were ligated with *Eco*RI and *Mse*I adapters using T4 DNA ligase (Gibco BRL, Gaithersburg,). 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 *Eco*RI primers were end-labelled with γ-[^{33}P]-ATP using T 4 polynucleotide kinase. Polymerase chain reaction (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 a 6% denaturing polyacrylamide gel. The gels were dried and exposed to Kodak Bio-Max X-ray film.

Data analysis

For each introgressed line, the number of introgressed AFLP marker bands was determined as previously described (Lashermes et al. 2000a). The 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 Trial 1 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 three to four 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 1993). The squared Mahalanobis distances between lines were calculated, and the significance of the variation among distances was determined by a Wilks' lambda test.

For Trial 2, diallel analyses were carried out on hybrid families. Data from the organoleptic analysis were processed according to the Griffing model for a half-diallel, adapted to unbalanced and non-orthogonal designs (Keuls and Garretsen 1977), discussed by Baradat and Desprez-Loustau (1997). OPEP software (Baradat and Labbé 1995) was used to estimate the components of variance and covariance for general combining ability (GCA). It was also used to calculate *F* tests for GCA considered as fixed effects. Ranking and comparison of the parental lines according to organoleptic scores and GCA values were carried out using Newman-Keuls tests. The rankings for organoleptic scores were compared with the GCA of the parents by a Pearson correlation coefficient.

Results

Amount of introgression and evaluation for chemical contents and BQ

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 (1.26–1.27%), with the lowest and highest values 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 that in the controls (7.43–7.66%). However, this extreme content was not beyond the range found for the chlorogenic acid content of Arabica coffee produced in Central America (7.5–8.3%; Guyot, unpublished data). Two lines (T17933 and T17937) had a lower chlorogenic acid content than the controls. With respect to the fat content, significant differences existed between the lines. One line (T17928)

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

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

Table 3 Beverage characteristics of Timor Hybrid-derived lines and cvs. Caturra, Catuai and Villa-Sarchi as non-introgressed controls. Data obtained from a panel of eight evaluators. Means followed by the same suffix in the same column are not significantly different at $P = 0.05$

	Year 1				Year 2			
	Overall standard[a]	Acidity[b]	Body[b]	Flavour[b]	Overall standard[a]	Acidity[b]	Body[b]	Flavour[b]
T17924	2.18b,c	2.21b,c	2.50a	2.50a,b	1.86b	1.64c	2.57a	2.93a
T17925	2.39a,b	2.18b,c	2.50a	2.82a,b	2.36a,b	2.36a,b,c	2.57a	2.93a
T17926	1.75c	1.75c	2.40a	2.40a,b	2.36a,b	2.64a,b	3.14a	3.50a
T17927	2.11c	2.00b,c	2.54a	2.75a,b	2.00a,b	1.86c	2.71a	3.14a
T17928	2.18b,c	2.11b,c	2.46a	2.75a,b	2.64a	2.64a,b	3.07a	3.07a
T17929	2.47a,b	2.33a,b	2.80a	2.91a,b	2.28a,b	2.14a,b,c	2.86a	2.71a
T17930	2.47a,b	2.50a,b	2.61a	2.75a,b	2.14a,b	2.14a,b,c	2.57a	3.21a
T17931	2.24a,b,c	2.14b,c	2.57a	2.76a,b	2.79a	2.57a,b	2.86a	3.35a
T17933	2.19b,c	2.14b,c	2.47a	2.62a,b	2.69a	2.39a,b	3.15a	3.07a
T17934	2.76a	2.71a,b	2.86a	2.91a,b	2.36a,b	2.14a,b,c	2.57a	2.85a
T17935	2.53a,b	2.57a,b	2.75a	2.82a,b	2.79a	2.86a,b	3.14a	3.14a
T17936	2.19b,c	2.09b,c	2.71a	2.62a,b	2.36a,b	2.57a,b	3.00a	3.21a
T17937	2.53a,b	2.46a	2.64a	2.96a,b	2.29a,b	2.07a,b,c	2.71a	3.50a
T17938	2.04b,c	1.93c	2.43a	2.29b	2.57a,b	3.07a	3.14a	3.07a
T17940	2.36a,b	2.25b,c	2.53a	2.57a,b	2.71a	3.07a	2.71a	3.43a
cv. Caturra	2.79a	2.81a	2.81a	3.13a	2.33a,b	2.33a,b	2.90a	3.04a
cv. Catuai	2.73a	2.99a	2.67a	2.60a,b	2.97a	2.87a,b	2.90a	2.84a
cv. Villa-Sarchi	2.60a	3.05a	2.86a	2.36a,b	3.04a	3.05a	2.81a	2.82a

[a] Overall standard, 0–5: 0 = unacceptable; 5 = excellent
[b] Scores based on a scale of 0–5: 0 = nil; 5 = very strong

Table 4 Discriminant analysis performed to classify 20 lines using squared Mahalanobis distances on the basis of NIRS wavelengths. Each sample was characterized by 52 wavelengths in the 1,104 to 2,448-nm interval. Lower matrix, squared 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	

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 (13.0–15.0%). The trigonelline content varied between 0.95% (T17930) and 1.14% (T17926). Seven lines had higher contents than the controls (1.02–1.03%) 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 (7.14–7.23%). Four lines had significantly lower values than the controls. However, deviations were slight (6.41-7.10%) and still within the range for Arabica from this region (6.2–9%).

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 with respect to the overall standard and 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 2 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, we calculated squared Mahalanobis distances between lines (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 wavelengths. Three introgressed lines could not be distinguished from one or two of the non-introgressed controls – 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 Fig. 1.

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 number of introgressed AFLP markers. It can be seen in Fig. 1 that line T17929 (one 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 intro-

Table 5 Comparison of performance[a] and GCA value[a] of parental introgression lines for beverage characteristics. Means followed by the same suffix in the same column are not significantly different at $P \leq 0.05$

	Performance of parental lines				GCA value			
	Overall standard	Acidity	Body	Aroma	Overall standard	Acidity	Body	Aroma
T8666	2.58a,b,c	2.36b	2.75a,b	2.56a	–0.006a,b	–0.003b,c	–0.025a,b,c	–0.024a
T8667	2.56a,b,c	2.53a,b	2.70a,b	2.72a	0.016a,b	0.034b,c	–0.051a,b,c	–0.002a
T17930	2.87a,b	2.86a,b	2.51a,b	2.82a	–0.079b,c	–0.117b,c	-0.007a,b,c	0.093a
T17931	1.97b,c	1.78c	2.34b	2.34a	–0.617c	–0.758c	–0.175b,c	–0.174a,b
T17933	2.81a,b	2.90a,b	2.99a,b	2.90a	–0.005a,b	0.015b,c	0.026a,b,c	0.043a
T18121	2.94a	3.18a	3.07a	3.25a	0.199a	0.197b,c	0.182a	0.024a
T18130	1.89c	1.86c	2.41a,b	2.42a	–0.317c	–0.495c	–0.201c	–0.253b
T18138	2.67a,b,c	2.66a,b	2.83a,b	3.08a	–0.092b,c	–0.113b,c	–0.078a,b,c	0.002a
T18140	2.78a,b	2.71a,b	3.00a,b	2.82a	0.265a,b	0.404a	0.088a,b	0.093a
T18141	2.50a,b,c	2.50a,b	2.72a,b	2.56a	0.178a,b	0.271a,b	0.089a,b	0.002a

[a] Scores for aroma, body, acidity, estimated using scales ranging from 0 to 5. 0 = nil; 5 = very strong. Preference score, 0 to 5. 0 = unacceptable, 5 = excellent. Data obtained from a panel of eight evaluators

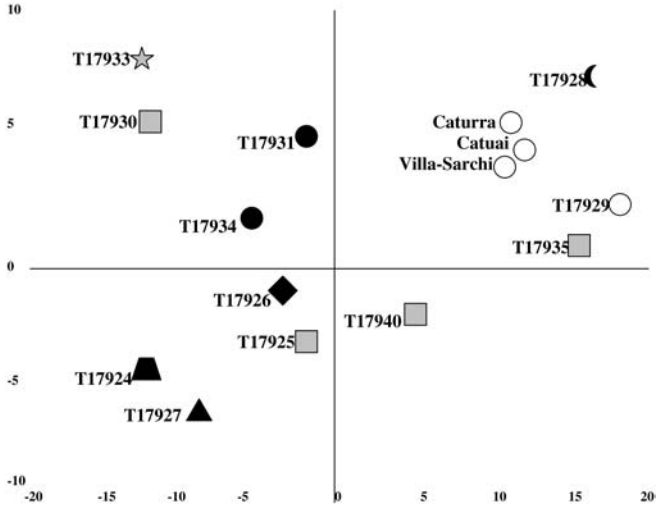

Fig. 1 Representation of 15 *Coffea arabica* accessions based on the squared Mahalanobis distance calculated on the near infrared spectroscopy spectrum from the Y3 samples. For each accession, the line number (centroid) represents the means for three to four samples on the two principal components. Differences, 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 color). *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* lines significantly lower than the non-introgressed controls for sucrose content and for beverage acidity; *trapeze* lines significantly lower than the controls for sucrose and chlorogenic acid contents and for beverage acidity and overall standard. The *black symbols* represent lines with 21–32 introgressed markers; the *grey symbols* represent lines with 5–20 introgressed markers; the *white symbols* represent lines with zero to one introgressed marker

gressed 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.

Relations between performance of the parental lines and their GCA for BQ

Significant differences were observed between the parental lines (Trial 2) for all beverage descriptors (Table 5). Analysis of the half-diallel revealed significant differences for the GCA of parental lines. Significant correlations were found between the performance of parents and their GCA, with $r = 0.78, 0.76, 0.81, 0.67$, respectively, for the overall standard, acidity, body and flavour ($P<0.05$).

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 one of the lines (T17929) 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 16 of the 22 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). With respect to Arabica, the conclusion drawn from most of the investigations published on the quality of introgressed lines is 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 some Timor Hybrid-derived lines 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, both of 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 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.

For the time being, NIRS analysis (Perez et al. 2001) does not appear to be an adequate tool for predicting the BQ of introgressed lines. However, it should be possible to form reference groups by compiling databases from the NIRS of the main cultivars and structuring them into characterized groups for BQ and for the main chemical contents. Once the base has been established, each new line will be assigned to a reference group. Lastly, for further breeding programmes based on crosses between introgressed lines, the associations found between the performance of the parental lines and the GCA for BQ means that it is possible to predict with reasonable accuracy the value of progenies from the performance of the parents.

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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