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Catalogue of cacao clones

selected by CATIE for commercial plantings

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Contents

Introduction

The growth of cacao production in Latin America is limited by the serious impact of diseases and poor performance of many plantations due to genetic and management reasons. The use of improved varieties in combination with appropriate agricultural practices would help to increase the production and control diseases in ways that are more effective, long lasting and economically and environmentally friendly. This has a particular importance for Latin America where cacao is often planted by small farmers of limited means, sometimes located in areas that are very isolated or very sensitive to environmental changes. Improved varieties could increase the living standards of these producers, which will consequenty contribute to a more stable supply of cacao for the industry, a win-win situation for producer families, chocolate manufacturers and ecosystems (Guiltinan and Maximova 2002).

CATIE's Cacao Genetic Improvement Program (hereafter referred to by the Spanish acronym PMG) has created improved varieties using the extensive genetic diversity contained in its International Germplasm Collection (IC3). The last 30 years of research have resulted in identification of moniliasis (or frosty pod rot) tolerant-clones with distinct genetic and/or geographic origins. These clones are being crossed progressively to obtain varieties with increased levels of resistance, thus exploiting the predominantly additive character that this trait has in cacao (Cervantes-Martínez *et al*. 2006).

These studies take on global relevance since moniliasis presents one of the most serious threats to modern cacao cultivation. The disease is currently confined to 13 countries of tropical America[1/] but it could spread to the major production centers in Western Africa and Southeast Asia placing the global chocolate industry at risk. On the other hand, the generation of highly resistant clones will allow cacao production in moniliasis-infested environments, where until recently the only alternative was to abandon or change the activity on the plantations, as it has been documented for different eras and countries of the region (Phillips-Mora and Wilkinson 2007).

Based on results from field trials conducted over the last 15 years, in 2007 the PMG selected a group of six high yielding, moniliasis-tolerant trinitario clones for distribution in Central America. The clones CATIE-R1, CATIE-R4, CATIE-R6, CC-137, ICS-95 T1 and PMCT-58 are now part of the genetic strategy of the Central American Cacao Project (Spanish acronym PCC) and other regional initiatives that are designed to fully modernize plantations in the region and improve incomes and living conditions of farm families.

Within the framework of the PCC, the clones are being established in a network of clonal gardens in Panama, Costa Rica, Nicaragua, Honduras, Guatemala and Belize, which will help define their range of adaptation and the existence of genotype by environment (G x E) interactions. Due to the wide range of climate and soil conditions that exists in Central America, the clones will conserve their experimental status until their adaptation in a specific agro-environment is corroborated.

1/ Belize, Colombia, Costa Rica, Ecuador, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Peru, Panama and Venezuela. The presence of the disease in Bolivia was corroborated by the first author in August 2012.

The objectives of this catalogue are to make the genetic, agronomic, morpho-physiological and molecular information that the PMG and its collaborators have generated for the six selected clones available to farmers, technical and scientific personnel, chocolate manufacturing companies and other interested parties. The document describes the genetic improvement strategy developed by CATIE and indicates the physical, environmental and agronomic conditions of the field trials involved in the process, as well as the reasons that justified the selection of the materials.

Morpho-physiological data will permit to distinguish the six clones, comparing them with other materials of interest and corroborating their identity. In cases where more convincing confirmation is required, the provided molecular information could be used. Finally, detailed descriptions are given with all the information available about the agronomic behavior of the clones: their productive potential, their natural and artificial reactions to moniliasis and black pod, and their industrial qualities.

"Since a successful variety is a rare combination, it is not easy to develop"

Briggs and Knowles 1967

Fruits of the clones selected by CATIE

CC-137

ICS-95 T1

PMCT-58

Section One
General information

Overview of Genetic Improvement in Cacao

Although it is commonly accepted that the future success and sustainability of cacao farming will depend largely on the capacity to create new varieties, very few advances have been made globally in this direction (Efron *et al.* 2003a). For example, until very recently, only 30% of the world's cacao came from improved varieties (Paulin and Eskes 1995) and less than 1% of the best clones were originated in the preceding 20 years (Lockwood 2003). This is surprising considering the broad genetic diversity of cacao in Latin America, which was collected intensively in the 1930s and conserved in collections that have not been systematically exploited.

One immediate consequence of the narrow genetic base of the commercial varieties is their high vulnerability to devastating diseases (Figure 1), which was evident, for example, with the appearance of witches' broom (*Moniliophthora perniciosa* Aime & Phillips-Mora) in Brazil in 1978 (Pereira *et al*.

Figure 1. Major diseases of cacao in Tropical America: A. Black pod (*Phytophthora palmivora*), B. Moniliasis or frosty pod rot (*Moniliophthora roreri*) and C. Witches' broom (*Moniliophthora perniciosa*).

1996) and moniliasis (*Moniliophthora roreri* Evans *et al*.) in Mexico in 2005 (Phillips-Mora *et al*. 2006; Phillips-Mora *et al*. 2007b).

The slow progress in the creation of new varieties can be attributed to different reasons:

- As in other perennial crops, genetic improvement in cacao is very slow; a single selection cycle frequently takes more than a decade and it is often necessary to complete two or more cycles before being able to release a new variety. Even for annual crops it has been estimated that the development of a new variety requires 10 to 20 years of work (Briggs and Knowles 1967).

- The most important evaluation parameters in cacao are measured when the plants reach maturity and several years of data are required in order to draw reliable conclusions.

- Most economically importance traits in cacao are multigenic and they often have complex inheritance that makes it difficult to handle them together. Therefore, it is essential that improvements be made in successive stages, which increases the duration of the process.

- Many improvement programs have had ephemeral or intermittent duration consistent with the international prices of the beans.

- Other programs have excessively emphasized the search for disease-resistant individuals, with this often becoming the sole objective of the program paying very little attention to improvements in production (Kennedy *et al*. 1987).

- The improvement programs have been based on a few genotypes from the Scavina series (SCA), Pound, Nanay (NA), Parinari (PA), United Fruit (UF), Iquitos Mixed Calabacillo (IMC) and the Imperial College Selection (ICS) selected during years 1940-1950, neglecting the extensive genetic diversity present in the germplasm collections (Lopes *et al*. 2011).

For a cacao improvement program to be successful it must:

a) Have a broad genetic base in accordance with its final goals.

b) Focus on resolving the main factors limiting the current as well as potential production.

c) Be compatible with the needs of farmers, and responsive to market demands.

d) Have a duration and continuity coherent with the objectives to be achieved.

CATIE's improvement strategy

CATIE, with support from the American Cocoa Research Institute (ACRI) and later from the World Cocoa Foundation (WCF), began a genetic improvement program in 1996. However, the design of disease inoculation methods and selection of tolerant clones initiated in 1980. The main goals of this program are the identification of sources of resistance to moniliasis and black pod (*Phytophthora palmivora* Butler) and the generation of high yielding, resistant varieties. On a global scale and due to their great relevance, these traits have received the most attention from breeders, followed by sexual compatibility and quality (Lopes *et al.* 2011).

The program has been implemented without interruption for 17 years and it has been strengthened in recent years with parallel projects in partnership with USDA/MARS (2002-) and Bioversity-CFC (2005-2009).

Figure 2 summarizes CATIE's genetic improvement strategy for obtaining superior progenies, clones and trees. The strategy follows four different routes (described below) that start with the germplasm contained in the International Collection:

Partial view of the International Cacao Germplasm Collection at CATIE. Replication located in La Montaña farm, Turrialba, Costa Rica.

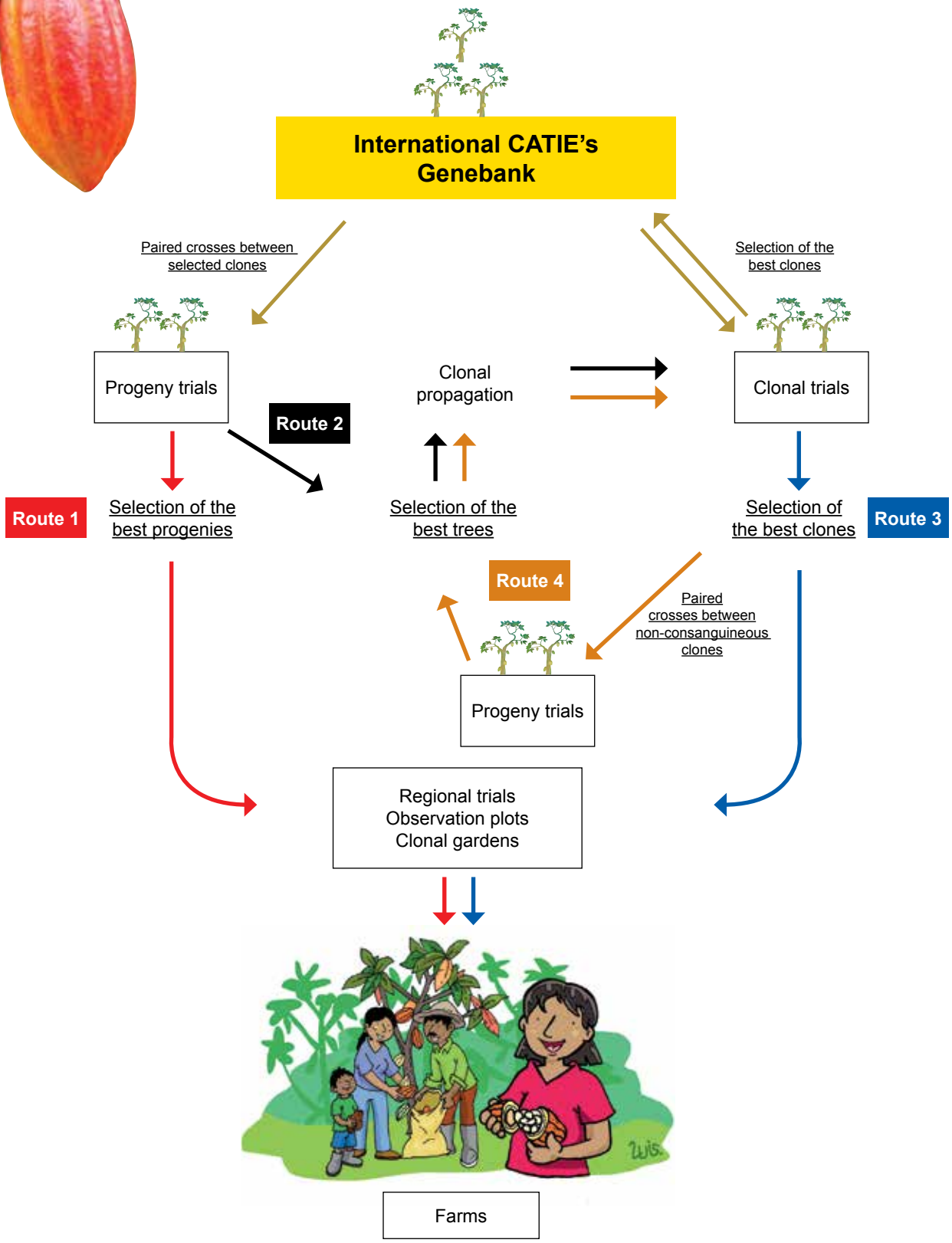


Figure 2. CATIE's improvement strategy and routes for obtaining superior germplasm.

- **Route 1:** Trials are conducted on progeny (hybrid families) obtained from the paired crosses (artificial pollination) of clones that possess ideal traits, the most important being good production and/or disease resistance (moniliasis, black pod and witches' broom). Based on the data collected over 5-7 years, the best progenies are selected and subsequently evaluated in regional trials or directly on producing family farms.

- **Route 2:** From the progeny trials, superior trees are selected that combine several desirable traits and/or accumulate genes favorable for a particular trait. Once these trees are identified, they are clonally propagated to preserve and / or to include them in clonal trials.

- **Route 3:** Clonal trials are set up that include the best pre-selected clones, superior cloned trees and national and international controls. Once at least five years of data have been obtained, the most outstanding materials are selected for their eventual distribution to producing families, but first they are established in clonal gardens, regional trials and/or observation plots that have the following objectives:

  - **Clonal gardens:** These are sources of vegetative material for propagation of clones. They can act eventually as observation plots or regional trials, in which case they should be established under a suitable experimental design, randomly assigning the position of the clones in the field.

  - **Multi-location or regional trials:** These are used to evaluate the behavior of the clones in different environments and/or under different types of management. The aim is to select the clones that have the best performance in a given environment and those that perform well in different sites.

  - **Observation plots:** These give present visual and numerical information about the performance of the clones in local conditions. Thus, both farmers and technical personnel can make decisions regarding the materials.

- **Route 4:** Progeny trials are established based on crossing pairs of pre-selected clones with very good general profiles that meet the following conditions: a) they are not closely related so that hybrid vigor or heterosis is expressed in their offspring, and b) the cross can potentially compensates the defects of one with the virtues of another in relevant traits. The idea is to produce superior trees that accumulate most of the desirable traits, which usually represent a very small proportion of the population. Once identified, these trees are cloned and evaluated in trials under appropriate experimental conditions for verification of their performance.

Breeding stages developed at CATIE

Consistent with the strategy indicated above, the PMG has developed the following activities: 1. Design and application of reliable artificial inoculation methods for identifying moniliasis- and/or black pod-resistant material; 2. Establishment of field trials based on this material; 3. Selection of the most outstanding individuals based on several years of data; 4. Characterization and evaluation of the selected material; and 5. Establishment of clonal gardens, multi-location trials and observation plots. Each stage is described in detail below.

1. Identification of sources of resistance

The first step in a genetic improvement program aimed at resolving the problem of diseases is to identify resistant individuals. The availability of broad genetic diversity and reliable methodologies for the selection of the materials are essential for this purpose. CATIE meets both requirements:

1. It has one of the two unique cacao genebanks with international status named the International Cacao Collection at CATIE (IC3) (Phillips-Mora *et al*. 2007a), which in February 2013 contained 1170 clones of diverse origins. In recent years the collection has been genetically enriched by the introduction of wild clones from the Reading University Intermediate Cocoa Quarantine Station (England) and from CIRAD in France, among others. This has increased the possibilities of identifying resistant materials with different geographic origins.

2. Furthermore, CATIE has developed efficient artificial inoculation methods for evaluating the response of the material to moniliasis (Sánchez *et al*. 1987; Phillips and Galindo 1988) and black pod (Phillips and Galindo 1989) (Figure 3). Similar research has not been conducted for witches' broom since the disease is absent in Costa Rica; its current range includes some Caribbean islands, South America and part of eastern Panama to the Canal. As a compensatory measure, international sources of resistance available in the Germplasm Collection (clones SCA-6, SCA-12 and CCN-51) were included within the PMG.

The process for identifying clones tolerant to moniliasis and black pod began at CATIE in 1980. After evaluating nearly 800 clones from the International Collection, it was concluded that resistance to moniliasis is an uncommon trait since only 10% of the materials have shown a resistant (2%) or moderately resistant (8%) response. For black pod the situation has been more favorable, with nearly a third of the clones showing high levels of resistance.

Figure 3. Artificial inoculation methods: A) **Black pod:** adult unripe fruits are inoculated using paper discs impregnated with a suspension of 160,000 spores/ml and the diameter of the lesions are measured 10 days later; B) **Moniliasis:** two-month old fruits are inoculated by spraying them with a suspension of 120,000 spores/ml. Percentage of internal area damaged is evaluating nine weeks after infection.

2. Establishment of field trials

In the last 17 years, the PMG has established 35 field trials in a 30.3-hectare area. Most of the trials are on La Lola Farm, which is in a traditional cacao farming area that combines all the conditions for cultivating cacao, as well as the development of diseases (Box 1). The rest of the trials as well as two of the three replications from the International Germplasm Collection (IC3) were established at CATIE, Turrialba, at 602 masl, 2,645 mm annual rainfall, and 22.5 °C average temperature.

The PMG is currently evaluating 17 clonal trials, 10 progeny trials and 5 segregant populations used for molecular studies in conjunction with USDA and MARS. The trials are evaluated monthly and by tree using parameters related to production, such as the number of healthy fruits and the fresh weight of the seeds, or the reaction to moniliasis and black pod for which the number of diseased fruits is counted. Other parameters usually evaluated are the height of jorquette emergence, the tilt angle of the branches and the trunk diameter.

L6 or "Experiment on disease tolerant clones": This is one of the PMG's most important clonal trials due to its relative antiquity and for being the source of the clones referred to in this catalogue. The trial was planted on La Lola Farm in 1998 and 1999. It consists of 42 clones planted on 1.5 hectares under a Randomized Complete Block Experimental Design with four replications and eight plants per replication for a total of 1,344 plants sown at a distance of 3 m x 3 m. The clones in this trial were selected from previous trials or from the genebank, mainly because they are tolerant to moniliasis, black pod or witches' broom and/or are highly productive. Clones with tolerance to moniliasis for which there was no information on their productive potential predominate in the trial.

Temporary shade for the trial consists of banana (*Musa* sp.) at a distance of 6 m x 6 m, which was gradually thinned until leaving only the permanent shade plants such as the guava (*Inga edulis*) and immortelle (poró, *Erythrina poepiggiana*), irregularly distributed in the area.

The cacao trees were given structural prunings at the beginning of the trial and periodic maintenance prunings. On a regular basis, 600 g of granular fertilizer formula 18-5-15-6-0.3-7 divided into four applications of 150 g were applied every 3 months. No disease control is carried out in the trial other than the cutting of diseased fruits at the time of the monthly evaluations, which are then left on the soil without applying any kind of treatment. Manual weed control is carried out every 2 months, complemented by 2 directed applications of paraquat (0.2 kg/ha) per year.

3. Selection of outstanding individuals

When the clonal or progeny trials accumulate at least 5 years of data, a first selection is made of the best clones, progeny or individual trees based mainly on their productive performance and reaction to moniliasis. This selection is corroborated as more information is accumulated, making the necessary adjustments. The trees selected are vegetatively propagated and established in a clonal trial along with other pre-selected clones and local or international controls such as CCN-51. For their part, the selected clones go on to more advanced stages of improvement, such as the establishment of clonal gardens, observation plots, multi-location trials or tests on producers' farms.

Box 1

CATIE'S LA LOLA FARM

La Lola is an experimental cacao farm that originally belonged to the *United Fruit Company*, which handed its administration over to the American Institute of Agricultural Sciences of the Organization of American States (OAS) in 1947, donating it to that same institute in 1962 (Anonymous 1962). It is located at 28 Millas de Bataán, Matina canton, Limón province, on the Atlantic coast of Costa Rica at 10°06' North latitude and 83°23' West longitude and an elevation of 40 masl. In accordance to the Holdridge life zone system, the area belongs to the tropical moist forest transition to tropical wet forest.

The soils at La Lola were formed from alluvial materials deposited by water currents. These consist mainly of large rocks, stones, gravel and a mixture of sand with small amounts of sedimentary material from erosion. There is significant variation in soil texture within the farm due to differences in the distribution of rocks, stones and gravels both horizontally and vertically. Most of the farm's soil (69%) consists of silty-clay, 21% coarse sand and 10% sandy-clay. The topography is classified as nearly flat (Bazán 1963).

The soil has low surface infiltration capacity and poor rainwater drainage, which are affected mainly by the soil's texture, structure and compaction; this, added to deficient soil aeration, is a limiting factor for cacao growth and production (Bazán 1963).

The region's climate can be defined as warm (24.5 °C average annual temperature), very rainy (3,560 mm average annual precipitation), with a marked decline in rainfall in the months of March and September. It has high relative humidity, considerable cloudiness with a few hours of sunshine, average solar radiation and an excess of water for most of the year (Jiménez 1986). The hottest months are May and June, while December and January are the coolest ones, although mean temperatures are similar year-round; the difference between the average temperature for the hottest month and the coolest month is less than 2 °C, however the differences between the monthly maximum and minimums is close to 10 °C (Jiménez 1986).

The farm is located in a region where cacao has been cultivated since the colonial period (Fonseca *et al*. 2001). The appearance of moniliasis in the area in 1979 led to the sucessive abandonment of farms, a situation that still persists today. In fact, on the farm's periphery there is a large number of abandoned plots and a permanent and very intense presence of moniliasis both within and outside of the farm, making it an ideal site for selecting resistant genotypes.

Cacao production in this region is bimodal, peaking in the months of April to May and October to January. Not many fruits are collected from June to September, meaning that few flowers are formed from January to April, five months prior to the second of these periods when a season of low temperatures occurs (Hardy 1961).

Selected superior clones at L6: The L6 trial has been systematically evaluated over the last 11 years. Data collection has started at the second year after planting and continues to date. The following parameters are evaluated monthly for each tree: number of healthy fruits, % of fruits affected by moniliasis, % of fruits damaged by black pod, and fresh weight of the seeds. The production of dry cacao in kilograms per hectare is estimated based on the fresh weight of the seeds multiplied by a factor of 0.38.

Table 1 summarizes data averages and response to diseases for the clones included in L6 trial for the following periods: a) the first 7 years, b) the 11 years available, and c) the last 5 years.

In 2007, using the first seven years of data, the following group of 6 trinitario clones were selected for highest production and tolerance to moniliasis: CATIE-R1, CATIE-R4, CATIE-R6, CC-137, ICS-95 T1 and PMCT-58. These were incorporated into the Central American Cacao Project (PCC) genetic strategy and today they are part of the different regional initiatives for genetic improvement of Central American plantations.

Clone ICS-95 T1[1/] was included on the list of clones selected for representing an international material with recognized track record in Latin America: good production and tolerance to moniliasis (Phillips-Mora *et al*. 2005). Its behavior was not precisely determined in L6, because half of the trees in the trial belonged to another clone, according to DNA tests done by the USDA in 2009. ICS-95 is considered to be a promising material in Peru (Evans *et al*. 1998) and it is recommended for planting in all producing areas of Colombia (Rondón 2000). It is tolerant to witches' broom in Colombia (Argüello 2000) and to moniliasis in Costa Rica (Phillips-Mora 1996), Colombia (Argüello 1997) and Peru (Evans *et al*. 1998). In fact, it showed tolerant behavior against seven strains of *M. roreri* that represented the genetic diversity of the fungus in Latin America (Phillips-Mora *et al*. 2005).

CCN-51 T2[1/] clone was not included in the selected group because of its high susceptibility to black pod and questionable quality, despite its good yields and certain degree of tolerance to moniliasis.

Even though the selection of the clones was made when the seventh year of production was completed, the results that were collected subsequently reinforced the decision. In fact, we found a very high correlation (99%) between the average cumulative production in the seventh year and to that in the eleventh year, and also with the average of the last five years of data (94%). In all cases clones CATIE-R1, CATIE-R4, CATIE-R6 and CC-137 had the highest in production. PMCT-58 was the only clone with small reduction in tree production in respect to prior years.

1/ At CATIE, clones that molecularly coincide with the reference clone are designated Type 1 (T1). This way they are differentiated from other clones that have the same name but their molecular profile is incorrect (Type 2 or T2). For example, CCN-51 T2 shares many traits with the original type, but its DNA profile does not completely coincide, therefore obligating its identification as Type 2.

The results for the incidence of moniliasis were also consistent between years (average of the first seven years vs. 11 years and average of the first seven years vs. the last five years), obtaining coefficients of correlation of 99% and 97% respectively. Clones CATIE-R1, CATIE-R4 and CATIE-R6 again demonstrated the best results. However, the incidences of moniliasis have increased in the last five years in all clones in the trial except CATIE-R6 and CATIE-R3.

Within the group of six clones, the most outstanding ones are CATIE-R6 and CATIE-R4, because they consistently showed the highest yields and the lowest incidence of moniliasis throughout all the years of evaluation. Their productive potential is notable even under conditions very favorable for moniliasis, which caused losses of 84% to 86% of the fruits during the last five years in international clones such as Pound-7 and CATIE-1000 (with recognized productive potential and resistance to black pod) and in the clones resistant to witches' broom, SCA-6 and SCA-12 (Table 1).

Table 1. Yield and incidence of diseases in 42 cacao clones from the L6 Trial at La Lola Farm. The averages included are: for the first 7 years, all 11 years, and the last 5 years of the available data.

Clone	Average for the first 7 years			Average for all 11 years			Average for the last 5 years		
	Yield (kg/ha/yr)	% moniliasis	% black pod	Yield (kg/ha/yr)	% moniliasis	% black pod	Yield (kg/ha/yr)	% moniliasis	% black pod
CATIE-R6	1018	5	0	1485	5	0	2363	4	0
CATIE-R4	977	7	1	1336	9	1	2070	12	1
CC-137	854	24	2	990	32	1	1321	43	0
CCN-51 T2	772	37	5	824	45	4	1034	56	2
CATIE-R1	745	10	8	1066	12	7	1674	15	6
PMCT-58	703	20	5	789	26	4	1036	35	2
ARF-22	667	49	1	756	54	0	1012	62	0
UF-273 T1	655	13	5	933	14	4	1395	16	3
EET-183	645	27	3	760	30	3	1038	33	2
CATIE-R2	640	9	7	839	12	6	1204	18	2
Árbol-81	634	45	1	732	47	1	976	48	0
CATIE-R7	576	11	7	807	14	7	1210	19	6
CATIE-R5	562	7	1	706	9	0	992	13	0
ARF-14	555	38	4	648	42	3	907	46	1
ARF-37	534	42	2	602	49	1	792	59	0
POUND-7	519	69	0	542	75	0	668	86	0
ICS-95 T1	516	21	7	636	26	6	926	32	4
ICS-43	511	22	9	641	28	7	890	39	5
IMC-60	394	30	3	455	39	2	597	51	1
EET-59	389	48	1	426	55	1	610	65	0

Clone	Average for the first 7 years			Average for all 11 years			Average for the last 5 years		
	Yield (kg/ha/yr)	% moniliasis	% black pod	Yield (kg/ha/yr)	% moniliasis	% black pod	Yield (kg/ha/yr)	% moniliasis	% black pod
CATIE-R3	389	19	3	506	19	2	748	19	1
ARF-6	379	20	5	447	28	4	599	39	3
UF-273 T2	363	31	13	505	35	9	752	43	1
CC-42	349	53	1	384	61	1	505	75	0
PA-169	312	12	1	377	17	0	540	25	0
SGU-84	292	24	5	305	30	4	357	39	2
CATIE-1000	285	69	1	298	76	1	372	85	0
BE-8	252	44	2	302	52	2	458	64	1
CC-240	210	33	6	378	37	4	692	42	0
RB-41	207	73	1	197	78	1	195	88	1
PMCT-82	202	44	1	267	48	1	399	54	0
A5-R2 (T3)	194	51	9	202	59	7	239	72	3
ICS-44	180	68	7	287	73	4	528	80	0
SCA-12	165	73	1	162	78	1	181	86	0
A-174(RETRO)	132	43	2	201	51	2	319	68	1
CC-252	105	33	3	119	42	2	154	59	1
UF-712	101	10	2	155	18	2	274	31	1
SCA-6	89	70	2	94	75	2	117	84	0
P-23	50	48	3	68	54	2	112	64	0
A-173(RETRO)	48	34	0	114	43	0	241	51	0
A-147(RETRO)	47	40	0	102	51	0	198	63	0
GU 133-N	29	12	0	61	14	0	108	18	0

CATIE-R1 showed good production despite having trees of very low stature. This trait, along with its self-compatibility, makes it a good candidate for planting in high density plantations.

Polyclone: It is recommended that trees of the six clones be planted randomly or in alternating rows to avoid phytosanitary and compatibility problems associated with genetic uniformity. In the field, the six clones behave like a polyclone that is characterized by having good average behavior in terms of production, disease tolerance, compatibility, and industrial quality. This implies that the comparative advantages of some clones compensate for the defects of others.

It is important to keep in mind that in the near future the PMG will release new materials from trials that are in different phases of evaluation, which will complement or replace some of the current clones.

4. Characterization and evaluation of the selected materials

The best materials of the PMG are characterized and evaluated systematically. The characterization consists of determining the existing variation using morphological, phenological, biochemical or molecular parameters that are little influenced by the environment. Additionally, the evaluation includes description of the variation in traits with agronomic importance, that are influenced by the environment (i.e. yield, quality, etc.). The main objective of the characterization is the identification of the genotypes, since the purpose of the evaluation is to determine their agronomic value. A descriptor is any trait or condition attributed to the clone or variety.

The six selected clones were subjected to a broad characterization and evaluation. Results of which will be described in the section two of this catalogue.

5. Establishment of clonal gardens, multi-location trials and observation plots

In 2008, the six selected clones began to be established in Panama, Costa Rica, Nicaragua, Honduras, Guatemala and Belize as part of the PCC improvement strategy that consists of the establishment of 5 hectares of clonal gardens and a multi-location trial of one hectare in each country. Some of the areas are still in the process of establishment.

The objectives of the clonal gardens are: to provide vegetative material for the subsequent multiplication of the clones, evaluate the behavior and adaptation of the materials in different agro-environments, and serve as demonstration plots for the farmers and other interested parties. The multi-location trials aim to evaluate in different environments the behavior and adaptation of a group of 30 clones, selected as potential good candidates for release in the near future.

In addition to the PCC strategy, the selected materials has been sown on plantations of small and medium-size producers in Panama, Costa Rica and El Salvador. As part of a joint initiative with Hershey and ECOM, the clones were introduced in Mexico in June 2012. The information that is obtained from these plantations will be relevant for determination of the range of adaptation of the materials to different agro-environments and the effect of different management conditions on the performance of the clones.

Section Two
Passport data and morphological characterization of the clones

Passport data and other general characteristics

Passport information such as country and institution of origin of the material and its pedigree is included. In addition, the typical tree growth habit is described for each clone based on a reference 4-year-old trees grown in the Mother Clonal Garden located in Turrialba, that were propagated by bud patch grafting. Information about the average trunk diameter is also provided based on data collected from 18 to 32 trees (14 years old) grown in the L6 trial at La Lola Farm and from 44 to 59 trees, 4 years old, located in the Mother Clonal Garden.

To facilitate the identification of the clones and their handling, planting and data collection, PMG has assigned a distinctive color to each one: CATIE-R1 (Green); CATIE-R4 (Red); CATIE-R6 (Yellow); CC-137 (White); ICS-95 T1 (Black) and PMTC-58 (Blue). Orange color was assigned to clone IMC-67, which is commonly used on the plantations as a pollen donor.

Morphological descriptors

The clones were characterized morphologically using a list of 51 descriptors: 8 for leaf, 22 for flower, 15 for fruit and 6 for seed. For the flower and seed descriptors, a minimum amount of 30 samples were used. The sample sizes were larger than 50 for leaf and fruit descriptors. Standard errors are included with the value of the descriptor, where appropriate. The descriptors used are explained in detail below.

Leaf descriptors: 1) The color of flush (6-7 days old) was observed and recorded under natural light. The coloration ranged from tones of green to different degrees of red, pink and/or brown pigmentation.

For following descriptors 50 mature leaves from the intermediate part of the trees were measured early during the morning hours (Figure 4): 2) leaf shape according to the scale proposed by Hartmann *et al*. (1981), 3) tip angle, 4) shape of the base, 5) leaf width, 6) leaf length, 7) petiole length, and 8) length from the base to the widest point of the leaf (LBW).

Flower descriptors: thirty fresh, open flowers with pearly white color pollen as an indicator of their freshness were randomly collected early in the morning. Flowers were manipulated and dissected using electronic vernier caliper, stereoscope, cover slips and slides (Figure 5). La following descriptors were recorded: 1) Pedicel length, 2) Pedicel width, 3) Sepal length, 4) Sepal width, 5) Ligule length, 6) Ligule maximum width, 7) Filament length, 8) Filament width, 9) Staminode length, 10) Staminode width, 11) Style length, 12) Style width, 13) Ovary length, 14) Ovary width, and 15) Number of rudimentary seeds (ovules) in the ovary. To count the rudimentary seeds, 30 recently open flowers were used. Each ovary was placed on a slide with a drop of water. A longitudinal cut was made under the stereoscope using a scalpel. Each rudimentary seed was then separated using fine needles.

Using a visual index, data were also recorded for anthocyanin intensity of: 16) Pedicel, 17) Sepal, 18) Ligule, 19) Filament, 20) Staminode, 21) Style, and 22) Ovary. The index values used were: 0 = absent, 3 = slight, 5 = intermediate and 7 = intense.

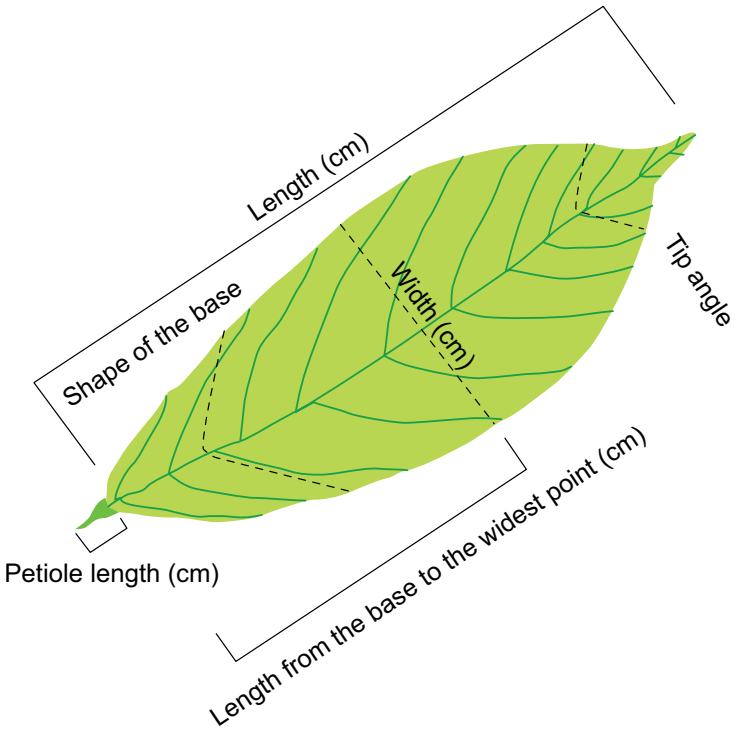


Figure 4. Morphological descriptors of cacao leaves.

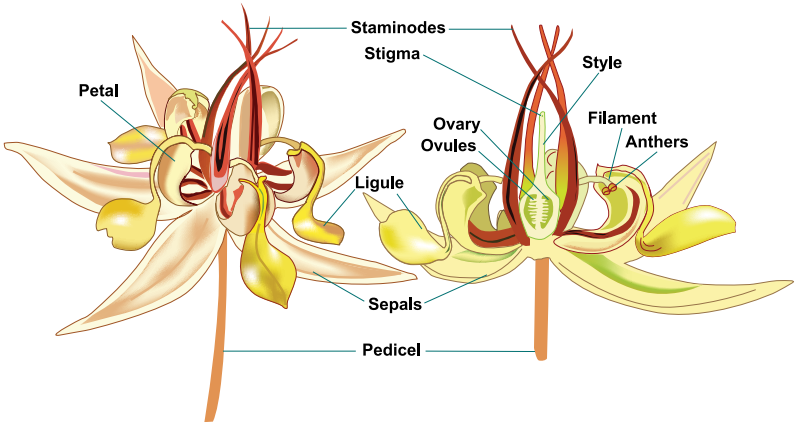


Figure 5. Floral structure of cacao (Aranzazu *et al.* 2008).

Fruit (pod) descriptors: For fruit characterization, a minimum of 50 fruits from the L6 Trial were collected during different seasons of the year for the period 2007-2010. Descriptors included: 1) Color at two months old fruits, 2) Color of ripe fruit; 3) Fruit shape (Figure 6), 4) Shape of the apex (Figure 7), 5) Shape of the fruit base constriction (Figure 8), 6) Fruit surface rugosity (roughness) (Figure 9), 7) fruit mesocarp hardness using a scale with the following values: 3 = soft, 5 = intermediate and 7 = hard. Similarly, the parameters that are shown graphically in Figure 10 were recorded: 8) Weight, 9) Length, 10) Diameter, 11) Length/width relationship, 12) Fresh weight of seeds per fruit; 13) Number of seeds per fruit, 14) Fruit wall thickness at ridge and 15) Depth of the furrow.

Seed descriptors: The fruits used for the pod characterization were also used for seed characterization. The seeds were scrubbed with sawdust to remove the mucilage (aril) and the integument, after which the following parameters were evaluated: 1) Cotyledon color, 2) Seed shape (Figure 11), 3) Shape in cross section (Figure 11), 4) Length, 5) Diameter, and 6) Thickness.

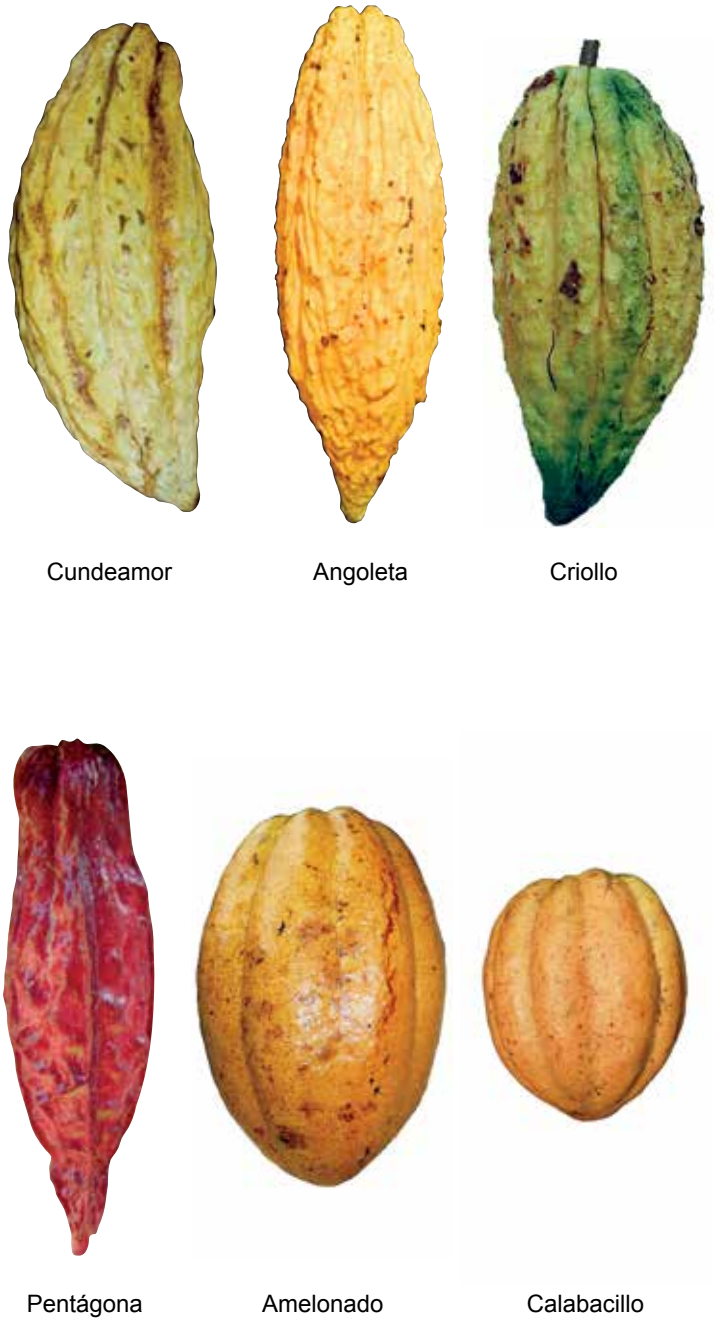


Figure 6. Shapes of cacao fruits.

Figure 7. Different shapes of the cacao pod apex.

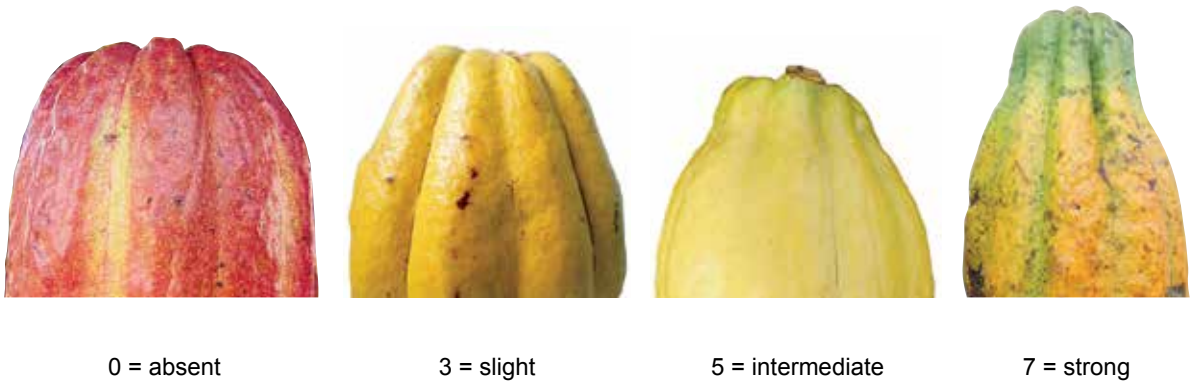


Figure 8. Different shapes of the fruit base constriction.

0 = absent

3 = slight

5 = intermediate

7 = intense

Figure 9. Fruit surface rugosity on cacao fruits.

Figure 10. Other morphological descriptors of the fruit: A. Fruit length (cm). B. Fruit diameter (cm). C. Apex form. D. Basal constriction form. E. Weight. F. Number of seeds per fruit. G. Fresh weight of seeds. H. Fruit wall thickness at ridge (cm). I. Furrow depth (cm).

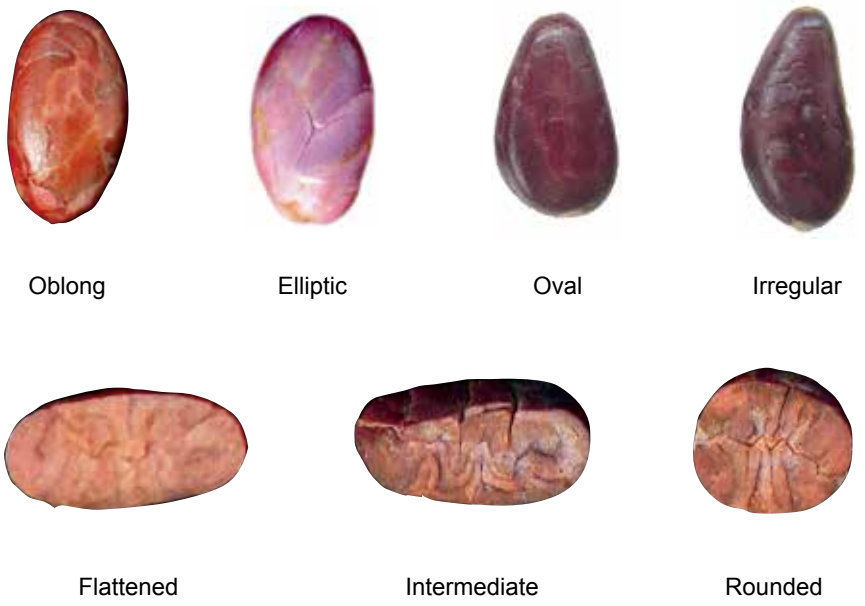


Figure 11. Seed form (above) and shape in cross section (lower part).

Results obtained

Passport data: All the clones except ICS-95 T1 were selected at CATIE, Costa Rica. The CATIE-R6 and CATIE-R4 clones are offspring originating from the cross "UF-273 T1 x PA-169". CATIE has demonstrated that this family produces precocious offspring that are high yielding and moniliasis-resistant. This has been corroborated by institutions, such as FHIA in Honduras, which have selected clones with similar traits using seed from the same cross supplied by CATIE in the 1990s.

According to observation made at the Clonal Garden of CATIE in Turrialba, the individual clones differ a great deal in the size and the vigor of the trees. Considering these characteristics, the order of the clones starting with the largest size and the most vigorous clone from left to right is as follow: ICS-95 T1 > CC137 > PMCT-58 > CATIE-R6 > CATIE-R4 > CATIE-R1. A similar order was observed when the trunk diameter was measured for the same trees: ICS-95 T1 (8.1 cm) > CC-137 (7.7 cm) > PMCT-58 (7.5 cm) > CATIE-R4 (7.6 cm) > CATIE-R1 (6.6 cm) > CATIE-R6 (6.1). This result agrees with the high correlation found by different authors between trunk diameter and vigor characters (Glendinig 1960; Mariano 1966; Peralta 1978; Moses and Enríquez).

Trunk diameter measurements in trees 14 years of age in L6 (La Lola Farm) gave a different order possibly due to the older age of these trees: CATIE-R4 (18.6 cm) > CC-137 (16.8 cm) > CATIE-R6 (16.7 cm) > CATIE-R1 (16.0) > ICS-95 T1 (13.3 cm) > PMCT-58 (12.2 cm).

Most distinctive morphological traits: Table 2 summarizes the morphological traits of the 6 clones. The most distinctive traits include those related to the color of the unripe fruits and the shape of the fruits. There are particularities that help distinguish one clone from the others. For example, CC-137 has sepals that are usually fused; PMCT-58 has a longer pedicel and ICS-95 T1 has longer staminodes. For its part, the large size of the seed of CC-137 helps distinguish it from the rest of the materials.

Table 2. Summary of morphological traits of 6 cacao clones selected by CATIE.

Morphological Descriptors			CATIE-R1	CATIE-R4	CATIE-R6	CC-137	ICS-95 T1	PMCT-58
Leaf	Flush color		Pale red with green	Pale red with green	Pale red with green	Light greenish-brown	Intense pink	Red with intense brown
	Leaf shape		Elliptic	Elliptic	Elliptic	Elliptic	Elliptic	Elliptic
	Tip angle		Cuspidate	Aristate	Aristate	Aristate	Cuspidate	Aristate
	Shape of the base		Obtuse	Cuneiform	Cuneiform	Cuneiform	Obtuse	Obtuse
	Leaf width (cm)		10.7	11.8	13.0	11.8	13.4	12.5
	Leaf length (cm)		31.9	30.4	33.8	32.5	34.4	38
	Petiole length (cm)		2.0	2.1	1.8	2.5	2.7	2.1
	LBW[1/]		11.3	11.9	17.1	11.8	17.6	16.4
Flower	Pedicel	Length (mm)	20.8	20.4	16.0	21.6	22.1	28.6
		Width (mm)	0.8	0.6	0.7	0.7	0.7	0.8
		AI[2/]	7	0	3	7	7	7
	Sepal	Length (mm)	8.5	8.4	7.0	8.7	8.2	9.7
		Width (mm)	2.6	3.1	3.0	2.9	3.2	2.6
		AI[2/]	5	0	0	3	5	5
	Ligule	Length (mm)	4.1	6.2	7.1	6.2	5.7	6.2
		Width (mm)	3.0	2.6	3.3	2.6	2.9	2.5
		AI[2/]	3	0	5	3	3	3
	Filament	Length (mm)	1.5	1.3	1.3	0.9	0.9	1.3
		Width (mm)	0.4	0.3	0.4	0.3	0.3	0.4
		AI[2/]	0	3	5	0	0	0
	Staminode	Length (mm)	6.0	6.0	5.8	5.7	8.3	6.7
		Width (mm)	0.3	0.3	0.4	0.3	0.3	0.5
		AI[2/]	7	7	0	7	7	7
	Style	Length (mm)	2.2	3.4	1.7	1.3	2.1	1.7
		Width (mm)	0.2	0.3	0.3	0.3	0.3	0.4
		AI[2/]	0	0	0	0	0	0
	Ovary	Length (mm)	1.3	1.4	1.8	1	1	2.7
		Width (mm)	0.9	1.1	0.9	1	1.5	1
		AI[2/]	3	0	0	0	0	3
	Number of rudimentary seeds (ovules)		41	44	41	30	33	39

Table 2 continued.

Morphological Descriptors			CATIE-R1	CATIE-R4	CATIE-R6	CC-137	ICS-95 T1	PMCT-58
Fruit (fruit)	Color	Unripe	Purple with eventual presence of green	Pale green with very soft red tones	Green with purple	Light green and furrows whitish	Dark purple	Purple with light green
		Ripe	Orange with yellow sectors	Yellow with orange and eventually red flecks	Yellow with orange and eventually red flecks	Yellow	Orange with yellow	Orange with yellow
	Shape	Fruit	Angoleta-Cundeamor	Cundeamor	Angoleta-Cundeamor	Amelonado-Angoleta	Angoleta-Acriollada	Amelonado
		Apex	Attenuate	Attenuate	Attenuate	Attenuate	Acute	Obtuse
		Basal constriction[3/]	5	5	3	3	3	3
	Mesocarp	Rugosity[4/]	5	5	3	3	5	3
		Hardness[5/]	3	3	3	3	5	3
	Other traits	Weight (g)	556.7	573.7	566.1	461.6	589.7	441.1
		Length (cm)	17.4	18.7	14.3	14.9	19.7	13.8
		Diameter (cm)	9.2	9.6	9.5	9	8.5	8.8
		L/D relationship (cm)	1.9	1.9	1.8	1.6	2.3	1.6
	Seeds	Fresh weight per fruit (g)	93.4	144.7	127.2	117.3	102	93.1
		Number of seeds per fruit	29	35	31	27	33	37
	Ridge	Thickness (cm)	1.7	1.5	1.6	1.4	1.7	1.5
	Furrow	Depth (cm)	1.3	1.1	1.2	1.1	1.2	1.1
Seed	Cotyledon color		Intense purple	Purple	Light purple	Intense purple	Light purple	Purple
	Shape		Oblong	Oval	Irregular	Oval	Irregular	Oval
	Shape in cross section		Intermediate	Rounded	Rounded	Flattened	Intermediate	Flattened
	Length (cm)		2.5	2.5	2.6	2.5	2.1	2.3
	Diameter (cm)		0.9	1.0	0.9	1.1	0.9	0.8
	Thickness (cm)		1.2	1.3	1.2	0.9	1.1	1.1

[1/] **LBW:** Length from the base to the widest point of the leaf

[2/] **AI:** anthocyanin intensity: 0 = absent, 3 = slight, 5 = intermediate, 7 = intense

[3/] **Basal constriction:** 0 = absent, 3 = slight, 5 = intermediate, 7 = strong

[4/] **Rugosity:** 0 = absent, 3 = slight, 5 = intermediate, 7 = intense

[5/] **Hardness:** 3 = soft, 5 = intermediate, 7 = hard

CATIE-R1

Country of origin: COSTA RICA
Institution: CATIE
Pedigree: "UF-273 T1 X CATIE-1000". CATIE-1000 was selected in the 1970s from the cross Pound-12 x Catongo for its good production and tolerance to black pod.

Appearance: Trees small in size, with moderate foliage and branches with semi-erect growth.
Trunk diameter: 16.0 cm ± 0.84 (14 year old trees, La Lola); 6.2 ± 0.20 (4 year old trees, Turrialba).
Distinctive color for clone: Green

LEAVES CATIE-R1

Flush color:	Pale red with green
Leaf shape	Elliptic
Angle shape	Cuspidate
Shape of base	Obtuse
Leaf width (cm)	10.7 ± 0.18
Leaf length (cm)	31.9 ± 0.53
Petiole length (cm)	2.0 ± 0.03
LBW[1/]	11.3 ± 0.22

[1/] **LBW:** Length from the base to the widest point of the leaf

FLOWERS CATIE-R1

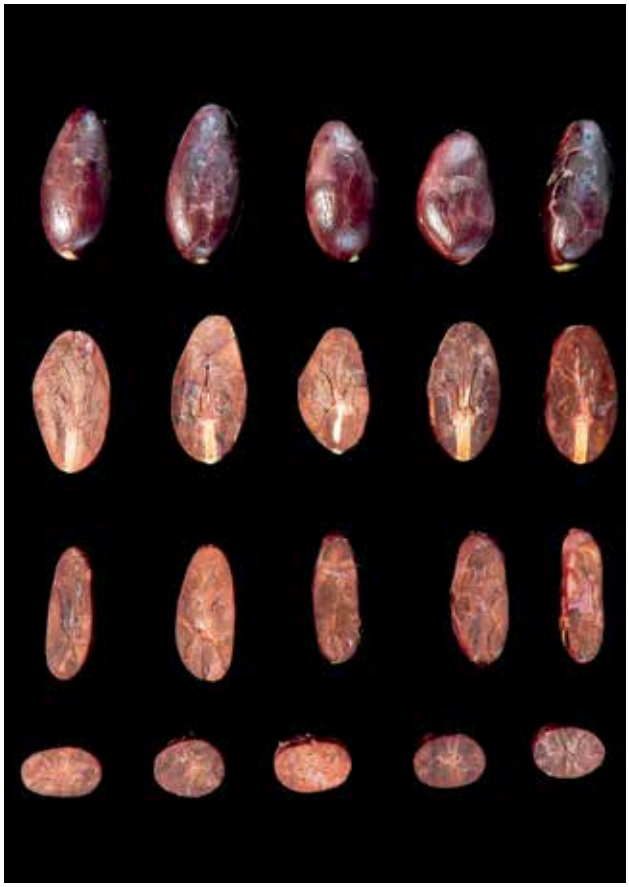
Floral Part	Length (mm)	Width (mm)	AI[1/]
Pedicel	20.8 ± 0.49	0.8 ± 0.49	7
Sepal	8.5 ± 0.11	2.6 ± 0.07	5
Ligule	4.1 ± 0.04	3.0 ± 0.06	3
Filament	1.5 ± 0.01	0.4 ± 0.02	0
Staminode	6.0 ± 0.08	0.3 ± 0.08	7
Style	2.2 ± 0.06	0.2 ± 0.05	0
Ovary	1.3 ± 0.03	0.9 ± 0.01	3
Number of rudimentary seeds (ovules): 41 ± 0.17			

[1/] **AI:** anthocyanin intensity: 0 = absent. 3 = slight. 5 = intermediate 7 = intense

FRUITS CATIE-R1

Color	Unripe	Purple with eventual presence of green
	Ripe	Orange with yellow sectors
Shape	Fruit	Angoleta-Cundeamor
	Apex	Attenuate
	Basal constriction[1/]	5
Mesocarp	Rugosity[2/]	5
	Hardness[3/]	3
Other	Weight (g)	556.7 ± 19.8
	Length (cm)	17.4 ± 0.27
	Diameter (cm)	9.2 ± 0.17
	L/D relationship (cm)	1.9 ± 0.03
Seeds	Fresh weight per fruit (g)	93.4 ± 3.82
	Number of seeds per fruit	29 ± 1.08
Ridge	Thickness (cm)	1.7 ± 0.03
Furrow	Depth (cm)	1.3 ± 0.03

[1/] **Basal constriction:** 0 = absent. 3 = slight. 5 = intermediate. 7 = strong.
[2/] **Rugosity:** 0 = absent. 3 = slight. 5 = intermediate. 7 = intense
[3/] **Hardness:** 3 = soft. 5 = intermediate. 7 = hard.



SEEDS CATIE-R1

Cotyledon color	Intense purple
Shape	Oblong
Shape of cross section	Intermediate
Length (cm)	2.5 ± 0.08
Diameter (cm)	0.9 ± 0.02
Thickness (cm)	1.2 ± 0.02

CATIE-R4

Country of origin: COSTA RICA
Institution: CATIE
Pedigree: "UF-273 T1 X PA-169"
Tree appearance: Trees of intermediate size, dense foliage and semi-erect branches.

Trunk diameter: 18.6 cm ± 0.76 (14 year old trees, La Lola); 7.6 ± 0.24 (4 year old trees, Turrialba)
Distinctive color for clone: Red

LEAVES CATIE-R4

Flush color	Pale red with green
Leaf shape	Elliptic
Angle shape	Aristate
Shape of base	Cuneiform
Leaf width (cm)	11.8 ± 0.18
Leaf length (cm)	30.4 ± 0.47
Petiole length (cm)	2.1 ± 0.09
LBW[1/]	11.9 ± 0.28

[1/] **LBW:** Length from the base to the widest point of the leaf

FLOWERS CATIE-R4

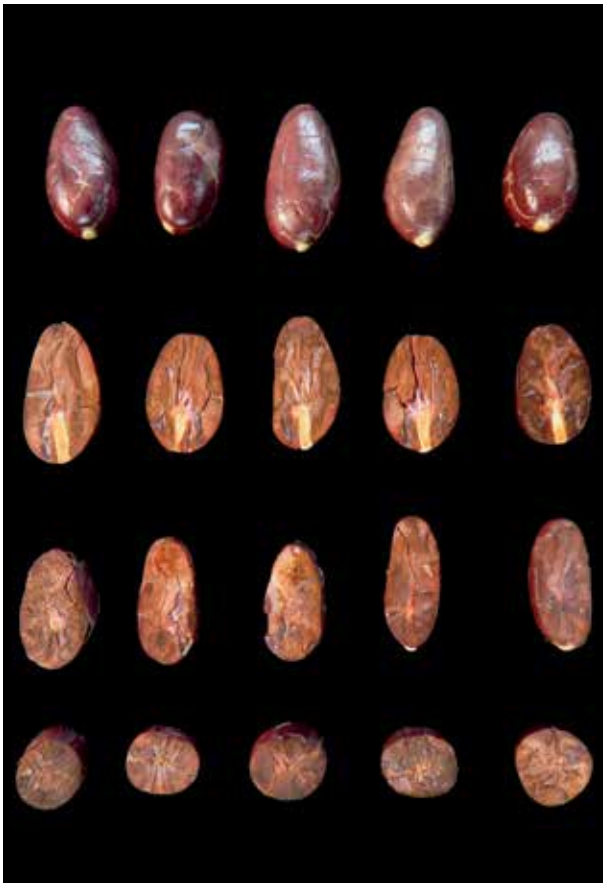
Floral Part	Length (mm)	Width (mm)	AI[1/]
Pedicel	20.4 ± 0.36	0.6 ± 0.25	0
Sepal	8.4 ± 0.07	3.1 ± 0.12	0
Ligule	6.2 ± 0.06	2.6 ± 0.07	0
Filament	1.3 ± 0.08	0.3 ± 0.06	3
Staminode	6.0 ± 0.07	0.3 ± 0.06	7
Style	3.4 ± 0.04	0.3 ± 0.07	0
Ovary	1.4 ± 0.04	1.1 ± 0.02	0
Number of rudimentary seeds (ovules): 44 ± 0.11			

1/ AI: anthocyanin intensity: 0 = absent, 3 = slight, 5 = intermediate 7 = intense

FRUITS CATIE-R4

Color	Unripe	Pale green with very soft red tones
	Ripe	Yellow with orange and eventual red flecks
Shape	Fruit	Cundeamor
	Apex	Attenuate
	Basal constriction[1/]	5
Mesocarp	Rugosity[2/]	5
	Hardness[3/]	3
Other	Weight (g)	573.7 ± 19.8
	Length (cm)	18.7 ± 0.25
	Diameter (cm)	9.6 ± 0.13
	L/D relationship (cm)	1.9 ± 0.02
Seeds	Fresh weight per fruit (g)	144.7 ± 5.70
	Number of seeds per fruit	35 ± 1.30
Ridge	Thickness (cm)	1.5 ± 0.04
Furrow	Depth (cm)	1.1 ± 0.02

[1/] **Basal constriction:** 0 = absent, 3 = slight, 5 = intermediate, 7 = strong.
[2/] **Rugosity:** 0 = absent, 3 = slight, 5 = intermediate 7 = intense
[3/] **Hardness:** 3 = soft, 5 = intermediate, 7 = hard.



SEEDS CATIE-R4

Cotyledon color	
Shape	Oval
Shape of cross section	Rounded
Length (cm)	2.5 ± 0.08
Diameter (cm)	1.0 ± 0.01
Thickness (cm)	1.3 ± 0.03

CATIE-R6

Country of origin: COSTA RICA
Institution: CATIE
Pedigree: "UF-273 T1 X PA-169"
Appearance: Trees of intermediate size, foliage dense and erect bushy and branches.

Trunk diameter: 16.7 cm ± 0.61 (14 year old trees, La Lola); 6.1 ± 0.37 (4 year old trees, Turrialba).
Distinctive color for clone: Yellow

LEAVES CATIE-R6

Flush color	Pale red with green
Leaf shape	Elliptic
Angle shape	Aristate
Shape of base	Cuneiform
Leaf width (cm)	13.0 ± 0.20
Leaf length (cm)	33.8 ± 0.48
Petiole length (cm)	1.8 ± 0.02
LBW[1/]	17.1 ± 0.28

[1/] **LBW:** Length from the base to the widest point of the leaf

FLOWERS CATIE-R6

Floral Part	Length (mm)	Width (mm)	AI[1/]
Pedicel	16.0 ± 0.15	0.7 ± 0.15	3
Sepal	7.0 ± 0.10	3.0 ± 0.04	0
Ligule	7.1 ± 0.04	3.3 ± 0.04	5
Filament	1.3 ± 0.03	0.4 ± 0.06	5
Staminode	5.8 ± 0.03	0.4 ± 0.03	0
Style	1.7 ± 0.03	0.3 ± 0.03	0
Ovary	1.8 ± 0.04	0.9 ± 0.01	0
Number of rudimentary seeds (ovules): 41 ± 0.12			

[1/] **AI:** anthocyanin intensity: 0 = absent, 3 = slight, 5 = intermediate 7 = intense

FRUITS CATIE-R6

Color	Unripe	Green with purple
	Ripe	Yellow with orange and eventual red flecks
Shape	Fruit	Angoleta-Cundeamor
	Apex	Attenuate
	Basal constriction[1/]	3
Mesocarp	Rugosity[2/]	3
	Hardness[3/]	3
Other	Weight (g)	566.1 ± 18.4
	Length (cm)	14.3 ± 0.24
	Diameter (cm)	9.5 ± 0.15
	L/D relationship (cm)	1.8 ± 0.02
Seeds	Fresh weight per fruit (g)	127.2 ± 4.46
	Number of seeds per fruit	31 ± 1.11
Ridge	Thickness (cm)	1.6 ± 0.03
Furrow	Depth (cm)	1.2 ± 0.02

[1/] **Basal constriction:** 0 = absent, 3 = slight, 5 = intermediate, 7 = strong.
[2/] **Rugosity:** 0 = absent, 3 = slight, 5 = intermediate, 7 = intense
[3/] **Hardness:** 3 = soft, 5 = intermediate, 7 = hard.

SEEDS CATIE-R6

Cotyledon color	Light purple
Shape	Irregular
Shape of cross section	Rounded
Length (cm)	2.6 ± 0.07
Diameter (cm)	0.9 ± 0.01
Thickness (cm)	1.2 ± 0.02

CC-137

Country of origin: COSTA RICA
Institution: CATIE
Pedigree: Open pollination of UF-12
Appearance: Trees of large size, leafy and robust. Open branches that tend to join together between rows.

Trunk diameter: 16.8 cm ± 0.92 (14 year old trees, La Lola); 7.7 ± 0.30 (4 year old trees, Turrialba).
Distinctive color for clone: White ◯

LEAVES CC-137

Flush color	Light greenish-brown
Leaf shape	Elliptic
Angle shape	Aristate
Shape of base	Cuneiform
Leaf width (cm)	11.8 ± 0.17
Leaf length (cm)	32.5 ± 0.52
Petiole length (cm)	2.5 ± 0.08
LBW[1/]	11.8 ± 0.18

[1/] **LBW:** Length from the base to the widest point of the leaf

FLOWERS CC-137

Floral Part	Length (mm)	Width (mm)	AI[1/]
Pedicel	21.6 ± 0.34	0.7 ± 0.40	7
Sepal	8.7 ± 0.05	2.9 ± 0.09	3
Ligule	6.2 ± 0.04	2.6 ± 0.08	3
Filament	0.9 ± 0.07	0.3 ± 0.06	0
Staminode	5.7 ± 0.08	0.3 ± 0.06	7
Style	1.3 ± 0.02	0.3 ± 0.06	0
Ovary	1.0 ± 0.01	1.0 ± 0.01	0
Number of rudimentary seeds (ovules): 30 ± 0.15			

[1/] **AI:** anthocyanin intensity: 0 = absent, 3 = slight, 5 = intermediate 7 = intense

FRUITS CC-137

Color	Unripe	Light green and furrows whitish
	Ripe	Yellow
Shape	Fruit	Amelonado-Angoleta
	Apex	Attenuate
	Basal constriction[1/]	3
Mesocarp	Rugosity[2/]	3
	Hardness[3/]	3
Other	Weight (g)	461.6 ± 13.8
	Length (cm)	14.9 ± 0.18
	Diameter (cm)	9.0 ± 0.07
	L/D relationship (cm)	1.6 ± 0.01
Seeds	Fresh weight per fruit (g)	117.3 ± 3.87
	Number of seeds per fruit	27 ± 0.78
Ridge	Thickness (cm)	1.4 ± 0.02
Furrow	Depth (cm)	1.1 ± 0.02

[1/] **Basal constriction:** 0 = absent, 3 = slight, 5 = intermediate, 7 = strong.
[2/] **Rugosity:** 0 = absent, 3 = slight, 5 = intermediate, 7 = intense.
[3/] **Hardness:** 3 = soft, 5 = intermediate, 7 = hard.

SEEDS CC-137

Cotyledon color	Intense purple
Shape	Oval
Shape of cross section	Flattened
Length (cm)	2.5 ± 0.08
Diameter (cm)	1.1 ± 0.02
Thickness (cm)	0.9 ± 0.02

ICS-95 T1

Country of origin: TRINIDAD AND TOBAGO
Institution: IMPERIAL COLLEGE
Pedigree: Unknown Trinitario x Criollo hybrid
Appearance: Trees with the largest size of the 6 clones, leafy and robust. Branches open with much foliage that rapidly closes the space between rows.
Trunk diameter: 13.3 cm ± 0.43 (14 year old trees, La Lola); 8.1 ± 0.27 (4 year old trees, Turrialba)
Distinctive color for clone: Black

LEAVES ICS-95 T1

Flush color	Intense pink
Leaf shape	Elliptic
Angle shape	Cuspidate
Shape of base	Obtuse
Leaf width (cm)	13.4 ± 0.20
Leaf length (cm))	34.4 ± 0.50
Petiole length (cm)	2.7 ± 0.04
LBW[1/]	17.6 ± 0.30

[1/] **LBW:** Length from the base to the widest point of the leaf

FLOWERS ICS-95 T1

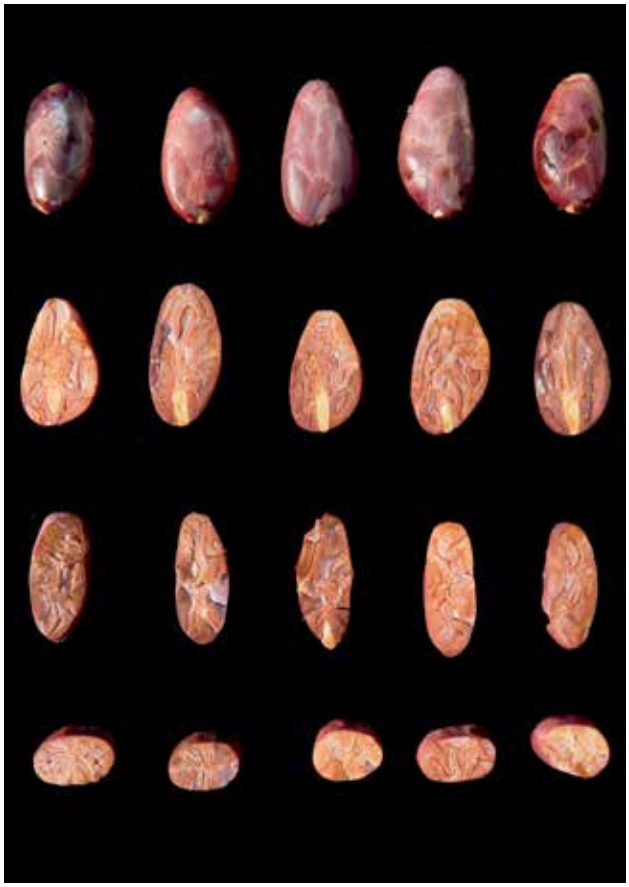
Floral Part	Length (mm)	Width (mm)	AI[1/]
Pedicel	22.1 ± 0.67	0.7 ± 0.71	7
Sepal	8.2 ± 0.60	3.2 ± 0.20	5
Ligule	5.7 ± 0.08	2.9 ± 0.20	3
Filament	0.9 ± 0.07	0.3 ± 0.05	0
Staminode	8.3 ± 0.50	0.3 ± 0.04	7
Style	2.1 ± 0.01	0.3 ± 0.03	0
Ovary	1.0 ± 0.02	1.5 ± 0.40	0
Number of rudimentary seeds (ovules): 33 ± 0.34			

[1/] **AI:** anthocyanin intensity: 0 = absent, 3 = slight, 5 = intermediate 7 = intense

FRUITS ICS-95 T1

Color	Unripe	Dark purple
	Ripe	Orange with yellow
Shape	Fruit	Angoleta-acriollada
	Apex	Acute
	Basal constriction[1/]	3
Mesocarp	Rugosity[2/]	5
	Hardness[3/]	5
Other	Weight (g)	589.7 ± 18.54
	Length (cm)	19.7 ± 0.26
	Diameter (cm)	8.5 ± 0.10
	L/D relationship (cm)	2.3 ± 0.02
Seeds	Fresh weight per fruit (g)	102.0 ± 2.93
	Number of seeds per fruit	33 ± 0.76
Ridge	Thickness (cm)	1.7 ± 0.03
Furrow	Depth (cm)	1.2 ± 0.02

[1/] **Basal constriction:** 0 = absent, 3 = slight, 5 = intermediate, 7 = strong.
[2/] **Rugosity:** 0 = absent, 3 = slight, 5 = intermediate, 7 = intense
[3/] **Hardness:** 3 = soft, 5 = intermediate, 7 = hard.



SEEDS ICS-95 T1

Cotyledon color	Light purple
Shape	Irregular
Shape of cross section	Intermediate
Length (cm)	2.1 ± 0.05
Diameter (cm)	0.9 ± 0.02
Thickness (cm)	1.1 ± 0.02

PMCT-58

Country of origin: COSTA RICA
Institution: CATIE
Pedigree: Trinitario hybrid of unknown parents
Appearance: Trees of intermediate size but with a lot of variation. Their branches are open.

Trunk diameter: 13.2 cm ± 0.51 (14 year old trees, La Lola); 7.5 ± 0.23 (4 year old trees, Turrialba).
Distinctive color for clone: Blue

LEAVES PMCT-58

Flush color	Red with intense brown
Leaf shape	Elliptic
Angle shape	Aristate
Shape of base	Obtuse
Leaf width (cm)	12.5 ± 0.19
Leaf length (cm)	38.0 ± 0.72
Petiole length (cm)	2.1 ± 0.05
LBW[1/]	16.4 ± 0.48

[1/] **LBW:** Length from the base to the widest point of the leaf

FLOWERS PMCT-58

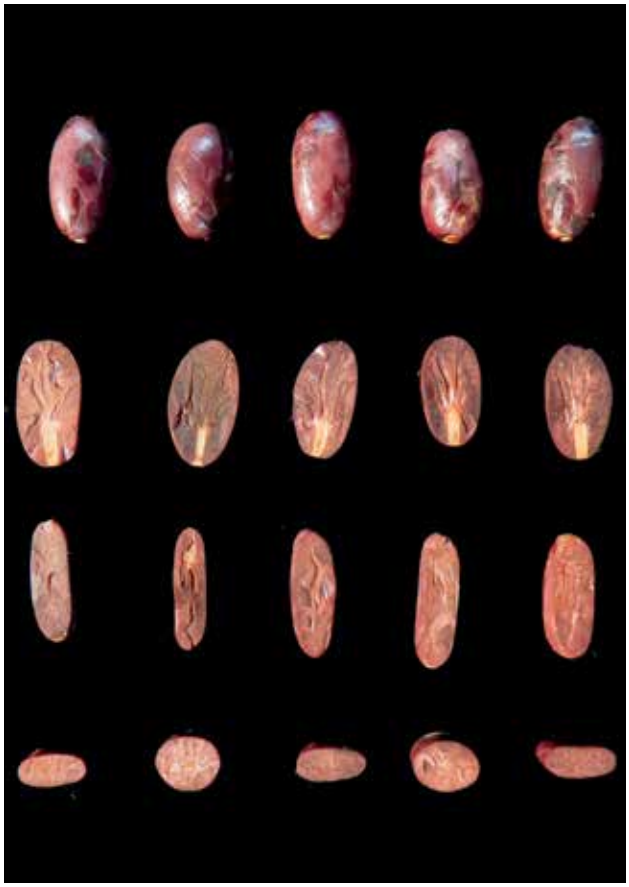
Floral Part	Length (mm)	Width (mm)	AI[1/]
Pedicel	28.6 ± 0.24	0.8 ± 0.17	7
Sepal	9.7 ± 0.12	2.6 ± 0.05	5
Ligule	6.2 ± 0.05	2.5 ± 0.05	3
Filament	1.3 ± 0.08	0.4 ± 0.05	0
Staminode	6.7 ± 0.07	0.5 ± 0.05	7
Style	1.7 ± 0.03	0.4 ± 0.01	0
Ovary	2.7 ± 0.02	1.0 ± 0.02	3
Number of rudimentary seeds (ovules): 39 ± 0.12			

[1/] **AI:** anthocyanin intensity: 0 = absent, 3 = slight, 5 = intermediate 7 = intense

FRUITS PMCT-58

Color	Unripe	Purple with light green
	Ripe	Orange with yellow
Shape	Fruit	Amelonado
	Apex	Obtuse
	Basal constriction[1/]	3
Mesocarp	Rugosity[2/]	3
	Hardness[3/]	3
Other	Weight (g)	441.1 ± 18.50
	Length (cm)	13.8 ± 0.24
	Diameter (cm)	8.8 ± 0.15
	L/D relationship (cm)	1.6 ± 0.02
Seeds	Fresh weight per fruit (g)	93.1 ± 4.48
	Number of seeds per fruit	37 ± 1.32
Ridge	Thickness (cm)	1.5 ± 0.02
Furrow	Depth (cm)	1.1 ± 0.02

[1/] **Basal constriction:** 0 = absent, 3 = slight, 5 = intermediate, 7 = strong.
[2/] **Rugosity:** 0 = absent, 3 = slight, 5 = intermediate, 7 = intense.
[3/] **Hardness:** 3 = soft, 5 = intermediate, 7 = hard.



SEEDS PMCT-58

Cotyledon color	Purple
Shape	Oval
Shape of cross section	Flattened
Length (cm)	2.3 ± 0.09
Diameter (cm)	0.8 ± 0.02
Thickness (cm)	1.1 ± 0.02

Section Three
Molecular characterization

The molecular characterization of the six clones was done by USDA-ARS in Miami in 2010. The analysis of the clones was performed using 18 microsatellite markers (or SSR) developed by CIRAD (Lanaud *et al*. 1999) and commonly used by USDA.

For each microsatellite the methodology allows visualizing the two alleles, one contributed by the female parent and the other contributed by the male parent. The genetic proximity between two individuals, whether individual trees or clones, is proportional to the number of similar alleles they have. Furthermore, the pattern of alleles that a specific material has is stable and allows its identification and differentiation from other genetically distinct individuals. This makes microsatellites a powerful tool for correcting errors in identification, which are very common in cacao. For example, primers have been used by USDA to molecularly corroborate the identity of all the trees of the Mother Clonal Garden of CATIE, which guarantees the distribution of pure propagation material from those trees.

Table 3 summarizes the results obtained from the molecular characterization. It can be seen that most of the microsatellites can individually distinguish between four or five of the six clones studied. Even clones CATIE-R4 and CATIE-R6 that present great genetic similarity as male siblings can be differentiated using any of six primers, including mtcCIR009, mtcCIR015 or mtcCIR025. The same result was observed with two clones that are apparently related genetically, CC-137 and ICS-95 T1. These can be differentiated using some of the seven polymorphic primers identified, for example mtcCIR024.

It is important to acknoledge that while molecular tools are very powerful, they are also costly and not very accessible to many people, so they should only be used when it is essential. Morphological characterization is simpler, it does not have high costs and it allows the identification/corroboration of some genotypes with a certain degree of reliability.

Table 3. Results of the molecular characterization using microsatellites.

Microsatellites	Alleles	CATIE-R1	CATIE-R4	CATIE-R6	CC-137	ICS-95 T1	PMCT-58
mtcCIR003	1	217	217	217	206	206	228
	2	241	271	271	217	217	271
mtcCIR006	1	228	228	228	228	228	228
	2	234	234	234	228	246	236
mtcCIR009	1	286	283	283	254	254	283
	2	286	286	283	286	286	286
mtcCIR015	1	246	232	232	232	232	250
	2	248	248	240	250	250	254
mtcCIR017	1	271	271	271	271	271	271
	2	281	287	287	271	281	271
mtcCIR018	1	331	335	335	331	331	335
	2	354	344	344	344	344	354
mtcCIR019	1	348	371	371	371	375	371
	2	371	377	377	375	375	377
mtcCIR021	1	142	142	142	153	153	149
	2	153	142	142	153	163	157
mtcCIR024	1	200	184	184	184	196	184
	2	200	200	200	184	196	196
mtcCIR025	1	145	128	128	145	145	130
	2	156	145	138	150	150	138
mtcCIR026	1	200	184	184	184	196	184
	2	200	200	200	184	196	196
mtcCIR029	1	163	161	161	157	157	163
	2	165	161	165	161	161	169
mtcCIR033	1	307	284	272	--	296	307
	2	309	308	308	--	344	344
mtcCIR074	1	257	--	--	261	251	--
	2	261	263	263	263	263	--
mtcCIR102	1	111	107	107	115	105	--
	2	115	117	117	117	117	--
mtcCIR172	1	138	124	138	124	124	124
	2	138	138	--	--	--	--
mtcCIR172	1	203	199	199	187	187	203
	2	211	211	203	211	211	211
mtcCIR244	1	260	260	260	243	243	243
	2	264	270	270	264	264	268

Section Four
Agronomic evaluation of the clones

Yield

The productive behavior of the 42 clones that comprise the L6 trial, as well as the reasons that justified the selection of the six clones described in this catalogue are already described in the first part of the catalogue (pg. 17). This section includes details on the productive behavior of the six materials based on data accumulated over 11 years. Also included are the results obtained for the SCA-6 and POUND-7 clones as moniliasis-susceptible controls.

Figure 12 shows the annual yield of the clones during the entire evaluation period. It can be seen that the materials show important fluctuations that are affected by the biannual behavior of the production, which is typical of cacao in the Atlantic zone of Costa Rica (Bazán 1972).

As expected, production has been increasing over the years, starting with values below 400 kg/ha in the third year after planting (first year of production) and reaching levels near 3,000 kg/ha for CATIE-R6 in the eleventh year. The highest production for the trial was attained between the ninth and eleventh years after planting, possibly coinciding with the productive maturity of the trees.

The CATIE-R6 and CATIE-R4 clones have shown the best production in the trial, on several occasions exceeding 2,000 kg/ha (Table 1). The CC-137 clone had notable behavior in the first years, but declined in recent years (Figure 12). For its part, CATIE-R1 had good production and it has even rebounded in the last year, while the ICS-95 T1 and PMCT-58 clones showed intermediate production and had a downward tend relative to the susceptible controls. The susceptible control SCA-6 did not exceeded 200 kg/ha and moreover, its production was reduced due to moniliasis. POUND-7 showed good production in the first 9 years, but its productive potential declined dramatically due to the disease infection, falling nearly to zero in the last year.

The declines in production shown in recent years by the CC-137, PMCT-58 and ICS-95 T1 clones are mainly due to an increase in moniliasis in the area. Given that these are clones of larger stature, it is probable that this reduction is also related to a deterioration in the yield caused by the intertwining of the tree crowns, as has been suggested by several authors (IPGRI 2000; Efron *et al.* 2003b, Lachenaud *et al.* 2005). To correct this situation, there should be intensive pruning and a change in the fertilization regimen, such that the foliage is renewed and the trees are revitalized. This was not done in the trial so as not to affect its experimental conditions.

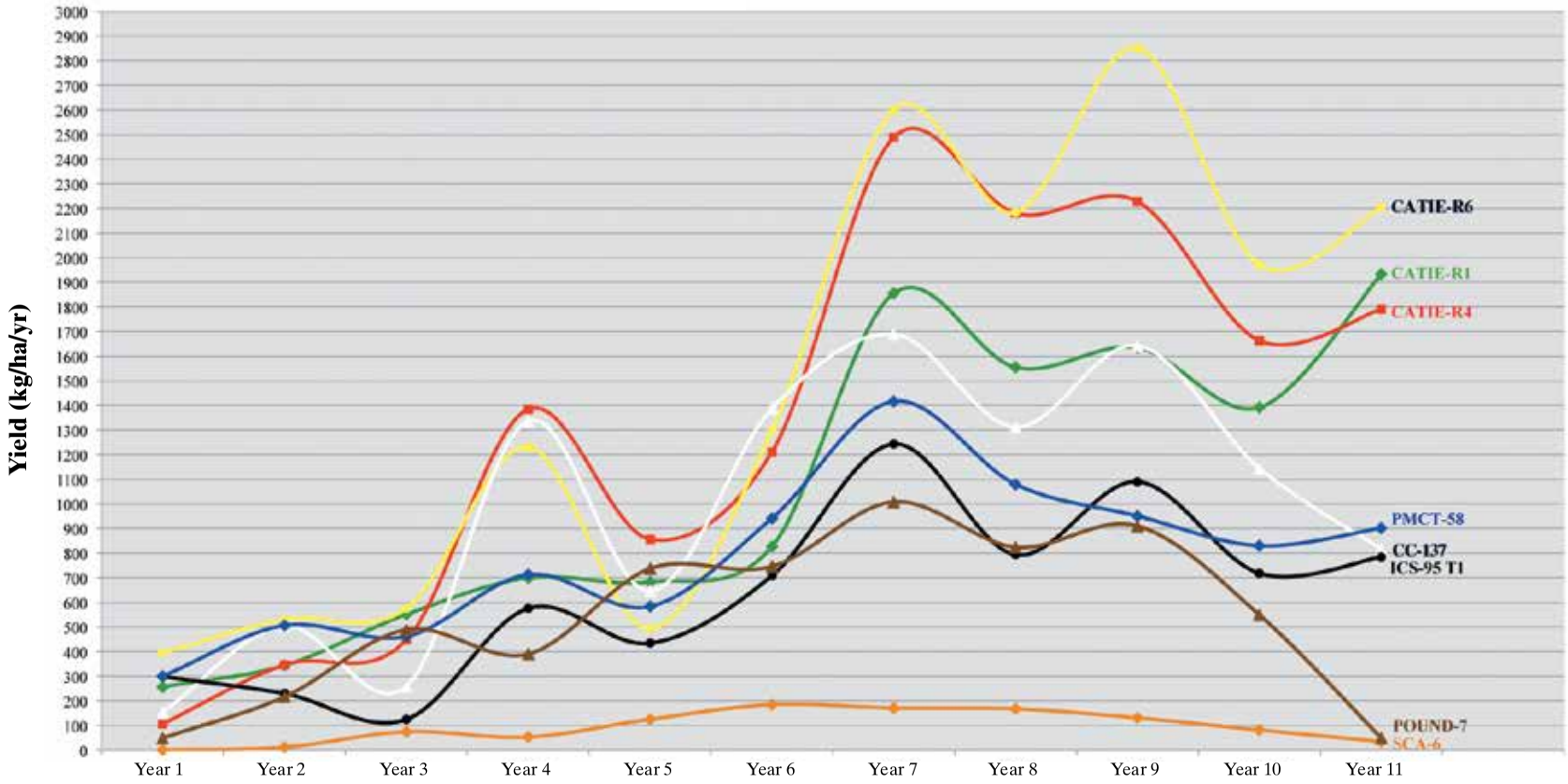


Figure 12. Average production for eight cacao clones evaluated by CATIE over a period of 11 years.

Figure 13 shows the average annual behavior of production adjusted to a calendar year. Under the conditions of La Lola Farm and in general of the Atlantic coast of Costa Rica, cacao production behaves bimodally with a production peak in April and another in November, which was common for all the clones.

Three groups of clones can be clearly distinguished, those such as CATIE-R4 and CATIE-R6 whose production is significantly greater in April, and CATIE-R1 and to a lesser degree PMCT-58, which increase their production at the end of the year. In the middle are CC-137 and ICS-95 T1, which show similar production in both peaks. This information could be useful for planning on-farm agricultural practices and to help predict production at a determined moment. However, it needs to be noted this results are based on averages achieved in specific environmental conditions.

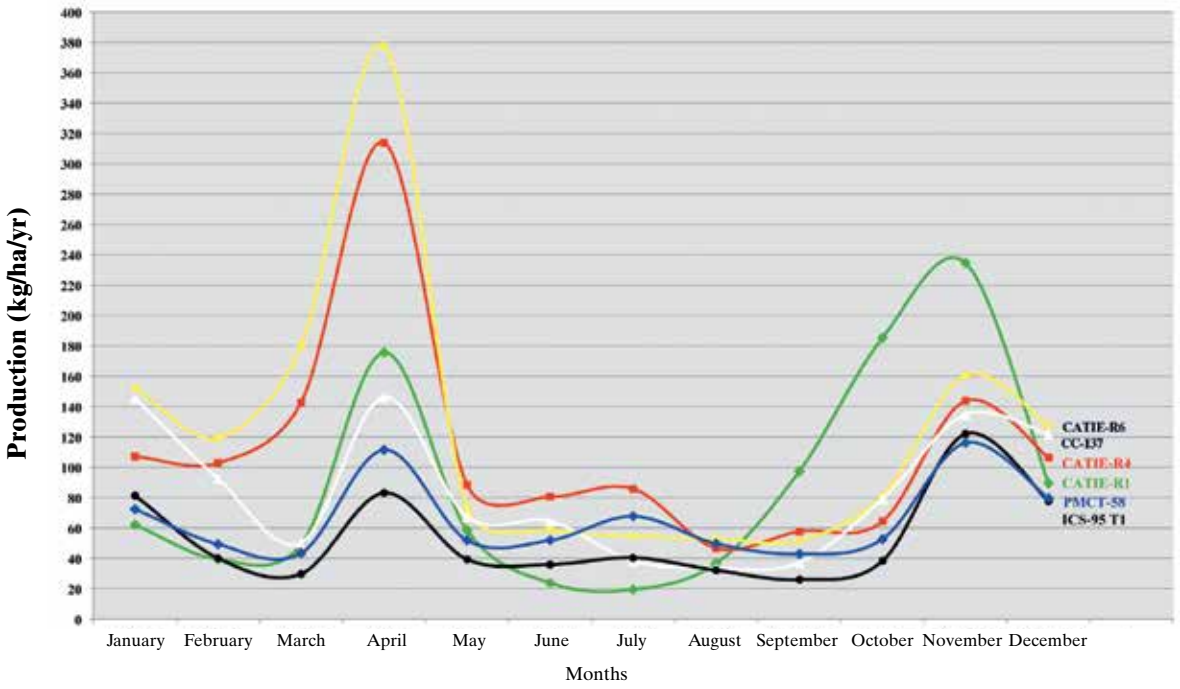


Figure 13. Monthly production (kg/ha/year) for 6 elite clones (average of 11 years of data).

Natural response to moniliasis and black pod infection

The reaction to diseases is also based on results obtained in L6 over 11 years. Considering that the natural incidence of black pod was very low in all the clones (< 7%) (Table 1), the discussion will focus on moniliasis, which is the most limiting factor for cacao production throughout the region.

The incidence of this disease has greatly fluctuated over the years, and it is directly associated with greater fruit production. These fluctuations were more pronounced for the susceptible clones SCA-6 and POUND-7 and less intense for the tolerant clones (Figure 14). The materials can be classified into two groups according to their behavior over the years:

- The first group consists of the CATIE-R clones that showed the lowest incidences over all the years of evaluation with values that did not exceed 25% of losses. CATIE-R6 stood out with very stable resistant behavior and average incidences below 10%. This demonstrates the potential of genetic resistance for reducing the impact of moniliasis even in areas of high infestation such as La Lola Farm. CATIE-R4 also had outstanding behavior, although its resistance is inferior to that of CATIE-R6, showing an unusual increase of incidence in the last year.

- The second group includes the clones CC-137, ICS-95 T1 and PMCT-58, which for several years had losses below 30%, but in the last two years they demostrated a considerable increase in disease incidence. This behavior can be explained by the fact that the materials have been subjected in recent years to a very intense disease pressure. The increase of disease incidence was a result of the concurrence of the following factors: very favorable environmental conditions; increased availability of young fruits; high inoculum pressure in the area; presence of large numbers of susceptible clones in the experimental area (L6); and total absence of moniliasis control.

The establishment of commercial plantations with a proportional mixture of the six clones (polyclone) planted at random or in alternating rows produces a compensatory effect among tolerant and susceptible clones, thus reducing the spread of diseases and the development of epidemics. This behavior is being corroborated in the clonal gardens established by the PCC in Central America (Figure 15), where the incidence of moniliasis and black pod is very low even though production has been increasing. It should be noted that genetic resistance should be part of a package of integrated disease management that promotes a favorable environment for the plant and an unfavorable one for pathogens, as well as the periodic elimination and proper disposal of diseased pods.

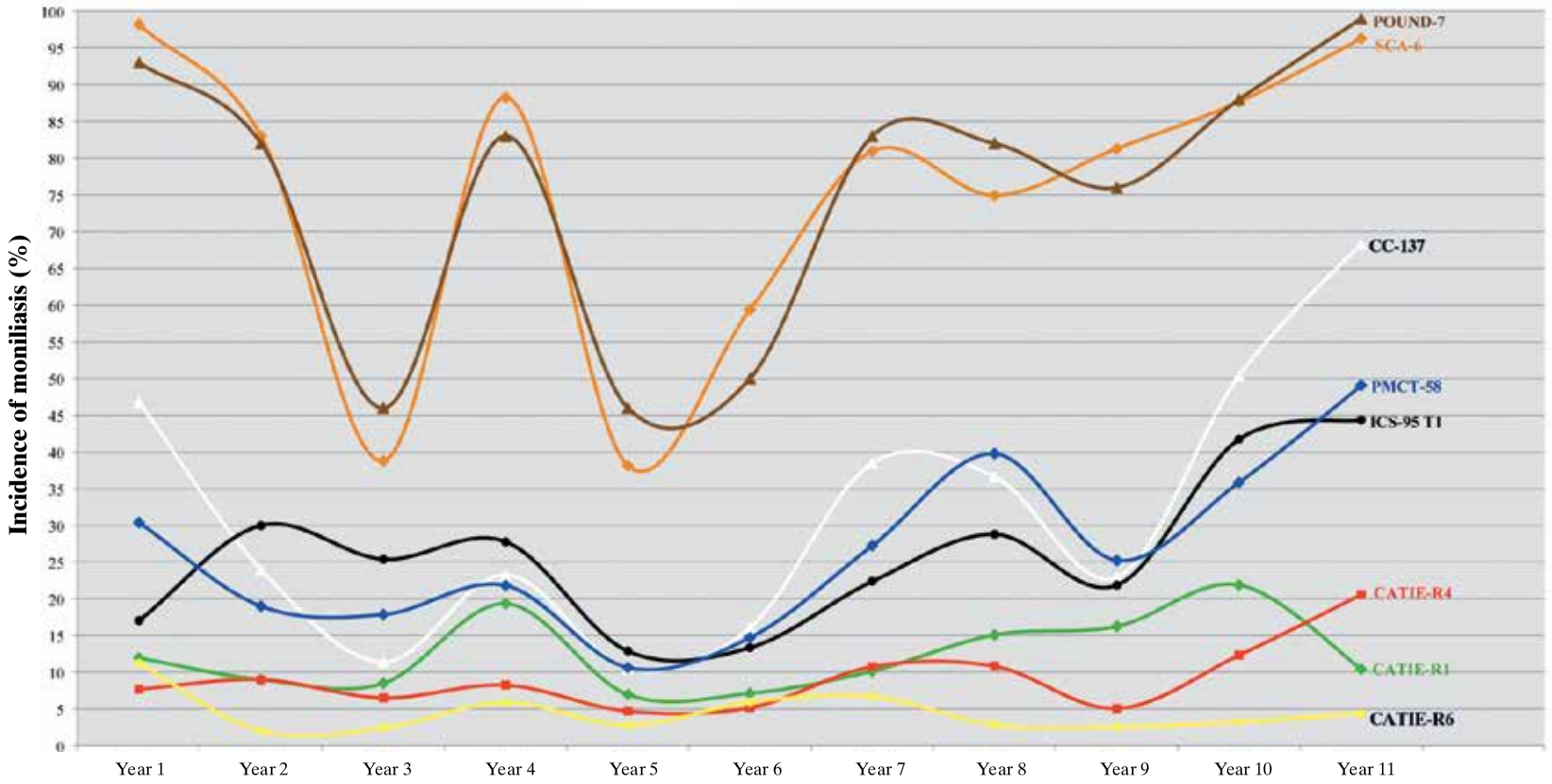


Figure 14. Average incidence of moniliasis in eight cacao clones over 11 years.

Figure 15. A. Partial view of the clonal garden of FHIA (Honduras), established in 2008. **B.** CATIE-R6 tree in production in the Clonal Garden of APPTA (Talamanca, Costa Rica), established in 2008. **C.** Mother Clonal Garden of CATIE (Turrialba, Costa Rica), established in 2007, showing plant size and vigor differences among the CC-137 and CATIE-R1 clones.

Artificial response to moniliasis and black pod infection

The artificial reaction to moniliasis was determined using the method of Sánchez *et al*. (1987) modified by Phillips and Galindo (1988) (Figure 3). The inoculations were done in 2011 on the trees of L6 located on La Lola Farm. Two resistant clones (CATIE-R4, CATIE-R6), one moderately resistant clone (CATIE-R1) and three moderately susceptible clones (CC-137, ICS-95 T1 and PMCT-58) were identified. Artificial inoculations provide great selection pressure due to applications of high concentrations of inoculum (1.2 x 10^5 spores/ml) and a moisture chamber to encourage infection; the results obtained by this artificial method are similar to those observed naturally (Table 1).

The artificial reaction to black pod is determined using the paper disc method designed by Phillips and Galindo (1989) (Figure 3). The CATIE-R6, CC-137 and ICS-95 T1 clones showed moderately resistant reaction, while CATIE-R1 and CATIE-R4 were susceptible. PMCT-58 proved to be highly susceptible. For the clones ICS-95 T1, PMCT-58 and CATIE-R4 the results of the inoculations differed from the behavior observed in the field, which was characterized with very low levels of black pod infection that did not allow determination of differences between the clones (Table 1). The high incidences obtained for these clones as a result of the artificial inoculations may be due to the use of a more aggressive isolate and very high concentrations of inoculum (1.6 x 10^5 spores/ml).

Fruit and seed indexes

The fruit index is the number of fruits required to obtain one kilogram of fermented and dried cacao (IPGRI 2000). It is influenced by genetic and environmental factors, plant age, position of fruits on the tree, and soil and fertility conditions (Soria 1966). For this reason, it is important to use a minimum of 20 fruits (IPGRI 2000) for its determination.

The seed index is the average weight in grams of 100 fermented and dried seeds taken at random (IPGRI 2000).

For the determination of both indexes, fruits from La Lola Farm picked in different seasons of the year in the period 2007-2010 were used. The seeds of these fruits were processed using the protocol that is summarized in Figure 16.

Figure 16. Stages in the fermentation and drying process: **A.** Harvest and identification of material. **B.** Fruit opening and beans removal from the fruits. **C.** Weighing the samples. **D.** Handling and labeling of sample. **E.** Fermentation in stepped boxes. **F.** Turning of cacao beans mass. **G.** Drying. **H.** Drying samples in wooden screens. **I.** Storage and labeling for shipment.

1. Seeds were extracted from fruits that showed adequate physiological ripeness and no damage. Placentas and foreign materials such as pieces of wall were removed.
2. Each individual sample consisting of seeds from a single clone with a weight not exceeding 1 kg was placed in a plastic mesh bag, 80 cm long x 80 cm wide with perforations of 0.5 mm. Each sample was labeled.
3. The plastic mesh bags were placed in the first (uppermost) box in the stacked fermenter, consisting of 5 boxes, 100 x 70 x 70 cm (length, width, height), stacked up like stairs. The bags were placed, alternating 5 to 8 bags with regular fermentation mass. Finally, the box was covered with plantain leaves and jute sacks to keep the temperature stable inside the fermenter.
4. Fermentation lasted 5 days, making the first turning of the mass (dumping the mass into the next lower box) at 48 hours following by turnings every day until the process was completed. During the five days, the temperature was taken at 6:00 and 14:00 hours for the purpose of monitoring and ensuring the success of the fermentation.
5. The drying of the samples was initiated begun immediately after completion of the fermentation phase. The samples were dried under sun light using the procedure recommended by Ed

Seguine of MARS: 3 hours of exposure the first day (10 am - 1 pm); 4 hours the second day (9 am - 1 pm), and 6 hours the third day (9 am - 3 pm); or alternatively in a solar dryer with transparent polyethylene sheets, after which the seeds were left openly exposed to the sun until reaching 7% moisture verified by means of a moisture meter. After each period of exposure to sun, the seeds were piled up and stored under a roof.

6. The seeds were weighed, packed in plastic bags and stored in a chamber at 5 °C until use.
7. The average weight of 100 seeds (seed index) was then determined. The fruit index was calculated based on the number of fruits that entered the process and the dry weight of all the seeds obtained.

Note: The procedure described was also used for the preparation of samples for the analysis of quality.

In all cases, the seed indexes for the six clones exceeded the minimum required by the industry, which is 1 g. The lowest values (1.2 g) corresponded to ICS-95 T1 and PMCT-58, and the highest (1.7 g) to CC-137. The CATIE-R clones showed intermediate values (1.3 – 1.5 g).

The fruit index was very favorable for CATIE-R4, with a value of 18. It was very high for CATIE-R1 (29) and intermediate for the clones CATIE-R6, CC-137, ICS-95 T1 and PMCT-58, which had values of 22 to 24 fruits.

Yield efficiency index

The yield efficiency index is a relationship that is calculated based on the production and the vigor of the tree (Eskes 1999). Trunk diameter is used as a measure of vigor, as it has a highly significant positive correlation with the vigor of the tree and the production of cacao plants (Glendining 1960; Mariano 1966; Peralta 1978; Moses and Enríquez 1979).

The object is to identify within specific populations, the trees or clones with the highest efficiency indexes, demonstrating that they are highly productive with moderate plant development. These materials would help establish more efficient and productive plantations, making use of high planting densities. Moreover, low growing plants facilitate phytosanitary management and shade regulation practices for the cacao and the associated causes trees. The intertwining of the tree crowns is also reduced, which has been mentioned as one of the for yield reductions in adult cacao plantations (IPGRI 2000; Efron *et al*. 2003b, Lachenaud *et al*. 2005).

The efficiency index was calculated for the six clones by dividing the average of the production for 11 years into the square of trunk diameter (cm), measured at 30 cm height 14 years after planting in the trial.

The efficiency index had the following behavior: CATIE-R6 (5.3) > PMCT-58 (4.4) > CATIE-R1 (4.0) > CATIE-R4 (3.8) = ICS-95 T1 (3.8) > CC-137 (3.7). This indicates that CATIE-R6 is the clone that had the best relationship between tree size and production, followed by PMCT-58. CC-137 had the lowest index.

All the agronomic variables described above are summarized in Table 4.

Table 4. Agronomic evaluation of six cacao clones selected by CATIE.

Clone		Natural incidence of diseases (%)				Artificial Reaction		Production (kg/ha/ year)		Indexes		
		Moniliasis		Black pod		Moniliasis[1/]	Black pod[2/]	Average for 11 years	Average for last 5 years	Fruit	Seed	Efficiency
		Average for 11 years	Average for last 5 years	Average for 11 years	Average for last 5 years							
Clones selected	CATIE-R1	12	15	7	6	MR[3/]	S	1066	1674	29	1.3	4.05
	CATIE-R4	9	12	1	1	R	S	1336	2070	18	1.5	3.81
	CATIE-R6	5	4	0	0	R	MR	1485	2363	24	1.4	5.34
	CC-137	32	43	1	0	MS	MR	990	1321	24	1.7	3.71
	ICS-95 T1	26	32	6	4	MS	MR	636	926	22	1.2	3.79
	PMCT-58	26	35	4	2	MS	HS	789	1036	27	1.2	4.35
Controls	CCN-51	45	56	4	2	MS	S	824	1034	18	2.1	4.45
	POUND-7	75	86	0	0	MS	R	542	668	25	1.2	2.21
	SCA-6	75	84	2	0	MS	HR	94	117	47	0.6	0.90
	UF-273 T1	14	16	4	3	R	HS	933	1395	31	1.3	5.00

[1/] Artificial inoculations of *M. roreri* using the methods of Sánchez *et al*. (1987) and Phillips and Galindo (1988).
[2/] Artificial inoculations of *P. palmivora* using the disc method of Phillips and Galindo (1989).
[3/] HS: Highly Susceptible, S: Susceptible, MS: Moderately Susceptible, MR: Moderately Resistant, R: Resistant, HR: Highly Resistant.

Section Five
Self and cross-compatibility

Self-compatibility is the ability of a plant or a group of genetically identical plants (clone) to fertilize its own flowers and produce fruits. Accordingly these plants can be classified as self-compatible, or in contrast, self-incompatible. Cross-compatibility refers to the ability of a plant or clone to fertilize flowers of another, genetically distinct plant or clone that classifies them as cross-compatible or not cross-compatible.

Under experimental conditions, a plant or clone is considered self-compatible or inter-compatible when artificial pollination leads to fruit set (fertilization and fruit formation) superior or equal to 30% in all flowers pollinated (Terreros *et al*. 1983; Royaert *et al*. 2011).

Compatibility is a desirable trait because it facilitates crossings and fruit set and it makes the planting of individual compatible clones possible in uniform areas. In contrast, incompatibility has been associated with lower production (Hardy 1961; Aranzazu *et al*. 2008).

Many clones useful in genetic improvement are self-incompatible, including some of the ones most resistant to diseases; this has caused some programs to make considerable effort trying to reverse this situation (Lopes *et al*. 2011). However, given that the self-incompatible materials usually have the capacity to cross with others (Wood and Lass, 1985), the situation in the commercial plantations can be easily corrected using appropriate planting designs that foster pollen exchange between cross-compatible clones.

Procedure

Self-compatibility and cross-compatibility of the six clones was determined following the standard protocol described by Martins *et al*. (1998) and Eskes *et al*. (2000), which is summarized below and illustrated in Figure 17. In addition, clone IMC-67 was included in the study as control, which is often used on clonal plantations as a universal pollen donor due to its apparent ability to easily fertilize other materials.

Mature flower buds are selected the day before in the afternoon hours and covered with a tube of transparent glass, fixed to the tree with a ring of modeling clay and a rubber band. The outer end of the tube is covered in advance with fine gauze to prevent the entry of insects, water, etc.

Pollinations are done in the morning between 6:00 and 11:00 am on days that are not cold (< 20°C) or rainy. Pollinations are performed by rubbing the anthers from a male parent on the stigma of the female parent flower that has two staminodes removed. The process is repeated with the other anthers until pollen grains are seen to remain adhered to the stigma.

Figure 17. Procedure for determining the self-compatibility and the cross-compatibility of the clones: **A.** Necessary materials. **B.** Placement of the glass tube to isolate the flower. **C.** Selection of adequate flowers. **D.** Preparation of the male parent flower. E. Pollination. **F.** Recently pollinated flower protected by the glass tube. **G.** Fruit set. **H.** Development of the fruit.

After pollination, the flowers are again covered with the tubes and individually labeled. Fifteen days later the percentage of flower retention (FR) is determined.

The artificial pollinations were carried out from September to November of 2008. For each combination (self-pollinations or cross-pollinations), 30 flowers divided into three replicates were manually pollinated. As mentioned earlier, the clones were classified as self or cross-compatible when the percentage of successful pollinations was equal to or greater than 30% (Royaert *et al*. 2011).

Self and cross-compatibility of the clones

Following the methodology indicated, the self-compatibility of the CATIE-R1, CC137 and ICS-95 T1 clones was corroborated, as was the self-incompatibility of the CATIE-R4, CATIE-R6, PMCT-58 and IMC-67 clones (Table 5).

Table 5. Matrix of self and cross sexual compatibility of seven selected clones.

Female parent / Male parent	CATIE-R1	CATIE-R4	CATIE-R6	ICS-95 T1	PMCT-58	CC-137	IMC-67
CATIE-R1	+[1/]	++	++	++	++	--	++
CATIE-R4	++	-	++	++	++	--	++
CATIE-R6	++	++	-	++	++	++	++
ICS-95 T1	++	++	++	+	++	--	--
PMCT-58	++	++	++	++	-	--	--
CC-137	++	++	++	--	++	+	--
IMC-67	++	++	++	--	--	--	-

[1/] (+) = Self-compatible; (-) = Self-incompatible; (++) = Cross-compatible (= 30%); (--) = Cross-incompatible (< 30%).

High levels of cross-compatibility were found among the clones evaluated (Table 5), which will allow planting the material in the field in random mixtures or alternating rows. Three rows of the same clone were also observed to function well in the APPTA Clonal Garden in Talamanca, Costa Rica.

The clones that showed the best cross-compatibility were CATIE-R1, CATIE-R4 and CATIE-R6. These clones can be successfully pollinated by any of the other six clones. They also have a good level of cross-compatibility when they act as male parent except when they are crossed with PMCT-58, CC-137 and IMC-67.

ICS-95 T1 is cross-compatible with all the clones except CC-137 and IMC-67, when acting as female or male parent. For its part, PMCT-58 is only cross-compatible with ICS-95 T1 when it acts as female or male parent and with CATIE-R clones when it acts as male parent.

The clones that have the lowest level of cross-compatibility are IMC-67 and CC-137. As female parents, they cannot be successfully pollinated by the rest, although they can fertilize flowers of CATIE-R clones. This is not inconvenient for CC-137 because it is self-compatible and, it does not depend on external pollen to produce fruits.

IMC-67 showed very low levels of inter-compatibility as female and as male parent, which contradicts the generalized belief that this clone could act as an universal pollen donor in the plantations. Accordingly, Cadavid-Vélez (2006) reported that IMC-67 shows incompatibility with the ICS-39, ICS-60, ICS-95 and SC-6 clones when it acts as female parent.

Section Six
Industrial quality and post harvest processing protocols

There is a growing worldwide demand for good quality cocoa, especially that associated with seals or distinctive geographic origins or genetics. The planting of good quality superior varieties along with proper post harvest practices will lead to benefits for the entire production chain, such as greater earnings for producing families and a more stable supply of fine cocoas for specialty markets. This would potentially increase the sustainability of the cacao plantations in Latin America, the existence of which has been seriously threatened by periodic drops in international cocoa bean prices.

Selection for quality become increasingly important in the improvement programs during the last 10 years, in line with the new market demands. For example, in Ecuador much emphasis has been placed on the development of varieties with the Arriba flavor associated with Cacao Nacional, and in Brazil they are working with high quality varieties to explore the European fine cocoa market (Lopes *et al*. 2011).

In 2005, CATIE began quality assessments of its advanced breeding lines in collaboration with companies and institutions of Europe and the United States such as Guittard, Mars, Chocolate Bernrain, Felchlin, Flor de Santos, Theo chocolat, University of Hamburg, Chocolates Halba, etc., which have evaluated samples prepared by the PMG following the fermentation and drying procedure described in the Section Four of this document (Fruit and Seed Indexes) (Page 50).

Most of the companies have evaluated the quality of the clones separately, while others have shown more interest in mixtures of the clones under the premise that most farmers will be selling blended product. On the other hand, the companies apply different evaluation standards that range from preconceived (the ideal cacao is that which resembles the cacao the company currently processes), to innovative, which aims to identify new types of cacao to expand the supply of differentiated chocolates.

The most commonly evaluated parameters have been the organoleptic quality of the materials using local tasting panels, pH, and the fat content of the seeds. At the University of Hamburg, they have completed more sophisticated evaluations analyzing the content of reducing sugars, caffeine, theobromine, free amino acids and polyphenols, among others (Jens 2011; Hegmann 2012). A synthesis of the results is presented below.

Ed Seguine (MARS) is a global expert on cacao quality and an important collaborator in the CATIE Genetic Improvement Program.

Individual analysis of the six clones

Jens (2011) as well as the Guittard company, indicate that all the clones individually have high quality potential. Accordingly, the CATIE-R4 and CATIE-R6 clones were selected among the best cacaos in the 2009 Salon du Chocolat event in Paris (Box 2), as discussed below. However, it is a fact that there are important differences among the clones, ranging from the acknowledged quality of PMCT-58 to the moderate quality of CC-137, a perception that is shared by companies such as Felchlin, Theo Chocolate and Chocolat Bernrain.

Box 2

"Cocoa of Excellence" in the *Salon du Chocolat* of Paris

Cocoa of Excellence is an initiative led by Bioversity International that promotes cacao diversity as a source of commercial opportunities for producing families and for industries. The goals of the initiative are: to increase knowledge throughout the supply chain of the opportunities existing for market differentiation; to give global recognition to *terroirs*[1/] and to outstanding producers of high quality cacaos; to expose chocolate manufacturers and specialty consumers to the spectrum of existing flavors; to foster links among producers of quality cacao and specialty chocolate manufacturers; and to stimulate the capacity of producer countries to seek, evaluate and produce specialty cacaos (http://www.cocoaofexcellence.org).

Every year, interested countries send cocoa bean samples to the competition that represent the genetic and geographic origins of their region. Only the best samples are transformed into chocolate to be subjected to the scrutiny of a panel of experts in the International Cocoa Awards competition that is held annually as part of the *Salon du Chocolat* of Paris. The jury selects the chocolates that stand out for having characteristic notes of cacao, sweet, floral, fruity, nutty, woody, spicy or other.

1/ Terroir (homeland) is a French word that denotes the special characteristics that the geography, geology and climate of a particular place impose on a particular variety. It refers to a clearly defined and homogeneous geographical area that imprints some noteworthy peculiarity to some agricultural product(s).

Based on the analyses done, the quality of the clones could be classified in descending order as: PMCT-58 > CATIE-R6 > CATIE-R1 > CATIE-R4 > ICS-95 T1 > CC-137, however, this classification could vary in accordance with the criteria for selection that each company applies.

In 2009, the University of Washington, at the request of the Theo Chocolate Company completed a chemical analysis of the volatile profiles of the six clones. The profile of PMCT-58 was found to be very complex in comparison with CC-137 in terms of concentration and presence of volatile compounds, which helps explain the differences in quality between them.

Based on the analyses done by Jens (2011) at the University of Hamburg, it can be concluded that the good quality of PMCT-58 appears to be based on the combined effect of different traits, including: a high fat content, an intermediate caffeine/theobromine relationship, a particularly high free amino acid content despite being associated with a relatively low content of reduced sugars (Table 6). In contrast, the moderate quality of CC-137 is possibly due to the combination of factors such as: a low fat content, a low free amino acid and reducing sugar content, and the high content of theobromine, caffeine and polyphenols.

Companies such as Guittard and Chocolat Bernrain found that CATIE-R6 has good quality potential, followed by CATIE-R4. In fact, during the 2009 Salon du Chocolat event in Paris, the jury selected these two varieties among the best chocolates in the competition. Thus, CATIE-R4 was ranked among the 10 best varieties in the notes for cacao, sweet, floral and fruity and CATIE-R6 in the notes for nutty and woody.

Table 6. Physical-chemical traits of seeds from five clones of the CATIE Genetic Improvement Program (Jens 2011).

Parameters	CATIE-R1	CATIE-R4	CATIE-R6	CC-137	PMCT-58
Average weight of fermented and dried bean (g)	1.25	1.30	1.35	2.00	1.15
Fat (%)	52.3	56.2	55.7	50.6	59.1
Caffeine (mg/g FFDW) [1/]	6.31	4.22	3.77	8.64	5.74
Theobromine (mg/g FFDW)	18.98	22.90	19.30	30.67	23.64
Theobromine/caffeine relationship [2/]	3.01	5.43	5.12	3.55	4.12
Free amino acids (mg/g FFDW) [3/]	16.85	14.43	14.18	9.60	23.77
Reducing sugars (mg/g FFDW) [4/]	2.059	1.787	2.046	1.492	0.836
Total polyphenols (mg/g FFDW) [5/]	55.19	52.13	52.45	64.17	62.83
Epicatechin (mg/g FFDW) [6/]	4.63	2.22	3.20	7.35	3.07
Catechin (mg/g FFDW) [6/]	0.16	n. d.	n. d.	0.32	n. d.

[1/] **Caffeine content:** A high content (more than 3 mg/g FFDW) is associated with a superior quality cacao. FFDW = fat-free dry weight.

[2/] **Theobromine/caffeine relationship:** Indicator used to differentiate between common cacao and premier quality cacao. If the coefficient is less than 8 and it is associated with a caffeine content above 3 mg/g FFDW, the cacao is assumed to be of high quality.

[3/] **Free amino acid content:** A high content is indicative of good aroma potential.

[4/] **Reducing sugars:** In addition to the free amino acids, the free sugars are also necessary for developing the special flavors of cacao during roasting. The amino acids react with the free sugars to form complex aromatic compounds that contribute to high aroma potential of the cacao.

[5/] **Total polyphenols:** Polyphenols have an influence on aroma, color and the anti-oxidant activity of cacao (Elwers *et al*. 2009). The products of the reaction of the phenolic substances are among the most important components of cacao flavor (Rohan and Connell 1964). Anti-oxidant activity has been associated with health benefits due to its anti-carcinogenic, anti-inflammatory, anti-thrombotic; vasodilator and other properties (Hii *et al*. 2009). The greater the amount of polyphenols, the greater the anti-oxidant activity. For example, forastero cacaos have been reported with more than 84 mg/g and criollo cacaos with a minimum of 40 mg/g (Hii *et al*. 2009). On the other hand, the high concentrations of polyphenols are the cause of the bitterness and astringency in cacao, which can also affect the aroma. This makes high concentrations of polyphenols in fine chocolates undesirable (Elwers *et al*. 2009). The brown color of unprocessed cacao seeds is due to the reaction between the phenolic compounds and the proteins or amino acids.

[6/] **Epicatechin and catechin:** Clapperton *et al*. (1994) correlated the flavor quality of different cacao samples with their epicatechin content.

CATIE-R1 is the clone that has demonstrated the most discrepancies in the evaluations provided by the different companies, ranging from those that consider it an excellent cacao (Theo Chocolate) to those that rank it as a cacao with limited potential (Chocolat Bernrain). It appears that for this clone in particular, adjusting the fermentation and drying process and roasting protocols to maximize its quality is very important.

The following evalutions were provided by Ed Seguine. The roasting temperature and process duration are indicated in parentheses:

CATIE-R1 (126°C x 25 min): Mild early acidity that is definitely fruit like. Very pleasant. It has middle cooked fruit / yellow fruit note. It could be seen also as a dried red fruit. It is moderate cocoa in mid-taste with some bitterness with the cocoa. Astringency is present but moderate. Ends with a very pleasant cocoa / browned fruit note along with mild bitterness.

CATIE-R4 (126°C x 25 min): It has an initial acidity that is a cross between fruit acid and a mineral acid (like Papua-New Guinea acidity). Has a shift to the center taste of a very aromatic and floral woody note like a fragrant cedar woody character. Chocolate is moderate with only moderate astringency but more bitterness. It is a very interesting flavor bean type.

CATIE-R6 (126°C x 25 min): Moderate up front acidity with mineral acid and fruit acid notes. Middle taste has some brown wood characteristics with moderate astringency and mild bitterness. It has a later browned fruit / dried fruit note. Has a middle-to-end chocolate base that is quite good. 126°C x 25 minutes is a good roast for this bean.

CC-137 (145°C x 14 min): Moderate up front acidity with a mix of fruit (citric) and mineral acid. Cacao is relatively low. It has low bitterness and moderate astringency. Some generic browned notes but this is not a particularly distinguished bean. It could be used for a very mild milk chocolate.

ICS-95 T1 (not available): Astringent up front followed by sharp acid but not as strong a the lower roasting temperature. Has a distinct bitter, browned character. Has some chocolate but not a lot. Late taste is mildly astringent but has a green forest note.

PMCT-58 (149°C x 13 minutes): Mild acidity early on that gives way to definite browned, dried fruit, leathery, dark raisin-like notes. Clean flavor. Has low astringency and only mild bitterness. Some chocolate flavor but is mild. Very interesting flavor profile. Based on the end taste, the end bitterness is coming from the roast rather than from the beans.

After analyzing samples of the clones CATIE-R1, CATIE-R4; CATIE-R6, CC-137 and PMCT-58, Jens (2011) concludes that they all have a high fat content, an optimum pH level, and an adequate content of methylxanthines (caffeine and theobromine), combined with an excellent blend between these two substances (Table 6). Some of the clones also have a high aroma potential due to their good level and proportion of reduced sugars and free amino acids.

Polyphenol content varied from 5.2 to 6.4%, which is considered adequately low (Jens 2011). Regarding the aroma precursors of aroma, the amount of reducing sugars in the CATIE-R clones are high and they are associated with a good content of free amino acids. This allows to predict a good aroma potential. The reduced sugar content in PMCT-58 is very low, hence it cannot achieve the full aroma potential associated with its high amino acid content. The same applies for CC-137, but in reverse.

The high theobromine concentration of CC-137 could be useful in the future due to the therapeutic uses this substance has as a vasodilator, diuretic, and cardiac stimulant. Theobromine also has an antitussive effect superior to that of codeine, and it is useful in the treatment of asthma, relaxing the respiratory muscles. Theobromine has also been identified as one of the components responsible for the aphrodisiac effect of chocolate.

Analysis of the mixture of the six clones

Considering all the studies done to date, it can be concluded that the mixture of the six clones selected by CATIE (polyclone) has good potential for quality. This has been recognized, even during the 2010 Salon du Chocolat event in Paris, where the mixture was classified among the 50 most outstanding chocolates of the competition. Consistent with this, the analysis done by Chocolates Halba of Switzerland in 2011 concluded that "the mixture of the six clones has good quality, not extremely fine but very good among the trinitario materials."

In 2012 Hegmann found that the mixture of the six clones from CATIE has high reduced sugar and free amino acid contents that are intimately related to the good quality of the final product. In comparing these results with samples from around the world included in the German Cocoa and Chocolate Foundation (2010). The study has concluded that the mixture has chemical characteristics that place it in the group of fine cacaos.

Improvement of pod harvest protocols

It is known that the aroma profile and the potential quality of a cacao variety are defined mainly by its genotype (Thompson *et al*. 2001); however, the fermentation and drying process of the beans also has a decisive role on the quality by fostering the formation of the precursors of aroma in the seeds. For this reason, CATIE has done studies jointly with other institutions and companies to determine the pod harvest processing conditions that maximize the quality of the clones.

In 2008, CATIE and the MARS Company studied the number of days of fermentation necessary for obtaining good quality cocoa from the CATIE-R1, CATIE-R4, CATIE-R6, CC-137 and PMCT-58 clones. Under the conditions in Turrialba (602 masl), all clones were best fermentated after 5 days, compared to 3, 4 and 6 days. According to information provided by the company, 5- days fermented cacao had excellent flavor and intense notes of dry fruit and wood.

The same study concluded that CATIE-R clones have similar flavor profiles and very similar seed sizes which would allow processing them together. Although PMCT-58 has smaller seeds, it could

be blended with the ones above. In contrast, CC-137 should be processed separate because it has different organoleptic characteristics and a seed size significantly larger than the others. Consistent with this, Jens (2011) recommends that to the extent possible, each clone be fermented separate, or at least that CC-137 be separated from the rest because it requires longer fermentation time due to its larger seeds. According to the results of MARS, it is likely that CC-137 could also require lighter but longer roasting that the rest of the clones.

Hegmann (2012) studied different fermentation and drying strategies to maximize the quality of the mixture of the six clones in two contrasting sites: La Lola Farm at 40 masl and CATIE in Turrialba at 602 masl. Based on the results of the chemical analyses (concentration of organic acids, free amino acids, reducing sugars and polyphenols) and the cut test, it was concluded that the best bean quality in both localities was obtained when the mixture was fermented in wooden boxes for 5 days, applying the first turning of the beans after two days and daily turnings for the following 3 days. The best drying was obtained by gradually exposing the samples to the sun: 3 hours the first day, 4 hours the second day and 6 hours on the following days until attaining 7% moisture. After each period of exposure, the seeds were piled up and stored under a roof.

In comparison with the wooden boxes, the use of Rohan trays (particularly at La Lola) for fermentation resulted in production of a larger proportion of violet beans, which is an indicator of insufficient fermentation (German Cocoa and Chocolate Foundation 2010). The trays are more exposed to the environmental conditions and facilitated the development of undesirable microorganisms (Hegmann 2012). Additionally, direct sun drying was better than using a solar dryer with transparent polyethylene sheets, because the latter cause escessively temperature increase resulting in very rapid surface drying of the beans. The councequence of which is that interior of the beans to remain moist, preventing the formation of some of the compounds associated with aroma and flavor. It also facilitates the colonization of microorganisms that deteriorate the quality of the bean.

To maximize the potential for quality of the materials it is recommended that the ideal fermentation and drying conditions, and post-harvest management of the beans in each production area should be studied locally. This should be complemented with studies on an industrial scale to optimize roasting conditions.

References

Anonymous. 1962. The La Lola Story: how United States private enterprise aids LatinAmerican agriculture (on line). Consulted: 15 Nov 2011. Available at: http://books.google.co.cr/books?id=WdkOAQAAIAAJ&pg=PP6&dq=united+fruit+company+COCOA+costa+rica&hl=es&ei=pWrRTpblMKnV0QHfxd0x&sa=X&oi=book_result&ct=result&resnum=1&ved=0CDAQ6AEwAA#v=onepage&q=united%20fruit%20company%20COCOA%20costa%20rica&f=false.

Aranzazu, F; Martínez, N; Rincón-Guarín, DA. 2008. Autocompatibilidad e intercompatibilidad sexual de materiales de cacao. Modelos para el empleo de los materiales de cacao más usados en Colombia utilizando los mejores porcentajes de intercompatibilidad. Unión Temporal Cacao de Colombia. Bucaramanga, Colombia. 24 p.

Argüello, O. 1997. Evaluación de materiales de cacao por resistencia a *Moniliophthora roreri* en Santander. Tercer Seminario Técnico de la Corporación Colombiana de Investigación Agropecuaria. CORPOICA. Bucaramanga, Colombia. p. 23-28.

Argüello, O. 2000. Variabilidad morfoagronómica de 59 árboles élite de cacao (*Theobroma cacao*) en Santander. In: Mejía LA; Argüello, CO. eds. Tecnología para el mejoramiento del sistema de producción de cacao. CORPOICA. Bucaramanga, Colombia. p. 50-54.

Bazán, R. 1972. Patrón de variabilidad de la producción de cacao en la zona Atlántica de Costa Rica. Tesis M. Sc. Turrialba, Costa Rica. Instituto Interamericano de Ciencias Agrícolas. 84 p.

Bazán, R. 1963. Soil survey of La Lola cacao farm. Master's thesis, Agriculture. Turrialba, Costa Rica. Inter-American Institute of Agricultural Sciences of the O.A.S. 127 p.

Briggs, FN; Knowles, PF. 1967. Introduction to plant breeding. Reinhold, USA. 426 p.

Cadavid-Vélez, S. 2006. Características de compatibilidad sexual de algunos clones de cacao y su aplicación en siembras comerciales. Compañía Nacional de Chocolates, Colombia. 28 p.

Cervantes-Martínez, C; Brown, JS; Schnell, RJ; Phillips-Mora, W; Takrama, JF; Motamayor, JC. 2006. Combining ability for disease resistance, yield, and horticultural traits of cacao (*Theobroma cacao*) clones. Journal of the American Society for Horticultural Science 131(2):231-241.

Clapperton, JF; Lockwood, G; Yow, STK; Lim, DHK. 1994. Effects of planting materials on flavour. Cocoa Growers' Bulletin 48:47-57.

Efron, Y; Epaina, P; Marfu, J. 2003a. Breeding strategies to improve cocoa production in Papua New Guinea. In: Bekele, F; End, MJ; Eskes, AB. eds. Proceedings of the International Workshop on Cocoa Breeding for Improved Production Systems. INGENIC. Accra, Ghana. p. 12-32.

Efron, Y; Epaina, P; Tade, E; Marfu, J. 2003b. The relationship between vigour, yield and yield efficiency of cocoa clones planted at different densities. In: Bekele, F; End, MJ; Eskes, AB. eds. Proceedings of the International Workshop on Cocoa Breeding for Improved Production Systems. INGENIC. Accra, Ghana. p. 92-102.

Elwers, S; Zambrano, A; Rohsius, C; Lieberei, R. 2009. Differences between the content of phenolic compounds in Criollo, Forastero and Trinitario cocoa seed (*Theobroma cacao*). European Food Research Technology. 229:937–948.

Eskes, AB; Engels, JMM; Lass, RA. 2000. Working procedures for cocoa germplasm evaluation and selection. IPGRI. Roma, Italia. 176 p.

Eskes, AB. 1999. Evaluation of vigour, yield and fruit and bean traits. Working procedures and Recording Sheets for the CFC/ICCO/IPGRI Project. Montpellier, France.

Evans, HC; Krauss, U; Rios-Ruiz, R; Zecevich-Acosta, T; Arévalo-Gardini, E. 1998. Cocoa in Peru. Cocoa Growers´ Bulletin. 51:7-22.

Fonseca, E; Alvarenga, P; Solórzano, JC. 2001. Costa Rica en el siglo XVIII. San José, Costa Rica. Editorial de la Universidad de Costa Rica. 463 p.

German Cocoa and Chocolate Foundation. 2010. Cocoa Atlas. 2010 Edition. Hamburg, Germany. 1 DVD.

Glendining, DR. 1960. The relationship between growth and yield in cocoa varieties. Euphytica 9:351-355.

Guiltinan, M; Maximova, S. 2002. The Penn State Program in the molecular biology of Cacao.

Hardy, F. 1961. Manual de cacao. IICA. Turrialba, Costa Rica. 439 p.

Hartmann, HT; Flocker, WJ; Kofranek, AM. 1981. Plant science grown development and utilization of cultivated plants. Prentice-Hall. New Jersey, USA. p 33.

Hegmann, E. 2012. The impact of different post-harvest management strategies on the quality potential of raw cocoa in Costa Rica. Tesis Mag. Sc. University of Goettingen and University of Kassel, Germany. 126 p.

Hii, CL; Law, CL; Suzannah, S; Misnawi Cloke, M. 2009. Polyphenols in cocoa (*Theobroma cacao*). Asian Journal of Food and agro-industry. 2(04):702-722.

IPGRI (International Plant Genetic Resources Institute, FR). 2000. Working procedures for cocoa germplasm evaluation and selection. In Eskes, AB; Engels, JMM; Lass, RA. eds. Proceedings of the CFC/ICCO/IPGRI project Workshop 1998. Montpellier, Francia. 176 p.

Jens, I. 2011. Die Bedrohung des Kakaoanbaus durch Pilzpathogene in Mittel- und Südamerika – Qualitätsanalysen an Rohkakao von teilresistenten Klonen (Amenaza a la cacaocultura causada por hongos patógenos en Centro y Suramérica: análisis de calidad de granos de clones parcialmente resistentes). Tesis Bach. Agr. Hamburgo, Alemania. Universidad de Ciencias Aplicadas Hamburgo. 62 p.

Jiménez, FO. 1986. Caracterización climática de la Finca "La Lola". CATIE, Turrialba, Costa Rica. 21 p.

Kennedy, AJ; Lockwood, G; Mossu, G; Simmonds, NW; Tan, GY. 1987. Cocoa breeding: past, present and future. Cocoa Growers´ Bulletin 38:5-22.

Lanaud, C; Risterucci, AM; Pieretti, I; Falque, M; Bouet, A; Lagoda, PJL. 1999. Isolation and characterization of microsatellites in Theobroma cacao. Molecular Ecology. 8:2141-2152.

Lachenaud, P; Sounigo, D; Clément, D. 2005. The compatibility yield efficiency relationship. INGENIC NEWSLETTER 10:13-16.

Lockwood, R. 2003. Who Needs Clothing? INGENIC Newsletter 8:2-4.

Lopes, UV; Monteiro, WR; Pires, JL; Clement, D; Yamada, MM; Gramacho, KP. 2011. Cacao breeding in Bahia, Brazil - strategies and results. Crop Breeding and Applied Biotechnology S1:73-81.

Mariano, AE. 1966. Relaciones entre algunas medidas de vigor y producción en cacao. Tesis Mag.Sc. Turrialba, Costa Rica. IICA. 23 p.

Moses, DD; Enríquez, GA. 1979. Calibrating varieties for yield of cocoa, as well as the relationships of several cacao features with the environment. In: Proc.7th. Cocoa Res. Conf. 1979. Douala, Camerún. p. 51-55.

Paulin, O; Eskes, AB. 1995. Le cacaoyer: strategies de selection. Plantations Recherche Développement 2:5-I S.

Peralta, JR. 1978. Resultados del primer año de evaluación de los efectos del raleo sobre cuatro híbridos de cacao (*Theobroma cacao*) de nueve años de edad. Tesis M.Sc. Turrialba, Costa Rica. IICA. 47 p.

Pereira, JL; Almeida, LCC de; Santos, SM. 1996. Witches' broom disease of cocoa in Bahia: attempts at eradication and containment. Crop Protection 15(8):743-752.

Phillips-Mora, W; Galindo, JJ. 1988. Evaluación de la resistencia de cultivares de cacao (*Theobroma cacao*) a *Moniliophthora roreri* Cif. Par. In Proc.10th. Cocoa Res. Conf. 1987. San Domingo, República Dominicana. p. 685-689.

Phillips-Mora, W; Galindo, JJ. 1989. Método de inoculación y evaluación de la resistencia a *Phytophthora palmivora* en frutos de cacao (*Theobroma cacao*). Turrialba 39(4):488-496.

Phillips-Mora, W. 1996. Studies at CATIE on moniliasis resistance (*Moniliophthora roreri* Cif. & Par.) Evans *et al*.). In: International Workshop on the Contribution of Disease Resistance to Cocoa Variety Improvement, INGENIC.1999. Bahía, Brazil. p. 111-117.

Phillips-Mora, W; Castillo, J; Krauss, U; Rodríguez, E; Wilkinson, MJ. 2005. Evaluation of cacao (*Theobroma cacao*) clones against seven Colombian isolates of Moniliophthora roreri from four pathogen genetic groups. Plant Pathology 54(3):483-490.

Phillips-Mora, W; Coutiño, A; Ortiz, CF; López, AP; Hernández, J; Aime, MC. 2006. First report of Moniliophthora roreri causing frosty pod rot (= moniliasis disease) of cacao in Mexico. Plant Pathology 55:584.

Phillips-Mora, W; Mora, A; Johnson, E; Astorga, C. 2007a. Recent efforts to improve the genetic and physical conditions of the International Cacao Collection at CATIE. *In* Proc.15th. Cocoa Res. Conf. 2006. San José, Costa Rica. p. 611-623.

Phillips-Mora, W; Ortiz, CF; Aime, MC. 2007b. Fifty years of frosty pod rot in Central America: Chronology of its spread and impact from Panama to Mexico. In: Proc.15th. Cocoa Res. Conf. 2006. San José, Costa Rica. p. 1039-1047.

Phillips-Mora, W; Wilkinson, MJ. 2007. Frosty pod, a disease of limited geographic distribution but unlimited potential for damage. Phytopathology 97(12):1644-1647.

Rohan, TA; Connell, M. 1964. The precursors of chocolate aroma: a study of the flavonoids and phenolic acids. Journal of Food Science 29:460–463.

Rondón, JG. 2000. Mejoramiento genético del cacao. *In*: Mejía, LA; Argüello, CO. eds. Tecnología para el mejoramiento del sistema de producción de cacao. CORPOICA. Bucaramanga, Colombia. p. 37-49.

Royaert, S; Phillips-Mora, W; Arciniegas-Leal, A; Cariaga, K; Brown, JS; Kuhn, DN; Schnell, RJ; Motamayor, JC. 2011. Identification of marker-trait associations for self-compatibility in a segregating mapping population of Theobroma cacao. Tree Genetics & Genome DOI 10.1007/s11295-011-0403-5.

Sánchez, J; Brenes, O; Phillips, W; Enriquez, G. 1987. Methodology for inoculating pods with the fungus Moniliophthora (Monilia) roreri. In Proc.10th. Cocoa Res. Conf. 1987. San Domingo, Dominican Republic. p. 467-471.

Soria, J. 1966. Obtención de clones de cacao por el método de índices de selección. Turrialba (IICA). 16(2):119-124.

Terreros, J; Chavarro, G; Ocampo, F. 1983. Determinación de los genotipos de incompatibilidad o compatibilidad en varios cultivares de cacao (*Theobroma cacao*). El Cacaotero Colombiano. 24:27-37.

Thompson, S; Miller, K; Lopez, A. 2001. Cocoa and Coffee. Food Microbiology: Fundamentals and Frontiers, 2nd edition. Edited by Doyle, M.P. *et al*. ASM Press. Washington. pp 721-730.

Wood, GAR; Lass, RA. 1985. Cocoa. 4th Edition. Cornwall, U.K. 620 p..

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Glossary of terms used in the document

Additivity or additive genic action: Type of gene action in which neither of the two alleles is dominant and therefore, both contribute equally to the production of qualitative traits.

Allele: Each one of the alternative forms that a gene may have that encodes for a specific trait.

Clone or clonal variety: Genetically identical plants obtained by asexual reproduction (grafting, cuttings, twigs, laying or *in vitro* culture). Cloning is the way to fix, preserve and reproduce the desirable traits that a particular individual possesses. The differences between plants of the same clone are due to environmental and management reasons, rather than genetic ones.

Sexual compatibility: The ability of a clone to accept or receive pollen from another for form fruits.

Phenotype: The physical expression of the genotype. There are two categories: qualitative phenotypes, which are described, and quantitative phenotypes, which are measured. The terms “phenotype” and “trait” are synonyms.

Propagation material: Any sexual (seeds, pollen grains, etc.) or asexual part of a plant (cuttings, buds, plant tissue, etc.) that can be used for the multiplication of a plant variety or clone.

Genetic improvement: A set of techniques and procedures ranging from simple plant selection to genetic engineering (not used in cacao) that ultimately aims to develop new plant varieties with desirable traits.

Variety breeder: Natural or legal person who obtains and develops a plant variety using a genetic improvement process.

Pedigree: The genealogical relationships of living beings to determine the way in which genetic traits are inherited or manifested.

Segregating population: Population of individuals obtained by crossing two pre-selected parents. The aim is to create offspring that show large phenotypic variation to obtain traits of interest for use in molecular studies called QTL (*Quantitative Trait Loci*).

Polyclone: A group of clones that are planted together to balance their advantages and disadvantages, producing plantations with good productive performance, tolerance to the main diseases, industrial quality, etc.

Improved or superior variety: Set of genetically similar plants obtained by applying some genetic improvement technique, which possess stable, homogeneous and distinctive structural and behavioral traits. These varieties are generally associated with an increase in performance or productivity, resistance to biotic and abiotic agents, quality, or adaptation to adverse conditions, etc. In cacao, because populations obtained from seeds show a lot of variability, the concept of variety is better suited to clones (clonal varieties).

Hybrid vigor or heterosis: The larger size, better productivity, higher resistance, etc. that hybrid plants have with respect to the parents that gave rise to them.