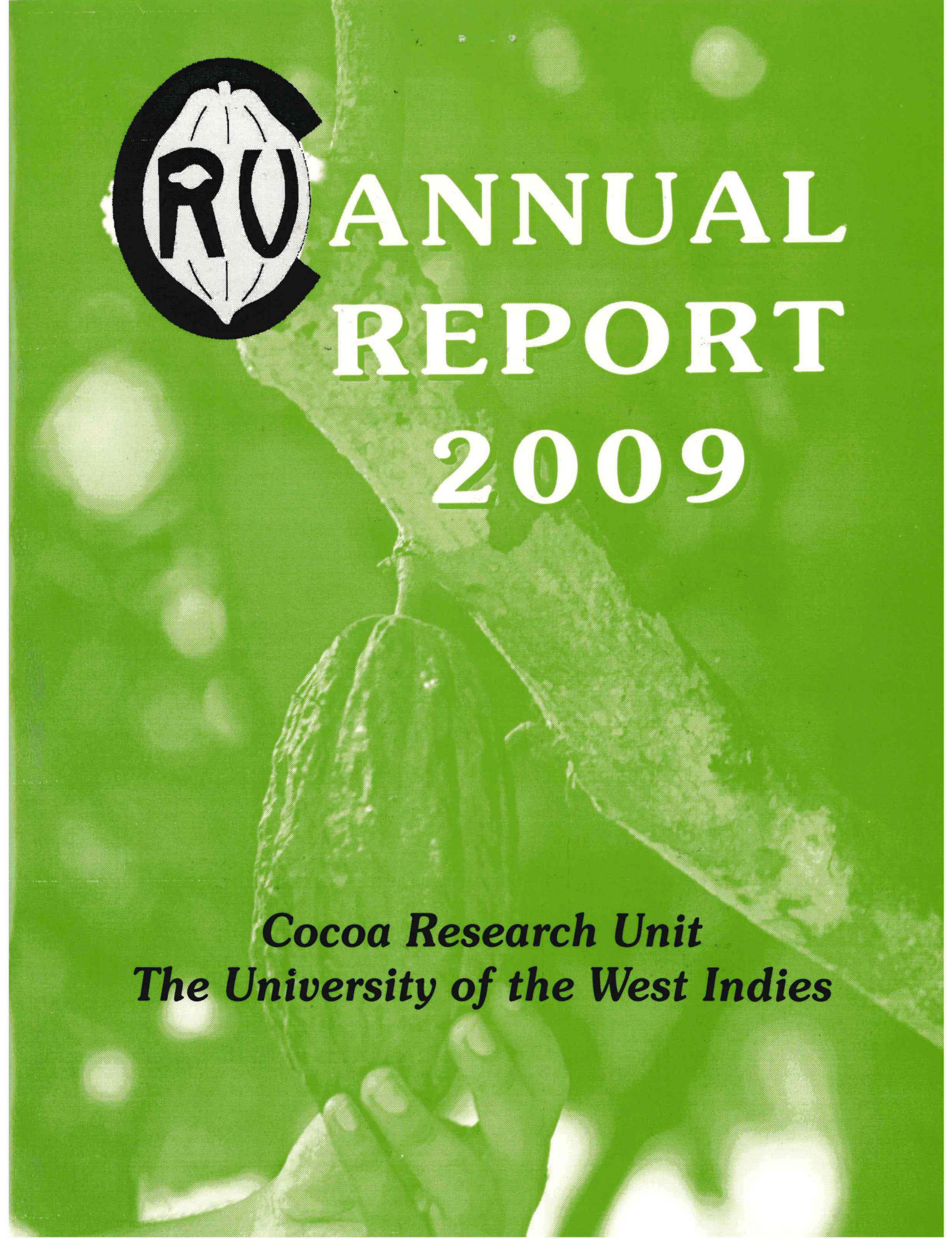

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Cover photograph. Trinitario cocoa pod at the ICG,T being examined by Valmiki Singh

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Preliminary insight into the biochemical composition, aroma volatile fingerprints and sensorial profiles of the Imperial College Selections over two crop years

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Introduction

The Imperial College Selections (ICS) accession group represents the origins of desirable flavour attributes for which Trinitario germplasm has gained its "fine or flavour" reputation and is a historically important and valuable genetic asset. Although ICS germplasm has been distributed globally across continents and used in many international breeding programmes, it still remains under utilised and is potentially a useful source of cacao material with quality traits of economic importance for the global cocoa industry. A project funded by the Dutch Ministry of Agriculture, Nature and Food Quality (LNV) and co-financed by three chocolate companies one in France, one in Switzerland and the other in USA was initiated in 2006 with the following aims:

- to identify ICS accessions that have potentially interesting flavour, near infrared (NIR) and volatile aromatic attributes (via solid phase micro extraction (SPME) coupled with gas chromatographic mass spectrometry (GC-MS));
- to highlight their potential for use in breeding programmes throughout the world.

The benefits of this project therefore accrue to a wide range of stakeholders in the production chain from farmers to final consumers.

This report follows on from Sukha et al., (2009) where we presented first impressions from physical and organoleptic quality attributes of selected Imperial College Selections (ICS) assessed on samples from the first crop year of the project. In that article we indicated that as results from the chemical analyses, such as chemical aroma profiles and polyphenols, become available in this project, these will be linked to the organoleptic results will provide a clearer picture of the potential value of the ICS clones. In this report we are providing a preliminary insight into linkages between the biochemical composition, aroma volatile fingerprints and sensorial profiles of the Imperial College Selections over two crop years.

Materials and methods

Beans were harvested from trees of ICS clones growing in the ICG,T. The beans were all processed according to standardised protocols in the same location; therefore any differences observed would be primarily due to genetic effects and not due to growing environment or processing activities or location.

Preparation of bean and cocoa liquor samples and organoleptic assessment

Micro fermentations were carried out according to Sukha et al. (2008) on 27 ICS clones from the working group, yielding 47 samples (27 samples in 2006/2007 and 20 samples in 2007/2008).

[1] CIRAD UMR QUALISUD, TA 95-B/16 34398, Montpellier cedex 5, France

Preparation of cocoa liquor samples and organoleptic assessments were carried out on 45 samples (25 samples in 2006/20071 and 20 samples in 2007/2008 crop years) according to Sukha et al. (2008) using a trained sensory panel of nine panellists. Liquor samples were tasted blindly over three repetitions. Individual flavour attribute scores were entered into a data template in Microsoft® Excel where mean flavour profiles and the standard errors of the mean (SE) were calculated. Principal Component Analysis (PCA) was performed on the pooled data (from both years) using XLSTAT version 2008.1.01 (Addinsoft, USA). Graphical representations of organoleptic results were carried out in Microsoft® Excel.

NIR assessments

Near infrared spectroscopy (NIRS) acquisitions were obtained on a Foss-Perstorp 6500 analyser using a spin cell. Spectral data were collected and processed using Winisi 1.5 software (InfraSoft International, Port Matilda, USA). A 3 g sub-sample of cocoa taken from 100 g of shelled, ground and sieved beans (0.5 mm) was analysed by diffuse reflectance from 400 nm to 2,500 nm in 2 nm steps. Data were saved as the average of 32 scans and stored as log(1/R) where R is the reflectance at each wavelength and 1 the reflectance of a standard ceramic reference. Spectrum acquisitions were done randomly, each sample was duplicated (two filled cells) and the average spectrum was stored. Statistical analyses were performed using Win-ISI II software and XLSTAT version 2008.1.01 (Addinsoft, France).

Theobromine and caffeine

Wet chemical method

After reflux extraction in water, caffeine and theobromine contents were determined by high-performance liquid chromatography (HPLC) using an Agilent system series 1100 with a UV-VIS diode array detector. The detection and quantification were performed at the maximum absorption wavelength (280 nm).

Near Infrared method

Chemical data and NIR fingerprints of 47 ICS clone samples were added to the CIRAD database (Davrieux et al., 2007) and Partial least square models were used to establish quantitative relations between NIR spectral bands (900 nm to 2,500 nm) and both caffeine and theobromine contents. Calibration statistics include the following parameters: standard deviation of the population, coefficient of determination (r^2), standard error of calibration, and the standard error of cross-validation (SECV). Caffeine and theobromine contents were then predicted for the whole set of samples using the new models. Assignment of samples to type (Criollo-like or Forastero-like) was then based on caffeine and theobromine contents. As part of the calibration development, PCA was used to extract the relevant information from the spectral matrix (n = 47). The generalised Mahalanobis distance (H) was calculated on the extracted PCs for each sample. This statistical distance is useful for defining boundaries of the population and a similarity index between spectra (Schenk, et al., 1996).

[1] There were insufficient quantities of beans from two ICS clones to make cocoa liquors for organoleptic evaluation. Due to sample availability, ICS 15 was also only present for the 2006 – 2007 crop year.

SPME assessments

A 3 g sub-sample of shelled and ground cocoa powder was placed into a 10 mL vial, which was capped with a PTFE/silicon septum. It was heated to 50°C for 15 minutes before the SPME fibre (DVB/Carb/PDMS) was introduced into the headspace surrounding the cocoa powder and left in at the same temperature for 45 minutes. Each extraction was done in triplicate. After every 20 extractions, a SPME fibre control was performed using a mixture of standards, during this control, extractions were done at 50°C with a pre-incubation duration of 15 minutes and an adsorption duration of 30 minutes (Laguerre et al., 2007).

Mass Spectrometer conditions

Mass spectra were registered using an Agilent 6980 gas chromatograph (Agilent Technologies, Palo Alto, CA) coupled with an Agilent 5973N quadrupole mass spectrometer (Agilent Technologies). The volatiles were thermally desorbed from the fibre in the GC-MS injector at 250°C operating in split/splitless mode for 4 minutes. They were quickly transferred to the mass detector through a DB-5MS fused silica capillary column operating at 220°C with helium (2 mL/min) as the carrier gas. The MS source temperature was 150°C, and the mass spectra were scanned in Electron Ionization (EI)+ mode with an excitation of 70 eV. The m/z[1] range used was from 45 to 190 at a rate of 8.17 scans/s. The global signal registered between 2.8 and 8 minutes was transformed by using Pirouette software v 3.1. (Infometrix Inc., Woodinville, WA). The global area was the mean abundance values of the mass fragments recorded between 2.8 and 8 minutes.

Results and Discussion

Organoleptic assessments

The composite average score for each flavour attribute over all samples of the 25 ICS clones revealed that the ICS clones in general terms were balanced in their cocoa and fruity flavour attributes with some acidity and floral notes and a moderate basal astringency. There were no significant crop year effects (data not presented).

Results from the PCA showed that the first three principal components explained 73.1% of the variation in the samples. A PCA plot of the first two principal components for the average flavour profiles over three repetitions for the 45 samples assessed over two crop years (Figure 1) revealed that principal components 1 and 2 accounted for 40.1 and 20.7% of the variation in the samples respectively.

For clarity, the "ICS" accession code has been omitted from the clone names in Figure 1 and the crop years are indicated by the suffix "1" and "2" respectively after the ICS clone number. Figure 1 shows a spread of the ICS clones over the main aromatic flavour attributes (fruity and floral), as well as, cocoa and nutty flavours. No distinct clustering around a particular flavour attribute was observed, however, a minority of samples were associated with astringency, bitterness and raw/beany/green (R/B/G) flavours. Clone ICS 15 was only available from the 2006-2007 crop year and appeared to be an outlying sample but closer examination of the individual average profile scores (data not presented) revealed that a combination of bitterness,

[1] Mass to charge ratio

astringency, cocoa and moderate fruity flavour, more than any specific aromatic attribute, accounted for the uniqueness of ICS 15 from the other samples.

The percentage contributions of the nine different flavour attributes to the first three principal components derived from XLSTAT are presented (with dominant contributions in bold) in Table 1. Nutty, acidity, floral and R/B/G flavours had the highest percent contribution to principal component 1, whilst bitterness, astringency and fruity flavours had the highest percent contributions to the principal component 2. Cocoa, "other" flavours and bitterness contributed most to principal component 3.

Table 1. Percentage contribution of different cocoa liquor flavour attributes to the first three principal components from the PCA analysis. Average flavour scores over three repetitions were used for each of the 25 ICS clones.

	Contribution of flavour attributes to the principal components		
Flavour attribute	**PC 1 (%)**	**PC 2 (%)**	**PC 3 (%)**
Cocoa	8.78	10.78	**29.61**
Acidity	**18.26**	0.04	2.40
Astringency	1.15	**24.91**	6.01
Bitterness	0.29	**27.00**	**20.34**
Fruity	10.55	**17.30**	0.14
Floral	**14.74**	7.22	12.18
Nutty	**21.54**	0.60	0.39
Raw/Beany/Green (R/B/G)	**13.55**	12.10	4.59
Other	11.14	0.07	**24.35**

A summary of the percentage contributions of the different ICS clones to the first three principal components derived from XLSTAT shows that ICS 15 from the 2006-2007 crop year which was the outlying sample in Figure 1 contributed most to principal components 1 and 2 in Figure 1 with 12.8 and 13%, respectively. ICS clones 48, 61, 62 and 65 from the 2007-2008 crop year contributed 10.3, 11 and 12% respectively to principal component 2. ICS 45 from the second crop year made the second highest contribution (18%) to principal component 3. By linking the trends from Table 1 and the percentage contributions of the different ICS clones to the first three principal components we can associate nutty, acid, floral and R/G/B flavours with ICS 15, as well as, astringent, bitter and fruity flavours with ICS 15 (from the 2006 – 2007 crop year) and ICS 48, 61, 62 and 65 from the 2007-2008 crop year. Clone ICS 45, also from the 2007-2008 crop year, was associated with cocoa, "other" flavours and bitterness.

A correlation matrix of the flavour attributes from the different ICS clones (with significant ($P \leq 0.05$) values in bold) (Table 2) revealed that cocoa was significantly correlated with nutty flavour (r = 0.586). Fruity and floral flavours were significantly correlated with acidity (r = 0.573 and 0.490 respectively). Additionally, nutty and R/B/G flavours were positively correlated with "other" flavours (r = 0.585 and 0.482 respectively). Cocoa flavour was negatively correlated (inversely related) with floral flavour (r = -0.674) whilst acidity was negatively correlated with nutty, R/B/G, "other" and cocoa flavours. Bitterness and fruity flavour were also

negatively correlated with each other (r = -0.358).

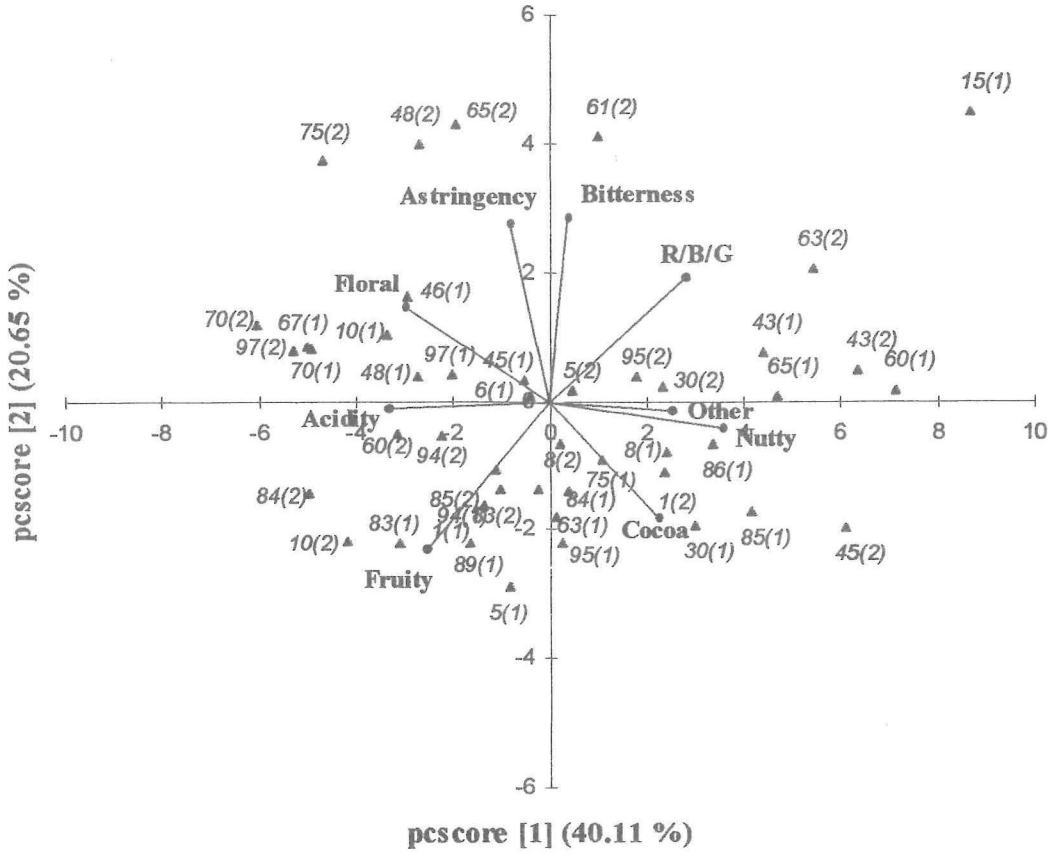

Figure 1. Principal component analysis plot of flavour scores averaged over three repetitions of tasting for ICS clones in the working group from the 2006-2007 (suffix 1) and 2007-2008 (suffix 2) cocoa crop years of the project.

Table 2. A correlation matrix of the flavour attributes from 25 ICS clones presented with significant ($P \leq 0.05$) values in bold.

Variables	Cocoa	Acidity	Astringency	Bitterness	Fruity	Floral	Nutty	R/B/G[1]	Other
Cocoa	1	**-0.309**	-0.144	-0.091	-0.075	**-0.674**	**0.586**	0.087	0.130
Acidity	**-0.309**	1	0.285	-0.051	**0.573**	**0.490**	**-0.598**	**-0.529**	**-0.431**
Astringency	-0.144	0.285	1	**0.366**	-0.136	**0.301**	-0.172	0.125	-0.089
Bitterness	-0.091	-0.051	**0.366**	1	**-0.358**	-0.010	0.054	0.248	-0.159
Fruity	-0.075	**0.573**	-0.136	**-0.358**	1	0.210	**-0.410**	**-0.609**	-0.242
Floral	**-0.674**	**0.490**	**0.301**	-0.010	0.210	1	**-0.618**	-0.273	**-0.313**
Nutty	**0.586**	**-0.598**	-0.172	0.054	**-0.410**	**-0.618**	1	**0.529**	**0.585**
R/B/G[1]	0.087	**-0.529**	0.125	0.248	**-0.609**	-0.273	**0.529**	1	**0.482**
Other	0.130	**-0.431**	-0.089	-0.159	-0.242	**-0.313**	**0.585**	**0.482**	1

[1]Raw/beany/green

Near infrared reflectance spectroscopy (NIRS) assessments

Principal components 1, 2 and 3 extracted from a PCA done on the 47 samples, explained respectively 85.6, 7.0 and 2.1% of total variation in the samples. No sample presented a distance H higher than 3 which indicated that no sample was atypical due to post-harvest process, growing conditions or measurement bias. No significant year effect on spectral fingerprint was observed in agreement with the organoleptic results.

New calibration models for caffeine and theobromine were developed by adding data from these ICS samples to the database previously established in CIRAD. The r^2 of regressions were 0.91 and 0.89 respectively and SECV were 0.048% for caffeine and 0.090% for theobromine. The distribution of the 25 ICS clones according to their caffeine and theobromine:caffeine ratio showed a wide distribution of the clones from Forastero-like to Criollo-like (Figure 2) confirming the expected diversity within these Trinitario clones. When these data were compared to the entire database at CIRAD that includes a wide variety of cocoa types sampled over many years (Figure 3) we see the ICS clones distributed across full range of the Trinitario genetic group. This confirms the efficiency of the relationship between caffeine and the theobromine:caffeine ratio to classify cocoa in the Forastero, Trinitario and Criollo genetic groups.

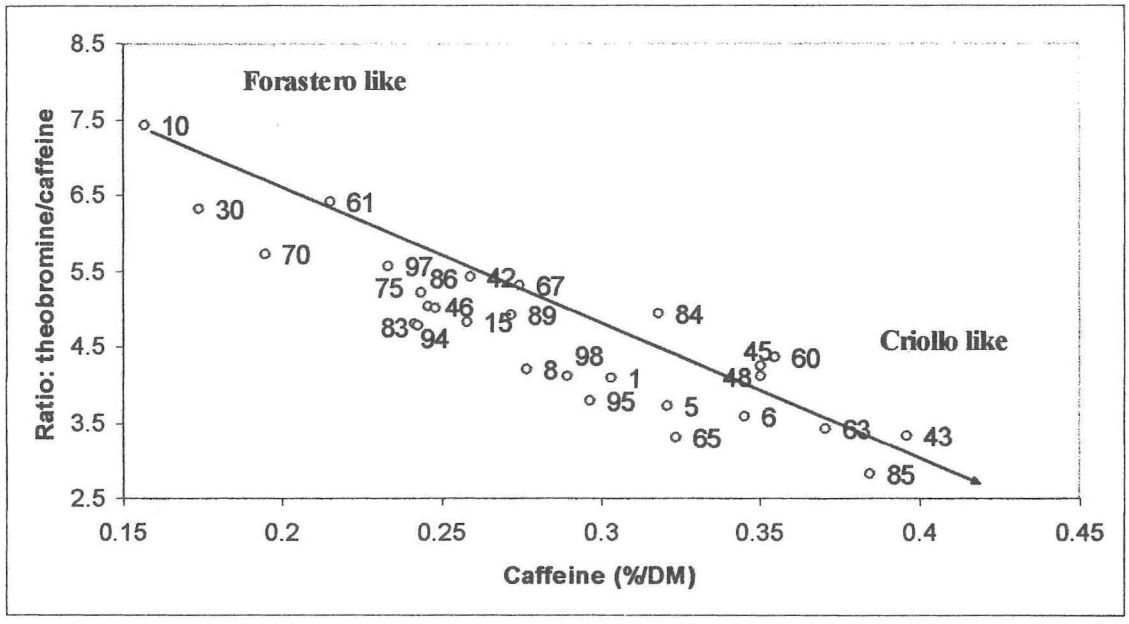

Figure 2. Distribution of 25 ICS clones according to their caffeine content and theobromine:caffeine ratios.

The maximum values observed for the theobromine:caffeine ratio were for ICS 10, 30, 61 and 70, typically indicating cocoa genotypes close to the Forastero group. At the opposite end of the spectrum, ICS 63, 43 and 85 presented caffeine and theobromine:caffeine ratios close to the Criollo group. The remaining ICS clones could be considered classical Trinitario genotypes. The NIR predicted values were year independent which was a satisfactory result that confirmed the

potential of NIR to accurately assess genetic traits related to cocoa quality when robust prediction models are set up on a representative database.

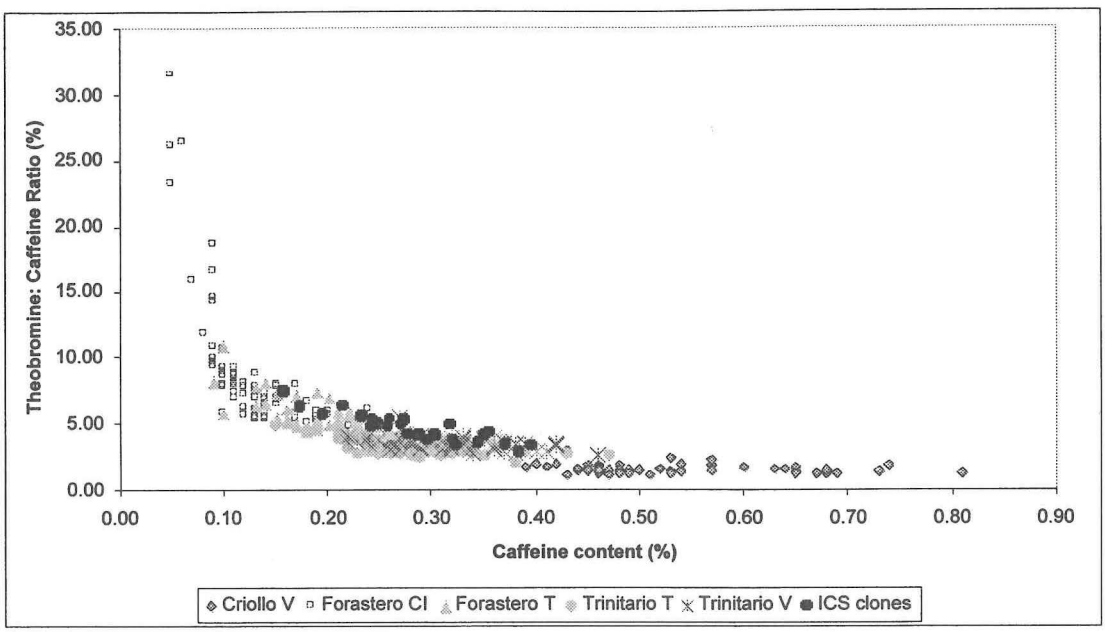

Figure 3. Separation of ICS clones according to their caffeine and theobromine:caffeine ratios among the entire data base of cocoa types analysed in CIRAD.

Solid Phase Micro Extraction (SPME) assessments

Principal component analysis performed on the entire database of 141 volatile fingerprints revealed that the first three principal components accounted for 55.9% of the variance in the data set. Since residual acetic acid at the end of drying contributes to most of the acidity found in cocoa liquors (Jinap and Dimik, 1990 and Jinap, 1994) this could cause discrimination in relation to the fermentation process. To avoid this, the ion m/z 60 (which is linked to the acetic acid content) was excluded from the PCA of volatile fingerprints. Contrary to the sensory and NIR analyses, a significant year effect on volatile fingerprint was observed (data not presented). Consequently, PCA for samples from each crop year separately was performed with 74 fingerprints for the first crop year and 60 for the second crop year. The first three principal components explained 65 and 62 % of the variance for the first and the second crop years respectively.

Some specific ICS clones formed distinct groups based on their volatile fingerprint analysis. For the first crop year, ICS 5, 30, 45, 63, 67, 70, 85, 97 and 98 were grouped; for the second year ICS 5, 10, 30, 65, 70, 85 and 97 were grouped. There were five ICS clones (data not presented) that seemed to have a specific fingerprint since they were discriminated from other clones each crop year.

It was not always possible to find associations between the volatile fingerprints of ICS clones and their sensory results. However clear examples of distinct sensory notes were found, such as ICS 70 with a floral note, ICS 43 and 45 with a nutty note and for ICS 30 with nutty and cocoa

flavour notes. Since the aim of this exercise was limited to differentiation of the ICS clones using different attribute assessments to find similar groups, we did not quantify different volatile compounds. As a result, linkages to specific aromatic compounds were not made; this forms another component of the project.

Nevertheless, the literature shows that cocoa and nutty flavours are developed mainly during roasting via the Maillard (non-enzymatic) reaction (Cros 1996). Flavour precursors such as peptides, free amino acids and reducing sugars derived from cocoa bean acidification and proteolysis participate in this reaction (de Brito et al. 2001 and 2004). Trimethylpyrazine and tetramethylpyrazine compounds noted for their roasted cocoa and nutty flavour attributes are also thermally derived both from roasting and during fermentation and drying from thermally initiated biochemical reactions, or by microbial synthesis (Reineccius et al. 1972 and Sukha et al. 2005). Fruity flavours on the other hand are generally based on the presence of esters derived from organic acids and alcohols which are themselves derived from sugar metabolism of the pulp whilst floral flavour has been linked to terpenes (Ziegleder, 1990; Biehl and Voigt, 1999 and Pino and Roncal, 1992).

The organoleptic and spectral (NIR) assessment results suggested that good diversity exists in the ICS accession group. These trends were also observed in the analysis of volatile fingerprints which showed an effect of crop year on certain volatile fingerprints whilst there was a specificity of ICS clones according to cocoa, nutty and floral flavours. Organoleptically, many of the ICS clones had a dominant fruity flavour characteristic associated with some acidity which can be considered typical of Trinitario beans from Trinidad (Sukha et al., 2008).

Conclusion

The literature does not cite any results, specific to these clones, of screening for organoleptic attributes and chemical attributes linked to aromatic properties despite the global distribution of ICS clones and their use in many international cacao breeding programmes. The impressions from the results presented here suggest that the ICS clones have potential value in terms of diversity within the Trinitario group showing a good range of interesting organoleptic and aromatic traits. The future direction of this work will be to identify specific ICS clones to highlight their potential for use in breeding programmes throughout the world. These results so far confirm that the ICS accession group provides a useful source of cacao material with quality traits of economic importance to the global cocoa industry.

Acknowledgements

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