Application de la spectrométrie Proche Infrarouge à la caractérisation des produits tropicaux
Application au cacao

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Conférence QualiREG 2013

De nombreux progrès réalisés dans le domaine des sciences agronomiques et agro-alimentaires reposent sur la l'analyse quantitative précise de la composition chimique de la matière organique.

Une grande diversité de méthodes a été proposée. Les méthodes traditionnelles sont basées sur des réactions chimiques ou biochimiques menées en laboratoire.

Des méthodes alternatives d'analyse exploitant les propriétés physico-chimiques des échantillons ont été proposées.

Ces méthodes basées sur les interactions entre la matière et les rayonnements électromagnétiques apportent des informations sur la composition chimique des produits.

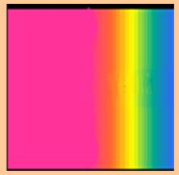
Ces informations sont directement liées à la qualité du produit et à son histoire.

Mesure de l'absorption de la lumière proche infrarouge par un échantillon

Frequency / Energy

\- Radio Micro + wavelengths

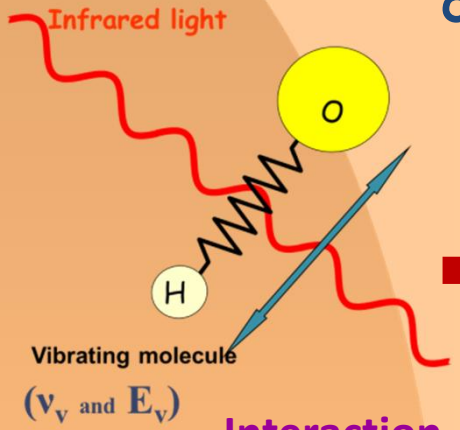
Ultra violet X-Ray -



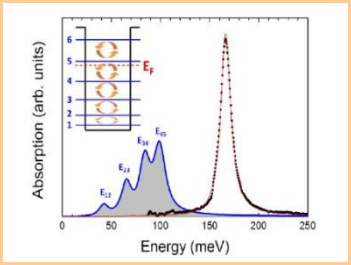
2500 - 800 - 400 nm

Basée sur les propriétés vibrationnelles des molécules et leur interaction avec la lumière.

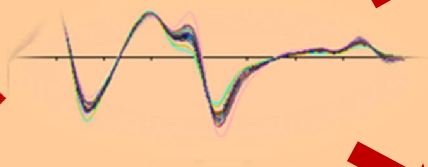
$E = Hv = h\ C/\lambda$



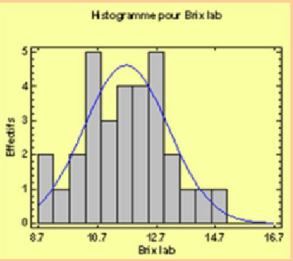
Interaction Absorption



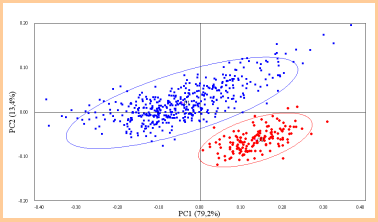
Composés majeurs



Spectres



Quantification



Qualification

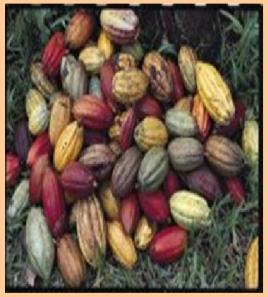
SPIR et Qualité des productions et des produits

- Spectre absorption = empreinte du produit liée à la chimie
- Qualité est liée à la composition chimique (organoleptique, texture, couleur, toxicité, spécificité….
- La composition chimique dépend de l'histoire du produit (de la culture à la commercialisation/consommation)

Cas du cacao

Culture

Récolte



Traitement post-récolte

fermentation

Séchage

Stockage
conditionnement
exportation

Transformation

Qualité et Questions posées

Origine géographique ?
Variétés ?
Niveau de transformation (niveau de fermentation, type de fermentation…) ?
Spécificité (teneur en matière grasse, « fruité », mâture…)
Etat sanitaire (moisissures, attaques d'insectes…)

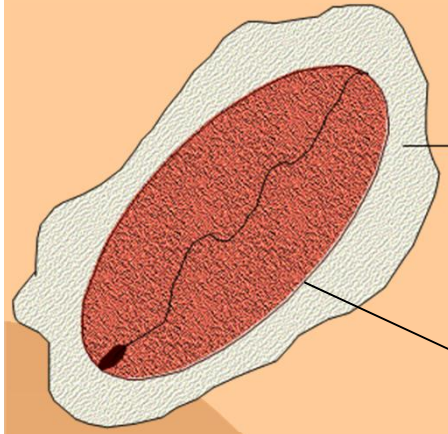
En fait un « panier » d' **attributs** pour une **qualité** définie pour un **produit**

Hypothèses :

- Il existe des « marqueurs » des attributs de qualité
- Ces marqueurs sont interprétables comme des empreintes digitales
- Des combinaisons de méthodes analytiques permettent de caractériser et d'interpréter ces empreintes
- On peut identifier les déterminants de ces attributs.

Cocoa fermentation Characterization

Clotilde Hue, Audrey Berghounou, Iván Samaniego, Fabrice Davrieux

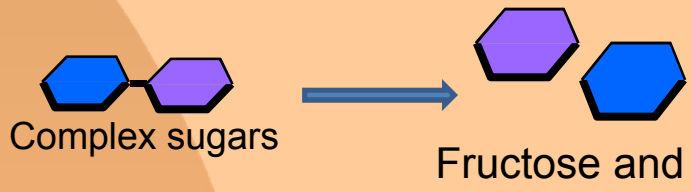


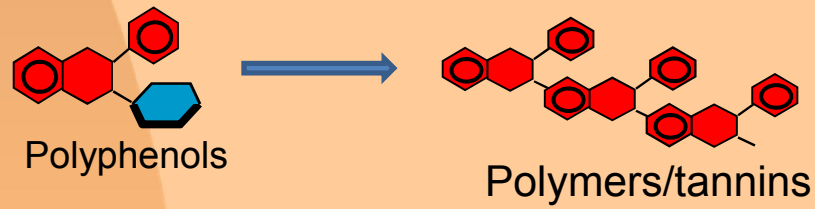
Pulp (Water, Citric acid, Pectin, Sugars)

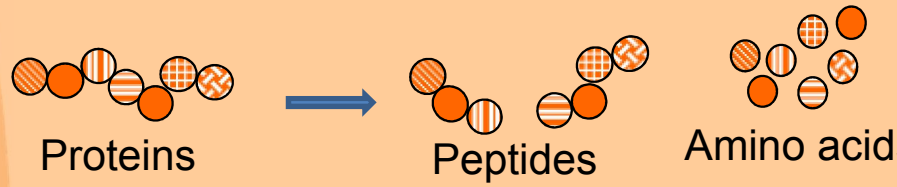
Cotyledon (Proteins, Fat, polyphenols)

In the Pulp, alcoholic fermentation then acetic, highly exothermic. Elimination of the pulp.

In the cotyledons. Penetration of acetic acid, death of the germ. Biochemical and enzymatic reactions.







?

Accumulation of NH_3

Material and methods

- 190 cocoa samples were prepared
- 6 origins, 7 fermentation stages, sun-dried

Cameroun, Ecuador, Ghana, Indonesia, Madagascar, Dominican Republic and Trinidad.

Fermentation stages: 0 days to 6 days, one sample every 24 hours
At least 7 samples per fermentation batch.

- Dried samples were shelled ground and sieved (<0,5 mm)
- NH_3 quantification by Conway traditional method

Ammoniac liberation by K_2CO_3, Fixation by boric acid, Titration by H_2SO_4

- Polyphenols (procyanidins) quantification by HPLC/UV detection
- **Near Infrared Spectroscopy**

Unshelled, ground samples
FOSS 6500 spectrometer.
Diffuse reflectance from 400 to 2500 nm.

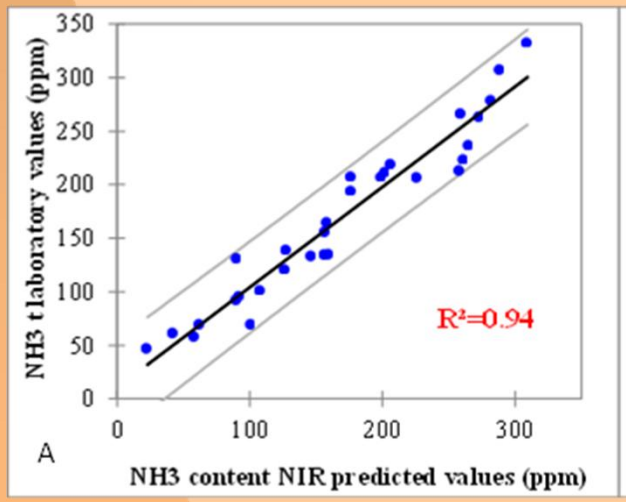
NH_3: Results / Calibration

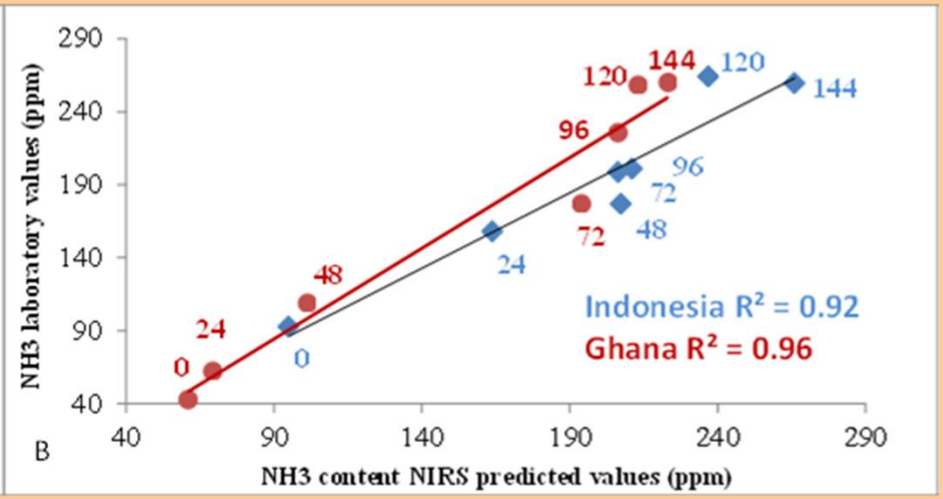
Two samples sets: 160 for **calibration**, 30 samples for **validation**

14 samples corresponding to 2 fermentation batches originated of two independent geographical origins and 16 samples randomly selected

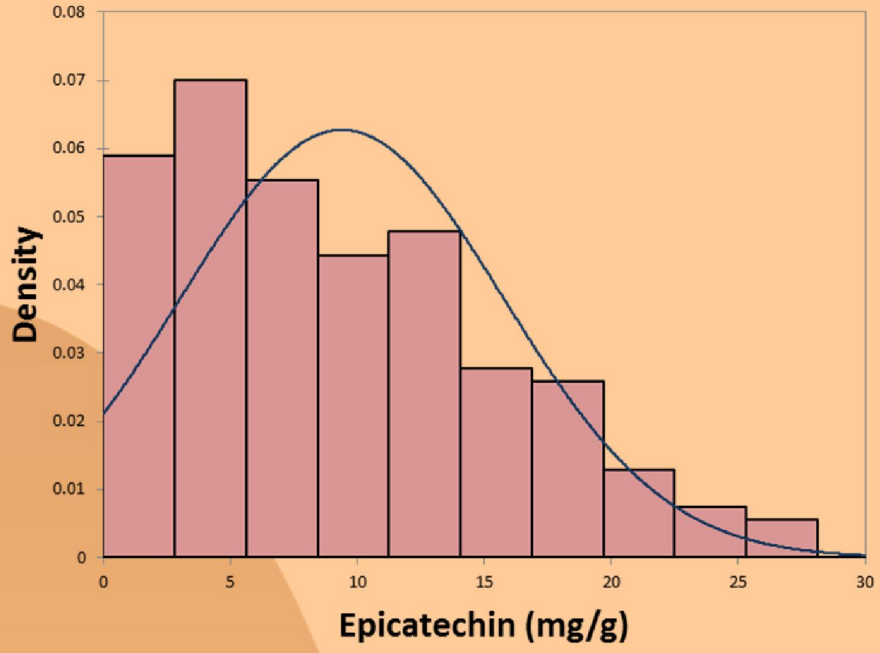
NH_3	N	Mean	Range	SD	SEC	R^2	SECV	Terms PLS	Outliers (N)	SEL
Calibration	154	183	25-441	102	16	0,975	24	13	6	6
	N	Mean	Range	SDval	**SEP**	slope	R^2p	**RPDp**		**SEL**
Validation	30	169	46-332	78	**20**	0,938	0,935	**3,9**		**6**

Validation: whole set and Indonesia and Ghana samples



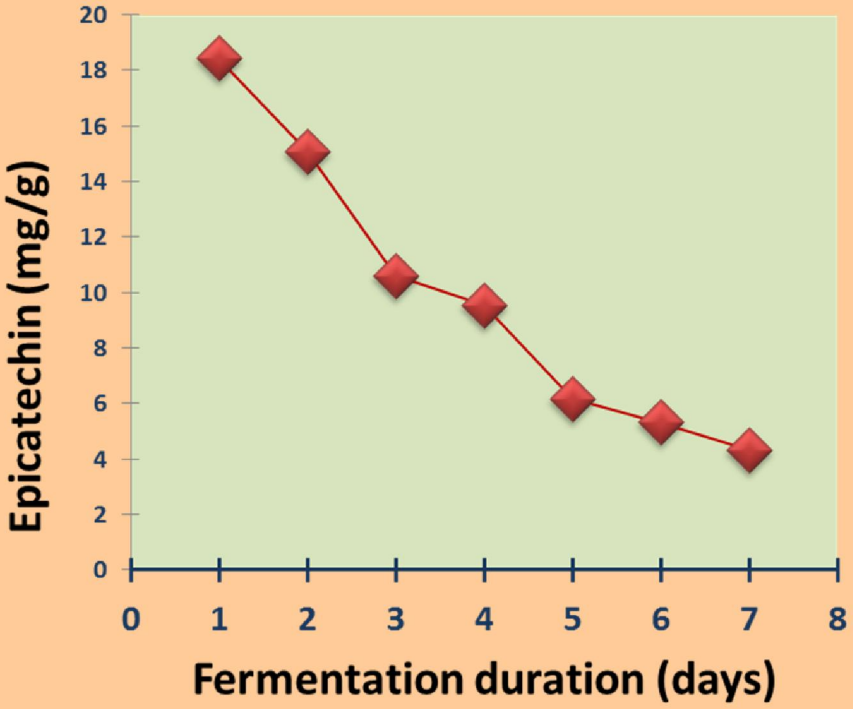


Polyphenols : Results /wet chemistry

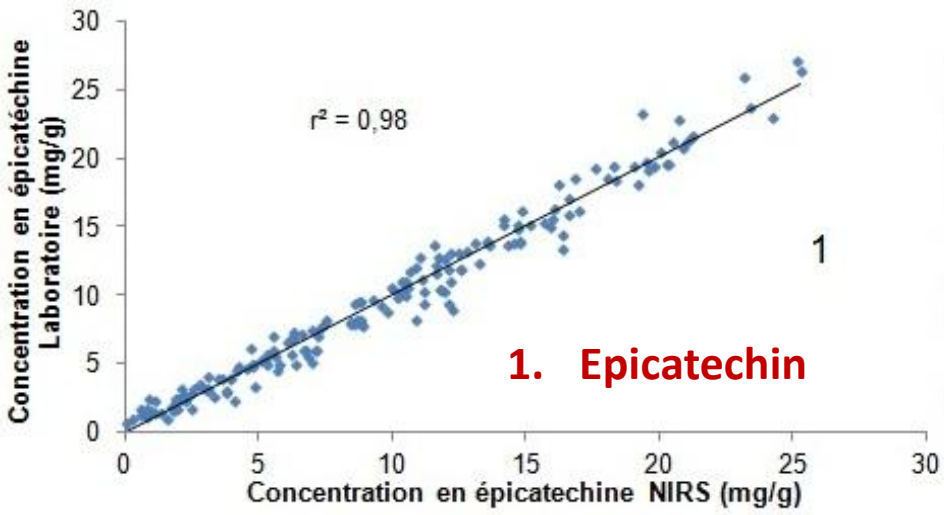


Histogram: Epicatechin

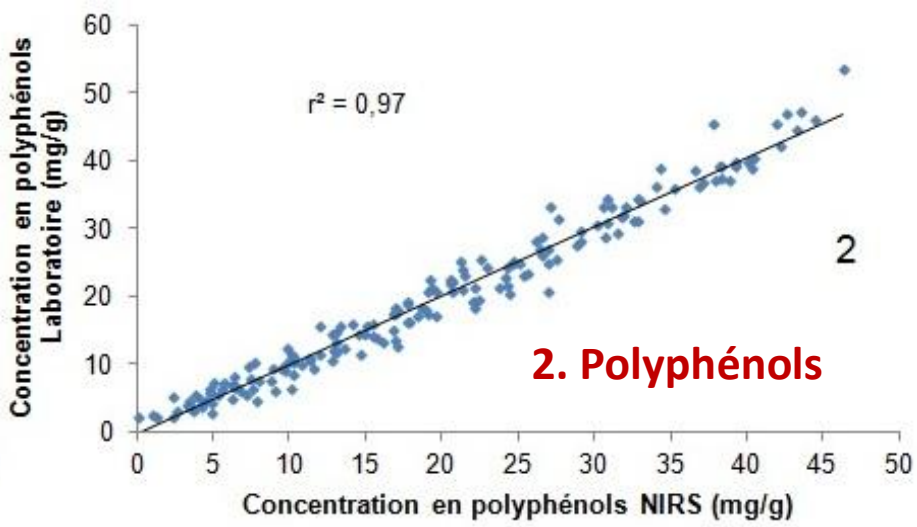
Evolution as a function of fermentation duration



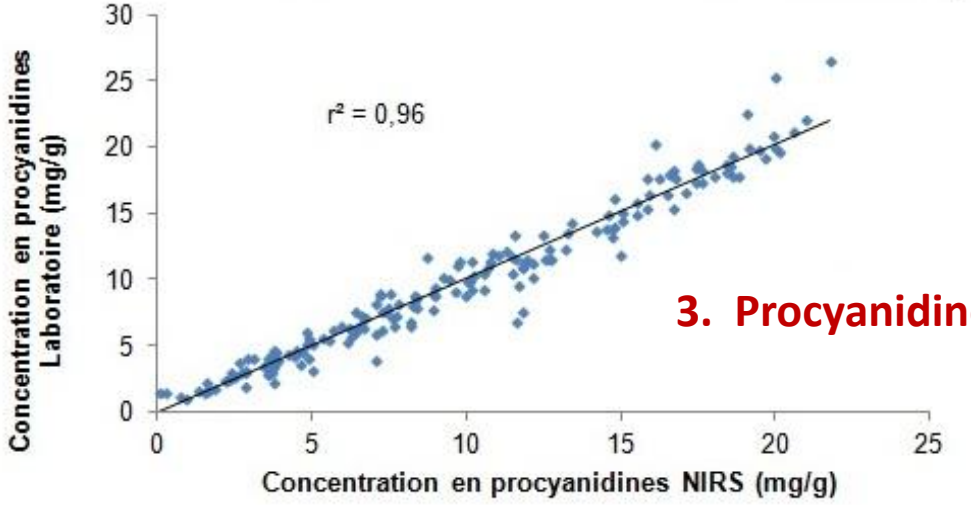
Polyphenols: Results / Calibration



1. Epicatechin

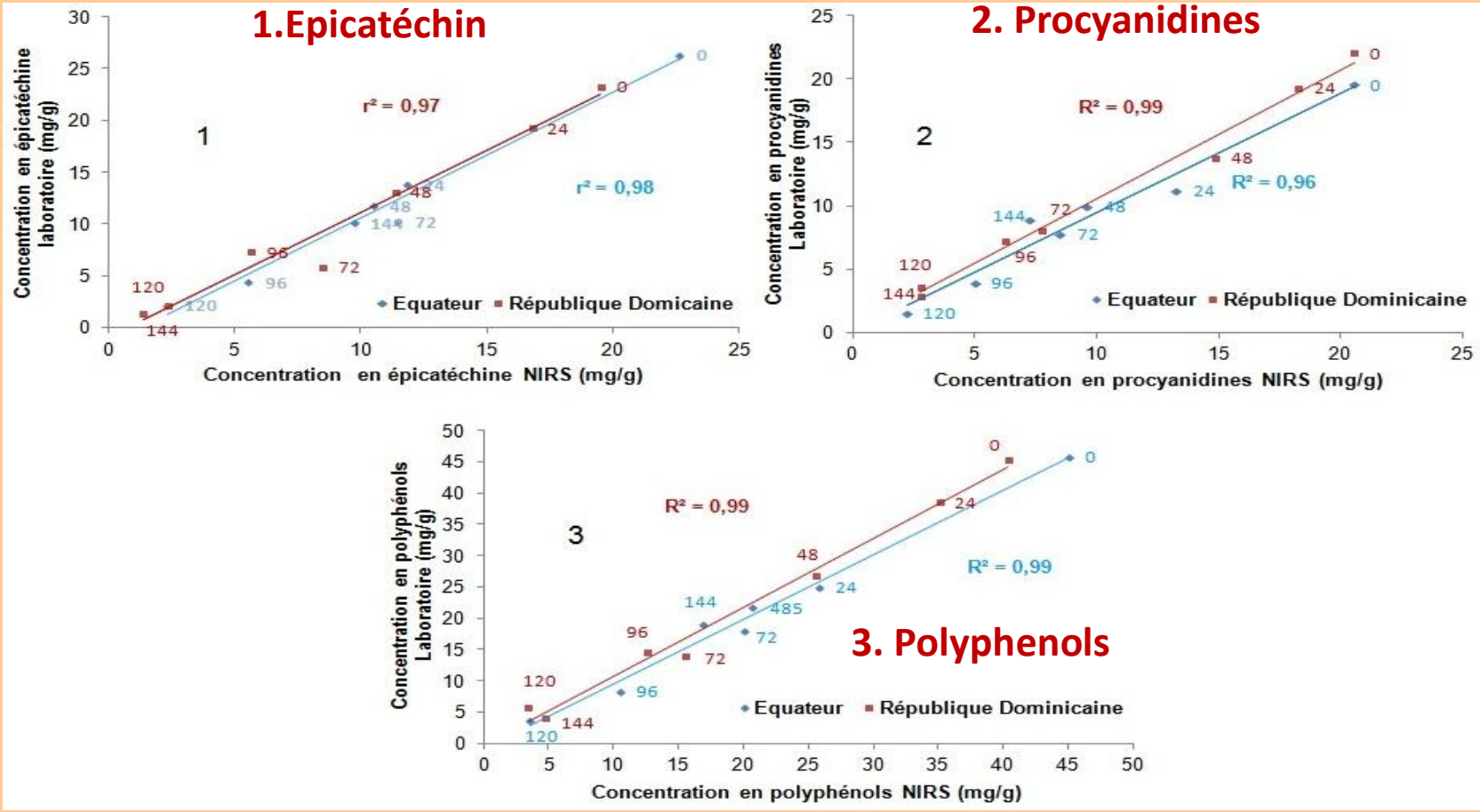


2. Polyphénols



3. Procyanidines

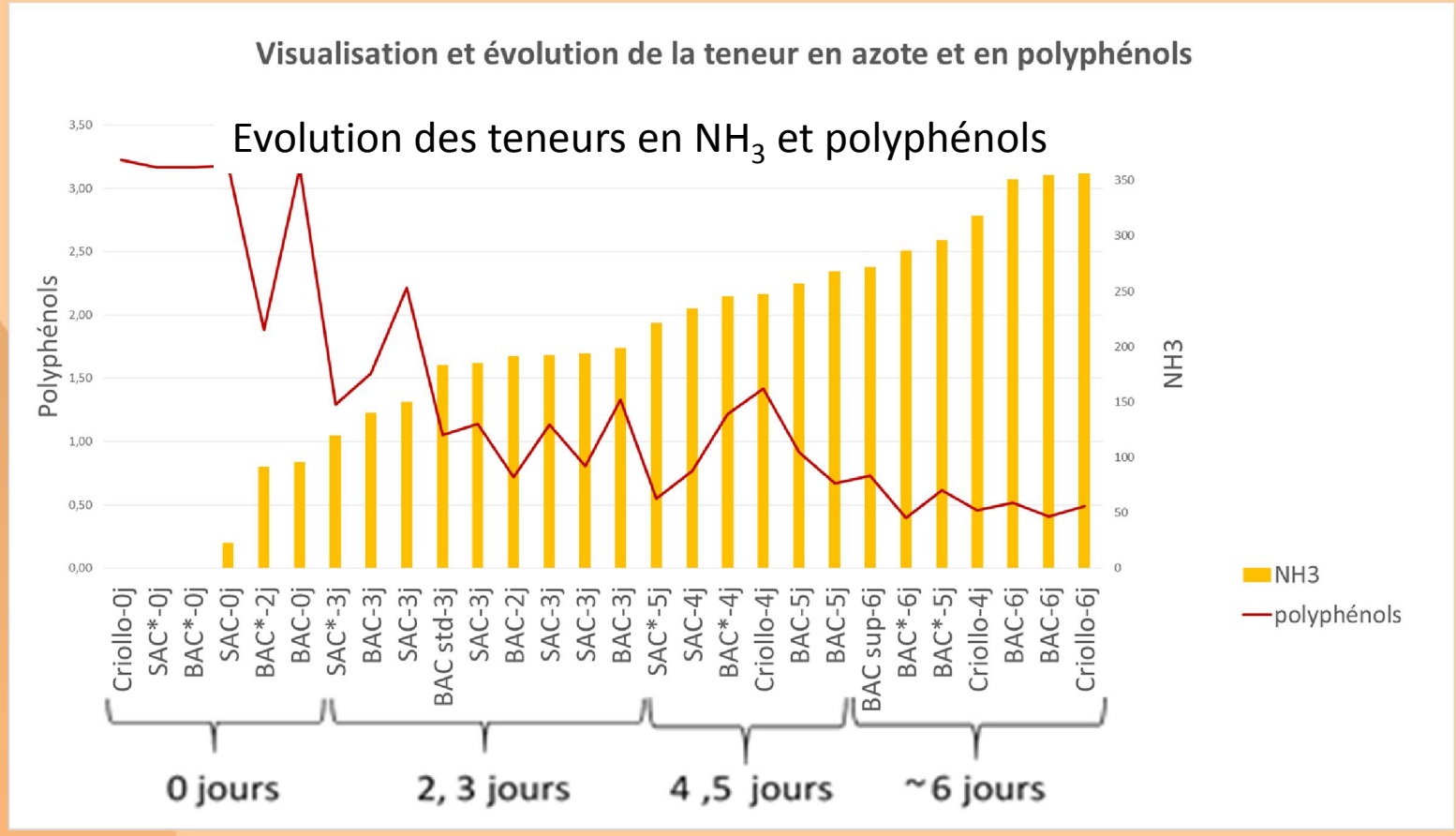
Validation: 2 independent fermentation batches



Standard errors of prediction (SEP) were respectively 1,98, 1,08 et 2,18 mg/g for Epicatechin, procyanidins et polyphenols

Caractérisation et éléments de différenciation des cacaos dans la vallée du Sambirano à Madagascar

Frédéric Descroix, Elisa Bousquet, Mathilde Hoarau, Fabrice Davrieux, 2013



PROJECT CACAO CCN51 VS NACIONAL

Objective: genotypes discrimination between CCN51 and Nacional

Nacional, Ecuador

CCN51
(Coleccion Castro Naranjal,)

	Cocoa Type		
Harvest Year	CCN51	Nacional	Total
2009	105	190	295
2010	117	142	259
2011	55	57	112
Total	277	389	666

666 samples were collected over 3 years in 6 different areas

	Cocoa type		
Province	CCN51	Nacional	Total
Azuay	15	14	29
Bolivar	54	67	121
Cotopaxi	22	23	45
El Oro	21	20	41
Guayas	58	112	170
Los Rios	103	149	252
No Indication	4	4	8
Total	277	389	666

DISCRIMINATION

Learning set and validation set

In order to test the discrimination the sample set (n = 636) was separated in two sub-sets: learning set and validation set.

To be representative of original repartition per year of the 2 genotype, **30% of the samples were selected** randomly per year for both genotypes.

191 samples (81 CCN51 and 110 Nacional) were selected as **validation** samples

The remaining **445** samples (186 CCN51 and 259 Nacional) were used for **calibration.**

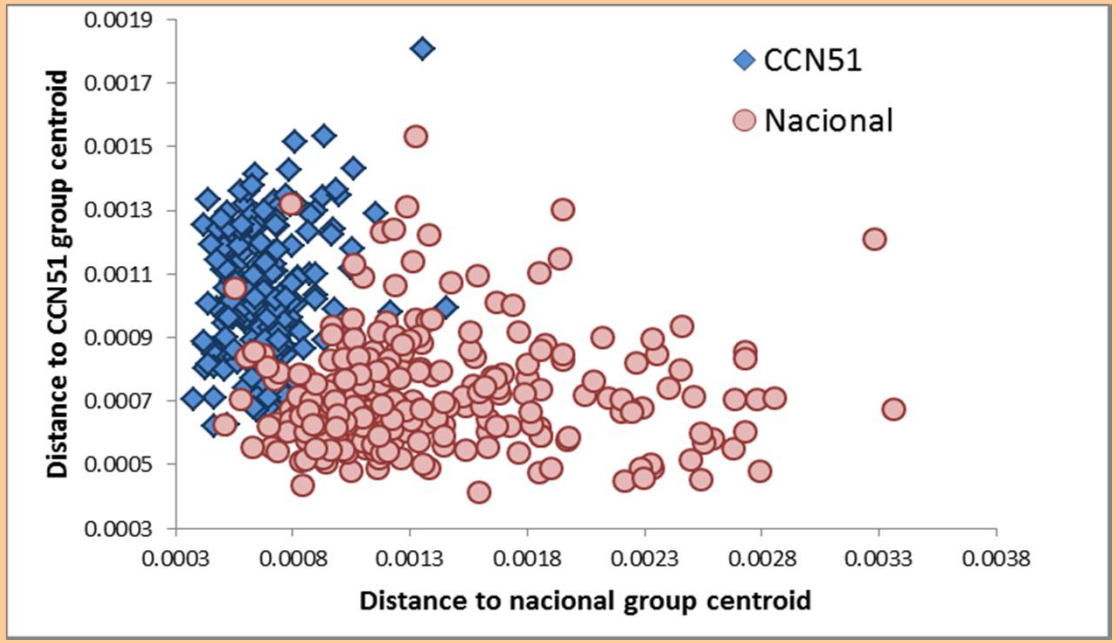
Harvet Year	Sample Set	CCN51	Nacional	Sub-total	Total	Percentage
2009	calibration	67	120	187		70.0%
	validation	29	51	80	267	30.0%
2010	calibration	81	99	180		70.0%
	validation	35	42	77	257	30.0%
2011	calibration	38	40	78		69.6%
	validation	17	17	34	112	30.4%
Total		267	369		636	

DISCRIMINATION

The SIMCA procedure applied on mean centered values ,with a probability threshold of 0,95. Nine components were retained for each group.

	CCN51	Nacional	No Match	total	Rate	Error
CCN51	176	10	0	186	94.62%	5.38%
Nacional	15	244	0	259	94.21%	5.79%
				445	94.38%	3.37%

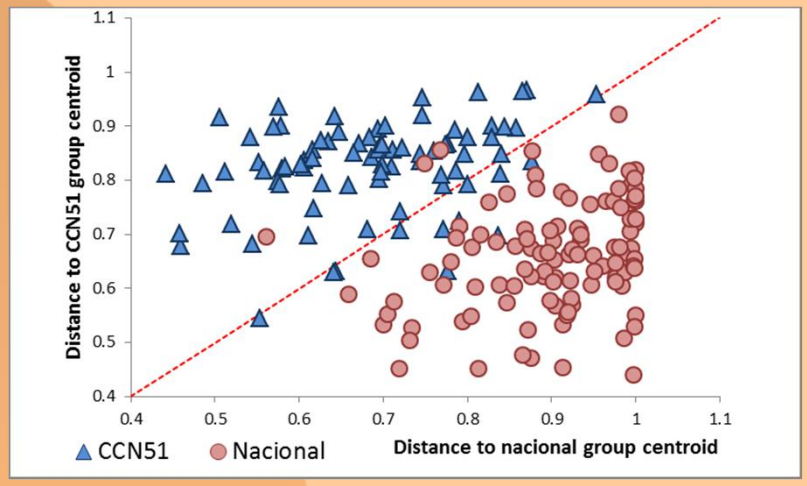
The classification rate for learning set was 94,38%: 10 CCN51 and 15 Nacional were misclassified



DISCRIMINATION

Validation

	CCN51	Nacional	No Match	total	Taux	Error
CCN51	74	6	1	81	91.36%	7.41%
Nacional	3	107	0	110	97.27%	2.73%
				191	94.76%	2.09%

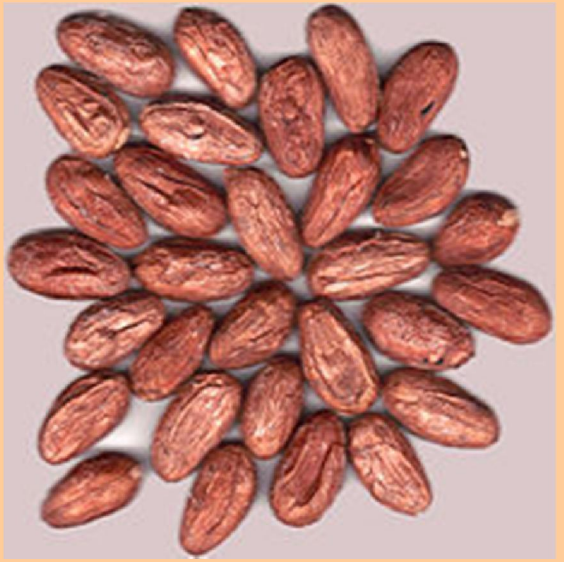


The correct classification rate was 94.76%.

The error was about 2%, with only 9 samples out of 191 misclassified

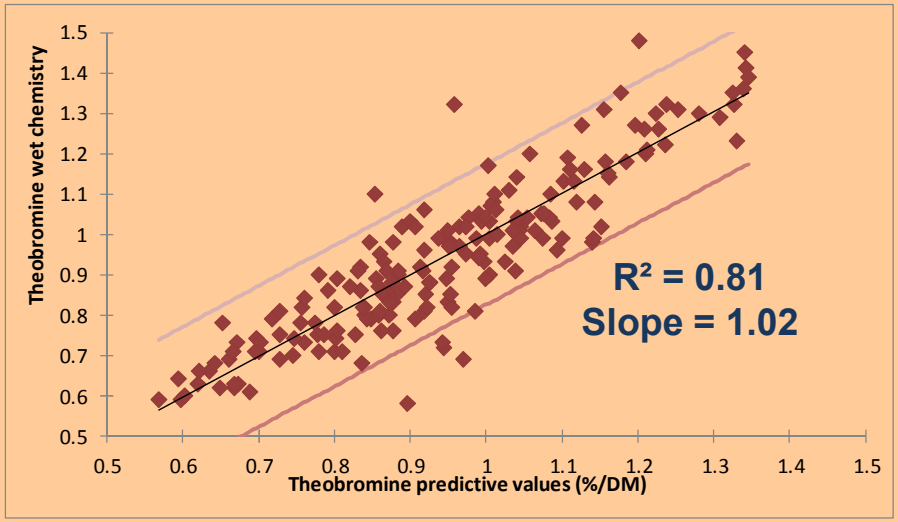
CHARACTERIZATION OF COCOA CLONES FROM DIFFERENT ORIGINS USING PURINE CONTENTS PREDICTED BY NIRS

F. Davrieux, S. Assemat, R. Boulanger, D.A. Sukha, B. Eskes, D. Paulin, E. Cros

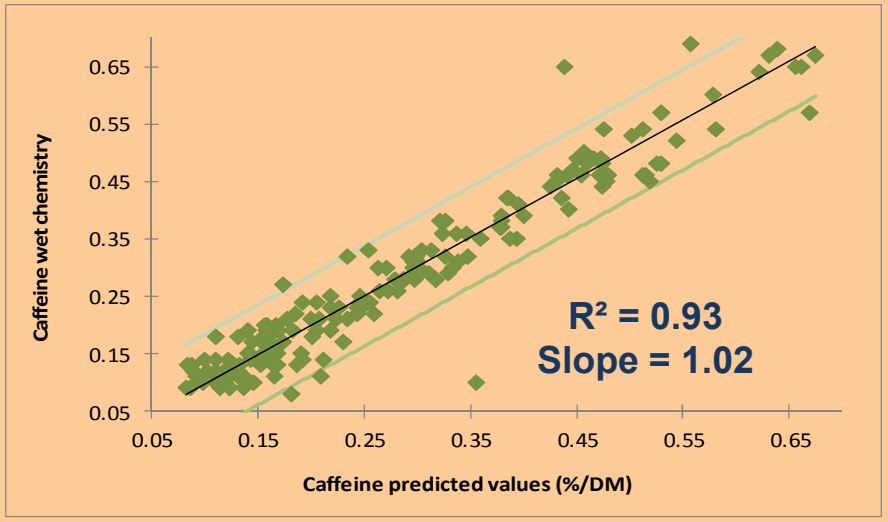


Cocoa: Caffeine and theobromine quantification

constituent	N	mean	SD	SEC	R^2	SECV
Theobromine	182	0.94	0.19	0.07	0.86	0.08
Caffeine	179	0.26	0.15	0.03	0.96	0.04

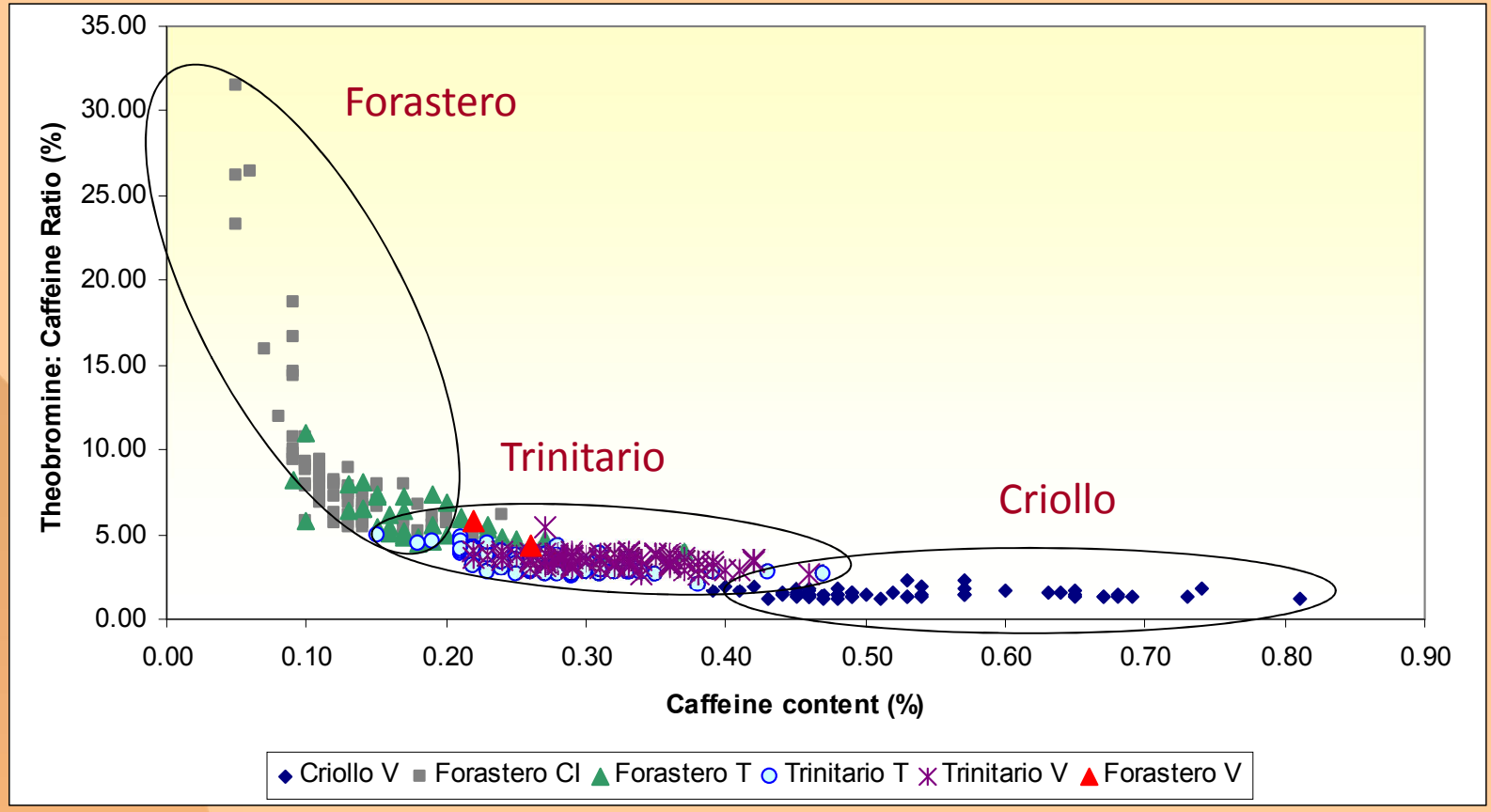


Theobromine



Caffeine

NIR and Purines data base: more than 322 cocoas



A strong relationship between cacao genotype and their relative caffeine and theobromine contents.

Plant Material

10 clones were collected in seven countries:

Brazil, Equator, Ghana, Côte d'Ivoire, Venezuela, Trinidad, and Papua New Guinea

The sampling period covered 3 cocoa crop years

116 samples were collected

All the cocoas were unfermented, to avoid any fermentation effect, and sun dried

Clone	Genetic assessment	Country of origin
AMAZ 15 /15	F-UPA	Peru
EET 59	F	Ecuador
GU 255 /V	Guiana	French Guiana
ICS 1	Trinitario	Trinidad
MAN 15 /2	F	Brazil
MOCORONGO	F-LA	Brazil
PA 107	F-UPA	Peru
PLAYA ALTA 2	F-LA	Venezuela
SCA 6	F-UPA	Peru
VENC 4 /4	F-LA	Venezuela

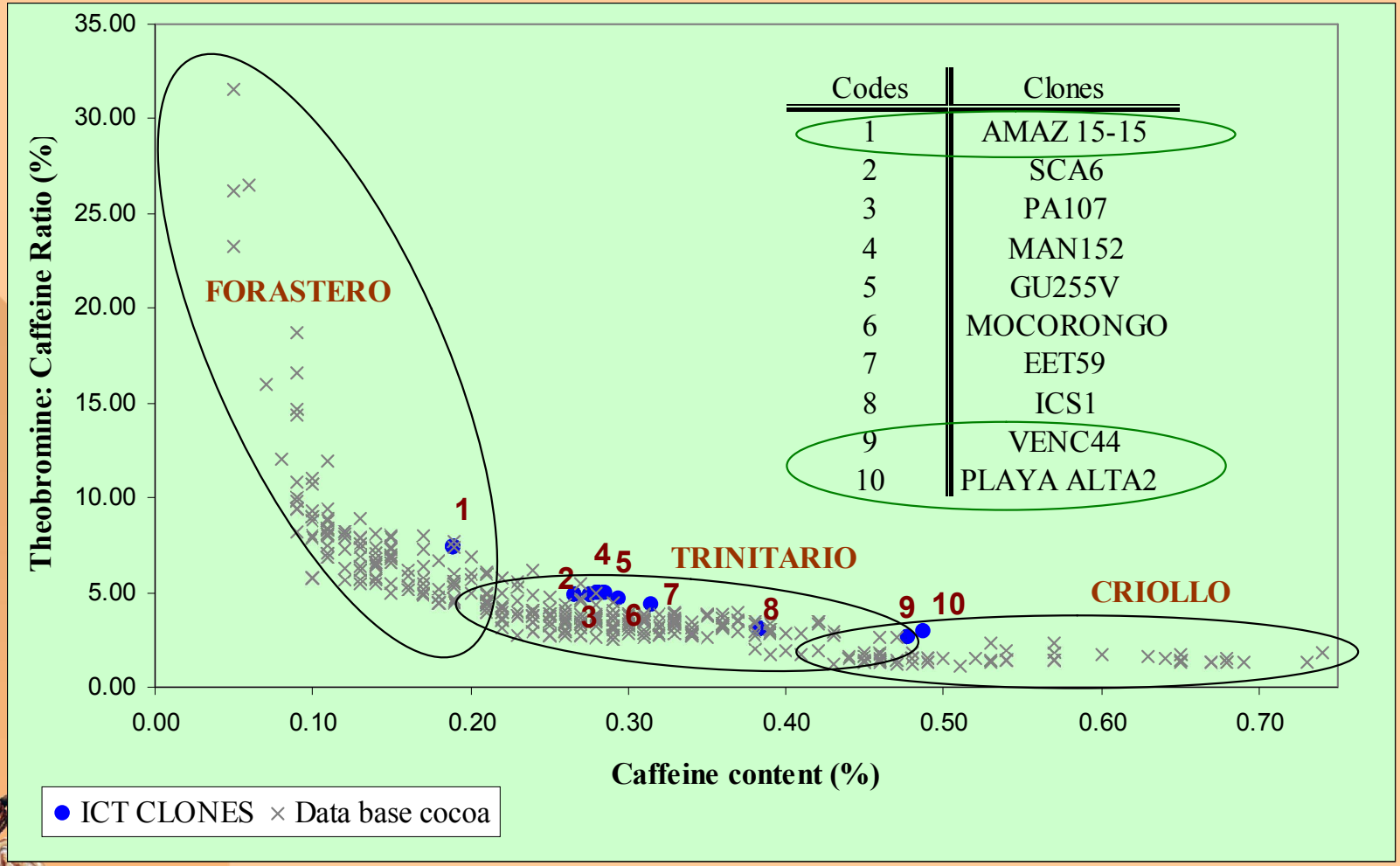
F: Forastero

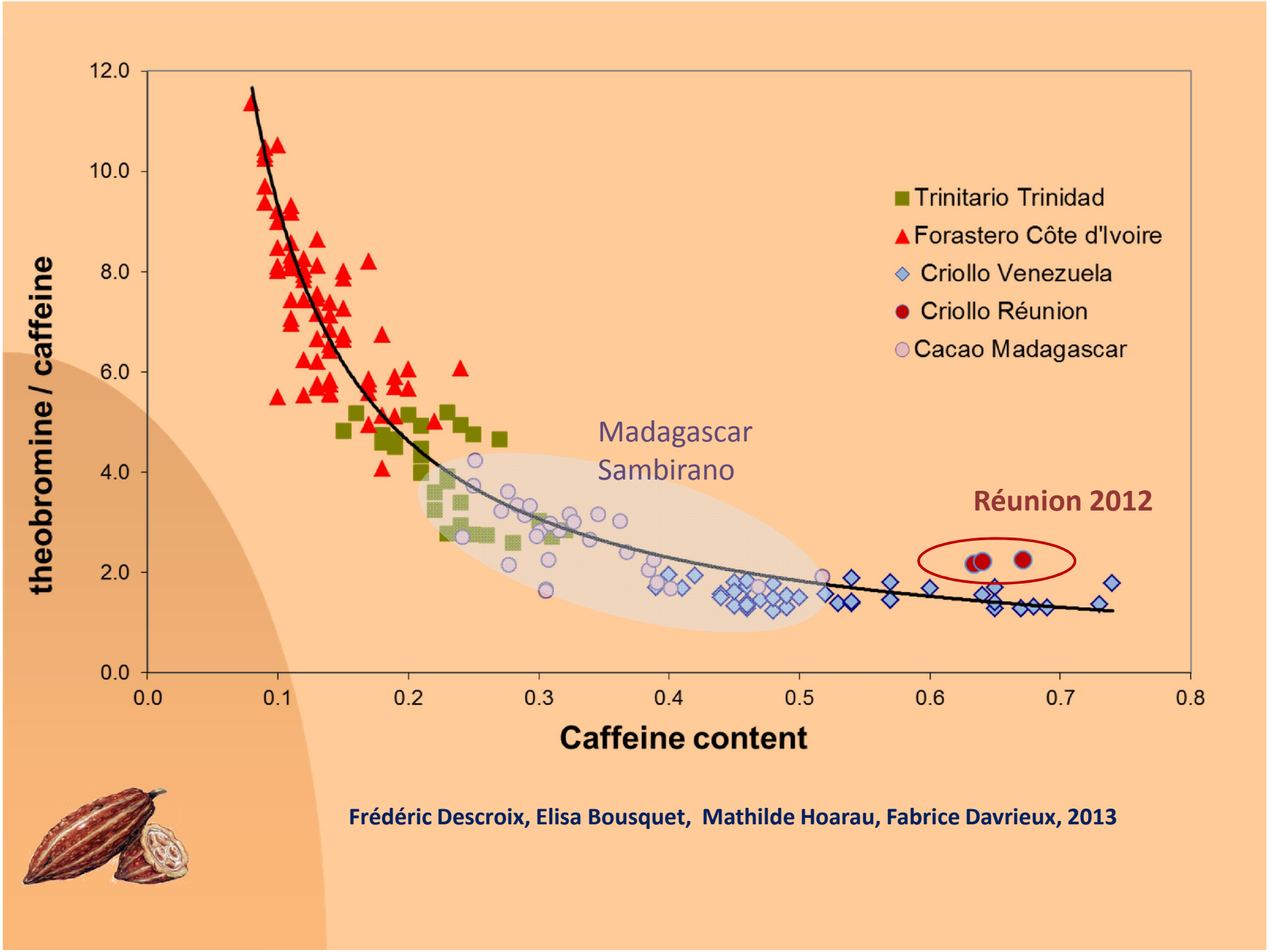
UPA: Upper Amazonian

LA: Lower Amazonian

RESULTS

ICT clones projections on CIRAD cocoa data base





Frédéric Descroix, Elisa Bousquet, Mathilde Hoarau, Fabrice Davrieux, 2013

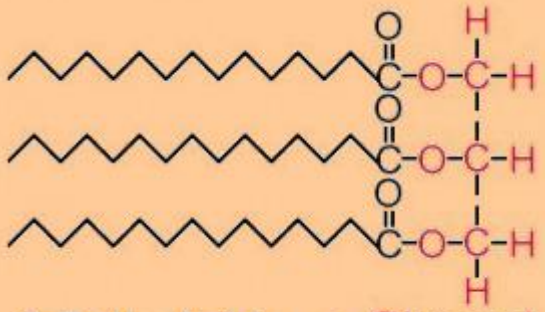
Cocoa beans quality through Free Fatty Acids (FFA) quantification

Insects damages (ephestia kuehniella)

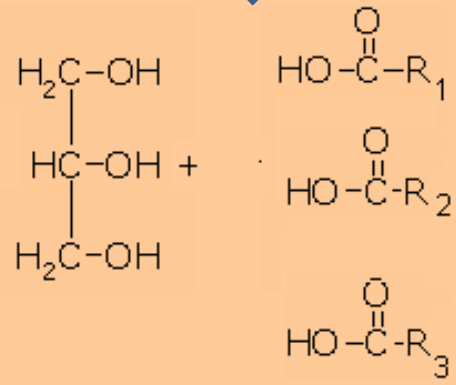
Drying, Storage, Handling

SHELL DAMAGES AND BROKEN BEANS

Triglycerides hydrolysis (lipases)

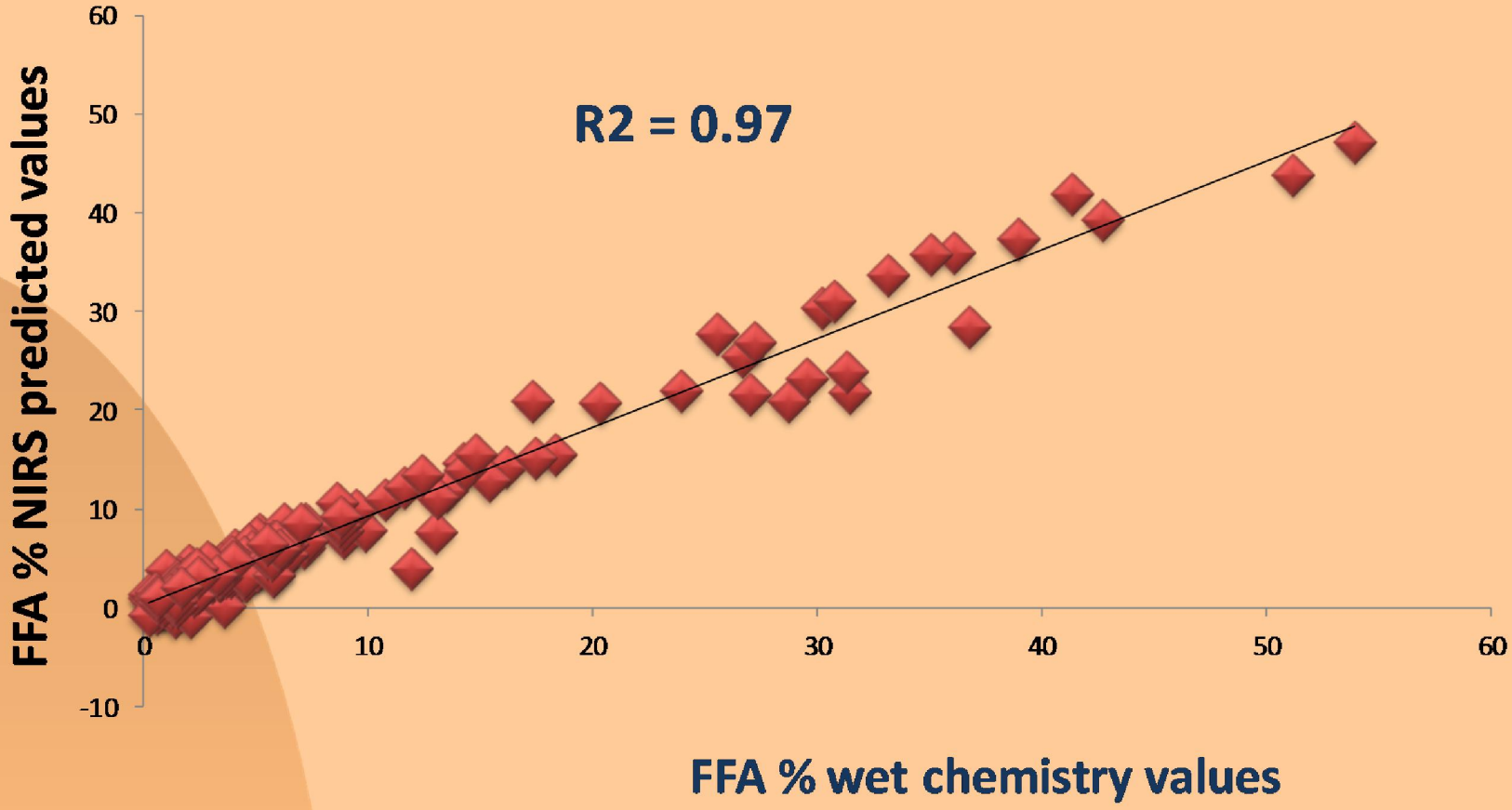


3 Fatty Acids + Glycerol



glycerol free fatty acid

Cocoa Free Fatty Acids (FFA) quantification



Standard Error of Cross Validation (SECV) = 1,07%

FFA financial Risk level > 3 % of Fat

55 samples in validation set

False Positive	Nirs value < 3%	« real » value > 3%	N = 2
False Negative	Nirs value > 3%	« real » value < 3%	N = 3

→ 90.9% of samples correctly classified (< 3%)

→ 3,6% of samples miss classified with loose of product

Conclusion

La spectroscopie proche infrarouge est un outil efficace pour caractériser la qualité d'un produit.

Cette caractérisation peut être appliquée à toutes les étapes d'élaboration du produit

Il est possible d'identifier des bio-marqueurs reliés à une qualité donnée

Il est possible de trouver les déterminants de ces bio-marqueurs

Thank you for our kind attention