

Update on Progress with Investments at IC3 and ICG,T made with Funds from the “Cocoa Conservation Initiative”

November 2022

The Curators of the designated International Cocoa Collections (IC3) at the Centro Agronomico Tropical de Investigacion y Ensenanza (CATIE) in Costa Rica and (of the International Cocoa Genebank, Trinidad (ICG,T) managed by the Cocoa Research Centre (CRC) at the University of the West Indies in Trinidad and Tobago greatly welcome your generous support to their work on improving the curation and conservation of the priceless cocoa material in their care. Since the inception of this initiative, the additional funding has allowed the Cocoa Research Association (CRA) to sign three contracts with CATIE for work programmes at IC3 and two contracts with CRC in Trinidad for work at ICG,T – all very specifically aimed at improving the security of the cocoa material in their care. Your support has funded their work as is described in the attached reports and has improved the conditions of the accessions in these two important International Cocoa Collections, though much still remains to be done. For your interest, I attach **two** reports from CATIE and **one** from CRC on their recent activities funded by your generous contributions. A second tranche of funding for continued activities at ICG,T/CRC is underway.

CRA and the curators of the International Cocoa Collections much appreciate your contributions to their vitally important work of maintenance and enhancement of these significant collections of wild cocoa material. The individual accessions in them have been assembled over many decades of expeditions to the centre of diversity of the species *Theobroma cacao* and represent a priceless resource for global genetic studies and cocoa breeding programmes. While almost certainly not encompassing the full range of the genetic diversity of the species *T.cacao*, the curators of these International Collections have placed their conserved material in the public domain thus making their accessions available on request to cocoa breeders worldwide. The use of such material in cocoa breeding programmes will, in the long-term, increase farmer's incomes, improve the sustainability of cocoa cultivation, and thus assist in achieving improved economic success and the sustainability of our industry. Thank you for your company's vision in supporting the improved security of these unique international cocoa resources. We are counting on your continued support.

Please feel free to circulate this note to any colleagues in your business who have interest in the important work of conservation of cocoa genetic resources. This document with its embedded Reports will be shortly posted on the CRA website and thus will then be a publicly available document.

Many thanks once more for your support for this very important work.

Tony Lass, MBE, Chairman, Cocoa Research Association (CRA) Ltd

November 2022



Attachment 1- CATIE
Report Cocoa Conser



Attachment 2_CATIE
report on CRA fundin



Attachment 3 _CRC
Report on Activities ur

**ANNUAL REPORT
(October 2020 – September 2021)**

***“The conservation of genetic resources in the
International Cacao Collection at CATIE (IC3)”***



Project Cooperation between the Tropical Agricultural Research and Higher Education Center (CATIE) and the Cocoa Research Association Ltd. (CRA)

Contractor: CATIE

Rolando Cerda Bustillos (Project Contact)
Allan Mata Quirós, Dominique Dessauw, Adriana Arciniegas Leal

Project financed by: CRA Ltd.
Michelle End (Project Contact)

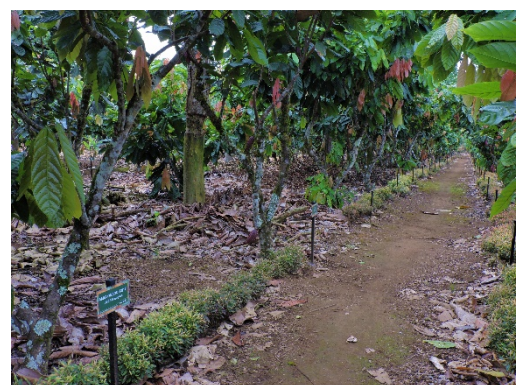
INTRODUCTION

The future of the cacao cultivation depends on the availability and access to the huge genetic diversity of the specie. Cacao genebanks have a very important role on this matter, due to their participation in the conservation and distribution of valuable cacao germplasm, the promotion of the chocolate industry and the development of new varieties with better characteristics, such as productivity, resistance to diseases and extreme conditions, and good quality.

The International Cacao Collection at CATIE (IC3) is one of the two most important international collections in the world. IC3 and the genebank in the University of West Indies in Trinidad and Tobago ensure global access to cacao germplasm through the conservation of their collections, both under the public domain, included in the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA).

The International Cacao Collection at CATIE (IC3) was established in Cabiria, Turrialba, in 1944 and since 2001-2002, the collection was replicated in two contrasting locations in Costa Rica: at La Montaña Farm, in Turrialba (600 m.a.s.l.) and at La Lola Farm, in Matina, Limón (40 m.a.s.l.). CATIE has done recent efforts to improve the physical and genetic characteristics of the collection, but these efforts require the commitment and support of many partners, including national and international players in public and private sectors.

The collection has a very active role in the distribution and exchange of cacao germplasm within tropical America and with other cacao-producing regions around the world. For instance, tolerant clones to moniliasis and black pod diseases identified at CATIE are periodically transferred to the Quarantine Station at Reading and other research institutes in many countries.



DESCRIPTION OF THE COLLECTION

- **Accessions and area**

Currently, the collection conserves 1250 accessions, represented by three trees each. IC3 is located in La Montaña Farm, Turrialba and replicated in La Lola Farm, Matina, Limón, in the Atlantic region of Costa Rica. In both sites, it comprises 10 ha (5 ha each).



- **Accessions rehabilitated and replanted**

Different factors can produce the death of some trees in the field, so, frequently, the status of the trees is updated, in order to avoid missing accessions. As soon as one died tree is detected, the clone is propagated by grafting to complete the three trees per accession.

During the period, more than 300 trees belonging 196 clones were grafted and replanted, in La Montaña as well as in La Lola. In every case when a missed tree is detected, sticks are collected in the surviving trees and bring to the nursery for the grafting process. Labeling is very important to avoid as much as possible any error in the replanting. The list of clones is as follow:

5E	CRIOLLO-65	ICS-95 T1	MOQ-6/95	RIM-39
AMAZ-11	DOM-25	ICS-95 T2	NA-111	RIM-41
AMAZ-15/15	DOM-3	IFC-5	NA-399	RIM-48
AMAZ-6/3	DR-1	IMC-14	NA-702	RIM-52
Amelonado-15	EBC-10/S-401	IMC-20	NA-824	RIM-68
Amelonado-17	EEG-48	IMC-33	Nacional-1(A11)	RIM-76
ARF-30	EET-395	IMC-38	Nacional-1(A7)	SC-13

B-7/14	EET-400	IMC-45	Nacional-4(A47)	SC-20
B-7/21	El Cuá	IMC-78	Nacional-4(A52)	SC-64
B-9/10-25	EQX-O	IMC-85	OC-61	SC-9
C-8756	EQX-Z	INIFAP-51	OC-77	SCA-11
Carmelo-2	FHIA-577	INFAP-70	P-22	SGU-104
CC-107	FILA-49	JA-10/12	P-7p	SGU-43
CC-11	FILA-61	JA-5/24	P-8p	SGU-50
CC-236	GDL-7	JA-6/2	PA-124	SGU-60
CC-252	GN-20	KER-6	PA-156	SGU-72
CC-37	GU 123-V	Lafi-7	PA-169	SGU-90
CC-44	GU 147-N	LCTEEN-302	PA-303	SIAL-339
CC-57	GU 166-N	LCTEEN-341/S-3	PA-51	SJ-1/19
CC-69	GU 183-G	LECTEEN-368	PBC-123	SJ-1/40
CC-9	GU 200-L	LCTEEN-37/G	Playa Alta-2	SPA-4
CHUAO-120	GU 221-H	LCTEEN-68	PMCT-10	SPEC-13845
CHUAO-24	GU 239-H	LCTEEN-D	PMCT-11	SPEC-194/15
CL-10/27	GU 255-V	LF-1	PMCT-13	SPEC-41/6-18
CL-13/4	Honduras-1	LP-4/20	PMCT-17	TAP-10
CNS-22	HY-2714202	LP-4/24	PMCT-29	TARS-1
CRINKLE LEAF	ICS-1	LP-4/8	PMCT-39	TARS-14
CRIOLLO-1	ICS-100	LV-17	PMCT-45	TARS-23
CRIOLLO-12	ICS-12	México-11(A1)	PMCT-49	TARS-34
CRIOLLO-13	ICS-134	México-11(A2)	PMCT-50	TRD-32
CRIOLLO-18	ICS-137	México-4(A4)	PMCT-53	TSH-516
CRIOLLO-19	ICS-27	México-4(A5)	PMCT-98	UF-601
CRIOLLO-22	ICS-39	México-7(A1)	RIM-10	UF-667
CRIOLLO-23	ICS-48	México-7(A2)	RIM-100	UF-677
CRIOLLO-28	ICS-5	México-7(A3)	RIM-105	UF-700
CRIOLLO-37	ICS-6	ML-1/2 Peso	RIM-15	UNAP-2
CRIOLLO-56	ICS-61	ML-4	RIM-19	VB-663
CRIOLLO-62	ICS-68	MO-20	RIM-2	YAMADA-LVD
CRIOLLO-63	ICS-91	MO-9	RIM-21	YAMADA-LVK
				Yucatán

• New accessions introduced

In February 2021, A group of 20 accessions were introduced from the International Cocoa Quarantine Centre at the University of Reading. The budwood were received in good conditions and immediately, it was proceed to do the graftings. Regular observations and maintenance of the plants were done during the following 4 months in the nursery to assure the higher survival rate and establish them in the field.

Out of the 20 clones, 5 clones were missed, so, they will be request in October, when a new group of clones will be received from Reading and 7 more clones from a clonal garden of the *Ministerio de Agricultura y Ganadería* of Costa Rica.

Clone Name	Established in the field	Clone Name	Established in the field
AM-1/57		PNG-139	X
COCA-3348/44		PNG-210	X
CRU-104	X	PNG-215	X
CRU-89	X	PNG-224	X
EET-28		PNG-336	X
ICS-42		PNG-360	X
IMC-33		SCA-10	X
IMC-85	X	SHRS-07	X
LP-4/8	X	SPEC-194/15	X
PA-126	X	TAP-12	X



- **Main activities developed**

- ✓ **Cacao and shade pruning**

Maintenance pruning for the cacao trees and shade species is applied every 6 months in the collection during the rainy season in Turrialba and La Lola. The practice involves the elimination of horizontal branches and the ones over 3 meters. This important practice stimulates the development of new branches to maintain the health and juvenility of the trees.



✓ **Elimination of chupons**

Chupons are eliminated every three months during the weed control application. This practice is very important, mainly in the juvenile plants established in the field.



✓ **Sampling and soil analysis**

Monitoring the soil fertility is very important to ensure the well growing, productivity and health of the trees. During the period, soil samples were collected in all the plots in La Montaña Farm and sent to CATIE's soil laboratory for the chemical analysis. Based on the results, actions were done to correct the deficiencies detected.

✓ **Fertilizer application**

Fertilization with 18-5-15 (NPK) is applied in the field every three months in February, May, August and November. 150 g per tree is applied per time, for a total of 600 g/tree/year.



✓ **Harvest**

Harvest was done every two weeks and monthly at the end of the period when decrease the production of pods. Fresh beans were sold to local consumers, and some clones were

fermented and dried in CATIE's facilities to continue the process of evaluation of quality that CATIE regularly do in order to identify some parameters of flavor in the accessions conserved in the genebank.



✓ **Herbicide application**

Although the weed control is done mainly manually, herbicide is used rationally, only in the plots where weeds grow faster than other plots. Just if necessary, herbicide is applied every 3-4 months.

✓ **Manual weeding control**

Manual control is the main way to control weeds. The weed control is usually applied every three months in all sections of the collection, especially in the gardens and areas surrounding the collection. Depending on the environmental conditions, more frequent applications are necessary.

✓ **Elimination of disease-infected pods**

Pods infected by the common diseases in the collection: Frosty Pod Rot (*Moniliophthora roreri*) and Black Pod Rot (*Phytophthora palmivora*) are regularly eliminated from the plantation every two weeks or every month, depending on the incidence. This practice permits a better control of the dissemination of pathogens.



✓ **Elimination of infected trees**

“Mal de machete” caused by *Ceratocystis cacaofunesta* is a disease affecting some trees in the field. When a tree is detected with the disease, is eliminated and replanted. Although is not usual, a few trees infected by *Rosellinia* were detected in some months ago, so they were completely eliminated and control practices were applied to avoid dissemination of the pathogen.

• **Accessions characterized**

In the CATIE’s genebank, most common activities related to characterization are: Artificial inoculations to test resistance, Compatibility test and quality tests.

✓ **Artificial inoculations**

Artificial inoculations with the pathogens *Moniliophthora rorer* and *Phytophthora palmivora* are routine done in the collection with the objective of identify the resistance sources to these diseases. Many clones have been already evaluated and categorized as resistant or susceptible in different ranges.

In the last year, 1415 pods were inoculated with *M. roreri* in 17 events and 283 pods in 12 events were inoculated with *P. palmivora*. The number of total trees evaluated were 511 and 138, respectively.

✓ **Compatibility test**

Self-compatibility is a character evaluated in the genebank. For this, mature flower buds are covered with a tube of glass, fixed to the tree with a rubber band. Artificial pollinations are done in the next morning between 6:00 and 11:00 am, by rubbing the anthers from a male parent on the stigma of the female parent flower until pollen grains are seen to remain adhered to the stigma.

After pollinations, the flowers are again covered with the tubes and fifteen days later is determined the percentage of flower retention. When retention is $\geq 30\%$, clones are classified as self-compatible.

A group of 19 clones belonging the series ML-, RIM- and SGU- were evaluated during the last year, as indicated as follow:

México-10	RIM -15
ML-1/2 Peso	RIM-13
ML-102	SGU-50
ML-103	SGU-54
ML-105	SGU-60
ML-106	SGU-67
ML-22	SGU-71

ML-4	SGU-82
ML-75	SGU-89
P-22	

✓ Quality test

Basic quality tests are done in the field and in the Quality Cocoa Laboratory of CATIE. After harvest, microfermentation are done by clone to obtain samples. Fermentation takes 6 days and then the samples are dry under the sun.

Then, the samples are brought to the laboratory where cut test is the first step followed by roasting, shelling, grinding and conching.

Small samples of cocoa liquor are prepared in the laboratory, where sometimes are used for organoleptic evaluations. In some times, samples are share to partners related to chocolate industry for chemical analysis.

Thirty-eight clones were completed to initiate the evaluations regarding quality, as mentioned below:

ARF-1	EET-381	MAN-12
ARF-6	EET-387	MAN-15/2
BE-10	EET-397	México-1(A3)
Búfalo-1	EET-408	México-1(A4)
CC-213	EET-45	NA-33
CC-225	FHIA-485	Nacional -1(A3)
CC-235	FHIA-707	Nacional-1(A3)
CC-262	ICS-1	Nacional-2(A19)
CCN-16	ICS-39	Nacional-2(A25)
CRIOLLO-15	ICS-4	RB-46
EEG-29	ICS-47	SCA-9
EEG-48	ICS-95	TSH-516
EET-103	LV-28	

Acknowledgements

We are grateful for the support from CRA, and the contributors to its Conservation Initiative, which complements the support from CATIE, and contributions from MOCCA ([MOCCA – Maximizando Oportunidades en Café y Cacao en las Américas](#) supported by USDA under its Progress for Food program) and the Embassy of France in Costa Rica and allows us to utilize and maintain these valuable genetic resources held in the IC3 for the service of the international community.

**FINAL REPORT
(October 2021 – October 2022)**

***“The conservation of genetic resources in the
International Cacao Collection at CATIE (IC3)”***

***“Characterization/evaluation of genetic resources in
the International Cacao Collection at CATIE (IC3)
2021-22”***

**Project Cooperation between the Tropical Agricultural Research and
Higher Education Center (CATIE) and the Cocoa Research Association
Ltd. (CRA)**

Contractor: CATIE
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Allan Mata Quirós, Adriana Arciniegas Leal, Mariela Leandro Muñoz

Project financed by: CRA Ltd.
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INTRODUCTION

CATIE's International Cacao Collection (IC3) has an important role in the conservation and utilization of the cacao germplasm worldwide. This genebank constitute the genetic pool for research not only at CATIE but also, at many different research centers among Central and South America, USA, Europe and others.

IC3 was established in 1944 in Turrialba at 600 m above sea level (m.a.s.l) and it was replicated in La Lola Farm in Matina, Limón at 40 m.a.s.l. It currently comprises 10 ha in both sites and contains 1262 accessions, representing all the genetic groups and the productive regions in the world. This diversity preserved in the IC3 is a valuable resource for the institution and for the users who access the public domain collection, due to its inclusion in the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA).

Assuring the conservation of the existing cacao materials is an important objective of CATIE, as well as the introduction of accessions with more diverse origin and interesting characteristics. In addition to this, the careful management of the established trees is a priority for the good maintenance of the field.

Agronomical practices for this maintenance include fertilizer application, shade regulation, cacao trees pruning, control of diseases and pests, replanting of missing trees, control of weeds, and harvesting, amongst others.

Distribution and characterization of the germplasm included in the IC3 is another important activity since the main priorities for genebanks are to: preserve the germplasm and assure its optimum use and generation of knowledge to share with the interested users in the world. In this document are reported the activities or products of the “The conservation of genetic resources in the International Cacao Collection at CATIE (IC3)” and the “Characterization/evaluation of genetic resources in the International Cacao Collection at CATIE (IC3) 2021-22” contracts between CRA and CATIE.

IC3 DESCRIPTION



Figure 1. *View of the International Cacao Collection of CATIE.*
A) La Montaña Site. B) La Lola Site

- **Accessions and area**

With the most recent introductions done during the last months, the collection conserves 1262 accessions, represented by six trees each. IC3 is located in La Montaña Farm, Turrialba and replicated in La Lola Farm, Matina, Limón, in the Atlantic region of Costa Rica. In both sites, it comprises 10 ha (5 ha each).



Figure 2. Location of both replicas of the CATIE's International Cacao Collection.

- **New accessions introduced**

Twenty-four clones were introduced from the International Cocoa Quarantine Centre at the University of Reading (ICQC,R) in October 2021. The material was shipped by DHL to CATIE, however, despite the effort and previous planning, custom arrangements were delayed and the budwood was held in customs for more than one week.

As soon as the material arrived CATIE, the grafting was carried out to try to assure the survival of most of the clones. Nevertheless, the rate of success was not as expected, and only 33,3% of the clones were reported as well grafted; so it will be necessary to plan a new introduction as soon as possible.

The list of clones introduced and the number of graftings done are indicated as follow:

CLONE	NUMBER OF GRAFTINGS	NUMBER OF SURVIVING PLANTS
AM-1/57 [POU]	3	1
AMAZ-1/5	3	0
COCA-3348/44 [CHA]	4	1
CRU-12	3	1
CRU-124	4	0
CRU-126	4	0
EET-28 [ECU]	3	0
EEG-8	5	1
ICS-42	3	0
IMC-58	4	0
LP-3/15 [POU]	2	0
PA-39 [PER]	6	4
PNG-153	4	0
PNG-218	2	1
PNG-224	5	0
PNG-296	6	0
PNG-340	5	0
SHRS-01	2	0
SHRS-02	4	1
SHRS-04	4	0
SHRS-05	4	0
SHRS-08	5	1
SHRS-09	2	0
SHRS-37	4	0



A group of Costa Rican clones were also introduced to the International Collection. These clones comprise 8 selected materials from a farm located in Guácimo, Limón and are considered clones with a very good potential for yield and good quality. The clones are well known in the country and were provided by a clonal garden of the *Ministerio de Agricultura y Ganadería* with the objective to preserve them and include them in the routine of evaluations related to artificial inoculations and quality.

The list of clones is indicated as follow:

List of clones introduced	
D1	F6
F3	F9
F4	F27
F5	F29

- **Main activities for maintenance of the genebank**

- ✓ **Cacao pruning**

It is one of the most important activities carried out in the collection. Since health and good vigor of the trees are essential to guarantee the conservation, pruning of the cacao trees are applied twice a year, after the peak of production.

Branches over 3-4 meters are eliminated, as well as the ones which cross over the other's trees, to stimulate the emerging of new branches to maintain the juvenility of the trees and the availability of budwood in case is necessary to propagate the genotype or for distribution.



- ✓ **Shade regulation**

Considering that the cacao trees are adult plants, high shading is not necessary in the collection. Pruning of the *Erythrina sp.* trees is done at the beginning of the rainy season, to avoid the excessive growing of those trees.

- ✓ **Elimination of chupons**

The elimination of chupons is applied every three months, considering the importance of this practice to regulate the soil moisture and avoid incidence of diseases.



✓ **Fertilizer application**

Six hundred grams per year of fertilizer 18-5-15 (N-P-K) is applied per tree, divided into 3 applications (200 g each). Program of fertilization was adjusted according to the production, flowering and weather conditions.

✓ **Manual weeding control**

Since manual control is the main way to control weeding, herbicide was not applied during this period. The weed control is usually applied every three months in all sections of the collection, especially in the gardens and areas surrounding the collection. According to the environmental conditions, more frequent applications are necessary.



✓ **Cacao harvest**

Harvest was done every two-three weeks depending on the production of pods; and monthly at the end of the period when production is low. Fresh beans were sold to local consumers, and some clones were fermented and dried in CATIE's facilities to continue the process of evaluation of quality that CATIE regularly do to identify some parameters of flavor in the accessions conserved in the genebank.

✓ Control of diseases

Pods infected by the common diseases in the collection: Frosty pod rot (*Moniliophthora roreri*) and Black pod rot (*Phytophthora palmivora*) are often eliminated from the plantation every 2-3 weeks or every month, depending on the incidence and the weather conditions. This practice permits a better control of the dissemination of pathogens.



✓ Grafting and replanting

After the regular review of status of the collection, 57 trees belonging 46 clones were planted to substitute missed trees. This is a very important activity carried out in the genebank to assure the conservation of the accessions through the complete number of trees per clone (three trees per location).

A group of more than 85 grafted plants are growing in the nursery to be established in the field in the next weeks to complete several clones.



- **Characterization activities**

- ✓ **Test for the reaction against diseases**

A routine program of artificial inoculations is executed in the IC3 with the objective to evaluate as many accessions as possible to corroborate the reaction against the pathogens *Moniliophthora roreri* and *Phytophthora palmivora* registered previously. From October 2021 to October 2022, 1193 pods have been inoculated in 27 events of artificial inoculation with *M. roreri* and 304 pods in 16 events have been inoculated with *P. palmivora*.

Tables 1 and 2 shows the list of events carried out for inoculations of both pathogens.

Table 1. List of events of artificial inoculation with *Moniliophthora roreri* carried out in the International Cacao Collection of CATIE

Number of event	Date	Number of evaluated clones	Number of inoculated pods
1	01-20-2021	49	157
2	02-03-2021	39	82
3	03-11-2021	37	116
4	04-02-2021	27	120
5	04-15-2021	12	43
6	04-28-2021	31	78
7	05-13-2021	27	55
8	05-26-2021	15	30
9	06-30-2021	14	24
10	07-14-2021	8	19
11	07-28-2021	8	29
12	09-22-2021	14	56
13	10-07-2021	14	34
14	10-20-2021	17	39
15	11-17-2021	13	33
16	11-17-2021	13	23
17	01-26-2022	8	15
18	03-16-2022	15	31
19	03-30-2022	17	40
20	04-21-2022	14	42
21	05-18-2022	12	28
22	06-01-2022	17	50
23	06-28-2022	6	12
24	07-03-2022	4	9
25	07-25-2022	4	8
26	08-11-2022	4	9
27	09-21-2022	5	11

Table 2. List of events of artificial inoculation with *Phytophthora palmivora* carried out in the International Cacao Collection of CATIE

Number of event	Date	Number of evaluated clones	Number of inoculated pods
1	01-25-2021	16	37
2	02-06-2021	6	11
3	03-06-2021	10	18
4	04-24-2021	19	47
5	05-08-2021	5	9
6	06-05-2021	7	13
7	07-03-2021	7	12
8	08-28-2021	11	18
9	10-23-2021	9	18
10	02-26-2022	9	16
11	03-24-2022	9	24
12	04-07-2022	4	7
13	05-05-2022	12	40
14	05-20-2022	3	14
15	09-22-2022	3	13
16	10-06-2022	4	7



Figure 3. Artificial inoculations in the International Cacao Collection of CATIE.
A) *M. roreri*. B) *P. palmivora*

Based on the obtained results, it is expected that during 2023, a list of clones tested for reaction to these two diseases will be published, with a classification in resistant/susceptible according to their characteristics, which will increase the knowledge about germplasm conserved in the genebank and, will facilitate prioritization for distribution to different research initiatives worldwide.

• Distribution of germplasm

Germplasm exchange is an active role of the genebank. Transfer of materials to different users, including research institutions, companies, farmers, and others is frequently done, not only in Costa Rica, but also, to several other countries. An intensive program of shipments of pods is in progress now, whereby pods of more than 300 clones are being transferred to MARS in USA for different studies carried out at the University of Florida.

Some of the clones already transferred to MARS are listed below:

1A	CC-80	GA-11	LCTEEN-37/G	PA-70[PER]	SCA-11
3C	CC-9	GC-29	MAN-15/2[BRA]	PAPAYO	SCA-6
4D	CCN-16	GHANA RED	MEXICO-10	PENTAGONA-2	SCR-2
6F	CCN-51	GS-46	MEXICO-14(A2)	PENTAGONA-17	SCR-5
7G	CL-1951	GU 133/C	MEXICO-3(A3)	PLAYA ALTA-2	SGU-32
ABC-146	COCA-3370/5	GU 140/S	MEXICO-5(A1)	PMCT-93	SGU-4
AMAZ-3/2	CRIOLLO-1	GU 349/N	MEXICO-5(A3)	PMCT-94	SGU-53
AMAZ-5/2[CHA]	CRIOLLO-35	HY-2714182	MEXICO-7	PMCT-95	SGU-90
Amelonado-16(3)	CRIOLLO-60	HY-2714184	MEXICO-7(A4)	PMCT-96	SIAL-163
ARF-10	CRIOLLO-64	HY-2714205	ML-106	PMCT-97	SIAL-339
ARF-22	CU-1	HY-2714207	MO-126	PMCT-99	SIAL-98
ARF-24	CU-3	ICS-10	Mutación Upala	PNG-197	SIC-2
ARF-42	DR-38	ICS-117	NA-246	POUND-12	SIC-28
ARF-7	EEG-25	ICS-134	NACIONAL-3A-41	POUND-16/A	SIC-802
ARF-9	EEG-29	ICS-75	NACIONAL-4(A51)	POUND-18	SPEC-1388
CAS-3	EEG-65	ICS-91	OC-61	POUND-7	SPEC-41/6-18
CATIE-1000	EET-395	ICS-95	P-19	PV-5	SPEC-54-1
Catongo Original	EET-401	IMC-11	P-20	RB-37	TSAN-792
CC-11	EET-407	IMC-20	PA-107[PER]	RB-48	TSH-1112
CC-121	EET-53	IMC-27	PA-121	RB-49	TSH-516
CC-45	EET-62	IMC-67	PA-13	RIM-8	UF-11
CC-47	EET-64	IMC-9	PA-39(170)	Santa Clara-3	UF-677
CC-49	EQX-100	INIFAP-51	PA-4	SC-20	UF-713
CC-57	EQX-93	IQ-1	PA-51	SC-4	YAMADA-LVE
CC-67	F-302	LCTEEN-37	PA-67	SC-61	YAMADA-LVH

Grafted plants of several clones have been already distributed to farmers in Costa Rica, to be established in plantations in all the cacao productive regions of the country. In many cases, there has been a particular interest in increasing the diversity in the farms and to have the opportunity of obtaining other types of cacao pods and flavors.

✓ **Organoleptic profiles**

Seven promising clones were characterized: CATIE-R5, CATIE-R52, CATIE-R58, CATIE-R73, CATIE-R78; CATIE-R91 and CATIE-R92, which include yield parameters, incidence of the two main cocoa diseases such as Frosty pod rot and Black pod, self-compatibility and intercompatibility. To date, sensory profile evaluations continue, which was evaluated at two times of the year. Finally, chemical analyzes were included to determine the content of theobromine, caffeine, free amino acids, total polyphenols, and fat, among others, for the main harvest season.

The results indicate that six of the seven clones characterized CATIE-R92, CATIE-R78, CATIE-R52, CATIE-R58, CATIE-R91 and CATIE-R5 registered an average yield of 1300 kg/ha/year. For resistance to diseases, CATIE-R92, CATIE-R58, CATIE-R91 and CATIE-R5 under artificial inoculation conditions show a high susceptibility to Black pod with an average incidence of over 20%, while when comparing the results with the natural incidence, its percentage of affectation is less than 8%, a situation that is still under evaluation for the reaction to cocoa Frosty pod rot. Regarding compatibility, a high intercompatibility ($\geq 50\%$) was demonstrated between the clones; and CATIE-R52, CATIE-R58, CATIE-R73 AND CATIE-R91 will be developed to be self-compatible with $\geq 30\%$ retention.

In the same way, the sensory profile evaluation of each of the characterized clones continues, however, this information will be documented in the next report that will be submitted, since the new profiles of season one (peak of secondary harvest) and two (main harvest peak), as well as the results of the chemical characterization will be included.

As an example, the sensory profile of CATIE-R92 in season 1 is indicated, which is characterized by its intense cocoa flavor, with notes of fresh and dried fruits. Sweet aftertaste with notes of cocoa and pecans. Sample with creamy texture.

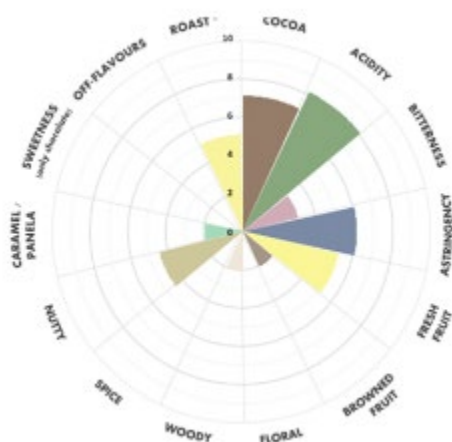


Figura 4. CATIE-R92 cacao clone sensory profile.

• Conclusions

- ✓ The 10 hectares of collection between La Lola and La Montaña have been conserved, which in total has 1,262 accessions and none have been lost, with good practices, replanting dead trees, and all the preservation and maintenance practices.
- ✓ The resistance of 66 accessions to Frosty pod rot, and 46 accessions to Black pod, for a total of 112 accessions has been evaluated.
- ✓ Quality characteristics of 7 promising clones have been evaluated, since these genotypes were prioritized due to their outstanding features that has been identified so far.

- ✓ The support of CRA has been key to conserve the entire collection in both sites and to advance with our research-characterization of diseases and quality evaluation, however, the characterization of more accessions is still vital for research and preservation purposes. Therefore, we appeal to CRA to continue to support CATIE in this task of conserving and generating data for the use of genetic resources in Latin America.

Report to Contributors to the Cocoa Conservation Initiative

(June 2021 – May 2022)

INTERNATIONAL COCOA GENE BANK, TRINIDAD

Rehabilitation and maintenance operations at the International Cocoa Genebank, Trinidad (ICG,T) under this funding proceeded as efficiently as was possible under the constraints of the COVID-19 pandemic in 2021/early 2022, with most of the scheduled activities being successfully completed. During the early part of this renewal work at the ICGT, several members of staff were unable to work in the field/greenhouses due to COVID-19 infections and quarantine periods. Notwithstanding this, major structural and sanitary pruning, fertilizing and termite treatments were completed in two fields with labels being replaced in two other fields. Field evaluation for Witches' Broom disease resistance and fingerprinting of at least 80 additional cocoa accessions was also completed. There are a few operations that have been delayed including the propagation of the 'at risk genotypes.' Initial delays due to the pandemic were compounded with changes in staff and difficulty in getting quality material and rooting of some clones. Notwithstanding this, propagation is continuing while preliminary investigations on improving the technique for rooting is being undertaken.

The current status of outputs proposed at the start of this period of funding in June 2021 with the constraints, limitations and solutions are listed in blue in Table 1. Negotiations for a further 12 months support to ICG,T are underway.

Table 1: Deliverables for Conservation Initiative Contributors for June 2021 – May 2022

Operation/Activities	Outputs	Current status	Constraints, limitations and solutions.
Structural pruning of cocoa trees. This has not been done for many years resulting in overgrown trees vulnerable to falling	Structural pruning of two fields 5B and 6B to reduce height to allow for better management of trees	Fields 5A and 6A have been pruned instead of 5B and 6B, which will be pruned in subsequent cycles. These 2 fields were pruned in preference since their requirements were more urgent.	A decision was made not to coppice any trees therefore the height of some trees were gradually reduced. Further reduction will be done in the next year.
Fertilisation of all pruned trees. Again has not been done systematically for years.	All trees in good growing conditions	Fertiliser application to all trees in 6A and 5A was completed. Nitrbor: 15%N:18%Ca:25%CaO +.3% Boron NPK 12:11:18+2.6Mg+B+Zn+Fe+Mn.	
DNA Fingerprinting of cocoa accessions using SNP technology.	Leaves from all individual trees of 80 accessions will be collected, DNA isolated and SNP genotyped, to identify mislabeled trees.	Leaves have been collected and packaged for DNA fingerprinting	DNA fingerprint results will be received, analysed and 'true to type' trees identified.

Operation/Activities	Outputs	Current status	Constraints, limitations and solutions.
Propagation of at risk cocoa accessions for infilling	Propagation by rooted cuttings and hardening of 250 at risk cocoa clones to be planted the following year.	Propagation of at risk clones via rooted cuttings has recently started. Cuttings from 30 clones have been set in the rooting propagator (Fig. 1). A total of 84 clones that were only located in the greenhouse having a mere one or two plants were transplanted into large pots to improve growth and provide cuttings for propagation	Work on the new Propagation greenhouse under the MOCCA Project was delayed due to COVID-19 but was completed in November 2021 allowing this activity to start. Collecting healthy material has been a constraint for some clones and survival during the rooting and hardening process is challenging for many clones. Different rooting solutions and systems are being investigated, as well as air-layering (Fig.2).
Re-labelling of cocoa trees	Replacement of labels on five thousand trees at ICGT with missing labels or illegible labels due to deterioration.	A total of 5000 trees have been re-labelled	
Field evaluation of cocoa clones for Witches' broom (WB) tolerance; and sanitation pruning. Urgent as many trees have been severely infected	Field evaluation of 500 cocoa clones for WB tolerance, urgent removal of all brooms and fungicide application to at-risk accessions. This year for reasons unknown, there has been a severe incidence of WB disease that has made the susceptible accessions severely stressed.	Sanitary pruning and field evaluation of 500 cocoa clones was completed in 2021. Sanitary pruning of fields 6A and 5A was completed with fungicide being applied twice during the period January to May.	Witches' Broom infestation is very high at the ICGT possibly worsened by the short dry season for the past 2 years. Pruning, encouraged the growth of many new shoots which are being infected by <i>Moniliophthora perniciosa</i> (WB disease) and initial fungicide applications have had limited success. Continual removal of brooms before basidiocarp formation and alternative fungicide applications are being evaluated.
Removal of termite nests and termiticide treatment of trees and around trees.	Two fields 6B and 5B subjected to termite treatment. Recently the incidence has increased and needs to be attacked	Termiticide treatment of field 6A and 5A was applied along the perimeter of fields 6A and 5A. On cocoa trees which were infested, the termite	

Operation/Activities	Outputs	Current status	Constraints, limitations and solutions.
	urgently to reduce problem	nests were removed and the trees and nests sprayed with a termiticide.	
Developing a method for molecular identification of two cocoa badnaviruses occurring on trees in the ICGT.	Developing a protocol to screen for two cocoa badnaviruses in collaboration with several researchers. Two viruses have been characterised but the level of incidence and threat to the genebank remains unknown. A screening protocol can be used to measure the incidence of badnaviruses and eventually identify the risk to ICGT.	LAMP and PCR-based screening tests have been developed in collaboration with scientists at the University of Reading and University of Arizona and are being used to screen for CaMMV and CYBBV on a selected set of ICG,T accessions.	A new project with University of Arizona, supported by USDA, is due to start later in 2022 and will permit wide-scale screening for BaDNA viruses within ICG,T once a CRC staff member has been trained
Upgrade of propagation greenhouse to allow hardening and growth for 6-8 months of propagated plants.	Construction of a 16ft x 50ft. hardening area adjacent to the propagation greenhouse	Propagation greenhouse and hardening area was completed in December 2021.	Full use of the facility was delayed since a faulty irrigation timer had to be replaced
Land clearing and preparation for duplication of 500 cocoa clones planted at ICGT	Preparation of 3 acre site for planting of 2000 trees from 500 clones.	Land clearing of a 3 acre site was completed (Fig. 3). The remaining heaps of vegetation will be burned. Ploughing and rotavating will be done next dry season since the plants are not yet ready for the field.	The Covid 19 pandemic generally impacted the workforce and the ease at which business is done. The selection and hiring of a contractor had to be repeated on three occasions.
GIS mapping of plots to support a georeferenced database	GIS mapping of plots at the ICGT	GIS mapping of plots, and individual trees within a plot, has been completed for two fields. The work is approximately 60% completed.	Should be completed by December, 2022
Building of database which will compile past and future work, making it readily available to researchers and management.	Developing a database for the ICGT collection and starting the populating of it.	The database development work undertaken by UWI's CITS (Campus IT Services) has been completed but the handover to CRC has not yet occurred. All the data from the triennial ICGT census (carried out in 2021/22) has been	Delays in the transfer of the database has prevented its completion. The data will be entered and validated.

Operation/Activities	Outputs	Current status	Constraints, limitations and solutions.
		checked, edited and prepared for populating the database.	

Figure 1: Cocoa cuttings in propagation bin



Figure 2: Air-layered cocoa branches



Figure 3: Cleared land to plant 500 cocoa clones

