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Comparison of bean biochemical composition and beverage quality of Arabica hybrids involving Sudanese-Ethiopian origins with traditional varieties at various elevations in Central America

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Summary For buyers of Arabica coffee (*Coffea arabica* L.) in Central America, elevation and variety are important indicators of quality. We compared coffee produced by three types of varieties established in various trials at elevations ranging from 700–1600 m in three countries (El Salvador, Costa Rica and Honduras). Arabica hybrids resulting from crosses of Sudanese–Ethiopian origins with either traditional varieties or with introgressed lines derived from the hybrid of Timor (*C. arabica* × *Coffea canephora* Pierre ex Froehn) were compared with traditional cultivars (TC). Effects of elevation and variety on bean biochemical composition (caffeine, chlorogenic acid, trigonelline, fat and sucrose) were evaluated by predictive models based on calibration of near-infrared (NIR) spectra and by chemometric analysis of the global NIR spectrum. Beverage quality tests were performed by a panel of ten professional cup-tasters. Experiment 1 was carried out on the slopes of the Poas volcano (Costa Rica) with the traditional cultivar ‘Caturra’. Experiment 2 compared the three varieties in a network of trials established in three countries of Central America. Significant linear regressions with elevation were observed in Experiment 1 with Caturra and in Experiment 2 for the traditional cultivars, and trends were established relating variation in biochemical compounds and cup quality to elevation. Convergence or divergence of the new hybrids in relation to these trends was observed. For the traditional cultivars, elevation had a significant effect on bean biochemical composition, with chlorogenic acid and fat concentrations increasing with increasing elevation. For the Arabica hybrids, elevation explained little of the variation in chlorogenic acid concentration and none of the variation in fat concentration. Nevertheless, Arabica hybrids had 10–20% higher fat concentrations than the traditional varieties at low elevations and similar fat concentrations at high elevations. The samples could be discriminated according to elevation based on NIR spectra; however, the spectra of the TC varieties were more strongly modified by elevation than the spectra of the hybrids. Nonetheless, this analysis confirmed homeostasis of the hybrids for which bean biochemical composition was less affected by elevation than that of the traditional varieties. The organoleptic evaluation, performed on samples originating from high elevations, showed no significant differences between Arabica hybrids and traditional cultivars. The new hybrid varieties with high beverage quality and productivity potential should act as a catalyst in increasing the economic viability of coffee agroforestry systems being developed in Central America.

Keywords: Coffea arabica, F1 hybrids, NIRS.

Introduction

Numerous factors affect coffee beverage quality including ecological conditions and agricultural practices. The role of soil types has been studied. Generally, the most acidic coffees are grown on rich volcanic soils. Climate and altitude play important roles through temperature and availability of light and water during the ripening period (Cannell 1974, 1985, Clifford and Wilson 1985, Guyot et al. 1996, Carr 2001, Decazy et al. 2003). The distribution of sunshine also has a strong influence on flowering, bean expansion and ripening. Shade decreases coffee tree productivity by about 20%, but reduces the alternate bearing pattern (Vaast et al. 2005). Shade positively affects bean size and composition as well as beverage quality by delaying berry flesh ripening by up to one month. Higher sucrose, chlorogenic acid and trigonelline concentrations in sun-grown beans than in shade-grow beans suggest incomplete bean maturation and account for increased bitterness and astringency of the coffee beverage (Muschler 2001). Physiological stresses such as over-bearing reduce bean size as a result of carbohydrate competition among berries during bean filling (Cannell 1985, Bertrand et al. 2004, Vaast et al. 2005).

Maturation also has a strong influence on coffee quality; Guyot et al. (1988) showed that yellow or green cherries of *Coffea canephora* Pierre ex Froehn harvested at the end of the picking season contain more mature beans (based on bean size, chemical composition and cup quality) than red cherries harvested at the begining of the picking season. However, for *Coffea arabica* L. in Costa Rica, early picking gives the best coffee (Vaast and Bertrand 2005). Post harvest techniques and the preparation of the beverage also strongly influence coffee quality. The biochemical composition of the coffee beans also appears to be influenced by genetic factors (Montagnon et al. 1998).

Comparisons of different varieties based on organoleptic evaluation and several scientific procedures indicate that similarities and differences are attributable to genetic traits (Fazuoli et al. 1977, Puerta 2000). In Central America, most buyers prefer the more traditional varieties (Bourbon, Caturra, Catuai, Pacamara) over the newer varieties derived from the 'Hybrid of Timor', an inter-specific hybrid between *C. arabica* × *C. canephora*. It has recently been shown that introgression via the 'Hybrid of Timor' confers resistance (rust and nematodes), but is sometimes accompanied by a substantial decrease in cup quality (Bertrand et al. 2003). Consequently, most national coffee organizations in charge of disseminating varieties in Central America have now suspended the distribution of these introgressed varieties. In the early 1990s, a regional breeding programme was undertaken based on the use of hybrid vigor in crosses between traditional Arabica varieties introgressed with or without the 'Hybrid of Timor' and Ethiopian or Sudanese trees of wild origin. Its aim was to produce high-yielding hybrids while improving coffee quality (Bertrand et al. 2005*a*). These hybrids are currently being tested in a trial network in Central America for their ecological adaptation, agronomic performances and quality characteristics, in comparison with traditional varieties. Here we report on the effects of variety and elevation on bean biochemical composition and cup quality.

Recently, near-infrared spectroscopy (NIRS) was proposed as a fast and nondestructive method for predicting chemical and physical properties in complex compositions like agricultural, horticultural and food products (Scanlon et al. 1999, Quilitzsch and Hoberg 2003, Velasco et al. 2004). In coffee, NIRS has been used successfully to predict the biochemical content of green beans (Guyot et al. 1993) and to authenticate coffee varieties (Downey and Boussion 1996, Bertrand et al. 2005*b*). We used predictive models based on NIRS to determine the chemical content of coffee beans. The global NIR spectrum was also used to compare the varieties.

Materials and methods

Experiment 1

Experiment 1 was carried out in 2003 on the traditional variety 'Caturra' planted at eight farms ranging in elevation from 900 to 1450 m a.s.l. on the slopes of the Poas volcano, Costa Rica. All the plots were aligned along a north–south transect with coffee trees grown in full sun at a density of 5000 trees ha^{-1}. Trees were 10–15 years old and were intensively managed, receiving 1000 kg ha^{-1} of 18,3,10,8,0.5 N,P,K,Mg,B fertilizer split annually into two applications in May and August, 250 kg ha^{-1} of N (as urea) in November and two foliar applications per year of copper hydroxide (1.5 kg ha^{-1}) to prevent leaf and fruit diseases such as coffee leaf rust (*Hemileia vastatrix*) and leaf and fruit brown eye spot (*Cercospora coffeicola*). 'Caturra' is a dwarf cultivar with a maximum height of 2.5 m after 4–5 yields. At all the farms, trees were managed on a 5-year rotational coppicing cycle, with every fifth row stumped at about 40–50 cm aboveground. Twenty-four trees were randomly selected within each plot to study the effects of elevation on bean characteristics and coffee beverage quality. Twelve trees were in their first year after coppicing and the other twelve in their third year after coppicing.

Experiment 2

Observations were made in 2003 in a network of trials established in 1999 and 2000 in three countries of Central America. Three to 14 varieties were tested in each trial using a randomised design with four blocks (replicates). The experimental plots comprised 10 trees per row. Planting density was 5000 trees ha^{-1}. Elevation ranged from 700 to 1600 m a.s.l. After 2 to 3 years, the coffee trees produced their first or second crop, respectively. The main characteristics of these trials are summarized in Table 1. In Costa Rica, the plants received 1000 kg ha^{-1} $year^{-1}$ of 18,3,10,8,0.5 N,P,K,Ca,Mg fertilizer split into two applications (May and August) and 250 kg ha^{-1} $year^{-1}$ of N (as urea) in November, along with two applications of copper hydroxide per year. In El Salvador and Honduras, the plants received 800 kg ha^{-1} $year^{-1}$ of 20,1,10 N,P,K fertilizer split into two applications (May and September) and 150 kg ha^{-1} $year^{-1}$ of N (as urea) in November, and one application of copper hydroxide per year.

Three types of varieties were tested: (1) five traditional cultivars (TC), 'Caturra', 'Pacas', 'Catuai', 'Bourbon' and 'Pacamara'; (2) eight Arabica hybrids of type F1-A : crosses of TC ('Caturra' or 'Catuai') with Ethiopian origins ('ET6', 'Rume Sudan', 'E531', 'ET15', 'E416', 'ET41' and 'Anfilo'); and (3) four Arabica hybrids of type F1-B: crosses between 'Hybrid of Timor' derived lines ('CR95' or 'T5296') and Ethiopian origins ('ET6', 'Rume Sudan' and 'ET25').

Berry harvest and processing

In both experiments, only healthy, ripe cherries from the upper branches of the trees were collected during the harvest peak in 2003. For each sample, 1 kg of coffee cherries was processed by the wet method (de-pulping, fermentation and drying) to obtain about 250 g of green coffee beans. The green coffee samples were screened through a size 17 sieve and defective beans were discarded. In Experiment 1, six composite samples were collected from 10 trees per plot. In Experiment 2, two composite samples were collected from two experimental plots per genotype.

Table 1. Characteristics of the trials (planting year, country, region, elevation, number of varieties tested and number of varieties per type) in Experiment 2. Abbreviations: TC = traditional cultivars; F1-A = clones of F1 hybrids (TC crossed with Sudanese-Ethiopian origins); and F1-B = clones of F1 hybrids (lines derived from Hybrid of Timor crossed with Sudanese-Ethiopian origins).

Elevation (m)	Planting year	Country	Region	No. varieties tested	No. varieties per type
700	1999	Costa Rica	Perez Zeledon	6	TC = 2; F1-A = 2; F1-B = 2
800	2000	El Salvador	Usulatán, San José	9	TC = 1; F1-A = 5; F1-B = 3
900	2000	Costa Rica	Naranjo	14	TC = 1; F1-A = 8; F1-B = 5
1100	2000	El Salvador	San Jorge	8	TC = 2; F1-A = 4; F1-B = 2
1100	1999	Costa Rica	Barva de Heredia	7	TC = 2; F1-A = 2; F1-B = 3
1200	2000	El Salvador	Usulatán, Los Pirineos	6	TC = 2; F1-A = 3; F1-B = 1
1300	2000	Costa Rica	La Hilda-Alajuela	11	F1-A = 7; F1-B = 4
1300	2000	El Salvador	Auchapán, El Milenio	6	TC = 2; F1-A = 3; F1-B = 1
1400	1999	Costa Rica	Sabanilla de Alajuela	3	TC = 2; F1-A = 1
1400	2000	Honduras	Marcala	8	TC = 2; F1-A = 4; F1-B = 2
1600	1999	Costa Rica	Santa María de Dota	4	TC = 2; F1-B = 2

Analysis of sensory characteristics

Beverage quality tests were performed on samples from all eight locations in Experiment 1 and from La Hilda in Costa Rica, Los Pirineos and El Milenio in El Salvador, and Marcala in Honduras in Experiment 2. After roasting the green coffee beans for 9–10 min at 220 °C, beverage quality was tested by a panel of ten cup-tasters on a 120 ml infusion prepared with 12 g of roasted coffee. The major taste and flavor attributes, aroma, body, bitterness and acidity were scored based on a scale ranging from 0 to 5 where: 0 = nil; 1 = very light; 2 = light; 3 = medium; 4 = strong; and 5 = very strong. An additional, overall preference score for beverage quality was based on the above attributes and also ranged from 0 to 5, where: 0 = unacceptable; 1 = bad; 2 = regular; 3 = good; 4 = very good; and 5 = excellent.

Biochemical analyses

Near-infrared reflectance (NIR) spectra were made with a scanning monochromator Nirsystems spectrophotometer (Model 6500, Perstorp Analytical, Silver Spring, MD) driven by NIRS2 (4.0) software (Intrasoft Int., Port Matilda, PA). The analyses were performed on green coffee (50 g) after fine grinding (< 0.5 mm). For each sample, 5 g of ground coffee was placed in a crystal cell. The samples were randomly scanned at 20 ± 1 °C within a week. For each sample, 32 scans were recorded in reflectance (R) mode, where R represents reflectance energy in the 900–2500 nm range in 2-nm steps (Downey and Boussion 1996). The log $(1/R)$ absorbance spectrum was obtained as the mean of these measurements. The mean quadratic error, estimated from two subsamples and based on the raw spectrum (log $1/R$), was less than 300 microns; this error was below the manufacturer's specifications and indicates satisfactory reproducability of the spectral measurements. Given these results, a single spectrum was acquired per sample. Caffeine, trigonelline, chlorogenic acid (5CQA), fat and sucrose concentrations were determined based on specific calibrations (Guyot et al. 1993). First, the concentration of each constituent (caffeine, trigonelline, fat, sucrose and chlorogenic acid) was determined on coffee beans by conventional methods for a set of 361, 323, 177, 331 and 267 independent samples, respectively. The reference analyses were acquired after extraction and purification. Caffeine, trigonelline and sucrose concentrations were determined by HPLC (UVV or electrochemical detectors). Chlorogenic acid concentration was determined by UV spectroscopy and fat concentrations by gravimetry. The NIR calibrations were obtained based on partial least squares regression (Table 2). The performances of the predictive models were tested by estimating the standard errors of prediction (SEP). These SEP values were close to the standard errors found in the laboratory (SEL), making it possible to apply the appropriate calibration equation in routine analysis (Davrieux et al. 2005).

Table 2. Calibration statistics for each coffee bean constituent. Abbreviations: No. = total number of samples used for calibrations; M = mean; SD = standard deviation of the concentration values; SEC = standard error of calibration; R^2 = coefficient of multiple determination; SECV = standard error of cross validation; 1-VR = estimate of the percentage of explained variance; SEP = standard error of prediction; and SEL = standard error of laboratory.

Constituent	No.	M (g kg^{-1})	SD	SEC	R^2	SECV	1-VR (%)	SEP	SEL
Caffeine	361	12.5	1.6	0.5	0.88	0.6	85	0.6	0.6
Trigonelline	323	9.9	0.9	0.4	0.82	0.5	72	0.5	0.3
Fat	177	143.1	17.1	4.5	0.93	4.9	92	5.4	5.0
Sucrose	331	73.9	9.4	4.3	0.79	5.3	68	5.0	4.0
Chlorogenic acid	267	76.4	8.0	3.5	0.81	3.9	76	3.9	3.0

Statistical analyses

Statistica software (2004; Statsoft, France) was used to perform all statistical analyses. In Experiment 1, following an analysis of variance (ANOVA), the mean biochemical and sensory values were compared by the Duncan test ($P \leq 0.05$). In Experiment 2, the three types of varieties were compared by ANOVA for the following elevational ranges (namely, 700–899 m, 900–1099 m, 1100–1199 m, 1200–1399 m and 1400–1600 m) for caffeine, trigonelline, fat, chlorogenic acid and sucrose concentrations. For sensory analysis of Experiment 2, each trial was separately analyzed by ANOVA and the mean values of variety types were compared by the Duncan test ($P \leq 0.05$).

In Experiments 1 and 2, linear regressions were calculated for caffeine, trigonelline, fat, chlorogenic acid and sucrose concentrations, with elevation as the independent variable. The regressions were calculated for the traditional cultivar ‘Caturra’ in Experiment 1, then for the TC varieties of Experiment 2, then for the F1-A types and the F1-B types of Experiment 2. The linearity of the regressions was tested as well as the homogeneity of the residual variances. The coefficients of regression were compared (Dagnélie 1975).

For the analysis of the global NIR spectra, chemometric processing consisted initially of a principal components analysis (PCA) based on the second derivatives of the spectra on the 900–2500 nm segment. For Experiment 1, a clustering analysis was performed on the individuals for the principal components (100% of the variability) by the squared Euclidian distance and the Ward’s method. For Experiment 2, the PCA was followed by a factorial discriminant analysis (FDA) with the principal components representing 99.9% of the variability. This FDA analysis was performed on the variety types grown at two elevational ranges (700–1200 and 1201–1600 m), which were taken as the classification factors.

Results

Influence of elevation on bean biochemical composition of the traditional cultivars

In Experiment 1 (Table 3), elevation had a significant effect on bean biochemical composition of the traditional cultivar ‘Caturra’. Chlorogenic acid concentration increased with increasing elevation above 1100 m. The reverse trend was observed for trigonelline. Caffeine and fat concentrations increased with increasing elevation, but then decreased at the highest elevations. Sucrose showed no clear trend with elevation. Similar trends were observed for the traditional varieties in Experiment 2 (Table 4). Caffeine and chlorogenic acid concentrations increased with increasing elevation above 1200 m. Fat concentration increased with increasing elevation, but decreased at the highest elevations. As in Experiment 1, sucrose displayed no clear trend with increasing elevation. Contrary to Experiment 1, elevation had no effect on trigonelline concentration.

Effect of variety type on bean biochemical composition

In Experiment 2, no significant effect of variety type was found on trigonelline or caffeine concentration (Table 4). Differences in chlorogenic acid concentration were observed between variety types only at the elevational range of 1200–1399 m. Differences in sucrose concentration were observed between variety types in various elevational ranges (700–899 m, 900–1099 m and 1200–1399 m). Beans of traditional varieties had higher sucrose concentrations at these elevational ranges than at the intermediate (1099–1199 m) and highest (1400–1600 m) elevational ranges. At any elevation, the variety types differed significantly in bean fat concentration. At elevations up to 1399 m, the F1-A hybrids had more fat than the TC varieties and the F1-B hybrids had an intermediate fat concentration. At 1400 m and above, fat concentrations of the TC varieties were similar to those of the F1-A hybrids, whereas the F1-B hybrids had significantly less fat.

Statistical relationships between biochemical compounds and elevation

Trigonelline concentration decreased significantly with increasing elevation in Experiment 1. The regression was significant, despite a low coefficient of determination ($R^2 = 0.31$). This relationship was not observed in Experiment 2 for either the TC varieties or the F1 hybrids (F1-A and F1-B). There was no significant relationship between sucrose concentration and elevation for the TC varieties or the hybrids. The chlorogenic acid concentration of the traditional varieties increased signifi-

Table 3. Effects of elevation on bean biochemical composition of the traditional cultivar ‘Caturra’ in Experiment 1. Within a column, means with different letters are significantly different according to the Duncan test ($P = 0.05$). Units = g kg^{-1}.

Elevation (m)	Trigonelline	Caffeine	Chlorogenic acid	Sucrose	Fat
900	8.4 b	11.1 d	76.1 c	73.9 b	140.7 e
1000	8.2 bc	11.2 d	76.3 c	74.2 b	145.8 d
1100	8.9 a	11.7 cb	81.9 a	74.1 b	142.5 e
1200	7.8 cd	11.7 cba	81.4 b	77.3 ab	151.2 c
1300	7.8 cd	12.6 cba	82.4 a	73.4 b	154.7 bc
1350	7.7 cd	12.9 ba	83.2 a	70.3 b	157.2 a
1400	7.5 d	13.1 a	82.5 a	75.4 ab	152.7 b
1450	8.0 c	12.5 b	82.4 a	81.3 a	146.5 d
F probability	0.0001	0.0001	0.0001	0.0001	0.0001

Table 4. Effects of elevation on bean biochemical composition (trigonelline, caffeine, chlorogenic acid, sucrose and fat; g kg^{-1}) of the different variety types. Within a column and elevational range, means followed by different letters are significantly different according to the Duncan test (P = 0.05).

Elevation (m)	Variety type	Trigonelline	Caffeine	Chlorogenic acid	Sucrose	Fat
700–899	TC	8.8 ± 0.3	11.3 ± 0.2	76.7 ± 1.1	74.4 ± 1.3 a	126 ± 3 c
	F1-A	9.3 ± 0.2	11.7 ± 0.1	80.0 ± 0.7	66.8 ± 1.1 b	152 ± 2 a
	F1-B	8.8 ± 0.2	12.0 ± 0.2	78.7 ± 0.8	69.7 ± 1.3 b	137 ± 2 b
	F probability	0.15	0.15	0.07	0.007	0.0001
900–1099	TC	9.7 ± 0.4	11.3 ± 0.3	77.9 ± 1.9	69.5 ± 2.4 a	129 ± 3 b
	F1-A	9.4 ± 0.2	11.7 ± 0.1	83.4 ± 0.7	64.0 ± 0.9 b	155 ± 1 a
	F1-B	9.0 ± 0.2	11.9 ± 0.1	83.2 ± 0.8	67.7 ± 1.1 b	143 ± 2 b
	F probability	0.16	0.49	0.89	0.04	0.0001
1100–1199	TC	9.1 ± 0.5	11.7 ± 0.3	79.9 ± 3.3	78.3 ± 2.5	139 ± 6 b
	F1-A	9.0 ± 0.2	12.1 ± 0.1	81.1 ± 1.7	73.6 ± 1.3	146 ± 3 a
	F1-B	9.2 ± 0.3	11.3 ± 0.2	86.7 ± 2.2	74.5 ± 1.6	140 ± 4 ab
	F probability	0.91	0.06	0.12	0.29	0.04
1200–1399	TC	0.87 ± 0.2	1.22 ± 0.2	80.2 ± 1.8 b	79.4 ± 2.1 a	143 ± 3 b
	F1-A	0.92 ± 0.2	1.22 ± 0.1	85.5 ± 0.8 a	69.0 ± 0.9 b	155 ± 1 a
	F1-B	0.82 ± 0.2	1.26 ± 0.2	84.1 ± 1.3 a	72.3 ± 1.5 b	144 ± 2 a
	F probability	0.25	0.08	0.0001	0.016	0.0001
1400–1600	TC	1.21 ± 0.4	1.21 ± 0.4	96.0 ± 0.2	71.5 ± 1.5 b	152 ± 2 ab
	F1-A	1.29 ± 0.2	1.29 ± 0.2	88.0 ± 0.1	72.6 ± 1.0 a	156 ± 1 a
	F1-B	1.26 ± 0.3	1.26 ± 0.3	91.0 ± 0.2	74.7 ± 1.1 a	146 ± 1 b
	F probability	0.29	0.29	0.08	0.21	0.02

cantly with increasing elevation (Table 5). Coefficients of regression were similar for 'Caturra' in Experiment 1 and the TC varieties in Experiment 2 (0.773 and 0.678, respectively) and the R^2 values were 0.58 and 0.43, respectively. Elevation explained little of the variation in chlorogenic acid concentration for the F1-A and the F1-B hybrids (R^2 values of 0.15 and 0.30, respectively). For caffeine, a significant linear regression was found for the TC in Experiment 1 ($R^2 = 0.61$) and for the F1-A hybrids ($R^2 = 0.25$) in Experiment 2. The higher the elevation, the higher the caffeine concentration. This relationship was not found for the TC and F1-B varieties in Experiment 2. Fat concentrations of the TC varieties in Experiments 1 and 2 were similar (Table 6). Their coefficients of regression were almost identical (0.65 and 0.64, respectively) and both had R^2 values of 0.39. For the traditional varieties, fat concentration increased with increasing elevation, whereas elevation had no effect on fat concentration of the hybrids (Figure 1; Table 6).

Discrimination of variety types based on NIR spectral analysis

In Experiment 1, the samples could be discriminated according to elevation based on their NIR spectra. At the highest level of the clustering analysis, two groups were observed (Figure 2). The first group included elevations between 900 and 1100 m. At the intermediate clustering level, 1100 m could be distinguished from the 900 and 1000 m elevations. The second group, which included elevations greater than 1200 m, could be split into two classes, a middle elevation (1200–1350 m) and a high elevation (1400–1450 m) with the exception of one sample at 1200 m.

In Experiment 2, at low elevations (700–1200 m), the Mahalanobis distances ($D^2 = 4.23$, $P = 0.001$) between the two hybrid

Table 5. Variations in chlorogenic acid concentration of coffee beans; comparison of regression coefficients, significance of these regression coefficients, nonlinearity probability and coefficients of determination for variety types with respect to elevation. Abbreviations: TC = traditional cultivars; F1-A = clones of F1 hybrids (TC crossed with Sudanese-Ethiopian origins); F1-B = clones of F1 hybrids (lines derived from Hybrid of Timor crossed with Sudanese-Ethiopian origins); and ns = not significant.

Treatment	Coefficient of regression	Probability associated with the significance of the coefficient of regression	Probability associated with the nonlinearity of the regression	Coefficient of determination (R^2)
Experiment 1 (TC)	0.77	0.001	ns	0.58
Experiment 2 (TC)	0.68	0.001	ns	0.43
Experiment 2 (F1-A)	0.44	0.01	ns	0.15
Experiment 2 (F1-B)	0.56	0.01	ns	0.30

Table 6. Variation in fat concentrations of coffee beans; comparison of regression coefficients, significance of these regression coefficients, nonlinearity probability and coefficients of determination for variety types with respect to elevation. Abbreviations: TC = traditional cultivars; F1-A = clones of F1 hybrids (TC crossed with Sudanese-Ethiopian origins); F1-B = clones of F1 hybrids (lines derived from Hybrid of Timor crossed with Sudanese-Ethiopian origins); and ns = not significant.

Treatment	Coefficient of regression	Probability associated with the significance of the coefficient of regression	Probability associated with the nonlinearity of the regression	Coefficient of determination (R^2)
Experiment 1 (TC)	0.652	0.00	ns	0.39
Experiment 2 (TC)	0.640	0.00	0.05	0.39
Experiment 2 (F1-A)	0.175	ns	0.00	0.01
Experiment 2 (F1-B)	0.348	ns	0.01	0.10

types, F1-A and F1-B, or between TC and F1-A (D^2 = 5.19, P = 0.004) or TC and F1-B (D^2 = 5.86, P = 0.004) were all significantly different. At high elevations (1205–1600 m), the Mahalanobis distances between the TC and the two hybrid types were also significantly different with D^2 values of 3.9 and 5.7, respectively. Furthermore, samples collected from the three variety types grown at high elevations differed significantly (P < 0.0001) from those collected at the lowest elevations. The first axis (Figure 3) represented 55% of the total variance and discriminated the majority of the samples grown at high elevations from the samples grown at the lowest elevations. The Mahalanobis distances between the TC samples collected at the two elevational ranges (D^2 = 13.9) were larger than the distances observed between the TC samples and those of the two other variety types at the same elevational range. The same pattern (although less marked) was observed for the F1-B samples (D^2 = 8.69). The Mahalanobis distance between the F1-A samples grown at the two elevational ranges was only D^2 = 5.8, indicating that the F1-A type was less affected by elevation than the TC and the F1-B variety type.

Beverage quality

Experiment 1 Samples originating from higher elevations (1350, 1400 and 1450 m) had higher beverage preference scores than samples from lower elevations (900, 1100, 1200 and 1300 m) (Table 7). The higher elevation samples also had higher acidities, lower bitterness values and more aroma than samples from the lower elevations. Samples from 1000 m were a notable exception, as they received good scores, lacked bitterness and were acidic with a good aroma.

Experiment 2 Cup quality was tested for variety types from four trials established in three countries at elevations between 1200 and 1400 m (Table 8). For the trial in Costa Rica, no significant difference was observed between the three variety types in either overall preference or body. However, the F1-A varieties were inferior to the F1-B varieties in aroma and acidity. No significant difference was found between variety types in the 'Los Pirineos' trial in Salvador (1200 m). For the 'El Milenio' site (1300 m) in Salvador, the F1-A and the TC varieties did not differ in cup quality. For Marcala in Honduras

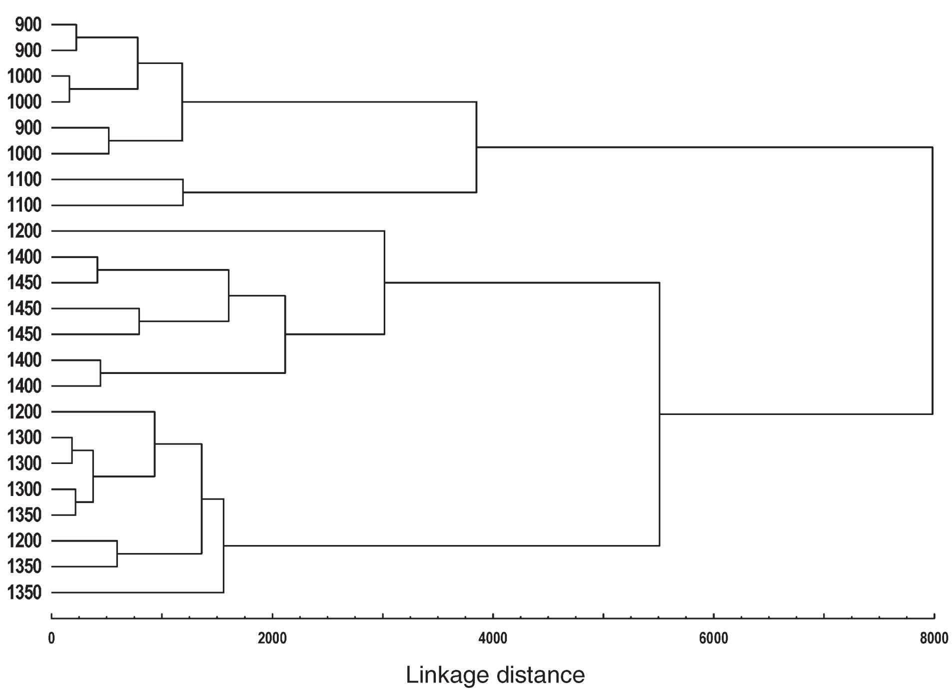

Figure 1. Tree diagram of elevation (m) and linkage distance for Experiment 1. The clustering analysis was performed using the squared Euclidian distance and Ward's Method.

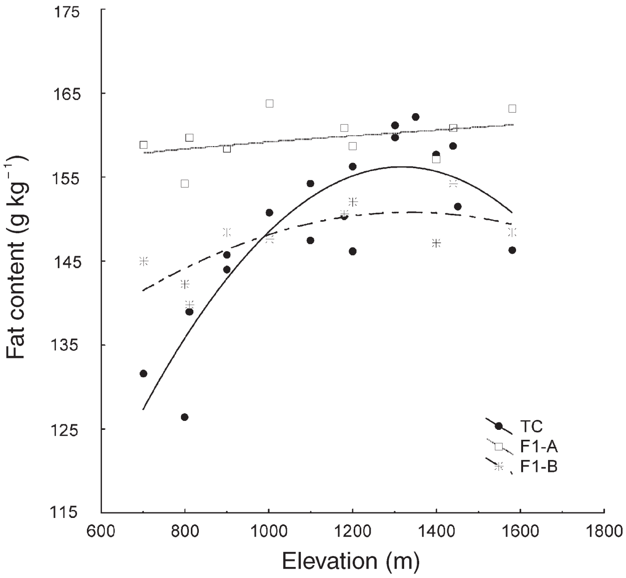

Figure 2. Effect of elevation on fat concentration (g kg^{-1}) of traditional cultivars (TC) and hybrids F1-A and F1-B. Regression curves were fitted by the least square method.

(1440 m), the F1-A type was superior to the two other types in acidity and body. The overall preference was significantly higher for the F1-A followed by the F1-B then the TC, whereas the TC was inferior to the F1-A in aroma.

Discussion

Traditional varieties are an unavoidable reference in Central America because they predominate in coffee plantations and contribute to the original taste that is sought after by coffee buyers in this region, and they have a long-standing reputation of producing high quality coffee. Therefore, it is important to compare bean biochemical composition and cup quality of the two types of new Arabica hybrids with traditional cultivars under the various ecological conditions of Central America before their release to farmers. Although the beneficial effects of elevation on coffee quality are common empirical knowledge, few scientific studies have documented these effects. Elevation has an obvious influence on temperature: it is generally accepted that, in the tropical climates of Central America, an increase in elevation of 100 m decreases the mean daily temperature by 0.8–1 °C (Rojas 1985). Temperature plays an important role in the phenologic cycle of coffee, particularly on berry development and ripening (Guyot et al. 1996). Experiment 1 was designed to document the beneficial effects of elevation on coffee quality: agricultural management, soil type and solar exposure were similar in all the plots, which were planted exclusively with the traditional cultivar 'Caturra'. Because significant linear regressions with elevation were observed in Experiment 1 with Caturra and in Experiment 2 for several TC varieties, trends could be established relating variation in biochemical compounds and cup quality to elevation. The convergence or divergence of the new hybrids in relation to these trends reflected their physiological functioning as it affected cup quality and bean biochemical composition.

Elevation was not a determining factor in explaining variation in caffeine and trigonelline concentrations of beans originating from the traditional varieties or from the F1 hybrids. However, the increase in chlorogenic acid concentration with increasing elevations appeared as a strong general trend in the traditional varieties. This trend was also noted for the F1 hybrids (F1-A and F1-B), but to a lesser degree because the beans

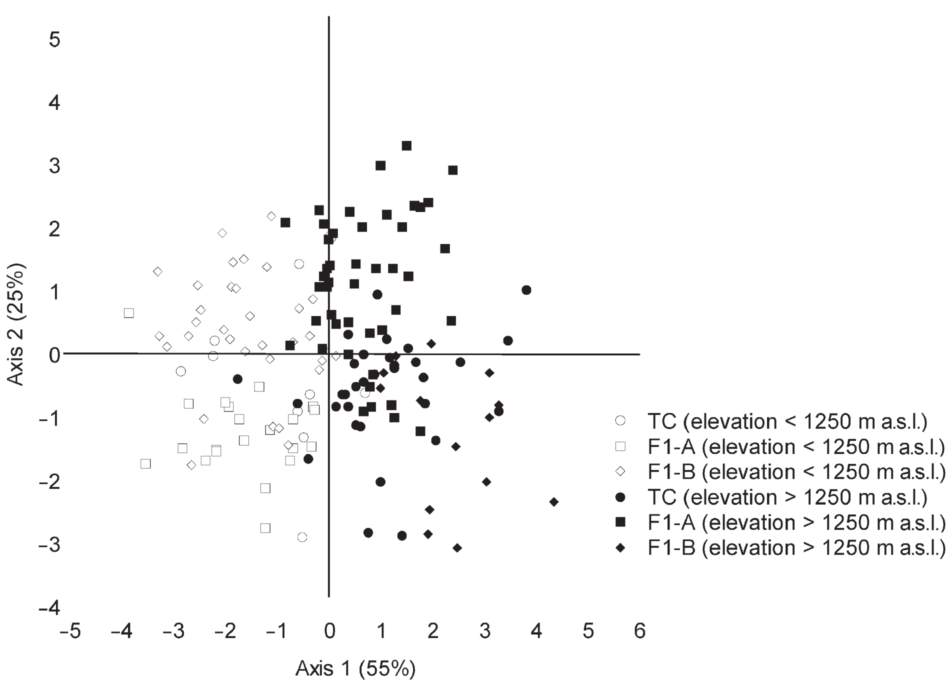

Figure 3. Graphic representation on the first two components (Axis 1 and Axis 2) of the factorial discriminant analysis (FDA) of three *Coffea arabica* variety types based on the squared Mahalanobis distances calculated from the near-infrared spectra of bean samples from Experiment 2. The circle, square and lozenge symbols represent the traditional cultivars (TC) and the hybrid varieties F1-A and F1-B, respectively. The samples collected at elevations ranging between 700 and 1200 m are represented by open symbols and the samples collected at elevations ranging between 1200 and 1600 m are represented by filled symbols.

Table 7. Effects of elevation on the traditional variety 'Caturra' beverage attributes (aroma, body, acidity and bitterness) and overall preference. Within a column and for the same elevation range, means followed by different letters differ significantly according to Duncan's test ($P = 0.05$). Scores for aroma, body, acidity and bitterness are based on a scale of 0–5, where 0 = null and 5 = very strong. Beverage preference score is based on a scale of 0–5: 0 = unacceptable; 1 = bad; 2 = regular; 3 = good; 4 = very good; and 5 = excellent.

Elevation (m)	Aroma	Body	Acidity	Bitterness	Preference
900	3.30 ab	2.76 ab	2.53 dc	1.93 a	2.53 c
1000	3.45 a	2.90 ab	3.32 ab	1.58 ab	3.12 ab
1100	3.30 ab	2.56 b	2.40 d	1.99 a	2.53 c
1200	3.00 b	2.58 b	3.32 ab	1.22 b	2.41 c
1300	3.53 a	2.85 ab	2.85 bc	1.53 ab	2.75 ab
1350	3.48 a	2.96 ab	3.37 a	1.55 ab	3.16 a
1400	3.51 a	2.83 ab	3.16 ab	1.51 ab	3.25 a
1450	3.61 a	3.19 a	3.41 a	1.35 b	3.38 a
F probability	0.01	0.06	0.0001	0.0001	0.0001

of hybrids tended to accumulate more chlorogenic acid than the beans of the TC varieties at lower elevations. For traditional cultivars, fat concentration increased sharply with increasing elevation, as observed by Decazy et al. (2003).

It is well documented that leaf to fruit ratios are higher at high elevations than at low elevations because leaf life span is longer (Vaast et al. 2004). Higher leaf to fruit ratios result in an increased carbohydrate supply to berries and higher bean fat synthesis. Furthermore, berry flesh ripening is delayed at the lower temperatures encountered at higher elevations, allowing longer and better bean filling (Vaast et al. 2006). Unlike the traditional cultivars, elevation did not significantly influence the bean fat concentration of the F1 hybrids. For these variety types, increased homeostasis combined with high heterosis has recently been demonstrated (Bertrand et al. 2005*a*). Consequently, these new varieties are exceptionally vigorous compared with traditional varieties. Therefore, it can be hypothesized that higher vegetative vigor resulted in higher leaf to fruit ratios and better carbohydrate supply to berries, irrespective of elevation. This was more evident for the F1-A hybrid type which produced less coffee than the F1-B type (Bertrand et al. 2005*a*). This hypothesis was confirmed by discrimination analysis of the NIR spectra. The spectra obtained from the TC varieties were more strongly modified by elevation than the spectra obtained from the F1-B and F1-A hybrids. Thus, the use of F1 hybrids should help to reduce variation in coffee bean fat concentrations and hence, undesirable variations in beverage quality. Decazy et al. (2003) observed that preference is positively linked to fat concentration. Heavy fruit bearing traditional coffee varieties with low leaf to fruit ratios tend to have strong biannual production and quality patterns. The year of high fruit load and production results, via carbohydrate and nutrient competition among berries, in lower bean size and cup quality and poorer vegetative growth that, in turn, leads to a lower fruit load during the following year, but improved quality (Vaast et al. 2006).

The organoleptic evaluation revealed no differences between the F1 hybrids and the traditional varieties. The F1 hy-

Table 8. Comparison of beverage attributes (aroma, body and acidity) and overall preference of three variety types of cultivars in four trials planted in three countries. Within a column and for the same trial location, means followed by different letters differ significantly according to Duncan test ($P = 0.05$). Scores for aroma, body, acidity and bitterness are based on a scale of 0–5, where 0 = null and 5 = very strong. Beverage preference score is based on a scale of 0–5: 0 = unacceptable; 1 = bad; 2 = regular; 3 = good; 4 = very good; and 5 = excellent. Abbreviations: TC = traditional cultivars; F1-A = clones of F1 hybrids (TC crossed with Sudanese-Ethiopian origins); and F1-B = clones of F1 hybrids (lines derived from Hybrid of Timor crossed with Sudanese-Ethiopian origins).

Country	Trial (elevation, m)	Variety type	Aroma	Body	Acidity	Preference
Costa Rica	Sabanilla (1300)	TC	3.33 ab	3.08 a	3.08 ab	3.01 a
		F1-A	3.06 b	3.13 a	2.81 b	2.86 a
		F1-B	3.42 a	3.27 a	3.35 a	3.22 a
Salvador	Los Pirineos (1200)	TC	3.13 b	3.13 a	3.16 a	2.75 a
		F1-A	3.32 b	3.00 a	3.02 a	2.96 a
		F1-B	3.73 a	3.19 a	3.27 a	3.15 a
Salvador	Milenio (1300)	TC	2.81 a	2.63 a	3.09 a	2.81 a
		F1-A	3.22 a	2.50 a	2.40 b	2.45 a
Honduras	Marcala (1440)	TC	2.90 b	2.86 b	2.50 b	2.40 c
		F1-A	3.38 a	3.32 a	3.28 a	3.44 a
		F1-B	3.20 ab	3.03 b	2.77 b	2.82 b

brids appeared to be inferior, similar or superior to traditional cultivars for certain attributes, such as acidity or aroma. With respect to overall preference, the F1 hybrids were always equivalent to the traditional cultivars, except at Marcala (Honduras) where they were superior. Because our cup quality assessment involved samples only from high elevations, it is essential to assess these new varieties at lower elevations. Recent results (P. Charmetant, unpublished data) indicate that the F1-B and F1-A hybrids are systematically superior to the TC varieties at elevations ranging 700 to 900 m.

In conclusion, after several decades of promoting intensively managed coffee systems planted in full sun, there is a renewed interest in managing Arabica coffee under shade in Central America. The presence of shade trees, especially leguminous species, improves soil fertility (organic matter content and nutrient cycling) and enhances coffee plantation sustainability (Beer et al. 1998, Soto-Pinto et al. 2000). Furthermore, coffee agroforestry systems help improve coffee farmers' revenues in the medium to long-term through diversification (timber production), production and marketing of high quality coffee and, eventually, payment of incentives for environmental services provided by these ecologically sound coffee systems (Vaast et al. 2004). Nonetheless, shifting from full-sun coffee monoculture to agroforestry systems will be relatively slow and expensive. Incorporating shade in fields planted either with traditional cultivars reduces yield by 20–30% under optimal conditions, which needs to be compensated by higher quality beans and higher coffee prices (Vaast et al. 2005). Use of these new hybrid varieties should act as a catalyst in increasing the economic and environmental sustainability of coffee agroforestry systems, given their high vegetative vigor, productivity and disease resistance combined with high cup quality. On the other hand, their use in intensive, full-sun systems might lead to increased negative ecological impacts because higher yields would have to be compensated for by additional fertilizer applications.

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