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OVERCOMING THE MAIN LIMITING FACTORS OF CACAO PRODUCTION IN CENTRAL AMERICA THROUGH THE USE OF IMPROVED CLONES DEVELOPED AT CATIE

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ABSTRACT

Cacao cultivation has been an important component of Central American culture and commerce since pre-Columbian times. However, during the last decades of the 20th century, cacao production declined considerably in this region due to the dramatic impact of pod diseases, the low yield-potential of the plantations, and the frequent periods of low prices. An expansion of the activity is currently underway encouraged by the improvement of international and local prices and the increased interest in the markets for Trinitario cacaos, which are prevalent in Central America. Success of these renovated efforts will depend on the ability to overcome the main biotic factors limiting cacao production: the incidence of frosty pod rot (FPR, *Moniliophthora roreri*) and to a lesser extent, black pod disease (BPD, *Phytophthora palmivora*), and the necessity to renovate/rehabilitate the unproductive and susceptible plantations using improved varieties. Clones resistant to FPR and/or BPD were identified in the International Cacao Genebank (IC3) by using artificial inoculation techniques developed at CATIE. They were incorporated in a breeding strategy initiated at CATIE in the late 1990's to generate superior clones and hybrid families possessing disease resistance, high-yield and good quality. This effort has been supported by the World Cocoa Foundation, USDA-ARS, and CFC/Bioversity. The first outstanding clones were recently released for farmer observation and eventual use in six Central American countries through a regional cacao project (PCC), which is executed by CATIE and supported by NORAD (Norway) and farmer organizations. A comprehensive characterization/evaluation of the released clones was previously performed by using different agronomic, morphological, and phytopathological parameters. Self- and cross-compatibility were also determined. The results were complemented with a quality study supported by Guittard Chocolate Co. The establishment of 30 hectares of clonal gardens and 6 hectares of experimental field trials are part of the regional strategy in progress.

INTRODUCTION

A successful cacao breeding program should be consistent with farmer needs and market demands, and be oriented to solve the current and potential biotic factors affecting cacao production in its area of influence. In this manner, improved germplasm could dramatically impact the lives of cacao farmers and contribute to a more reliable supply of cacao beans for chocolate manufacturers, a win-win situation for industry, the developing world and the ecosystem (Guiltinan & Maximova, 2002).

In Central America, more than half of the cacao production currently takes place in isolated, rural areas on small-scale subsistence farms of fewer than 5 hectares. The crop is seriously affected by the impact of diseases and the low-yielding potential of most plantations due to genetic and management reasons. Frosty pod or moniliasis (FPR, *Moniliophthora roreri*) is the major problem causing 30-100% yield losses. This extremely destructive neotropical fungus is present now from Panama to Mexico (Phillips-Mora *et al*., 2007b), with a recent confirmation of its presence in El Salvador (Phillips-Mora, unpublished), the only country that remained free of the disease in this region.

Genetic control of moniliasis combined with adequate agronomic practices seems to be the most viable strategy to obtain efficacious, durable and low-cost disease management for the farmers. In 1996, CATIE with the support of the World Cocoa Foundation initiated in Costa Rica a novel breeding program focused on the selection of superior clones and hybrid families possessing disease resistance and high-yielding ability. Quality is an increasingly important factor in obtaining premium prices for Central American beans. Over the past five years the breeding program has significantly increased selection pressure for quality characteristics.

This paper aims to summarize both the strategy performed by CATIE to generate/distribute superior germplasm for farmer use in Central America, and the information collected regarding the morpho-physiological, phytopathological, agronomic and industrial characterization/evaluation of the first released clones.

BREEDING STRATEGY AT CATIE

To be successful, a breeding program must have a logical sequence whereby each step of the breeding work leads to the next step until the final goal of releasing improved planting materials to farmers is achieved (Efrom *et al*., 2005). During the last years, the following successive steps have been conducted progressively at CATIE: 1. Design and application of reliable artificial inoculation methods to identify resistant genotypes against moniliasis and black pod diseases; 2. Establishment of progeny and clonal trials under a strategy of recurrent selection; 3. Selection of outstanding genotypes based on several years of data; 4. Comprehensive characterization/evaluation of selected materials using morpho-physiological, phytopathological, agronomic and quality parameters; 5. Establishment of clonal gardens, observation plots, and multi-location trials in different environmental conditions.

<u>Identification of sources of disease resistance</u>

The first step in a breeding program emphasizing disease resistance is to categorize appropriate sources of resistance. To reach this objective, it is indispensable to have an adequate source of cacao genetic diversity and confident methodologies to identify resistant genotypes. CATIE met both requirements:

a) The CATIE international cacao genebank (IC3), one of only two worldwide, currently contains 1107 accessions with different geographic and genetic origins (Phillips-Mora *et al*, 2007a). A continuous strategy for the physical and genetic enrichment of this genebank has been implemented during the last six years, with more than 300 wild clones introduced from the Reading Intermediate Cocoa Quarantine Station (UK), CIRAD (France), and several countries in Latin America.

b) CATIE also has developed and implemented effective artificial inoculation methods to test the reaction against moniliasis (Sánchez *et al*, 1987; Phillips and Galindo 1988), and black pod **(**Phillips & Galindo, 1989). Since witches' broom (*Moniliophthora perniciosa*) is not present in this region except south of the Panama Canal, screening for this disease has not been carried out at CATIE. To compensate this situation, international resistant clones available in IC3 such as SCA-6, SCA-12 and CCN-51 were incorporated in the breeding program.

Since the early eighties inoculation events have been routinely executed in IC3. Moniliasis inoculations are performed using the methodology summarized by Phillips-Mora *et al*. (2005). So far, 746 clones have been tested. The clones were classified as follows: resistant 2%, moderately resistant 8%, moderately susceptible 23%, and susceptible 67%. The resistant and moderately resistant clones are: ACT-211; AMAZ-3-2; AMELONADO-15(6); ARF (2, 5, 6, 33, 37); BE-8; CC-240; CHUA0-120; CL-19/10; Criollo (8, 14, 43, 66); EET (75, 129, 401, 407); EQX-69; GC-29; GU (123-N, 125-N, 147-N, 171-N, 254-A); HY-2714184; ICS (10, 75, 95); IMC (27, 54, 55, 60); Laranja; México-(10, 12A1, 14A1); ML-102; NA-756; Nacional-1 (A3, A4, A6, A13, A14); Nacional-2 (A19, A27, A38); P-23; PA (44, 67, 169, 303); Playa Alta-2; PMCT (12, 15, 16, 44, 46, 48, 51, 53, 82); Santa Clara-3; SC-24; SGU-84; UF (273 T1, 613, 712). The continuous introduction of wild clones into IC3 has permitted the identification of novel sources of resistance within various geographic origins, which are being successively incorporated into the breeding program.

The Paper Disc Method (Phillips & Galindo, 1989) is used to test the reaction of the clones to black pod. To date, 819 clones have been evaluated obtaining the following distribution: Highly Resistant 4%, Resistant 12%, Moderately Resistant 15%, Moderately Susceptible 18%, Susceptible 15%, and Highly Susceptible 37%. The following clones were rated as highly resistant: APA-5; ARF (12, 14, 22, 24, 31, 32); BE-4; Criollo-34; EET (59, 272); ICS-47; México-4A1; ML-103; Nacional-3A41; PA (4, 51); PMCT (23, 35, 37, 46, 92, 93, 99); Pound-7; RB-46, and SNK-12. Notice that clone PMCT-46 registered a high tolerance against both pathogens.

Establishment of progeny and clonal trials

With the support of the World Cocoa Foundation, USDA/MARS and the CFC/Bioversity Project, during the last twelve years, CATIE's Breeding Program established 28 field trials in 27 hectares of land and two contrasting environments in Costa Rica: La Montaña Farm in Turrialba (602 m.a.s.l., 2645 mm, 22.5 ºC) and La Lola Farm in Limón (40 m.a.s.l., 3560 mm, 24.5 ºC). Six segregating populations for molecular studies, 532 clones, and 292 hybrid families are now under evaluation. Trees are individually evaluated every month using parameters related to precocity, vigor, yield capacity and disease resistance.

The clonal trials have the following objectives: fix and preserve the outstanding features obtained in individual trees; test the performance of new clones under replicated and uniform conditions, and select superior clones for farmer use. Goals of the progeny trials are: exploit heterosis; accumulate resistant genes against moniliasis; combine disease resistance (to moniliasis and other pathogens) with yield attributes; select superior hybrid families for farmer use, and identify outstanding trees for cloning.

Selection of outstanding genotypes

In 2007 a group of six clones were selected for farmer evaluation in Central America. The clones are part of a clonal trial (L6) established at the La Lola farm in 1998-9 under a Completely Random Block design with four replications and eight plants per replication. L6 comprises 42 clones, some of which were previously identified as tolerant to moniliasis or black pod using artificial inoculations. The trial also includes clones resistant to witches' broom and local controls. The number of healthy and diseased pods and the fresh weight of seeds were evaluated monthly and by tree starting two years after the sowing of the plants in the field. Yield in kg/ha/year was estimated from the fresh weight of seeds. Nine years of data are currently available and summarized in Table 1, only for the most relevant clones.

Table 1: Yield and disease incidence of cacao clones from the L6 trial. CATIE, 2009.

CLONE	Average of 9 years of data (July 2000 - June 2009)			Average of the last 3 years of data
	Yield (kg/ha/year)	Moniliasis (%) [3/]	Black pod (%) [3/]	Yield (kg/ha/year)
***CATIE R-6**	1360	5	0.1	2538
***CATIE R-4**	1251	9	0.2	2272
***CC-137**	1000	27	0.2	1547
***CATIE R-1**	941	14	3.2	1678
UF-273 Type1 [1/]	891	13	0.6	1194
CCN-51 Type2 [2/]	861	43	0.1	1265
***PMCT-58**	777	25	0.9	1144
ARF-22	757	55	0.7	1167
CATIE R-2	731	14	0.0	1106
EET-183	722	25	0.0	1137
CATIE R-7	707	15	0.7	1230
CATIE R-5	676	9	1.8	1076
***ICS-95 Type1**	607	23	0.3	1037
POUND-7	587	74	2.2	908
CATIE R-3	478	19	0.1	815
EET-59	444	51	0.1	778
IMC-60	431	37	1.8	656
PA-169	358	15	2.3	659
SGU-84	313	28	0.0	409
CATIE-1000	301	79	0.0	422
BE-8	292	48	0.2	527
ICS-44	232	73	1.1	633
RB-41	215	77	0.3	243
SCA-12	176	77	0.5	231
UF-712	134	24	2.0	288
SCA-6	100	77	0.1	155
P-23	67	56	0.6	133
GU-133-N	48	19	0.1	98

***** Clones selected for farmer evaluation.
[1/] Type 1 means that the clone fits the true type as determined by USDA-ARS using microsatellites markers.
[2/] CCN.51 Type 2: Although this clone shares many morpho-physiological characteristics with the true type, it is an off-type as determined by USDA-ARS.
[3/] Natural incidence of moniliasis or black pod diseases.

Selection of the six clones was based on their yield capacity and disease tolerance to moniliasis. The most outstanding clones are two CATIE´s selections (CATIE R-6 and CATIE R-4) which consistently registered the highest yields and the lowest natural incidences of moniliasis and black pod since the beginning of the experiment. These clones have a remarkable yielding potential (>2200 kg/ha/year, average of last three years) despite the fact that they grow under conditions of high natural pressure of moniliasis, which causes over 70% pod losses in the international clones Pound-7, SCA-6 and SCA-12 (Table 1). CATIE R-6 and CATIE R-4 originated from the cross "UF-273 Type1 x PA-169", which has been observed at CATIE as producing improved progenies in terms of yield and moniliasis resistance. Since UF-273 Type1 and PA-169 are moniliasis-resistant clones with different genetic backgrounds, this result constitutes a clear example of the predominating additive gene action of moniliasis resistance (Cervantes-Martínez *et al*., 2006), and of the expression of hybrid vigor in cacao.

The other selected materials are mainly clones obtained in Costa Rica in different periods: CC-137, CATIE R-1, PMCT-58 and ICS-95 Type1. ICS-95 was included in the list due to its good performance in Latin America as reported by different authors (Phillips-Mora *et al*., 2005). This clone has been widely used in tropical America over a long period, and as a standard variety against which local varieties were assessed for yield. ICS-95 has been reported as tolerant to moniliasis and witches' broom in South America. Due to its good yielding potential, it is also considered to be a promising material in Peru and recommended for new plantings in all the cacao-producing areas of Colombia.

Despite its good yielding potential, CCN-51 Type2 was not selected due to its low quality profile and intermediate susceptibility to moniliasis. UF-273 Type1 was not considered in the original list; however, it is tolerant to moniliasis and has increased its yielding potential during the last years. For this reason, it has become a good candidate for future releasing.

Comprehensive characterization/evaluation of selected materials

To complement the information on yield and disease reaction described in the previous section, a comprehensive characterization/evaluation of the six selected clones was carried out taking into consideration the following parameters:

Morphological parameters

The color of the ligule, filament and ovary were recorded for flowers. The following parameters were measured for fruits: color of mature and immature pods; weight, length, diameter, basal constriction, apex shape, mesocarp roughness and hardness, thickness of ridge, and depth of the furrows. One of the most distinctive characteristics was the color of the immature fruits. It was red with small green areas in CATIE R-1; pale green with tenuous to intense red blushes in CATIE R-4 and CATIE R-6; green in CC-137, whitish red in ICS-95, and light red with green sectors in PMCT-58.

Self- and cross (inter)-compatibility

Thirty flowers per clone divided into three replications were pollinated artificially to determine the self- and cross-compatibility of the six clones and one control (IMC-67). Controlled pollinations were carried out from September through November 2008 using a standard protocol which is summarized by Martins *et al*. (1998) and Eskes *et al*. (2000). Clones were rated as self-compatible when the percentage of successful pollinations was $\geq$ 20%. Self-compatibility of clones CATIE-R1, CC-137 and ICS-95 and non-self compatibility of clones CATIE-R4, CATIE-R6, PMCT-58 and IMC-67 were corroborated.

Most clonal combinations were found to be inter-compatible (74%), with the best clones being CATIE R-1, CATIE R-4 and CATIE R-6, which are compatible against all other clones except CC-137 when used as the female parent. The exception was CATIE R-6, for which all combinations were successful. Apparently, this clone could become an important source of pollen for all others. PMCT-58 and ICS-95 are inter-compatible against all clones except IMC-67 and CC-137.

CC-137 was the least inter-compatible clone, in particular when acting either as female or in combination with IMC-67 and ICS-95. However, under the assumption that CC-137 is self-compatible, its low level of inter-compatibility is not expected to seriously affect the yield potential of this clone.

IMC-67, used as female or male, was not inter-compatible against PMCT-58, ICS-95, and CC-137. This contradicts the common belief that IMC-67 could be used as a universal donor of pollen in commercial plantations. Cadavid-Vélez (2006) also reported that IMC-67 showed maternal incompatibility against clones ICS-39, ICS-60, ICS-95 and SC-6.

Artificial reaction to black pod

Artificial reaction to black pod was determined using the Paper Disc method (Phillips & Galindo, 1989). Three clones were found to be moderately resistant, two susceptible and one highly susceptible (Table 2). This occurred despite the fact that all clones were recorded as resistant under natural incidence conditions (Table 1). The high concentration of inoculum used in artificial inoculations (1.6×10^5 spores mL^{-1}) could explain this disparity.

Table 2. Characteristics of six selected clones

Clone	Artificial reaction to *P. palmivora*	Self-compatibility	Indexes		Quality traits	
			Pod	Seed	%fat	Quality
CATIE R-1	Susceptible	yes	29	1.3	56	High
CATIE R-4	Susceptible	no	18	1.5	60	Intermediate
CATIE R-6	Mod. Resistant	no	24	1.4	57	Intermediate
CC-137	Mod. Resistant	yes	24	1.7	54	Low
ICS-95 Type1	Mod. Resistant	yes	22	1.2	55	Intermediate
PMCT-58	Highly Susceptible	no	27	1.2	58	High

Quality traits

Cacao samples were fermented and dried by using standard protocols. The following parameters were analyzed by Guittard Co.: fat content, cut test, aroma, flavor, and color. In general, all genotypes showed a good fat content with a maximum of 60% for CATIE R-4 (Table 2). Organoleptic analyses indicate a very good potential of the clones. These results were complemented with those obtained by Chocolat Bernrain AG, Felchlin, Flor de Santos, Theo Chocolate, which considered PMCT-58 as the best clone and CC-137 the worst one.

Seed and pod indexes

Seed and pod indexes were determined using the same samples prepared for quality analyses. Pod index is quite high for CATIE R-1 and PMCT-58; intermediate for CATIE R-6, CC-137 and ICS-95, and very good for CATIE R-4 (Table 2). Seed indexes surpass the minimum established for the industry (1 g).

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

The six clones were recently released for farmer observation and eventual use in six Central American countries through the "Central American Cacao Project," which is implemented by CATIE and supported by NORAD (Norway) and local farmer organizations in Panama, Costa Rica, Nicaragua, Honduras, Guatemala and Belize. The breeding component of the project includes the establishment of one mother clonal garden in CATIE, Costa Rica, and FHIA, Honduras, to supply planting materials for the establishing of 5 ha of clonal gardens (total =30 ha) and 1 ha of a multi-locational field trials (total = 6 ha) in each of the six participant countries. The main goals of the clonal gardens are to evaluate the behavior and adaptation of the clones in different Central American agro-environments; to serve as observation parcels for farmers, and to provide budwood for new plantings. In fact, different initiatives are currently underway in Talamanca, Costa Rica, to provide plants of the selected clones to small farmers in this area.

DISCUSSION

Although it is commonly accepted that the future success and sustainability of cacao cultivation will depend largely on the availability of improved cacao planting material (Lass, 2005), and that there is more than adequate genetic variability in cacao for breeders to achieve vast improvement in yield, very little progress has been achieved in cacao breeding to date (Efron *et al*., 2005). In Central America, as in other regions, the narrow genetic base of most commercial plantations and their vulnerability to highly destructive pathogens make the development of new varieties with disease tolerance and high yields imperative.

In spite of its relatively short existence, CATIE's breeding program has been successful in exploiting the genetic diversity of *T. cacao* included in its international genebank to generate novel cacao varieties. A group of outstanding clones was recently released for farmer observation and use in Central America, and many other clones and hybrid families are in different phases of selection. This germplasm is expected to become an important source of improved material in Central America for both new plantings and the renovation of the current cacao fields, characterized mainly for their low yielding potential and susceptibility to moniliasis disease. Since moniliasis imposes a substantial threat for cacao cultivation not only in surrounding areas (Brazil, the Caribbean, etc) but also on a global scale, the results presented here acquired global relevance.

CONCLUSIONS

The breeding strategy developed at CATIE has been successful for obtaining high yielding and disease- resistant genotypes. The following clones were selected for farmer observation and utilization due to their good performance: CC-137, CATIE R-1, CATIE R-4, CATIE R-6, ICS-95 Type1, and PMCT-58. However, many other clones and hybrid families are in different phases of selection. Results clearly support the fact that it is feasible to improve cacao production in Central America using selected resistant genotypes.

Artificial methods of inoculation were proven to be effective tools for both determining the reaction of cacao genotypes to diseases and selecting resistant genotypes. Resistance to moniliasis and black pod segregate independently in cacao. However, it is feasible to obtain individuals possessing simultaneously high levels of resistance to both diseases. The additive gene action of moniliasis resistance and on the expression of hybrid vigor for yield and moniliasis resistance in cacao was confirmed.

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