

Genotypic and Environmental Effects on Coffee (*Coffea arabica* L.) Bean Fatty Acid Profile: Impact on Variety and Origin Chemometric Determination

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In a previous study, the effectiveness of chlorogenic acids, fatty acids (FA), and elements was compared for the discrimination of Arabica varieties and growing *terroirs*. Since FA provided the best results, the aim of the present work was to validate their discrimination ability using an extended experimental design, including twice the number of location \times variety combinations and 2 years of study. It also aimed at understanding how the environment influences FA composition through correlation analysis using different climatic parameters. Percentages of correct classification of known samples remained very high, independent of the classification criterion. However, cross-validation tests across years indicated that prediction of unknown locations was less efficient than that of unknown genotypes. Environmental temperature during the development of coffee beans had a dramatic influence on their FA composition. Analysis of climate patterns over years enabled us to understand the efficient location discrimination within a single year but only moderate efficiency across years.

KEYWORDS: Coffee; lipids; fatty acids; authentication; variety; genotype; geographical origin; environment; temperature

INTRODUCTION

While many efforts have been made in the last 10 years to find efficient methods for the chemometric discrimination of the two cultivated coffee species *C. arabica* (Arabica coffee) and *C. canephora* (Robusta coffee) (1–5), very little has been done for the authentication of Arabica varieties and geographic growing origins through chemical profiling (6, 7). Still, there is a critical need for efficient methods to determinate small coffee *terroirs*, whose emergence on the market is expanding rapidly, and to distinguish beans of traditional Arabica varieties from those of modern introgressed varieties, since some introgressed lines may show undesirable traits, even if most of them show a beverage quality similar to traditional varieties (reviewed in ref 8).

In a previous study (9), we therefore investigated for the first time the possibility of discriminating high-quality traditional lines from introgressed lines in three contrasting Colombian environments, chosen to mimic small coffee *terroirs*. To this end, three chemical families, fatty acids (FA), chlorogenic acids, and elements, were compared. Among the three chemical families tested, bean FA were the most efficient in discriminating both Arabica varieties and environments. The overall proportion of correct classification of varieties obtained with FA (79%) was similar to that obtained by Bertrand et al. (7) on the basis of near-IR spectra (76%). Similarly, when the location was used as the criterion, the

proportion of correctly classified samples was very high (90%) (9). Such efficient determination of the variety and origin has been demonstrated in other oily fruits and beans such as almond (10), hazelnut (11), and olive (12).

Although our preliminary work (9) revealed that bean FA is a good candidate for efficient discrimination of both varieties and *terroirs*, it also raised critical issues that are addressed here. Because the comparison of three chemical families implied a limit in terms of the number of samples that could be analyzed, our previous study was performed using a limited number of samples. One could thus logically wonder whether the classification would have been as efficient with a larger experimental design. Therefore, using the same methodology—combining PCA and DA of known samples to estimate percentages of correct classification—the first aim of the present study was to test the discriminating value of bean FA with a design containing twice the number of genotype \times location combinations than was used in our previous study (9).

In addition, a second year of evaluation provided the opportunity to test, for the first time in coffee, the effect of the year and its interaction with the effects of the genotype and the location on the coffee bean FA composition. Indeed, this is a prerequisite to assess the predictive value of discriminant functions through cross-validation over years.

Finally, how the environment influences the FA composition of coffee beans remains a key issue to investigate. The ability to discriminate a very large number of *terroirs* greatly depends on

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Table 1. Fatty Acid Composition (Percent of Total Fatty Acids) and Lipid Content (Percent Dry Weight) of Green Beans in the Six Different Genotypes Studied in 2005^a

fatty acid		genotype					
		BGB.1033	BGB.1076	BGB.1040	BGB.1140	BGB.1219	Caturra
16:0	palmitic acid	31.2–33.1	32.1–34.4	31.7–34.0	32.0–33.9	32.8–35.3	31.7–33.8
18:0	stearic acid	5.9–7.5	6.1–7.9	6.1–8.0	6.0–8.2	6.0–7.8	6.6–8.3
18:1 n-9 cis	oleic acid	8.7–10.7	8.9–11.4	8.8–10.7	8.4–10.0	9.0–11.5	8.1–9.2
18:1 n-7	<i>cis</i> -vaccenic acid	0.4–0.6	0.4–0.6	0.4–0.6	0.4–0.6	0.5–0.6	0.4–0.5
18:2 n-6 cis	linoleic acid	43.8–45.9	41.8–44.3	42.3–44.7	42.8–45.4	40.8–44.9	43.6–45.1
18:3 n-3	linolenic acid	1.5–1.7	1.4–1.6	1.5–1.7	1.3–1.6	1.5–1.6	1.5–1.6
20:0	arachidic acid	2.2–2.6	2.3–2.8	2.3–3.0	2.4–2.8	2.3–2.8	2.6–2.9
22:0	behenic acid	0.5–0.7	0.6–0.7	0.5–0.7	0.6–0.7	0.5–0.7	0.6–0.8
lipid content (% DW)		12.7–15.3	14.2–15.5	14.2–15.2	13.6–16.4	14.3–16.4	15.0–16.8

^aThe interval given corresponds to the minimum and maximum mean values found in environments studied.

the number of environmental factors influencing bean FA composition and whether the *terroirs* show a stable typology for these factors over time. In many cold-tolerant lipid-rich fruit/seed species, including annual oilseed crops such as sunflower (13, 14), soybean (15), flax (16), rapeseed (17), and tree species such as olive (18) and hazelnut (19), the temperature during the development of fruit/seeds and, to a lesser extent, precipitation have been shown to have a dramatic influence on the final FA composition. For instance, one feature that is common to this category of plants is an increase in seed polyunsaturated FA content with decreasing temperature. To our knowledge, the influence of climatic conditions on seed FA has rarely been studied in cold-sensitive species. Coffee is a perennial plant originating from Ethiopian highland forests whose growth and metabolism is largely depressed by temperatures lower than 17–18 °C (20). The final objective of the present work was therefore to test whether responses to temperature variations similar to those reported in cold-tolerant species exist in coffee and, if this is the case, whether response patterns are the same among varieties. These results are discussed in terms of their impact on the chemometric discrimination of coffee varieties and growing origins.

MATERIALS AND METHODS

Materials. Two experimental designs were used in the present study. The first experimental design (in 2005) included five Colombian locations (Naranjal, Paraguaicito, Rosario, Tambo, and Pueblo Bello) in full combination with six *Coffea arabica* L. genotypes (Caturra, BGB.1033, BGB.1076, BGB.1040, BGB.1140, and BGB.1219). The second experimental design included three Colombian locations (Naranjal, Paraguaicito, and Rosario) in full combination with two years of harvest (year 1, 2003; year 2, 2005) and four genotypes (Caturra, BGB.1033, BGB.1076, and BGB.1040). Year 1 data were reported in a previous study (9). The variety Caturra was selected to represent high-quality traditional varieties. The five introgressed lines (BGB.1033, BGB.1076, BGB.1040, BGB.1140, and BGB.1219) derived (at least generation F5) from hybridizations between the Timor Hybrid C1FC-1343 (*C. arabica* × *C. canephora*) and Caturra were selected for their high yield, quality, and resistance to rust. They were chosen because they belong to different introgression genealogies. Locations were chosen to represent contrasting agroecological conditions in Colombia. Samples were collected during the harvest peak (245 days after flowering ±10 days), using healthy ripe cherries. For each sample, 1 kg of cherries was processed by the wet method (pulping, fermentation, and drying) to obtain approximately 250 g of green coffee beans. The samples of green coffee were screened through a size 17 sieve (17/64 in.), and the most defective beans were discarded.

Storage and Preparation of Coffee Samples. For each sample, coffee beans were dried and stored over silica gel in a hermetic plastic box placed at room temperature in the dark. The coffee beans were reduced to a fine powder in an analytical grinder (IKA A15, Germany). The coffee powder was stored over silica gel in a hermetic plastic box at 4 °C. The water content of the powder was estimated after complete drying of 0.2 g

aliquots in an oven at 105 °C overnight. Water content was measured in triplicate using a totally random experimental design.

Fatty Acid Composition Determination. Total lipids were extracted from 2 g samples of dried powder using a modified Folch method with methylene chloride instead of chloroform. Roughly, the bean material was homogenized for 30 s in 10 mL of methylene chloride/methanol (2:1) using an IKA (Staufen, Germany) T25 Ultraturrax prior to filtration onto a glass filter cup (pore size 4). The residue was extracted again with 10 mL of methylene chloride/methanol (2:1) under the same conditions. The filtrate was transferred in a 60 mL glass separatory funnel and washed with 4 mL of 0.73% NaCl solution by vigorous hand shaking. After the resulting mixture had separated into two phases, the lower phase was recovered. All steps were performed at room temperature. Extracted lipids were dried under nitrogen at 40 °C and then dissolved in 1 mL of methylene chloride/methanol (2:1) and stored at –20 °C until further analysis. Fatty acid methyl esters (FAMES) were prepared according to the ISO-5509 standard. Lipid extracts were first saponified with 4 mL of a 0.5 M methanolic solution of sodium hydroxide at 90 °C for 10 min and then methylated with 5 mL of 14% BF₃ methanolic solution at 90 °C for 3 min. GC analyses were performed using an HP 6890 system with flame ionization detection (FID). A Famewax capillary column (RESTEK, France), 30 m × 0.25 mm × 0.25 μm, was used. The analyses were carried out in program temperature mode from 185 to 225 °C at 4 °C/min and then in isothermal mode for 10 min at 225 °C. Helium was used as carrier gas at 40 cm s⁻¹. Both the injector and detector were at 230 °C. FAMES were identified by comparing their retention times with those of the fatty acid methyl ester standards (Supelco) and were quantified as percentages of total FA (w/w). For each genotype–environment combination studied, the fatty acid composition was analyzed in triplicate (from three different lipid extracts).

Statistics. All data analyses were carried out using the Statistica package Version 7 from Statsoft (Tulsa, OK). Analysis of variance (ANOVA) was used to test the effects of the genotype, the environment, the year, and their interactions on the fatty acid composition of the bean. Estimates of variance components were obtained using the variance decomposition procedure. All effects were considered random. Principal components analysis (PCA) and factorial discriminant analysis (FDA) were used to study genotype and location classification. Factorial scores of PCs explaining more than 2% of total variance were used to calculate discriminant function models. Year 1 and year 2 data were used one after another as the training set, the remaining data set being the test set. The relationships between climatic parameters and lipid variables were analyzed by linear regression.

RESULTS AND DISCUSSION

Fatty Acid Composition and Lipid Content. Seed fatty acids were analyzed by GC in 30 genotype × location combinations. Sixteen peaks were identified and quantified in the chromatograms of the corresponding samples. None of the peaks was exclusive to a location, a genotype, or a genotype × location combination. Consequently, only the eight FAs that were quantifiable accurately (Table 1)—i.e. when their relative percentage was higher than 0.5%—were retained for multivariate statistics.

Table 2. Significance and Coefficient of Correlation ($p < 0.05$) between Mean Environmental Factors, Quantified over the Last 5 Months before Harvest and Bean Fatty Acid Composition and Lipid Content, Estimated in 2005, over the Six Different Genotypes Studied^a

	palmitic 16:0	stearic 18:0	oleic 18:1	vaccenic 18:1 n-7	linoleic 18:2	linolenic 18:3	arachidic 20:0	behenic 22:0	lipid content
min temp (°C)	-0.46***	0.79***	0.51**	-0.81***	-0.54***	-0.61***	0.86***	0.71***	0.41*
av temp (°C)	-0.62***	0.82***	0.62***	-0.82***	-0.52***	-0.53***	0.85***	0.74***	0.45*
max temp (°C)	-0.64***	0.79***	0.61***	-0.77***	-0.49***	-0.52***	0.83***	0.76***	0.38*
rel humidity (%)	ns	-0.58***	ns	0.52***	ns	ns	ns	ns	-0.48**
precipitation (mm)	ns	ns	ns	ns	ns	ns	ns	ns	ns
sunlight (h)	ns	ns	ns	ns	ns	ns	ns	ns	ns

^aLegend: ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Overall, bean FA compositions found in the present study are in complete agreement with those reported previously in *C. arabica* (4, 9). Although the experimental design used in the present work was 2 times larger, the range of variation over environments or genotypes of each individual FA was of the same level of magnitude as that obtained previously (9). Bean lipid contents were also in agreement with data reported previously in *C. arabica* (5). The influence of the environment on the bean lipid content was variable among the six genotypes studied (Table 1). It had a marked effect in BGB.1140 and BGB.1033 (min–max difference of about 2.5%), while its influence was only moderate in BGB.1219 and Caturra and even much lower in BGB.1040 and BGB.1076.

Influence of Climatic Parameters on Individual Fatty Acid and Lipid Contents. The influence of six climatic variables (relative humidity, precipitation, sunlight, and minimal, average, and maximal temperatures) on the bean content of each FA was first tested by linear regression by combining data of the six genotypes studied. Mean values of climatic variables were calculated over the last 5 months of seed development, since it has been shown that this is the period when lipids accumulate in the seed (21). The three temperature variables were highly significantly correlated to all FA percentages (Table 2). In contrast, precipitation and sunlight were correlated to no bean FA. Relative humidity had a significant—but limited—influence on stearic and vaccenic acids only. The bean lipid content was correlated to the temperature and relative humidity of the growing environment (Table 2). However, the corresponding correlation coefficients and probability levels were slightly significant.

The slope of the regression equations obtained with temperature variables differed among FAs. A positive relationship between temperature and FA content was observed for stearic, oleic, arachidic, and behenic acids, showing that acyl chain elongation increased with high temperatures. In contrast, linoleic and linolenic acid contents were negatively correlated to temperature, showing that acyl desaturation increased at low temperature. Identical results were obtained when regressions were performed at the level of each individual genotype. In contrast, the bean lipid content was not significantly correlated to any of the six climatic variables tested when analyses were performed at the genotype level ($p > 0.05$). The bean lipid content was therefore not included in further statistical approaches.

Environmental temperature has previously been shown to play a crucial role in the final FA composition of seeds in many cold-tolerant crops such as sunflower, soybean, and rapeseed (13–15, 17) and in the model plant *Arabidopsis thaliana* (22). This role was confirmed here for the first time in a cold-sensitive tree species. Moreover, the response of each individual FA to temperature variations in coffee did not differ from that observed in the cold-tolerant species cited above. For instance, an increase in polyunsaturated FA (18:2 and 18:3) and a decrease in oleic acid with decreasing temperature are mechanisms shared by all these species, independent of their habit and the topography of the area

where they are cultivated. It is therefore plausible that the effects of temperature on bean FA composition are governed by ancestral mechanisms of cold acclimation, which are still functional in most—if not all—plants.

Temperature-mediated modulation of membrane content in polyunsaturated FA is believed to maintain suitable levels of membrane fluidity, thus enabling cell survival across a wide range of growth temperatures (23). Investigations into the mechanisms of temperature-dependent modulation of FA composition in membrane and storage lipids have provided evidence of control at both transcriptional and post-transcriptional levels (15, 24), as well as regulation through the concentration of oxygen in the seed (25) and the stability of FA biosynthetic enzymes (26), which both depend directly on environmental temperature. Interestingly, a recent study demonstrated that in cotton, another cold-sensitive subtropical crop, leaf linoleic acid content also increased at low temperatures, associated with a higher transcription of the gene that encodes the enzyme that converts oleic acid in linoleic acid, $\Delta 12$ desaturase (27).

Although variations in seed FA composition have mainly been related to temperature, significant relationships with other environmental factors, such as rainfall, have also been reported in oilseed crops (28–30). The climatic factors other than the three temperature variables tested here (sunlight, precipitation, and relative humidity) show no or very little significant correlations with percentages of FA in coffee beans. It is not yet clear whether these factors have no influence on seed FA composition in coffee or that the environments tested were not sufficiently contrasted in the case of these parameters.

Discriminant Analysis of Varieties and Locations Based on a 1 Year Data Set. PCA was first used to set up noncorrelated variables that contained the maximum of the initial variance. Factorial scores of the first six PCs, which explained 48.1, 17.9, 14.3, 8.6, 6.5 and 2.7% of the total variance, respectively, were used to calculate the discriminant function models. Significant classifications were obtained when either the genotype or the location was used as the criterion, as estimated by the p value (< 0.001) associated with Wilk's λ coefficient (0.003 and 0.011, respectively). The percentage of correct classifications was very satisfactory whatever the criterion employed: 97% and 86% for locations and varieties, respectively. Two and three significant canonical functions were obtained using the location and variety discriminant models, respectively ($p < 0.05$). The scatter plot of locations presented in Figure 1 was chosen to illustrate the efficiency of bean FA composition for the discrimination of known samples. The first canonical function allowed excellent separation of Tambo, Naranjal, and Paraguaicito sample groups from one another and from a group formed by Rosario and Pueblo Bello samples. The second canonical function accurately separated the two locations that could not be discriminated by the first canonical function (Rosario and Pueblo Bello).

Regarding the first objective of this work, the above results, obtained using a significantly higher number of variety \times location

combinations, therefore confirm that bean FA composition may be of great value for the authentication of traditional varieties and for the determination of coffee *terroirs*. It might have been expected that increasing the number of varieties and locations studied would have led to lower classification efficiency. In contrast, the proportions of well-classified samples were even slightly higher than those obtained previously (79% and 90% for varieties and locations, respectively (9)). It is also worth mentioning that the overall proportion of correct variety classification obtained with FA was higher than that (76%) obtained by Bertrand et al. (7) on the basis of near-IR spectra. Moreover, location classification was nearly as accurate as that obtained with elements, which exhibit outstanding effectiveness for the determination of origin (6, 9), likely due to the fact that the mineral and trace metal composition of beans reflects the varying compositions of the soil in which the plants grow.

Year Effect and Its Interactions with Genotype and Location Effects. In order to assess the stability over years of classifications based on bean FA composition, the effects of the genotype, the location, and the year and estimates of variance components were analyzed by ANOVA and variance decomposition using the set of genotype \times environment combinations common to the 2 years of study (present work and ref 9). This approach has been used in many annual oilseed species (31, 32) but is used here for the first time in coffee.

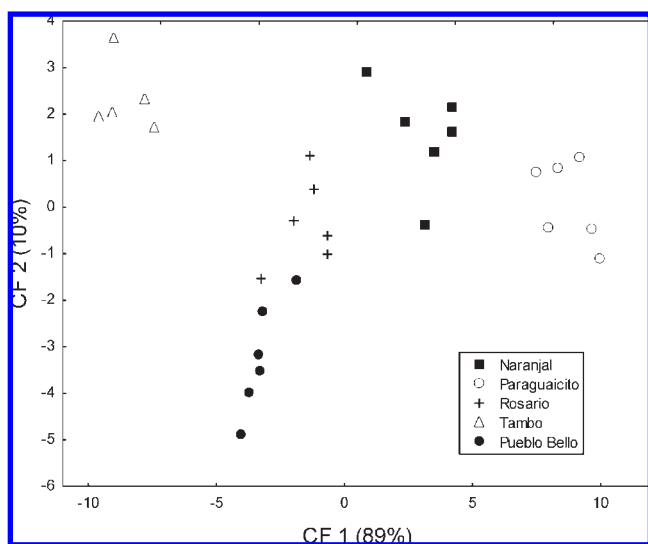


Figure 1. Scatter plot of canonical scores for the first two canonical functions resulting from the discriminant analysis of the five locations studied in 2005 (Naranjal, Paraguaicito, Rosario, Tambo, and Pueblo Bello) based on bean fatty acid composition.

The results obtained for the six major FA (relative percentage higher than 1%) of coffee beans are given in **Table 3**. The year effect was never significant and always accounted for a very low proportion of total variance. In contrast, the genotype always explained a significantly high percentage of total variance, from 21% for palmitic acid to 71% for linoleic acid. The interactions of the genotype with the location or the location and the year always accounted for a low proportion of total variance and were rarely significant. Consequently, in nearly all situations, the ranking of genotypes was independent of the year and the location, as clearly illustrated by the scatterplot of the 24 genotype \times location \times year combinations studied (**Figure 2**). A high genotype effect together with low contributions of $G \times L$ and $G \times Y$ interactions suggest that bean FAs are likely to provide functions enabling discrimination of varieties independent of the year of study.

The effect of the location contributed significantly to total variance (about 50%) for palmitic and stearic acids only (**Table 3**). For these two FAs, the classification of locations did not depend much on the year (data not shown). In contrast, no significant effect of the location was observed for linoleic and arachidic acids, suggesting that these two FAs would not be of great value in determining the location. In the case of oleic and linolenic acids, the location \times year interaction significantly accounted for a high percentage of total variance, while the effect of the location was not significant (**Table 3**). Accordingly, for these two FAs, the classification of the locations depended on the year of study. With regard to the location effect, inconsistent patterns of response were thus observed among the main FA. As regards the achievement of location discriminant functions that remain valid over years, the situation therefore appears less favorable than for the authentication of varieties.

Validation of Discriminant Functions across Years. In order to avoid misleading classification abilities, discriminant functions have to be suitably validated. Because the number of samples studied was not high enough to perform a random sampling of a training set and a test set, a different approach was used: year 1 and year 2 data sets were used one after another as the training set, the remaining data set being the test set. Whatever the criterion of classification—the variety or the location—all known samples were correctly classified.

The authentication of traditional varieties remained very satisfactory when the classification functions were applied to unknown samples: the percentage of correct classifications was 100%, with the exception of one year 1 sample of an introgressed variety, which was misclassified when the year 2 data set was used as the training set. This efficient prediction ability of the variety (traditional versus introgressed) across years is congruent with the high contribution of the genotype to total variance and its limited interactions with other factors, as detected by ANOVA (**Table 3**).

Table 3. Effects of the Genotype, the Environment, the Year, and Their Interactions on Coffee Bean FA Composition (FA Higher than 1% of Total FA): Three-Way Analysis of Variance over Three Different Locations (Naranjal, Paraguaicito, and Rosario), Four Different Genotypes (Caturra, BGB.1033, BGB.1076, and BGB.1040), and Two Years (2003 and 2005) and Estimates of Variance Components (VC) Expressed as Percentages of Total Variance^a

effect	df	palmitic acid		stearic acid		oleic acid		linoleic acid		linolenic acid		arachidic acid	
		MS	VC	MS	VC	MS	VC	MS	VC	MS	VC	MS	VC
year (Y)	1	3.0	2	0.4	1	0.8	0	0.4	0	0.0	0	0.1	1
genotype (G)	3	3.7*	21	2.0*	31	8.5*	53	20.0*	71	0.1*	34	0.2	27
location (L)	2	12.2*	46	5.3*	58	2.9	1	2.4	4	0.1	8	0.3	26
Y \times G	3	0.2	2	0.0	0	1.1**	14	1.7	10	0.0	0	0.0	0
Y \times L	2	2.2**	19	0.3*	6	2.8***	29	0.8	2	0.0***	42	0.1	7
G \times L	6	0.3	3	0.0	0	0.1	0	0.6	2	0.0	7	0.1	15
Y \times G \times L	6	0.1	2	0.0***	3	0.1***	3	0.4***	9	0.0*	4	0.0***	18

^aLegend MS, mean square; df, degree of freedom; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

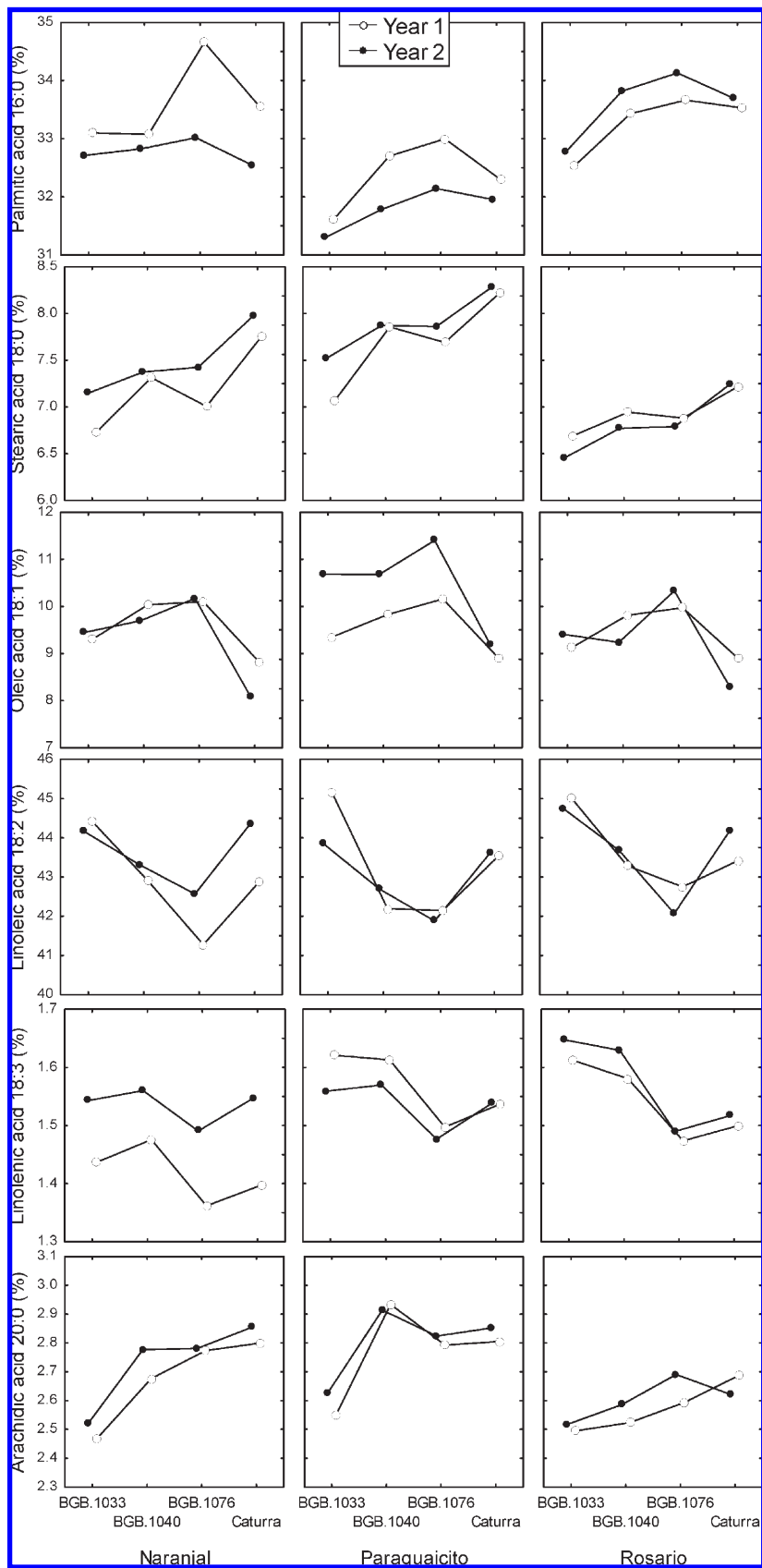


Figure 2. Effects of location (Naranjal, Paraquacito and Rosario), genotype (BGB.1033, BGB.1040, BGB.1076, and Caturra), and year (year 1, 2003; year 2, 2005) on the mean content in the six major fatty acids of beans.

Though the percentage of correct classifications was very high for most locations, the determination of the origin of unknown samples was overall less efficient. For example, Paraquacito

samples were largely misclassified when the year 2 data set was used as the training set. The misclassified samples did not have the same origin, depending on the year used as the training set

(Naranjal and Paraguaicito for year 1 and year 2, respectively), suggesting that functions differed for their main contributors. Since we demonstrated the dramatic influence of environmental temperature during the development of coffee seeds on their final FA composition, one can logically wonder whether climate patterns were stable over years in the three locations investigated. Monthly patterns of the mean average temperature in the period 2003–2005 clearly indicated that environmental temperature may be a pertinent parameter to distinguish *terroirs* since, within a given year, between-location differences remained stable. However, they also show that each year may be characterized by a yearly pattern, which was common to the three locations but differed among years. These two general climatic features provide a sound basis to understand how bean FA provided efficient location discrimination within a single year (e.g., **Figure 1** and ref 9) but only moderate efficiency across years.

In conclusion, validation tests across years revealed that prediction of unknown locations was less efficient than that of unknown varieties. We also showed that the environmental temperature during the development of coffee beans had a dramatic influence on their FA composition. Analysis of climate patterns over years, together with the effect of temperature on the bean FA composition, enabled us to understand the efficient location discrimination within a single year but only moderate efficiency across years.

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Supporting Information Available: **Table S1**, giving agroclimatic characteristics of the five Colombian coffee growing regions studied (altitude, latitude, longitude, annual mean temperature, sunlight, precipitation, and relative humidity of years 2003 and 2005), **Table S2**, giving discriminant analysis of the five locations studied (Naranjal, Paraguaicito, Rosario, Tambo, and Pueblo Bello) based on bean fatty acid composition in 2005 for the probability of significance, as assessed by Wilks's λ and corresponding F value, and classification efficiency, as determined by percentages of correct classification, **Table S3**, giving discriminant analysis of the six genotypes studied (Caturra, BGB.1033, BGB.1076, BGB.1040, BGB.1140, and BGB.1219) based on bean fatty acid composition in 2005 for the probability of significance, as assessed by Wilks's λ and corresponding F value, and classification efficiency, as determined by percentages of correct classification, **Table S4** giving the eigenvalue and χ^2 significance (p) of the significant canonical functions resulting from the discriminant analyses of the six genotypes and the five locations studied on the basis of bean fatty acid composition in 2005, **Table S5**, giving discriminant analysis of known (training set) and unknown (test set) varieties, with year 1 and year 2 data used one after another as the training set, **Table S6**, giving discriminant analysis of known (training set) and unknown (test set) locations, with year 1 and year 2 data used one after another as the training set, and **Figure S1**, giving monthly mean average temperature variations in Paraguaicito, Naranjal, and Rosario in 2003, 2004, and 2005. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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