Cacao Crop Germplasm Vulnerability Statement
Approved by the CCCGC on November 18, 2020

Summary
The purpose of this Crop Vulnerability Statement (CVS) for *Theobroma cacao* is to communicate periodic assessments of the challenges that cacao face, particularly from reduced genetic diversity resulting from genetic erosion, pests and diseases. The vulnerability status of *T. cacao* worldwide is moderate to high depending on areas of production or at *in situ* locations. Even though there are a few breeders developing new commercial cultivars and molecular tools to improve it, there are several threats and challenges including new diseases, pests, and changing climate combined with industry needs and consumer demands, with a limited number of improved cultivars in production. Collection of genetic resources is key to reducing crop vulnerability resulting from genetic erosion and uniformity, and for providing crop breeding and research programs with novel traits and underlying genes to satisfy evolving demands.

The genus *Theobroma* is in the family Malvaceae (Whitlock and Baum, 1999). Cacao is one of 22 species of *Theobroma* and is indigenous to the Amazon, South and Central America regions. *T. cacao* is one of several tropical fruit tree species within the Malvaceae that produces fruit with significant monetary value. Cacao is a valuable crop worldwide but 90% of the crop is typically produced by small holders with less than 5 ha, mainly in Africa, for whom linkages with international markets should be enhanced as a way to improve their livelihoods (ICCO, 2018; Gayy and Tsowou, 2016). In 2018, estimated global production was 4.45 million metric tons (FAOSTAT, 2018, ICCO, 2018). Revenue generated from cocoa products in the USA was valued at US$3,371.3 million in 2019 (Candy Industry, 2019). Cacao is an important cash crop grown throughout the humid tropics, predominantly by small holder farmers, with about 6.5 million hectares planted with the crop in more than 57 countries. Cacao is considered a very important food crop worldwide and the most important cash crop in West Africa (Omont, 2001).

Total production of cocoa beans has increased in recent years (ICCO, 2020). Cacao grown under intensive management requires substantial inputs of nutrients, pesticides, fungicides, and water to attain high yield and quality. There are several diseases and insect pests of economic importance for cacao. Since managing pests and diseases with chemical applications can be expensive and can be hazardous to human health and the environment, breeding for disease resistance offers the most sustainable and effective means of management. The development of cacao varieties with greater resistance to insect pests and diseases is necessary for the cacao industry to succeed (Gutierrez et al., 2016). In addition, research efforts should focus on abiotic stress tolerance since global climate change models predict a decrease in cacao yields throughout much of the neotropical regions mainly due to higher than normal temperature throughout the growing season (Gateau-Rey et al., 2018).

The development of drought tolerant varieties is expected to increase yields by >15% in the majority of countries. Thus, cacao farmers will likely benefit if breeders include selection for drought and heat tolerance. If irrigation water becomes less available and rainfall becomes more infrequent, it will also be important to improve water use efficiency in cacao. In addition, cacao breeders will need to develop cultivars with higher nitrogen use efficiency in order to offset the

increasing cost of nitrogen fertilizer and minimize the pollution of groundwater contamination by nitrates.

The United States Department of Agriculture (USDA) cacao program focuses on close cooperation with industry partners and with producing countries in several different areas of research: 1) The establishment of a global cacao molecular identification center in Beltsville, Maryland, to develop and use internationally standardized molecular probes for Deoxyribonucleic Acid (DNA) fingerprinting of all major germplasm collections of *T. cacao* in the Americas and other regions in the World; 2) The establishment of a USDA germplasm collection center in Mayaguez, Puerto Rico as a repository characterization and evaluation site for diverse accessions of *T. cacao*; 3) The establishment of a quarantine center in Miami, Florida to assist in the introduction of new elite disease-resistant accessions to the collection in Mayaguez, PR; 4) An in-depth molecular program of gene discovery, genetic mapping, gene expression and examination of population genetics of *T. cacao*, located at both Miami, Florida, and at Beltsville, Maryland; 5) The use of biocontrol agents, as part of an integrated pest management (IPM) strategy against the fungal pathogens causing diseases of *T.cacao*, located at Beltsville, Maryland (Saunders et al., 2001); and 6) Safety duplication of a core collection at the Pacific Basin Agricultural Research Center, National Clonal Germplasm Repository (PBARC, NCGR) in Hilo, Hawaii. Work is carried out in collaboration with other collection centers such as CATIE in Costa Rica, University of Reading in United Kingdom, and Cocoa Research Centre in Trinidad and Tobago. The sequencing of the cacao genome in 2010 opened new opportunities to utilize genomics for cacao improvement (Argout et al., 2011). The release of the cacao genome sequence has enabled cacao breeders to carry out more efficient research and to accelerate the breeding process in an effort to release superior cacao cultivars.

The cocoa tree produces recalcitrant seeds which cannot be preserved. The genetic resources of the cocoa tree are therefore preserved in the field. The most severe and critical threat to cacao germplasm collections would likely come from a natural disaster (i.e. hurricane, extreme drought or flooding), or from an overwhelming pest or disease infestation. Although *T. cacao* germplasm collections are replicated (backed up) at international cacao collections, restoring full genebank function could involve a slow and high-priced recovery, probably requiring temporarily abandoning non-critical (but important) genebank services such as distributions.

Introduction to the crop

Botanical features and ecogeographical distribution

The genus *Theobroma* is within the family Malvaceae and has 22 species. The chromosome number of *Theobroma cacao* L. is 2n=2x=20. Over 17 wild relatives of cacao and *Herrania*, a closely related genus, also share the same number of chromosomes (Glicenstein and Fritz, 1989). *Theobroma cacao* is the only species which is cultivated widely for economic value, the other better known species in the genus being *T. bicolor* and *T. grandiflorum*. An inventory of wild species is being developed by USDA-ARS and other organizations to ensure their long-term conservation.

Cacao is an understory fruit-tree species native to South and Central America (Motamayor et al., 2002). It is entirely neotropical in origin and its natural distribution is in the tropical lowland rainforests having a restricted geographical distribution, which extend from the Amazon basin through Southern Mexico (18°N to 15°S) (Cuatrecasas, 1964; Purseglove, 1968).

The flowers are formed on the trunk and branches, a habit referred to as cauliflorous or truncate. The flowers are only produced on wood of a certain minimum physiological age, which is usually two or three years old under good growing conditions and they are quite small, about 15 mm in diameter.

Most diploid wild and cultivated relatives are either self-incompatible or self-compatible. The latter suffer greatly from inbreeding depression, so a uniform commercial crop is almost exclusively accomplished by clonal propagation.

T. cacao domestication has been previously reported to have occurred in Mesoamerica (Motamayor et al., 2002) However, recent findings by Zarrillo et al. (2018) indicate that the cacao center of diversity was located on the upper Amazon region of northwest South America as was previously reported (Clement et al., 2010; Motamayor et al., 2008), which was also the center of domestication. Cacao varieties identification has been mainly classified by group using the terms ‘Forastero’, ‘Criollo’ and ‘Trinitario’ (Motamayor et al., 2002; Clement et al., 2010; Motilal and Butler, 2003), however, current research (Motamayor et al., 2008) proposed a new classification with 10 groups based on genotyping with 106 microsatellite markers

Genetic pool for cacao breeding

Wild relatives of cacao are genetically rich and diverse in traits that may be of economic value (CacaoNet, 2012). Most of this germplasm is not sexually compatible with cultivated cacao. Unlike many other crop plants, hybrids between wild and cultivated cacao can look much like ordinary breeding lines, however, they are of lesser quality and survivability is an issue. On the other hand, crosses between wild relatives (i.e. *T. bicolor* x *T. grandiflorum*) has been reported to have been very successful and productive (Cuatrecasas, 1964).

Some wild cacao relatives can be crossbred with the cultivated cacao, either directly or by applying strategies that allow the circumvention of hybridization barriers (Medina and Laliberte, 2017). In effect, enhanced cacao germplasm has made important contributions to disease

resistance, enhanced yield, and improved quality through plant breeding for over the last century (Bekele and Phillips-Mora, 2019). However, despite all these apparent advantages, use of enhanced germplasm is not easy to accomplish in practice, as witnessed by the fact that a small proportion of the genetic diversity in genebanks has been incorporated into advanced breeding lines.

Primary products and their added value

The global cocoa beans market size was estimated at USD 9.94 billion in 2018 and is projected to expand at a rate of 7.3% from 2019 to 2025. A rapidly growing chocolate industry in emerging economies including China and India is expected to boost the demand for cocoa beans as intermediates. The rising importance of cacao products coating in the processing of vegetables, fruits, and cereals for improved flavor is projected to increase the product demand further (Crandview Research, 2018).

Cacao butter, liquor and powder are the primary products of cacao. Secondary products include soft drinks and alcohol, animal feed and fertilizer. After fermentation and processing, many other important products can be produced.

In 2018, cocoa butter was the largest product segment and accounted for more than 50% of the global share of cocoa products, including production of chocolate. This product is also used as a lubricant in pharmaceutical industry. It is also used as anti-oxidant, flavor enhancer, and preservative in food and beverage industry. Furthermore, it is used as an aroma enhancer and humectant in the formulation of cosmetic goods.

Domestic and international crop production

United States

Cacao is not a main cash crop in the US. It is produced only in a couple of areas of the US, specifically in Hawaii and to a lesser extent in Puerto Rico and is harvested year around. The vast majority of the production is in Hawaii, accounting for around ~99% of US production in 2018. Yield per acre fluctuates widely among locations, and growing seasons, with the highest yields in the summer crop in both locations.

Total production in 2018 in Hawaii and Puerto Rico was estimated at around 17.0 and 5.0 tons, respectively. In 2013, there were 15,700 kg of cocoa beans harvested in Hawaii. Of that, 12,200 kg were grown on Oahu, 2, 700 kg on the Big Island, 285 kg on Maui, and 52 kg on Kauai (Hawaii Cacao Growers Association, 2019).

Although Puerto Rico and Hawaii have emerging cacao industries, the majority of production is in Africa (72.3%), the Americas (18.3%), and Asia (9.4%) (ICCO, 2018). This means that the multi-billion-dollar American chocolate industry depends largely on foreign countries for its cacao beans supply. In 2018, the U.S. was the worldwide leading chocolate importer, accounting for 18 % of total global imports (~$1.5 billion). In the same year, U.S. chocolate product exports achieved a record value of $1.1 billion and currently the U.S. is the second main global chocolate candy exporter, accounting for approximately 14 % of global trade (USDA-FAS, 2013).

The U.S. chocolate and confectionery industry is a principal consumer of key U.S. agricultural commodities. For every dollar of cocoa imported, between one and two dollars of domestic agricultural products are used in the making of chocolate, and this represents more than 70,000 jobs maintained in the US economy. U.S. consumers spent $22 billion on chocolate in 2017, which is approximately 12 pounds of chocolate consumption per person (Candy-Industry, 2019).

Three U.S. companies are ranked among the top 10 global confectionery corporations based on sales in 2016: MARS, Inc. ($18,000 million), Mondelēz International ($12,900 million) and Hershey Co. ($7,461 million) (Candy-Industry, 2019). In addition, important U.S. agricultural commodities such as milk, sugar, almonds, peanuts, and corn syrup sweeteners are used by the chocolate industry.

International

Cocoa world production in 2019 was estimated at 4.849 MT. Côte d'Ivoire and Ghana are by far the world's largest producers of cacao with 2.19 million and 850,200 tonnes, respectively. Other major cacao producing countries include Ecuador, Cameroon, Nigeria, Indonesia, Brazil and Papua New Guinea, in that order **(Annex 1)**.

The global chocolate market was valued at around USD 103.28 billion in 2017 and is expected to reach approximately USD 161.56 billion in revenue by 2024. The global chocolate market finds its scope in North America, Europe, Asia Pacific, Latin America, and the Middle East & Africa (Zion Market Research, 2018).

Cacao production around the world is sustained by small scale farmers and is currently affected by biotic and abiotic factors. The beans (seeds) obtained from the pods (fruits) are used by the confectionary industry as the main ingredient of chocolate as well as in the food and beverage, cosmetic, and pharmaceutical industries. Although diseases and insects are responsible for most of the production loses, environmental factors such as drought and high temperatures have also severely affected global cacao production.

Urgency and extent of crop vulnerabilities and threats to food security

Genetic uniformity in the "standing crops" and varietal life spans

It is generally agreed that the cultivated cacao in Neotropical areas, Africa and Asia have a constricted genetic base (Soria, 1959; 1978). Studies comparing modern with historical cultivars has not been able to detect significant genetic improvements in yield or pest and disease resistance during the last 20 years (Bekele and Phillips-Mora, 2019). The results show that a century of cacao breeding has not resulted in significant genetic advances for these traits. In spite of this, current cacao production in South America has a much more diverse cultivar base than it did several decades ago. With some exceptions the use of CCN51 by several Latin American countries has been increasing, leading to more genetic uniformity in the fields.

Most yield improvements have resulted from better management practices and a shift to production in geographic regions with higher yield potential. Genetic gains for yield have been less than expected in comparison, although recent cacao varieties have produced significant

economic benefits in terms of increased marketable yield, pest and disease resistance, and improved seed number and quality. Looking forward, it appears possible that genetic improvement will make a greater contribution to productivity increases, but only if growers, processors, and commerce develop, release and adopt novel varieties and especially if the growers adopt better crop management techniques. There is an urgency to increase production through increases in yield per ha.

In a multisite experiment in Puerto Rico, Irizarry and Rivera (1998) studied the yield potential of 1320 trees representing five interclonal cacao full sib families (UF-668 x Pound-7, IMC-67 x UF-613, EET-400 x SCA-l2, SCA-6 x EET62, and IMC-67 x SCA-12) over a period of 8 years (1986 to 1993) of production at two locations and 4 years (1986 to 1989) at a third location. Trees were 4 years old when first harvested. All parental clones used in the generation of the full-sib families belong to various populations of the Forastero cacao group. The controlled-pollinated seed from these families was introduced from the Cacao Improvement Program at the 'Centro Agronomico Tropical de Investigacion y Ensenanza' (CATIE), Turrialba, Costa Rica. In a second experiment, Irizarry and Goenaga (2000) grafted scionwood from the 40 highest-yielding trees obtained from these families at the three locations above onto an open-pollinated rootstock (EET-400) with resistance to Ceratocystis wilt *(Ceratocystis .fimbriata)* and evaluated these clonal selections under full sunlight and intensive management at Corozal, Puerto Rico, during 4 years of production. In 2009, nine of the best 40 selections were released (Goenaga et al., 2009) as clones yielding over 2000 kg/ha/yr of dry beans and are currently being used to establish a cacao industry in Puerto Rico.

Threats of genetic erosion *in situ*

The ecosystems in which *T. cacao* and its wild relatives grow are becoming threatened due to inadequate land cultural practices, deforestation, ranching, fires, urbanization, and infrastructure expansion such as road development and climate change (Medina and Laliberte, 2017**).** However, in recent decades no field level research has been conducted on habitat shifts and conservation status within their ecosystems and natural habitats (Bekele and Phillips-Mora, 2018).

Among important taxa, those with the most urgent need for conservation typically have a limited geographic range (Cuatrecasas, 1964). While collecting expeditions should concentrate on adding to the genetic diversity that is already found in existing germplasm collections, re-gathering of populations held in genebanks would offer an opportunity to evaluate genetic loss in the field and genetic degradation in genebank collections (Bekele and Phillips-Mora, 2019). Although longstanding and strong programs have been successful in maintaining genetic diversity and expanding diversity through collection at TARS (Tropical Agriculture Research Station) and SHRS (Subtropical Horticulture Research Station) (see Annex 5), supplementary studies on this subject are needed.

The wild relatives of cacao thus include two different groups of germplasm. The first is the large spectrum of wild populations which grow simultaneously in the Amazonian rainforest, ranging from French Guiana to Bolivia. Wild cacao, therefore, could be used in breeding or in commercial production, either as progenitors or as clones (Motamayor et al., 2008).

A significant challenge with gathering cacao wild relatives is that they are often found in similar areas where they hybridize readily and distort species boundaries (Medina and Laliberte, 2017). Wayward isolation in these hybrid populations may allow them to survive in habitats that are more extreme than those of either of their parents. It is important then to include naturally occurring hybrids when collecting, but to keep them isolated and, when feasible, clearly mark them as such. Explanatory data on habitat, spatial distribution, ecology, geography and surroundings, such as threats and preservation efforts, is also critical. Ongoing evolution, mediated by gene flow between cultivated and wild species, occurs in the center of cacao origin and should be more thoroughly documented (Bekele and Phillips-Mora, 2018). Not much is known about what happens after gene flow has occurred between wild and cultivated relatives in agricultural settings. Offspring must pass a series of critical natural and human selection steps in order to become viable new landrace varieties.

Current and emerging threats and needs

As an important worldwide crop, cacao is exposed to major constraints due to both biotic and abiotic stresses (Phillips, et al., 2013).

Losses in yield as a result of diseases are commonly 30% or more (Ploetz, 2007). Furthermore, drought cause yield reductions between 50 to 80% (ICCO, 2012). Average harvest loss due to several pest and diseases, based on data available from 2017-2018, ranged from 40% in Africa to 30% in Asia and the Americas (ICCO, 2018).

Intensively managed cacao requires significant cultural inputs such as fertilizer, pesticides, fungicides, and water to maintain high (<2,000 kg/ha/yr) of dry bean and high quality. Many factors contribute to a decline in production of cocoa beans worldwide, including insect infestations, social pressures to grow other crops, economic issues that discourage the long-term commitment of small acreage farmers to grow the crop, and societal pressures to destroy rain forest environments.

Major diseases of cacao

At present , around thirty percent of the world annual cacao production is lost to pests and diseases (Ploetz, 2007). The main losses in cacao are caused by four diseases: Black Pod Rot (BPR), caused by different *Phytophthora* species of which *P. palmivora* is the most common; frosty pod (FP), caused by *Moniliophthora roreri*, Witches' Broom (WB), caused by *Moniliophthora perniciosa;* and Cacao Swollen Shoot Virus (CSSV), caused by a member of the genus Badnavirus. BPR occurs in all cacao growing regions of the word. Whereas WB is only found in cacao production areas in South America, and certain countries in Central America and the Caribbean (Grenada, Panama, Saint Lucia, Saint. Vincent, Trinidad and Tobago). FPR, is established in most of Central and South America (not including Brazil), and recently in Mexico, and in Jamaica in the Caribbean. In contrast, CSSV is present only in the West African region, including Cote d'Ivoire, Ghana, Liberia, Nigeria, Sierra Leone, and Togo (Bowers et al., 2001; Bailey and Meinhard, 2016).

Black Pod, *Phytophthora* spp.

Black pod rot disease is the most destructive disease in cacao due to its global distribution and serious impact on pod yield and quality. It is caused by a fungus of the genus *Phytophthora* which is represented in all cocoa growing areas. It is caused by several species, the most important of which are *Phytophthora palmivora*, *P. megakarya*, *P. citrophthora*, *P. capsici*, and *P. tropicalis*. *P. palmivora* is present in all cacao producing countries and responsible for between 20 and 30% of yearly worldwide yield losses as well as for 10% of tree deaths (Brasier and Griffin, 1979; Guest, 2007a). *P. megakarya*, only found in West Africa, is the most virulent species and has the potential to cause significant damage if it were to continue to spread (Gregory, 1974). *P. citrophthora*, *P. capsici*, and *P. tropicalis* have only been reported on cacao in the western hemisphere, but sometimes they can cause localized epidemics (Guest, 2007b).

Phytophthora spp. can infect the pod, stem and trunks (stem cankers), flower cushions, leaves (blight), shoots, seedlings (blight), and roots. The pod symptoms are manifested as external brown spots that quickly blacken; internally, the beans become infected and necrotic within a few weeks (Gregory, 1974; Surujdeo-Maharaj et al., 2016).

The most significant economic loss arises from the infection of pods in the two months before they reach ripeness. Pods infected at this stage can result in total loss for the farmer. Cultural techniques for reducing disease incidence and severity include, shade reduction, improving air circulation, regular harvesting, removal of old pods and use of chemicals. Spraying with a copper fungicide is the standard control measure, but it is expensive and never completely effective. The use of other chemicals is possible, and cultural techniques can reduce the level of infection. The long-term solution must lie in breeding for resistance or tolerance to the disease and in obtaining a better understanding of the disease propagation and spread. There has been considerable research effort in many countries on breeding, and some cultivars offer a high degree of resistance have been identified, but much have not been distributed to farmers.

Although work has been carried out with regards to determining genetic markers for traits. These markers have not been validated in segregating populations to be effectively employed in breeding programmes. The replacement of susceptible cultivars by ones showing durable resistance to the pathogen is the ultimate solution for the elimination of the disease.

Frosty Pod Rot, ***Moniliophthora roreri***

M. roreri, is the causal agent for the disease known as frosty pod rot (FPR), or Monilia pod rot. FPR causes up to 80% crop losses in some areas since the beans of infected pods are nearly always destroyed (Phillips-Mora et al., 2006).

Monilia pod rot was first classified in the genus *Monilia* as an ascomycete, but the identification of dolipore septa led to it being re-classified as a basidiomycete, and a new genus *Moniliophthora* was created (Evans et al. 1978). *M. roreri* has a long latent period (7 weeks) (Phillips-Mora and Wilkinson 2007) during which it colonizes pods and various degrees of malformation may develop. This is followed by a shorter necrotrophic stage where lesions develop on the pod and the fungus begins sporulating (Bailey et al., 2013; Evans 2007; Evans et al., 2002). External symptoms have been observed as early as twenty–one days after infection.

M. roreri only invades green pod tissues, subsequently growing between the parenchyma cells of the cortex and producing conidia both within and on the surface of the host tissue.

There are no standard recommendations for control, but the simplest, and perhaps the most cost effective control method proposed for Ecuador, is the removal of the sources of primary inoculum, mummified hanging pods, during the intercrop or dry season period, thus substantially reducing the level of inoculum available and delaying the onset of infection at the start of the following wet season. Other cultural practices involve surface drainage, weeding should be regular and timely, frequent mild pruning of cocoa trees to remove chupons and to keep the trunk and main branches clear and pruning every six months. Chemical methods such as fungicides have been discouraged due to their ineffectiveness.

At least five cultivars have been identified at CATIE that have resistance to *M. roreri*. These include CATIE series "R clones (R1- R6), 'ICS43', 'UF273', 'UF712', and 'EET75'. Only 3% of the screened clones of the CATIE collection have been identified as resistant to FPR (Phillips-Mora and Castillo, 1999). Studies examining the inheritance of FPR resistance have indicated that the inheritance may be recessive and polygenic, since the majority of the progenies were classified as susceptible (Phillips-Mora and Castillo, 1999). Five QTLs for FPR resistance have been reported in LG2, LG7 and, LG8, (Brown et al., 2007). The previously described F_1 and F_2 populations ('Pound 7' × 'UF 273') are also segregating for FPR resistance and the confirmation or detection of new QTLs or confirmation of previously identified QTL associated with FPR resistance is in process. More recently CATIE selected a group of six high yielding FPR tolerant clones for commercial use (Phillips-Mora et al., 2013).

Witches' Broom, ***Moniliophthora perniciosa***

The witches broom disease, caused by the fungus *M. perniciosa*, is endemic to Bolivia, Brazil, Ecuador, Colombia, Peru, and Venezuela, currently limiting cocoa production in these countries. Witches broom disease was first reported in 1895 in Surinam where it caused severe losses in cacao plantations. Currently, it occurs in all cacao regions of South America, in certain Caribbean islands (Trinidad and Tobago; Saint Lucia; Grenada, St Vincent; and Grenadines) and in Panama.

The most obvious symptoms, which give rise to its name, are the characteristic shoots or brooms, caused by the hypertrophic growth of an infected bud. The fungus attacks flower cushions and branches, and to some degree the pods. Symptoms on the pods depend on the age and size of the infection.

Phytosanitary pruning and removal and burning of diseased material will reduce the amount of inoculum and thus the level of infection but will never eradicate the pathogen. In addition, fungicide applications have been recommended but have not been effective. The ultimate solution for the control of witches' broom is universal planting of material resistant to the disease but, in the interim, alternative strategies must be developed to reduce the impact of this disease.

Several QTLs associated with WB resistance have been identified (Brown et al. 2005; Faleiro et al. 2006; Queiroz et al. 2003). Queiroz et al. (2003) using an F_2 population derived the cross of Scavina 6 (resistant) by ICS 1 (susceptible) found a QTL with a dominant effect responsible for 35 % of phenotypic variation for resistance. Later, Brown et al. (2005) utilizing a comparable

population, found two QTLs with dominant effects. One major QTL is located on LG9 and a minor QTL on LG1. A QTL meta-analysis was employed by Lanaud et al. (2009) which showed the significance of the LG9 region associated with resistance to witches' broom and black pod. Recently, Royaert et al. (2016) using the F_1 population 'TSH1188' × 'CCN 51' identified seven QTLs associated with WB resistance on five different chromosomes utilizing SNP markers and a multi-trait QTL analysis using an outbreeding full-sib family. They also stated that the major QTL located on LG9is derived from 'Scavina 6' since this clone is in the pedigree of 'TSH 1188'. Recently the collaborative USDA-ARS/MARS/INIAP program developed 39 clones that were selected under WB disease pressure in Pichilingue, Ecuador.

Cacao Swollen Shoot Virus (CSSV)

CSSV is a serious constraint to the production of cocoa in West Africa (from Cote d'Ivoire to Cameroon), particularly in Ghana where the disease was first recognized in 1936 (Posnette 1940; Stevens 1936). CSSV is not found in Central and South America. Several different strains of the virus exist and can cause defoliation, dieback of the plant and severe yield losses (Ameyaw et al., 2017; Chingandu et al., 2017). In susceptible varieties such as West African Amelonado, the most severe strains of the virus can kill the plant within 2-3 years.

Virus infections cannot be cured but their spread can be restricted by destruction of the virus sources. Removal of infected trees and destruction of the infected material has slowed the spread and eliminated some outbreaks. The use of insecticides has proven not to work.

Thus far, the Ivory Coast has managed to avoid major CSSV-related damage. Although the country has been dealing with the disease for many years, its impact was limited to central regions of the country. The disease is starting to show up in southern and western areas of the country where 60% of cocoa production is located. Over the past two years, Ghana has lost 14% of its cocoa production largely to CSSV. A similar level of losses in Ivory Coast production would reduce world production by 320,000 tonnes. That's almost 20% of global 2018/19 production (GROW Intelligence, 2019).

Vascular Streak Dieback (VSD)

VSD is caused by the fungus *Oncobasidium theobromae*. It was first described in the 1960s in Papua New Guinea where it caused heavy losses of trees. The disease has spread and is now found throughout south-east Asia. An initial symptom is leaf chlorosis on the second or third flush from the tip. The fungus may spread internally to other branches or the main trunk, usually causing death of the tree. When an infected leaf falls during the rainy season, hyphae emerge and develop into a basidiocarp. Basidiospores are discharged at night after heavy rains. The spores are dispersed by wind and require high humidity for survival. Genetic variation for resistance to VSD is known to exist. In crosses between Trinitario female parents and Amazonian male parents, all disease ratings had a significant general combining ability (GCA) while only the parents of dead plants were found to have significant specific combining ability (SCA). Gene effects are additive indicating that selection for progeny resistant to VSD is effective (Tan and Tan 1988). Breeding for VSD resistance has been a major objective of the Coconut and Cacao Institute (CCI) breeding program and genetic resistance is limiting losses in commercial plantings. In Indonesia, sources of VSD have been identified and tested in participatory plant breeding programs. Out of 49 clones that were tested only eight clones, including 'DRC 15',

'KA2 106' and a local Sulawesi selection, 'VSD2Ldg' were identified as VSD resistant. (McMahon et al. 2010). Samuels et al. (2012) collected and isolated pure cultures of *O. theobromae* obtained from infected cacao plants sampled from Indonesia, extracted their DNA, and performed a Phylogenetic analysis of their ITS sequences. They placed *O. theobromae* close to *Ceratobasidium anastomosis* groups AG-A, AG-Bo, and AG-K and proposed a new combination *Ceratobasidium theobromae*. In addition, a PCR-based protocol was developed to detect and identify *O. theobromae* in plant tissue of cacao, enabling early detection of the pathogen in plants (McMahon, and Purwantara, 2016).

***Ceratocystis* wilt *(Ceratocystis cacaofunesta)* or 'Mal de Machete' or Mal de Choroni disease**

CW is caused by the fungus *Ceratocystis cacaofunesta*, formerly *C. fimbriata*. Early symptoms are withered leaves remaining on the tree, followed by branch necrosis and finally the death of the plant. The pathogen can be transported through the vascular tissue producing necrosis at different levels in the trunk and branches. The pathogen mainly infects the trunk and the branches through wounds. Trunk boring insects from the genus *Xyleborus serve* as vectors for the disease. This disease is very important in the Caribbean and Central and South America and has been reported to cause the death of 50% of the trees in cacao plantations (Iton 1966). Resistance to *Ceratocystis* in the cacao cultivar 'IMC67' is controlled by a single gene. The cultivar 'IMC67' is often used as a rootstock, as it is homozygous for the resistance conferring allele, which acts in a dominant manner. Open pollinated seedlings of this cultivar contain at least one copy of the allele and are resistant. This gene has not been associated with any molecular marker. The resistant allele/s in 'IMC67' were derived from 'Pound18', one of the original seedlings collected by J.F. Pound in the headwaters of the Amazon, and the IMC (Iquitos Mixed Calabacillos) are seedlings of 'Pound18'. The localization of this gene on the genome map will be a high priority. In contrast, Santos et al., (2012) using 143 F_2 individuals originated from the selfing of hybrid-clone 'TSH 516' ('SCA 6' × 'ICS 1') reported that the inheritance of the resistance was quantitatively inherited and that two QTLs located on LG3 and LG9 were associated with the resistance.

Neither chemical control of the beetle or the fungus, nor destruction of infected material, has proven to be a successful control method. The removal and burning of all infected branches and dead trees have been recommended but this may in fact disturb the beetles and infected debris and result in the spreading of the spores to healthy trees. The most useful technique is the prevention of' the spread of the fungus by minimizing damage at pruning and harvesting. Sterilizing the cutlass after completion of pruning on each tree by incorporating a fungicide in the scabbard or painting fungicide paint on all large exposed surfaces may be useful.

Abiotic (environmental extremes, climate change)

Climate Conditions

The natural habitat of the cocoa tree is in the lower canopy of an evergreen rainforest, and climatic factors, particularly temperature and rainfall, are important in encouraging optimum growth. Cocoa plants respond well to relatively high temperatures, with a maximum annual average of 30 - 32ºC (86-89.6 ºF) and a minimum of 18 - 21ºC (64.4-69.8 F). Discrepancies in the yield of cocoa trees from year to year are affected more by precipitation than by any other environmental factor (Gateau-Rey et al., 2018). Trees are very sensitive to soil water deficits. Precipitation should be

abundant and well-spread throughout the year. Annual rainfall between 1500 mm and 2000 mm is generally ideal. Drought periods, where rainfall is less than 100 mm per month, should not surpass three months.

A hot and damp environment is indispensable for the ideal growth of cocoa trees. In regions that produce cacao, the relative humidity is generally high: regularly as much as 100% during the day, falling to 70-80% during the evening. The cocoa tree will make optimum use of any light available and traditionally has been grown under shade. Its natural environment is the Amazonian rainforest which provides natural shade trees. Shading is indispensable for good development in a cocoa tree's early years.

Climate change

Climate change predictions indicate that increasing temperatures and decreasing water availability will result in a substantial worldwide cacao yield reduction of up to 32% by 2050 (Gateau-Rey et al., 2018). Access to diverse crop genetic resources should benefit selection and breeding of varieties with adaptive traits for abiotic along with biotic stress tolerance (Beebe et al., 2011; Kisoudis, et al., 2014). Since plants under abiotic stress are affected physiologically and biochemically, adaptation to stress can be facilitated by morphological, physiological and biochemical responses (Saibo et al., 2009). Adaptive traits may include deeper root systems, stomatal control/conductance and the efficient use of water and translocation of nutrients within the plant. Changes in weather patterns that result in hotter summers, "El Nino" and "La Nina" phenomena resulting on altered rainfall patterns and availability will affect where and what types of cacao can be grown (Abdulai et al., 2018).

Severe threats to cacao wild genetic resources in South America, where most of the wild cacao species are found, include mining, overgrazing, expansion of exogenous livestock (such as cattle and goats), deforestation, expanding agriculture, and habitat loss in general. The regions under greatest threat to crops and their wild relatives include the tropical highlands of South America, and the neotropics. Cacao is sensitive to drought (Wood and Lass, 1985). In Indonesia, over 60% yield losses were reported during drought events caused by "El Nino" (Keil et al., 2008). Furthermore, in Ecuador, 19% of the area planted in late 1990's was lost to drought events caused by "El Nino" (Vos et al., 1999). In Bahia Brazil, Gateau-Rey et al., (2018) reported yield losses > than 80% due to drought. A strong positive El Nino Southern Oscillation (ENSO) signal has been linked to dry weather in West Africa and weak cacao bean yields. Likewise, strong winds, which rise from the Sahara Desert between November and March, can cause mid-crop cacao production to fall (Rodriguez et al., 2019; Schroth, 2016).

The effects of climate change on cacao has been receiving increasing attention within the research community. The World Cacao Foundation (WCF) member companies issued a statement in 2017 to end deforestation and forest degradation in partnership with other organizations. Thirty-five companies are part of this pledge which encourages agricultural intensification and increased productivity in environmentally suitable areas with the goal to grow more cocoa, but on less land (Confectionary News, 2017). Abiotic stress factors, such as drought, flooding, high temperature and excessive solar radiation, pose a threat to sustainable cocoa production (Oyekale et al., 2009). There is a need to optimize water use, soil and microclimatic conditions through cultivation of improved, stress tolerant planting material (Padi

et al., 2013). This is particularly urgent since Baligar et al. (2008) concluded that cocoa is ineffective in limiting transpiration in dry air compared with other rain forest trees.

Changing climate conditions may also affect water availability during the growing season, causing some regions to experience drought, and other regions, with poorly drained soils, to be waterlogged (Bassett et al., 2011). Drought stress causes wilting, leaf yellowing, advanced leaf fall and premature fruit ripening or fruit drop. Sunburn may also cause leaf and fruit scorching. Young trees are more sensitive to these stresses. In contrast, waterlogged trees can easily become infected by Phytophthora root and crown rot, which leads to premature fruit ripening, a decrease in quality, and overall orchard decline.

A focus on breeding for drought and salt tolerance, and adaptation to fluctuating temperatures will be important. Identification of drought or waterlogging resistant rootstocks may be the most promising solution to this potential threat.

Cacao Breeding

Cacao breeding began in Trinidad in 1930 with the hiring of a geneticist, Dr. J.F. Pound, who was given the task of developing cacao populations in Trinidad with resistance to WB disease and high productivity. Subsequently, breeding was started in West Africa at the West African Research Institute (WARI) where a major goal was developing resistance to CSSV. Currently, significant breeding programs exist in Brazil, Costa Rica, Mexico, Trinidad, Ecuador, Ghana, Nigeria, Cameroon, Cote d'Ivoire, Indonesia, Malaysia, PNG, India, the Philippines, USA, and Vietnam.

Cacao is grown commercially in two different ways, as clones and as seedlings. The breeding methods used have differed depending on the type of planting material provided to farmers. In the Caribbean, South Asia, and parts of South and Central America, cacao is usually propagated using clones. For example, the breeding program in Trinidad has concentrated on the development of new clones using single tree selection for low pod index, resistance to BP and resistance to WB. The potential yield of the Trinidad Select Hybrids (TSH) is 2000kg/ha while the average yield of unimproved seedlings is approximately 300 kg/ha. In Brazil breeding for WB and *Ceratocystis* wilt is also an important breeding goal (Lopes et al. 2011). In this case the clones represent a significant improvement in yielding ability. In South Asia, the programs at the Indonesian Coffee and Cocoa Research Institute (ICCRI) and Papua New Guinea Cocoa and Coconut Institute (CCI) and at the Malaysian Cocoa Board (MCB) have concentrated on the development of clones with resistance to CPB, VSD and BP and a number of productive new clones have been selected in these programs.

In West Africa, seedlings are used to establish commercial cocoa farms. At CRIG (previously WARI) in Ghana, Upper Amazon parents are crossed with Trinitario parents and the full-sib families are evaluated for productivity, resistance to CSSV, and resistance to BP. Once good families have been identified the parental clones are placed in seed gardens. Most of the Upper Amazon parents are self-incompatible, so seed production is by natural pollination in bi-clonal gardens, and the seed is distributed to farmers as a hybrid family. Unfortunately, in West African programs many of the original Upper Amazon clones used as parents in the 1940s and 1950s are

still being used today without any improvement through recurrent selection. For many reasons, average yields in West Africa are still around 300 kg/ha.

In 2009, the USDA-ARS released nine high-yielding clones (Goenaga et al., 2009) which are currently being used to establish a cacao industry in Puerto Rico and Hawaii. These clones are also being evaluated in Costa Rica and soon in Colombia and The Philippines. Most cacao breeding programs have only made modest gains in the genetic improvement of cacao. One of the main reasons is the lack of localized standard production procedures and the fact that many of the programs are severely underfunded and not able to conduct proper phenotypic evaluation. Misidentification of parents has also been a significant problem, and many of the full-sib families evaluated from select parents have significant pollen contamination (Schnell et al., 2005). The advantage of clones is the distribution of homogeneous material to growers and even to combine the advantages of the rootstock with that of the grafted clone. While the sowing of seeds is more heterogeneous, each plant differing from the others.

Breeding programs worldwide share similar goals, with differences largely based on regional or international consumer preferences and the specific biotic and abiotic challenges in the local areas of production (Bekele and Phillips-Mora 2019). In 1955, the U.S. Department of Agriculture initiated a program for the quarantine and distribution of cacao cultivars as a service to the cacao research centers located in the tropics (Fisher et al. 1960). This activity was divided between two locations, with the USDA-ARS Miami Station serving as the quarantine facility and the USDA-ARS Mayaguez, PR station maintaining the germplasm. This activity was partially supported by the American Cocoa Research Institute and continued until 1992 when Hurricane Andrew destroyed the quarantine facilities at Miami. In 1998, the USDA-ARS revived the cacao genetics program based at the SHRS in Miami and Mayaguez and on 2004 the shipments of cacao material that was released from quarantine was reinitiated. The primary goal of the program is to develop a biotechnology-based approach to solving the destructive disease problems. In addition, the USDA-ARS and Mars Inc. have collaborated in an international cacao genetics project that feeds information into national and regional breeding programs to increase the efficiency of developing superior varieties for cacao farmers worldwide. Cacao breeding through traditional methods is a slow process and although much variability exists, most breeding work has utilized only a narrow genetic base (Hunter 1990; Motamayor et al. 2002). Cacao has a long generation time with a minimum of five years between generations. More realistically, the interval between generations is eight to ten years. Therefore, it is important that breeding objectives be clearly stated and trials well designed. Breeding for disease resistance is an important goal in cacao breeding programs around the world; however, yield and its components are also major objectives in any breeding program.

Currently clonal performance and breeding trials are located in Central and South America, West Africa, and South Asia. All these trials are conducted within each country’s National Agricultural Research Systems (NARS) experiment stations and private industry partner's facilities, such as MARS, Inc. In the Americas, the breeding trials are located in Ecuador, Colombia, Costa Rica, and Brazil. Collaborative research projects were also started in the early 2000's. Specific Cooperative Agreements with CATIE and INIAP have been in place. For example, INIAP has been releasing clones adapted to the conditions in Ecuador and CATIE

clones have been planted in seven countries across Central America. The shared benefits of the research are that these groups are working on common solutions to the diseases and pests that affect cacao production in the Western Hemisphere.

In Asia, breeding trials were initiated in 2004 with the objective to study and identify molecular markers linked to Vascular Streak Dieback (VSD), BP, Longicorn (trunk borer), productivity, quality and other agronomic traits, and associate.

To date, most cacao breeding programs have only made modest gains in the genetic improvement of cacao.

Cacao genetic resources are essential to maintaining and improving agricultural productivity. However, politics, lack of long-term funding, lack of sufficient diversity to reduce vulnerability to pests and diseases, complexities of treaties for germplasm sharing, etc., (Rubenstein, et al., 2005) are limitations for breeding programs whose objective is to provide growers with superior varieties.

Conservation and propagation

Ex situ cacao germplasm collections have been traditionally conserved as field grown trees and they are thus susceptible to severe weather, diseases and insects. Cacao is raised from seed. Seeds will germinate and produce healthy plants when taken from pods not more than 15 days underripe. Grafting (top and side), rooted cuttings, and budding are traditional vegetative propagation techniques applied to cacao (Wood and Lass, 1985). In addition, a large proportion of plants have been and are still generated from seed. Clonal multiplication guarantees identical multiplication of the initial genotype, while seed multiplication requires keeping a larger number of individuals to ensure that the intragenetic variability is maintained in the case of heterozygous accessions.

I*n vitro* plant propagation techniques have been developed (Nievenenak et al., 2008; Noah et al., 2013) and are being employed for plant propagation (Goenaga et al., 2015; Maximova et al., 2008; Urrea Trujillo et al., 2011) of specific varieties as well as used for maintaining backup germplasm banks for collections. *In vitro* propagation techniques can be applied for medium-term storage and distribution purposes and are being optimized for cryotherapy (Quainoo, et al., 2008) and for long-term cryopreservation backup (Adu-Gyamfi et al., 2018; Fang et al., 2009; Fang and Wetten, 2011).

Cryo storage protocols have been adopted to back up accessions maintained in the International Cocoa Intermediate Quarantine Centre at the University of Reading as an effort of *in situ* cacao germplasm conservation (Adu-Gyamfi and Wetten, 2012), however, further research is needed on phenotypic, genetic and epigenetic variation resulting from cryo storage (Adu-Gyamfi, et al., 2016).

The development of molecular markers in cacao

Many methods have been used to develop molecular markers (Irish et al., 2010; Livingstone et al., 2011; Kuhn et al., 2010; Motilal et al., 2011; Perry et al.,1998; Santos et al., 2015; Zhang et al., 2012; Zhang et al., 2006). More than 600 microsatellite markers have been developed and mapped by CIRAD and USDA-Miami. More recently, the sequencing of two cacao genomes, the Belizean Criollo genotype B97-61/B2 (Argout et al., 2011) and a Forastero genotype Matina 1-6

(http://www.cacaogenomedb.org/) has substantially increased the amount of resources available for the development of new molecular markers that can help in the development of new cacao varieties. More recently, SNPs have been developed and used for comparative genomic studies, consensus genetics maps, marker assisted breeding, and for determining off-types in clonal collections (Allegre et al., 2012; Kuhn et al., 2010; Livingstone et al., 2012; Livingstone et al., 2011; Takrama et al., 2012).

Genomic selection

Although problems in identification of resistant genotypes will remain, the recently released cacao genomes (http://www.cacaogenomedb.org/) will aid in their discovery by providing high-throughput genotyping techniques that will accelerate the process. In spite of improvements in genotyping capacities, much interest still exists in phenotyping, characterizing, and evaluating existing germplasm and in the acquisition of "wild" germplasm (Giron et al., 2004). Errors in the identification of genotypes in clonal cacao collections is of concern (Motilal, L. and Butler, D., 2003; Perry et al.,1998; Santos et al., 2015; Sounigo et al., 2000; Zhang et al., 2009). Traditionally, identification has relied on phenotypic traits that could easily distinguish accessions. However, the advent of genotypic fingerprinting techniques allows for accurate identification of accessions. Several of these techniques have been applied successfully in cacao genotyping, including AFLPs (Perry et al., 1998) and SSRs (Ferraz dos Santos et al., 2016; Irish et al., 2010; Kuhn et al., 2010; Motilal et al., 2011; Perry et al.,1998; Santos et al., 2015; Zhang et al., 2012; Zhang et al., 2006). Development of SNPs for genotyping and molecular breeding of crops is increasing (Abdelgadir, 2015; Myles et al., 2010; Padi et al., 2013) and in cacao SNP's would provide a larger set of markers for genotyping with improved genome coverage. SNPs also provide a method for unambiguous, non-platform dependent, direct fingerprint comparisons (Cosme et al., 2016; Ji et al., 2012). Genetic markers are also being used in cacao genomic research for providing an avenue for improvement through the identification and characterization of markers linked to disease resistance (Kuhn et al., 2010; Schnell and Priyadarshsn, 2015; Schnell et al., 2007; Schnell et al., 2005).

Production/demand (inability to meet market and population growth demands)

Cocoa price volatility is not new, as commodity prices are often unstable. However, the current rise in demand coupled with any disruption to or inadequate supply of cocoa could dramatically impact the price of cocoa products (Cocoa butter, chocolate, etc.). There were 5.2 million tonnes of cocoa beans produced in 2017 supplying an ever-increasing worldwide demand for chocolate. The global chocolate market was valued at USD 103.28 billion in 2017 and expected to grow to around USD 161.56 billion by 2024.

The Ivory Coast is by far the largest cocoa producing country in the world supplying over 30% of the world's cocoa beans at 2,034,000 tonnes in 2017. The cocoa industry is hugely important to the economy of the country as it accounts for 40.2% of its export income. However, they face many challenges to that sustainability due to the problems of climate change and deforestation and disease, and the ongoing crisis of child labor.

While Ghana is the second biggest cocoa producing country in the world with 883,652 tonnes in 2017, generating about $2 billion in foreign exchange annually, it has faced the problems of child labor and deforestation.

Indonesia expanded rapidly into the cocoa industry over the last few decades to become the third biggest cocoa producer in the world, despite the fact they hardly grew cocoa before the 1980s. Indonesia produced 659,776 tonnes of cocoa in 2017 making it their fourth largest agricultural export. However, the cocoa industry in Indonesia is going through a downturn with production output decreasing to around 220,000 tonnes during 2019/2020. This is due to ageing trees, lower productivity, lower domestic consumption and farmers switching to more profitable crops such as palm oil.

Other countries, such as Colombia, Peru and Ecuador face issues of their own. They must confront the trade and high prices offered by the Coca (*Erythroxylum coca)* plant.

Accessibility (inability to gain access to needed plant genetic resources because of phytosanitary/quarantine issues, inadequate budgets, management capacities or legal restrictions)

Theobroma cacao is listed as an import prohibited item, meaning that special permits and careful testing by federal quarantine in Miami is required. All cacao material coming into the USA is required to enter a two-year quarantine at SHRS Miami prior to being shipped and added to the TARS collection. Procedures (SOP) followed during quarantine at SHRS is included in **Annex 2**.

Accessibility

Theobroma cacao L. and other species (wild crop relatives) of potential interest in breeding are not native to the US. Access to these novel plant genetic resources depends on legal and phytosanitary requirements in the US and abroad. International regulations affect movement of germplasm between countries and also limit or control the extent of access to genetic resources (CBD, 1992). The International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) governs movement of plant genetic resources between signatory countries (ITPGRFA, 2009). Cocoa (*Theobroma cacao*) is not included in the list in Annex 1 of the species and genera concerned by the ITPGRFA and therefore does not form part of the Multilateral System. The two International Collections of CATIE (Costa Rica) and Cocoa research Unit (CRU) (Trinidad and Tobago) voluntarily put their cocoa accessions under article 15 of ITPGRFA. The other national collections exchange very little genetic resources of cocoa. Material transfer agreements, patents, or restrictive agreements may impede acquisition and distribution of valuable germplasm. Access to *cacao* genetic resources varies on a country-by-country basis, and in some cases, significantly limits the ability to collect and conserve *cacao* species, including the primary crop wild relatives. Expeditions to source countries may also be limited by budgets or conflicts.

Significant and diverse cacao germplasm is conserved and available from cacao International Collections (CATIE, Costa Rica; CRU, Trinidad and Tobago; ICQC, Reading, UK).

Status of plant genetic resources in the NPGS available for reducing genetic vulnerabilities

Germplasm collections and ex situ reserves

USDA-ARS-SHRS in Miami and USDA-ARS-TARS in Mayaguez have been the recipients of donations from collection trips by well-known cacao germplasm collectors like Frank Pound. and other institutions such as CATIE, Costa Rica; Trinidad and Tobago, Cocoa Research Centre, as well as Ecuador, Colombia and Mexico.

The USDA-ARS-NPGS *Theobroma spp*. collection is maintained by the TARS in Mayaguez, PR (**Annex 3, Figure 1**). The cacao collection was established around 1939 as part of the USDA research station; in 1984 it became a clonal repository in the National Plant Germplasm System (NPGS) (Ayala Silva et al., 2010; Ayala Silva et al., 2013). Many of the varieties in the cacao collection were provided by foreign countries (CATIE, Costa Rica; RCU, Trinidad and Tobago) (Motamayor et al., 2008). Many of the species represented in the collection were obtained through plant exploration trips by Pound (1936) in the early 1940's. A backup collection in two-gallon containers and consisting of two clones per accession is also maintained in a shadehouse at the main location (Annex 3, Figure 1). All accessions are labeled with QR2 codes with the row and tree number, Plant Introduction number (PI), TARS inventory number, variety name, and genus and species. About 50 accessions have been backed up at the USDA Pacific Center for Genetic Resources Preservation (PCGRP) in Hilo, HI, US.

Holdings

Specifics of the TARS and SHRS holdings are fully documented and open for public view and ordering through GRIN GLOBAL (GRIN, 2020). The collection consists of about 300 clonal accessions kept and distributed as budwood, seeds, pollen, DNA, and leaves. The collection includes representative holdings of most cacao cultivars and several wild relatives.

Acquisitions

The NPGS genebank acquires stocks by donation from collectors, exchange with other genebanks, collections by private collaborators, of germplasm already present at TARS/SHRS collected and identified by genebank staff and research cooperators.

Collection size and priority is at the discretion of the curator, with direction provided by the Crop Germplasm Committee (CGC). Due to the limited budget and field space, the acquisition of new materials must be prioritized, and it is a decision that is based on existing genetic gaps, potential risk of genetic erosion of the material, novelty, desirability and need in the user community, quality passport data, and the freedom to distribute. Materials are not acquired that have intellectual property rights or that are regulated GMO's.

Maintenance

The permanent field collection is maintained as living trees. The cacao germplasm collection at the USDA-ARS TARS site in Mayaguez, PR consists of 283 accessions (**Annex 4**) (six plants of each accession) arranged in a RCBD with three blocks and two plants per accession. Plants are irrigated through a micro-sprinkler system and are monitored for disease and insect pests. Regular removal of diseased pods reduces inoculum and removal of ripe pods eliminates sites for future infections. Plants are established in full sun and management practices are followed according to Irizarry and Goenaga (2000) and Irizarry and Rivera (1998). Fertilization is carried out every four months with 10-5-20 plus 1% trace elements.

Pruning of the cacao collection is done on a routine basis by removing chupons and branches that are dead, broken or located low to the ground and a yearly pruning carried out with tractor-mounted topper-hedger.

Regeneration

The TARS cacao collection is maintained as living trees. Plants are regenerated when needed from budwood and grafted onto 'Amelonado' rootstock. The cacao collection is planted with six replicates of each clone and plants lost can be replaced from material maintained as back up in the shade house. Also, approximately 95% of the accessions are backed up in greenhouses with two grafted potted plants per field accession. A backup core collection site is currently set up at the PBARC in Hilo, HI. A total of nine locally developed and selected cacao germplasm accessions have already been successfully backed up and several accessions have been recently grafted. A small percentage of accessions is also maintained at the SHRS in Miami, FL, although unpredictable cold weather has affected this backup collection site in the past. Efforts will continue to collect Criollo genotypes around the island of Puerto Rico to increase genetic diversity in the collection (Cosme et al., 2016).

The cacao germplasm collection established at TARS is unique in that many of the American, African and Asian-confined insect and disease pests are not present. The only disease of consequence is black pod caused by a worldwide distributed soil-borne and pod-infecting Oomycete (*Phytopthora palmivora*) (Irish et al., 2006). To exclude the potential introduction of devastating diseases, all cacao germplasm introductions abide by the safe movement guidelines that require extensive and detailed quarantine procedures. Most cacao scionwood received at the TARS has been through at least one quarantine site at either the Intermediate Cocoa Quarantine Center at the University of Reading (ICQC, R), England and/or the USDA-ARS SHRS, Miami, FL.

Distributions and outreach

The main goal of TARS is to comply with orders within a week of receipt. However, under certain circumstances all orders cannot be delivered that fast, because, for instance, the material (pods, budwood, leaves) may not be ready for distribution. For non-research requests distribution are limited to 2-3 budwood pieces with requestors asked to assist with shipping charges.

All cacao distributions adhere to the safe movement guidelines (End, 2014). All distributions are inspected before shipping by an APHIS inspector and a phytosanitary certificate is submitted for all international or national shipments. Distribution of germplasm to cacao growing regions is via budwood intended for grafting at requesting sites. Budwood is treated with insecticides/fungicides according to IPGRI procedures for the safe movement of cacao germplasm (End, 2014). Pods are distributed as seed source for rootstocks or for display and are treated with disinfectants and inspected by APHIS. Germplasm is distributed as pods, budwood, seeds, leaves, flowers, pollen, fruit, and DNA, and requests are received from the Genetic Resources Information Network (GRIN, 2018). A total of 1500 orders comprising over 85,000 cacao propagules were distributed between 2013 and 2018 (**Annex 5**). This material was distributed to national and international collaborators, researchers and farmers.

Data access
All passport and phenotypic data are stored in GRIN GLOBAL (GRIN, 2018). Gene, sequence, marker, diversity, and trait locus data for *Theobroma* cacao is available through the Cacao Genome Database (CGD) https://www.cacaogenomedb.org/. CGD is a curated and integrated web-based interactive database that provides centralized access to cacao genomics, genetics, and breeding data and analysis tools to facilitate breeding and research. Other key websites relating to the cacao industry and genetic resources are listed in Annex 6.

Passport information
Passport data are recorded in GRIN Global and are publicly available (GRIN, 2018). Passport data usually include collection site, general description of the site and the accessions, latitude, longitude, GPS coordinates, elevation, and habitat information. Other information recorded in GRIN Global include accession number (PI and/or TARS/MIA number), collector name, date when accession was received, backup status, accession name, availability, narrative (about the accession), source history (development or collection information), pedigree, observation (phenotypic and genotypic data), and vouchers of the accessions (digital images).

Genotypic characterization data
Approximately 95% of the accessions in the *cacao* collection have been genotyped using AFLP's, SSR's, and SNP's (Boza et al., 2012; Zhang et al., 2012).

Over 600 microsatellite markers have been developed and mapped by CIRAD and USDA-Miami (Brown et al. 2005; Lanaud et al., 2009; Lanaud et al., 1999; Pugh et al., 2004). Sequencing of two cacao genomes, the Belizean Criollo genotype B97-61/B2 (Argout et al., 2011) and a Forastero genotype, Matina 1-6 (http://www.cacaogenomedb.org/) has substantially increased the amount of resources available for development of new molecular markers that can help in the development of new cacao varieties. In cacao, SNPs have been developed and used for comparative genomic studies, consensus genetics maps, marker assisted breeding, determining off-types in clonal collections (Allegre et al., 2012; Kuhn et al., 2010; Boza, et al., 2012; Livingstone et al., 2012; Livingstone et al., 2011; Takrama et al., 2012).

Phenotypic evaluation data
Since 1984, TARS scientists have been collecting data from formal and informal research originating in-house, with specialist cooperators, or from the applicable published cacao research literature.

The NPGS cacao collection has been characterized for collection of traits of botanical, horticultural, and breeding interest (Irish et al., 2010). Phenotypic traits relating to production and breeding, disease (black pod), morphology (fruit traits and colors), and phenology (flowering time, harvest season, and production description) have been collected over the years. Approximately 160 accessions in the collection have been phenotype using descriptors set by Bioversity International (Engels et al.,1980). Currently, about 130 accessions are being characterized.

Little characterization has been conducted on flavor traits of accessions in the collection. The African Cocoa Initiative (ACI-II) has labs and panels established at Cacao Research Institute of Ghana (CRIG), Centre National de Recherche Agronomique (CNRA), Cocoa Research Unit (CRU), and the Indonesian Coffee and Cocoa Research Institute (ICCRI). Scientists at these institutions are using the same equipment to process beans to liquor under standardized conditions and each lab has a panel using the same the chocolate evaluation guidelines under the Cocoa of Excellence (CoEx) program (Cocoa of Excellence Organization, 2017). As part of this program, periodic ring tasting evaluations / video training sessions are offered to maintain their calibration. Panels often rely on GC/MS instrumentation, to maintain calibration and uniformity. Establishing collaborations with these institutions will be sought to evaluate flavor.

Goals and emphases

To help breeders and others make informed use of the cacao germplasm, TARS, SHRS and Sustainable Perennial Crops Laboratory (SPCL) actively pursue various germplasm research projects to develop genetic knowledge about the collection and traits important to cacao variety improvement. Currently, TARS in-house research focuses on characterizing fruit quality and phenology traits of cacao in the permanent collection and using next-generation SNP markers to evaluate the diversity in the collection (Cosme et al., 2016; Zhang et al., 2012).

Other goals include:

Expand collection of "Criollo" cacao by collecting across the island of PR and, obtain cacao wild relatives from other genebanks or country collections. These are characterized as they arrive to the genebank.

Significant accomplishments

Extensive distribution (over 85,000 items (2014-2019) of cacao germplasm (Table 1) and associated information to researchers and stakeholders in the US and worldwide has significantly improved the scientific knowledge about cacao and the *Theobroma* genus and has helped speed up the use of cacao germplasm for the expansion of new cacao varieties and the resurrection of cacao farming in Puerto Rico.

- First Report of Black Pod on cacao in Puerto Rico (Irish et al., 2006)
- Release TARS series of cacao germplasm selections (Irizarry and Rivera, 1998); Goenaga et al., 2009).
- In 2010, the repository started conducting workshops on cacao planting for PR, which resulted in the revival of the cacao cultivation in PR. As a result, over 50 small cacao farms and at least three fine chocolate brands have been established.
- Plant exploration for Cacao "Criollo" native to Puerto Rico rendered a total of 135 accessions. Twenty of them were selected through DNA and were added to the collection and undergoing evaluation (Cosme et al., 2016).
- Comparing yield performance and bean quality of cacao propagated by grafting and somatic embryo-derived cuttings (Goenaga et al., 2015).

For more information and details visit our home page at: https://www.ars.usda.gov/southeast-area/mayaguez-pr/tropical-crops-and-germplasm-research/

Staffing

The TARS website (https://www.ars.usda.gov/southeast-area/mayaguez-pr/tropical-crops-and-germplasm-research/) lists and describes specialties of research personnel. In brief: Dr. Ricardo Goenaga is the Research Leader and plant physiologist responsible for field research and Dr. Tomas Ayala Silva is responsible for curatorial service, collecting, evaluation and characterization and genebank management. Numerous associates in the form of students and specialist collaborators from Puerto Rico, other states, federal, and international also take part.

Facilities and equipment

The in-house facilities in Mayaguez consists of 200 acres of land; laboratories and offices for research and support for five scientists; 13 screenhouses; a plant propagation unit; a cold storage unit; and provides additional office space for the local USDA Natural Resources Conservation Service (NRCS) (**Figure 1, Annex 3**). In addition, a 130-acre farm located in Isabela is utilized to support our research programs. A 58-acre research farm located at St. Croix, United States Virgin Islands serves as a quarantine and seed regeneration site for several crops. A research support agreement with the University of Puerto Rico (UPR) permits conducting work at the various research farms belonging to the UPR-Agricultural Experiment Station.

REFERENCES

Abdulai S,Vaast P, Hoffmann MP, Asare R, Jassogne L, Van Asten P, Reimund P, Rötter P, Graefe S, 2018 Global Change Biology 24 (1) 273 DOI: 10.1111/gcb.13885

Adu-Gyamfi R and Wetten A 2012 Cryopreservation of cocoa (Theobroma cacao L.) somatic embryos by vitrification. Cryoletters: 33:494-505

Adu-Gyamfi R, Wetten A, Rodríguez López, M C 2016 Effect of Cryopreservation and Post-Cryopreservation Somatic Embryogenesis on the Epigenetic Fidelity of Cocoa (*Theobroma cacao* L.) PLOS ONE 11(7): 0158857. https://doi.org/10.1371/journal.pone.0158857

Akaza JM, Kouassi AB, Akaffou DS, Fouet O, N'Guetta AS-P, Lanaud C 2016 Mapping QTLs for black pod (Phytophthora palmivora) resistance in three hybrid progenies of cocoa (Theobroma cacao L.) using SSR markers International Journal of Scientific and Research Publications 6:298-311

Allegre M, Argout X, Boccara M, Fouet O, Roguet Y, Berard A, Thevenin JM, Chauveau Al, Rivallan R, Clement D, Courtois B, Gramacho K, Boland-Aug A, Tahi M, Umaharan P, Brunel D, Lanaud C 2012 Discovery and mapping of a new expressed sequence tag-single nucleotide polymorphism and simple sequence repeat panel for large-scale genetic studies and breeding of *Theobroma cacao* L. DNA Res 19:23-35

Ameyaw GA, Chingandu N, Domfeh O, Dzahini-Obiatey,HK, Gutierrez OA, and Brown J K 2017 Variable detection of Cacao swollen shoots disease-associated badnaviruses by PCR amplification International Symposium on Cocoa Research (ISCR), Lima, Peru, 13-17 November, 2017

Argout X, Salse J, Aury J-M, Guiltinan MJ, Droc G, Gouzy J, Allegre M, Chaparro C, Legavre T, Maximova SN, Abrouk M, Murat F, Fouet O, Poulain J, Ruiz M, Roguet Y, Rodier-Goud M, Barbosa-Neto JF, Sabot F, Kudrna D, Ammiraju JSS, Schuster SC, Carlson JE, Sallet E, Schiex T, Dievart A, Kramer M, Gelley L, Shi Z, Berard A, Viot C, Boccara M, Risterucci AM, Guignon V, Sabau X, Axtell MJ, Ma Z, Zhang Y, Brown S, Bourge M, Golser W, Song X, Clement D, Rivallan R, Tahi M, Akaza JM, Pitollat B, Gramacho K, D'Hont A, Brunel D, Infante D, Kebe I, Costet P, Wing R, McCombie WR, Guiderdoni E, Quetier F, Panaud O, Wincker P, Bocs S, Lanaud C. 2011 The genome of T*heobroma cacao*. Nature Genetics 43:101-108

Ayala-Silva T, Schnell RJ, Meerow AM, Goenaga R, Zee F. 2004 Current status of the subtropical and tropical germplasm repositories of the National Plant Germplasm System. Proc Florida State Hort Soc 117: 182-187

Ayala Silva T Schnell II, R., Meerow AW, Goenaga RJ, Zee FT, Irish BM, 2013 The National Plant Germplasm System: The Subtropical and Tropical Fruit Genebanks. Acta Hort.

975:43-54

Bailey BA, Meinhardt LW 2016 Cacao diseases: A history of old enemies and new encounters. Switzerland: Springer. 633 pp

Bailey BA, Crozier J, Sicher RC, Strem MD, Melnick R, Carazzolle MF, Costa GGL, Pereira GAG, Zhang D, Maximova S, Guiltinan M, Meinhardt L 2013 Dynamic changes in pod and fungal physiology associated with the shift from biotrophy to necrotrophy during the infection of *Theobroma cacao* by *Moniliophthora roreri*. Physiol Mol Plant Pathol 1:84-96

Basset Y, Lamarre GPA, Ratz T, Segar S, Decaëns T, Rougerie R et al., 2017 The Saturniidae of Barro Colorado Island, Panama: A model taxon for studying the long-term effects of climate change? Ecol Evol 7:9991–10004 John Wiley and Sons.

Beebe SE, Ramirez J, Jarvis A, Blair MW 2011 Crop Adaptation to Climate Change. In book: Crop Adaptation to Climate Change. DOI: 10.1002/9780470960929.ch25

Bekele F, Phillips-MoraW 2019 Cacao (*Theobroma cacao* L.) Breeding. Chapter 12 In: Adv Plant Breed Strategies Vol 6: Industrial and Food Crops. Springer–Verlag, New York

Bowers JH, Bailey BA, Hebbar PK, Sanogo S, Lumsden RD 2001 The impact of plant diseases on world chocolate production. Plant Health Progress, 10, doi:10.1094/PHP-2001-0709-01-RV

Boza EJ, Motamayor JC, Amores FM, Cedeño-Amador S, Tondo CL, Livingstone DS, Schnell RJ, Gutiérrez OA 2014 Genetic characterization of the cacao cultivar CCN 51: its impact and significance on global cacao improvement and production. J Am Soc Hort Sci 139:219-229

Brasier CM, Griffin MJ 1979 Taxonomy of '*Phytophthora palmivora*' on cocoa. Transactions of the British Mycol Soc 72:111-143

Brenes OE, Enriquez GA 1984a El análisis cluster en la evaluación de la resistencia a *Monilia roreri*. In: APS (ed) The American PhytopatholSoc Caribbean Division Annual Meeting. APS, San José, Costa Rica

Brenes OE, Enriquez GA 1984b Evaluación de la resistencia a Monilia roreri de cultivares de cacao. In: APS (ed) The American Phytopathol Soc Caribbean Division Annual Meeting. APS, San José, Costa Rica

Brown JS, Phillips-Mora W, Power EJ, Krol C, Cervantes-Martinez C, Motamayor JC, Schnell RJ 2007 Mapping QTLs for resistance to frosty pod and black pod diseases and horticultural traits in *Theobroma cacao* L. Crop Sci 47:1851-1851

Brown JS, Schnell R, Motamayor J, Lopes U, Kuhn DN, Borrone JW 2005 Resistance gene mapping for witches' broom disease in *Theobroma cacao* L. in an F2 population using SSR markers and candidate genes. J Am Soc Hort Sci 130:366-373

CBD 1992 Convention on Biological Diversity. Concluded in Rio de Janeiro, Brazil 5 June 1992, entered into force 29 December 1993. http://www.biodiv.org/convention/articles.asp

Cacao Net 2012 A Global Strategy for the Conservation and Use of Cacao Genetic Resources, as the Foundation for a Sustainable Cocoa Economy (B. Laliberté, compiler). Bioversity International, Montpellier, France.

Candy-Industry 2019 Global top 100 candy industry. BNP Media,Troy, MI

Chingandu, N., Kouakou, K., Aka, R., Guttierez, O. A., and Brown, J. K. 2017b. Unexpected genome variability at multiple loci suggests *Cacao swollen shoot virus* comprises multiple, divergent molecular variants. J Emerg Virol Dis 3(1): doi http://dx.doi.org/10.16966/2473-1846.128.

Clement CR, de Cristo-Araújo M, d'Eeckenbrugge,GC, Alves Pereira A, Picanço-Rodrigues D 2010. Origin and domestication of native Amazonian crops. Diversity 2:72–106.

Cocoa of Excellence Organization. 2017. Chocolate evaluation guidelines. http://www.cocoaofexcellence.org/about-us

Confectionary News. 2017. The final cut: what can the chocolate industry really do halt cocoa deforestation? https://www.confectionerynews.com/Article/2017/09/27/Cocoa-deforestation-What-can-the-chocolate-industry-really-do?utm_source=copyright&utm_medium=OnSite&utm_campaign=copyright

Cosme-Reyes SM, Cuevas HE, Zhang D, OleksykTK, Irish BM 2016 Genetic diversity of naturalized cacao (*Theobroma cacao* L.) in Puerto Rico. Tree Gen Genomes. doi: 10.1007/s11295-016-1045-4

Crandview Research 2018 Cocoa Beans Market Size, Share & Trends Analysis Report By Application (Cosmetics, Confectionery, Pharmaceuticals), By Product (Butter, Powder, Liquor), By Distribution Channel (Online, Offline), And Segment Forecasts, 2019-2025. https://www.grandviewresearch.com/industry-analysis/cocoa-beans-market

Cuatrecasas J 1964 Cacao and its allies. A taxonomic revision of the genus Theobroma. Contributions from the United States National Herbarium 35:379-605

End M J, Daymond A J, and Hadley P 2014 Technical guidelines for the safe movement of cacao germplasm (Revised from the FAO/IPGRI Technical Guidelines No. 20). Global

Cacao Genetic Resources Network (CacaoNet), Bioversity International, Montpellier, France

Enríquez GA, Soria J 1984 Mejoramiento genético para resistencia a cinco enfermedades del cacao. Revisión de literatura. CATIE, Turrialba, Costa Rica

Evans HC 1981 Pod rot of cacao caused by Moniliophthora (Monilia) roreri. Commonwealth Mycological Institute., Kew, Surrey

Evans HC 2007 Cacao diseases-the trilogy revisited. Phytopathol 97:1640-1643

Evans HC 2016a Frosty Pod Rot (*Moniliophthora roreri*). In: Bailey BA, Meinhardt LW (eds) Cacao Diseases: A History of Old Enemies and New Encounters. Springer International Publishing, Cham, pp 63-96

Evans HC 2016b Witches' Broom Disease (*Moniliophthora perniciosa*): History and Biology. In: Bailey BA, Meinhardt LW (eds) Cacao Diseases: A History of Old Enemies and New Encounters. pp 137-177 Springer International Publishing, Cham

Evans HC, Holmes KA, Phillips W, Wilkinson MJ 2002 What's in a name: Crinipellis, the final resting place for the frosty pod rot pathogen of cocoa? Mycologist 16:148-152

Evans HC, Stalpers JA, Samson RA, Benny GL 1978 On the taxonomy of Monilia roreri, an important pathogen of *Theobroma cacao* in South America. Can J Bot 56:2528-2532

Faleiro FG, Queiroz VT, Lopes UV, Guimarães CT, Pires JL, Yamada MM, Araújo IS, Pereira MG, Schnell R, Filho GADS, Ferreira CF, Barros EG, Moreira MA 2006 Mapping QTLs for witches' broom (*Crinipellis perniciosa*) resistance in cacao (*Theobroma cacao* L.). Euphytica 149:227-235

Fisher H, Haun J, Ackerman W 1960 Cacao seedling production and distribution through plant quarantine. Cacao 5:1-8

Fang J, Wetten A, Adu R, Wilkinson M, Rodríguez C 2009 Use of secondary somatic embryos promotes genetic fidelity in cryopreservation of cocoa (Theobroma cacao L.). Agri d Food Sci, 18: 152-159

Fang J, Wetten A 2011 Importance of structural integrity of somatic embryos for long-term cryopreservation of cocoa (*Theobroma cacao* L.) germplasm. Afr J Agri Res 6:3954-3961

FAO 2009 Standard Material Transfer Agreement of the International Treaty on Plant Genetic Resources for Foord and Agriculture (SMTA of the ITPGRFA) Rome. (http://.itpgrfa.net/International/)

Fisher HH, Soderholm PK, Kahn RP 1967 U.S. Department of Agriculture cacao clone collection. pp 34-34

Gateau-Rey L, Tanner EVJ, Rapidel B, Marelli J-P, Royaert S 2018 Climate change could threaten cocoa production: Effects of 2015-16 El Niño-related drought on cocoa agroforests in Bahia, Brazil. PLoS ONE 13 (7): e0200454. https://doi.org/10.1371/journal.pone.0200454

Gayi, S. K. and Tsowou, K. 2016. Cocoa industry: Integrating small farmers into the global value chain. United nations conference on trade and development. United nations, NY. https://unctad.org/en/PublicationsLibrary/suc2015d4_en.pdf

Glicenstein LJ, Fritz PJ 1989 Ploidy Level in *Theobroma cacao* L., J Heredity 80 (6) 464-467, https://doi.org/10.1093/oxfordjournals.jhered.a110899).

Goenaga R, Irizarry H, Irish B 2009 TARS series of cacao germplasm selections. HortScience 44:826-827

Goenaga RJ, Guiltinan M, Maximova S, Seguine E, Irizarry H 2015 Yield performance and bean quality traits of cacao propagated by grafting and somatic embryo-derived cuttings. HortScience. 50:358-362.

Grattapaglia D, Resende MD 2011 Genomic selection in forest tree breeding. Tree Genet Genom 7:241-255

Gregory PH 1974 Phytophthora disease of cocoa. Longman, New York; London

GRIN GLOBAL 2018 Germplasm Resources Information Network [Internet]. Beltsville (MD): United States Department of Agriculture, Agricultural Research Service. [accessed12/20/2018]. Available from: http://www.ars-grin.gov/

GROW Intelligence 2019 As Cocoa Harvest Begins, Risks Emerge From Major Producing Countries. https://www.gro-intelligence.com/insights/articles/as-cocoa-harvest-begins-risks-emerge-from-major-producing-countries. 24 October 2019

Guest D, Keane P 2007 Vascular-streak dieback: A new encounter-disease of cacao in Papua New Guinea and Southeast Asia caused by the obligate basidiomycete Oncobasidium theobromae. Phytopathol 97:654-1657

Guest D 2007 Black pod: Diverse pathogens with a global impact on cocoa yield. Phytopathol 97:1650-1653

Gutiérrez OA, Campbell AS, Phillips-Mora W 2016 Breeding for Disease Resistance in Cacao. In: Bailey BA, Meinhardt LW (eds) Cacao Diseases: A History of Old Enemies and New Encounters. pp 567-609 Springer International Publishing, Cham

Hansen AJ 1961 Resistencia y susceptibilidad de tipos de cacao a P. palmivora. Cacao 6:9-10

Hartmann HT, Kester DE, Davies FT JR, Geneve RL 2002 Plant propagation - principles and practices. New Jersey: Prentice Hall. 880pp

Hawaii Chocolate and Cacao Association (HCCA) .Last visited November, 2019. https://hawaiicacao.com/

Hayes BJ, Bowman PJ, Chamberlain AJ, Goddard ME 2009. Invited review: Genomic selection in dairy cattle: Progress and challenges. J Dairy Sci 92:433-443

Heffner EL, Sorrells ME, Jannink JL 2009 Genomic selection for crop improvement. Crop Sci 49:1-12

Holliday P 1954 The control of black pod and witches' broom disease in Trinidad. Reunion del Comite Tecnico Interamericano del Cacao 5a, IICA (5RTIC/DOC 15-Fitop/2), Turrialba, Costa Rica

Hunter JR 1990 The status of cacao (Theobroma cacao, Sterculiaceae) in the Western Hemisphere. Econ Bot 44:425-439

ICCO Quarterly Bulletin of Cocoa Statistics 2018 Vol. XLV, No.3, Cocoa year 2018/19.

ICCO. 2020 Quarterly Bulletin of Cocoa Statistics (2020), Vol. XLVI, No.1, Cocoa year 2019/20

ICCO. 20012 Frequently asked questions. https://www.icco.org/faq/57-cocoa-production/123 how-many-smallholders-are-there-worldwide-producing-cocoa-what-proportion-of-cocoa-worldwide-is-produced-by-smallholders.html

Irish BM, Goenaga R, Park S, Kang S 2006 First report of *Phytophthora palmivora*, causal agent of black pod, on cacao (*Theobroma cacao* L.) in Puerto Rico. Plant Dis 91:1051

Irish, B.M., Goenaga, R.J., Schnell II, R.J., Motamayor, J., Brown, J.S., Zhang, D. 2010. Cacao (Theobroma Cacao L.) Genetic Resources Research At The USDA-ARS Tropical Agriculture Research Station. Proceedings of the International Cocoa Producer's Conference. PP. 417-423

Irizarry H, Goenaga R 2000 Clonal selection in cacao based on early yield performance of grafted trees. J Agric Univ PR 84:153-164

Irizarry H, Rivera E 1998 Early yield of five cacao families at three locations in Puerto Rico. J. Agric Univ PR 82:163-171

Jannink JL, Lorenz AJ, Iwata H 2010 Genomic selection in plant breeding: from theory to practice. Briefings in Funct Genomics 9:166-177

Keil A, Zeller M, Wida A, Sanim B, Birner R (2008) What determines farmers' resilience towards ENSOrelated drought? An empirical assessment in Central Sulawesi, Indonesia Climatic Change 86:291–307 DOI 10.1007/s10584-007-9326-4

Kuhn DN, Figueira A, Lopes U, Motamayor JC, Meerow AW, Cariaga K, Freeman B, Livingstone DS, Schnell RJ 2010 Evaluating *Theobroma grandiflorum* for comparative genomic studies with *Theobroma cacao.* Tree Genet Genom 6:783-792

Laliberté B, End M, (Eds). 2015 Supplying new cocoa planting material to farmers: a review of propagation methodologies. Bioversity International, Rome, Italy. 200pp

Lanaud C, Fouet O, Clément D, Boccara M, Risterucci AM, Surujdeo-Maharaj S, Legavre T, Argout X. 2009 A meta–QTL analysis of disease resistance traits of Theobroma cacao L. Mol Breed 24:361-374

Lanaud C, Risterucci AM, Pieretti I, Falque M, Bouet A, Lagoda PJL 1999 Isolation and characterization of microsatellites in *Theobroma cacao* L. Mol Ecol

Livingstone DS, Royaert S, Stack C, Mockaitis K, May G, Farmer A, Saski C, Schnell R, Kuhn D, Motamayor JC 2015 Making a chocolate chip: development and evaluation of a 6K SNP array for *Theobroma cacao*. DNA Res 22:279-291

Livingstone DS, Freeman B, Motamayor JC, Schnell RJ, Royaert S, Takrama J, Meerow AW, Kuhn DN 2012 Optimization of a SNP assay for genotyping Theobroma cacao under field conditions. Mol Breed 30:33-52

Livingstone DS, Motamayor JC, Schnell RJ, Cariaga K, Freeman B, Meerow AW, Brown JS, Kuhn DN 2011 Development of single nucleotide polymorphism markers in *Theobroma cacao* and comparison to simple sequence repeat markers for genotyping of Cameroon clones. Mol Breed 27:93-106

Lopes UV, Monteiro WR, Pires JL, Clement D, Yamada MM, Gramacho KP 2011 Cacao breeding in Bahia, Brazil: strategies and results. Crop Breed Applied Biotech11:73-81

McMahon P, Purwantara A 2016 Vascular Streak Dieback (*Ceratobasidium theobromae*): History and Biology, 307−336. In Bailey BA Meinhardt LW(eds.), Cacao Diseases: A History of Old Enemies and New Encounter. Springer International Publishing AG, Switzerland

Medina V, Laliberte B 2017 A review of research on the effects of drought and temperature stress and increased CO2 on *Theobroma cacao* L., and the role of genetic diversity to address climate change. Costa Rica: Bioversity International

Meinhardt LW, Rincones J, Bailey BA, Aime MC, Griffith GW, Zhang D, Pereira GAG 2008. *Moniliophthora perniciosa*, the causal agent of witches' broom disease of cacao: what's new from this old foe? Mol Plant Pathol 9:577-588

Meuwissen TH, Hayes BJ, Goddard ME 2001 Prediction of total genetic value using genome-wide dense marker maps. Genetics 157:1819-1829

Miranda S, da Cruz H 1953 Fighting brown pod disease in Bahia. 7th Cocoa Conference, London, p 120

Mooleedhar V 2000 A review of vegetative propagation methods in cocoa in Trinidad and the implications for mass production of clonal cocoa plants, Proc tech meeting: state of knowledge on mass production of genetically improved propagules of cocoa, 19-23 October 1998, Ilhéus, Bahia, pp. 122-125

Motamayor J, Mockaitis K, Schmutz J, Haiminen N, III D, Cornejo O, Findley S, Zheng P, Utro F, Royaert S, Saski C, Jenkins J, Podicheti R, Zhao M, Scheffler B, Stack J, Feltus F, Mustiga G, Amores F, Phillips W, Marelli J, May G, Shapiro H, Ma J, Bustamante C, Schnell R, Main D, Gilbert D, Parida L, Kuhn D 2013. The genome sequence of the most widely cultivated cacao type and its use to identify candidate genes regulating pod color. Genom Biol 14:r53

Motamayor JC, Lachenaud P, Mota JWDS, Loor R, Kuhn DN, Brown JS, Schnell RJ 2008 Geographic and genetic population differentiation of the Amazonian chocolate tree (*Theobroma cacao* L). PLoS ONE 3:e3311

Motamayor JC, Risterucci AM, Lopez PA, Ortiz CF, Moreno A, Lanaud C 2002 Cacao domestication I: the origin of the cacao cultivated by the Mayas. Heredity 89:380-386

Motilal L, Butler D 2003 Verification of identities in global cacao germplasm collections. Genet. Resour. Crop Ev 50: 799-807

Motilal LA, Zhang D, Umaharan P, Mischke S, Pinney S, Meinhardt LW 2011 Microsatellite fingerprinting in the International Cocoa Genebank, Trinidad: accession and plot homogeneity information for germplasm management. Plant Genet Resour Charac Utiliz 9: 430-438

Motilal LA, Zhang D, Mischke S, Meinhardt LW, Boccara M, Fouet O, Lanaud C, Umaharan P 2016 Association mapping of seed and disease resistance traits in *Theobroma cacao* L. Planta 244:1265-1276

Omont H 2001 IPGRI - Information sheet on Cocoa. Commodity Chains. http://www.fao.org/docs/eims/upload/216251/Infosheet_Cocoa.pdf

Padi FK, Ofori A 2016 Cacao Seed Purity and Genotype Influence on Seedling Growth under Peasant-Farmer Conditions in Ghana, J Crop Improv 30: 493-515

Phillip s-Mora W, Castillo J 1999 Artificial inoculations in cacao with the fungi Moniliophthora roreri (Cif. Par) Evans et al. and *Phytophthora palmivora* (Butl.) Butler. In: CATIE (ed) Actas IV Semana CientíficaTurrialba Logros de la investigacion para un nuevo milenio. CATIE, Turrialba. Costa Rica

Phillips-Mora W, Ortiz CF, Aime MC 2006 Fifty years of frosty pod rot in Central America: Chronology of its spread and impact from Panama to Mexico. Proceedings 15th International Cocoa Research Conference [San José, Costa Rica, 9-14 October 2006.

Phillips-Mora W, Wilkinson MJ 2007 Frosty Pod of Cacao: A Disease with a Limited Geographic Range but Unlimited Potential for Damage. Phytopathol 97:1644-1647

Phillips-Mora W, Arciniegas-Leal A, Mata-Quiros A, Motamayor-Arias JC 2013 Catalogue of cacao clones selected by CATIE for commercial plantings. Technical series. Technical manual / CATIE; no, 105. 68 p.

Ploetz RC 2007 Cacao diseases: important threats to chocolate production worldwide. Phytopathol 97:1634-1639

Pound FJ 1936 The completion of selection. Fifth Annual Report on Cacao Research 1935. Government Printing Office, Port of Spain, Trinidad, pp 7-16

Pugh T, Fouet O, Risterucci AM, Brottier P, Abouladze M, Deletrez C, Courtois B, Clement D, Larmande P, N'Goran JAK, Lanaud C 2004 A new cacao linkage map based on codominant markers: development and integration of 201 new microsatellite markers. Theor Appl Genet 108:1151-1161

Purseglove JW 1968 Tropical Crops Dicotyledons, Longman Scientific and Technical. John Wiley and Sons Inc., New York.

Queiroz VT, Guimaraes CT, Anhert D, Schuster I, Daher RT, Pereira MG, Miranda VRM, Loguercio LL, Barros EG, Moreira MA 2003 Identification of a major QTL in cocoa (*Theobroma cacao* L.) associated with resistance to witches' broom disease. Plant Breed 122:268-272

Risterucci AM, Paulin D, Ducamp M, N'Goran JAK, Lanaud C 2003 Identification of QTLs related to cocoa resistance to three species of Phytophthora. Theor Appl Genet 108:168-174

Rodriguez-Medina C, Arana AC, Sounigo O, Argout X, Alvarado GA, Yockteng R 2019 Cacao breeding in Colombia, past, present and future. Breed Sci 69:373–382.

Royaert S, Jansen J, da Silva DV, de Jesus Branco SM, Livingstone DS, Mustiga G, Marelli JP, Araújo IS, Corrêa RX, Motamayor JC 2016 Identification of candidate genes involved in Witches' broom disease resistance in a segregating mapping population of *Theobroma cacao* L. in Brazil. BMC Genomics 17:107

Rubenstein, K.D. Heisey, P., Shoemaker, R., Sullivan, J., and Frisvold, G. 2005. Crop Genetic Resources: An economic appraisal. https://www.ers.usda.gov/webdocs/publications/44121/17452_eib2_1_.pdf?v=3443.3

Samuels GJ et al. 2012 Vascular Streak Dieback of cacao in Southeast Asia and Melanesia: in planta detection of the pathogen and a new taxonomy Fungal biology 116:11-23 doi:10.1016/j.funbio.2011.07.009

Saunders JA, Hemeida AA, Mischke S 2001 USDA DNA fingerprinting programme for identification of Theobroma cacao accessions. In: Proceedings of the international workshop on new technologies and cocoa breeding, 16–17 October 2000, Kota Kinabalu, Malaysia, pp 108–114

Saibo NJM, Lourenço T, Oliveira MM 2009 Transcription factors and regulation of photosynthetic and related metabolism under environmental stresses. Annals of Botany 103:609–623

Schroth G, LaÈderach P, Martinez-Valle AI, Bunn C, Jassogne L 2016 Vulnerability to climate change of cocoa in West Africa: Patterns, opportunities and limits to adaptation. Sci Total Environ 556: 231-241.

Silva TE, Cidade LC, Alvim FC, Cascardo JC, Costa MG 2009 Studies on genetic transformation of *Theobroma cacao* L.: evaluation of different polyamines and antibiotics on somatic embryogenesis and the efficiency of uidA gene transfer by *Agrobacterium tumefaciens*. Plant Cell Tiss Organ Cult 99:287 -298

Soria VJ 1959 Notes on the variability of cacao types in some Nicaraguan plantations and comments on their genetic constitution. Cacao 4 (2):1–18

Soria VJ 1978 The breeding of cacao (*Theobroma cacao* L.). Trop Agric Res Ser 11:161-168

Sukha DA 2000 Potential value added products from Trinidad and Tobago cocoa. Proc. of : Seminar/Exhibition on the revitalization of the Trinidad and Tobago cocoa industry – Targets, Problems and Options. The Association of Professional Agricultural Scientists of Trinidad and Tobago (APASTT), Faculty of Science and Agriculture, The University of the West Indies, St. Augustine. 20th September, 2003. p. 69-73

Surujdeo-Maharaj S, Sreenivasan TN, Motilal LA, Umaharan P 2016 Black pod and other Phytophthora induced diseases of cacao: history, biology, and control. In: Bailey BA,

Meinhardt LW (eds) Cacao Diseases: A History of Old Enemies and New Encounters. pp 213-266 Springer International Publishing, Cham

Surujdeo-Maharaj S, Umaharan P, Butler DR 2004 Assessment of resistance to witches -broom disease in clonal and segregating populations of *Theobroma cacao*. Plant Dis 88:797-803

Surujdeo-Maharaj S, Umaharan P, Butler DR, Sreenivasan TN 2003 An optimized screening method for identifying levels of resistance to *Crinipellis perniciosa* in cocoa (*Theobroma cacao*). Plant Pathol 52:464-475

Takrama J, Dadzie A, Opoku S, Padi F, Adomako B, Adu-Ampomah Y, Livingstone III D, Motamayor J, Schnell R, Kuhn D 2012 Applying SNP marker technology in the cacao breeding programme in Ghana. African Crop Sci J 20:67-75

USDA-FAS 2013 International agricultural trade report: chocolate candy. USDA-FAS Office of Global Analysis, Washington, DC

Vos R, Velasco M, Labastida E 1999 Economic and social effects of" El Nino" in Ecuador, 1997/1998. ISS WorkPap Series General Ser. 292: 1-55.

WCF 2017 Economic profile of the U.S. chocolate industry. World Cocoa Foundation

Wheeler BEJ, Mepsted R 1988 Pathogenic variability amongst isolates of *Crinipellis perniciosa* from cocoa (*Theobroma cacao*). Plant Pathol 37:475-488

Whitlock BA, Baum DA 1999 Phylogenetic relationships of *Theobroma* and *Herrania* (Sterculiaceae) based on sequences of the nuclear gene Vicilin. Syst Bot 128-138

Wood GA, Lass RA 1985 Cocoa. 4th edn, John Wiley & Sons, London

Zarrillo, S., Gaikwad, N., Lanaud, C. *et al.* 2018 The use and domestication of *Theobroma cacao* during the mid-Holocene in the upper Amazon. Nat Ecol Evol 2, 1879–1888 .

Zhang D, Martínez WJ, Johnson ES, Somarriba E, Phillips-Mora W, Astorga C, Mischke S, Meinhardt LW 2012. Genetic diversity and spatial structure in a new distinct *Theobroma cacao* L. population in Bolivia. Genet Resour Crop Evol 59:239-252

Zhang D, Mischke S, Johnson ES et al., 2009 Tree Gen Genomes 5:1. https://doi.org/10.1007/s11295-008-0163-z

Zion-Market-Research 2018 Global Chocolate Market Expected to Reach USD 161.56 Billion By 2024: https://www.globenewswire.com/news-release/2018/10/22/1624439/0/en/Global-Chocolate.html.

APPENDIX

Annex 1

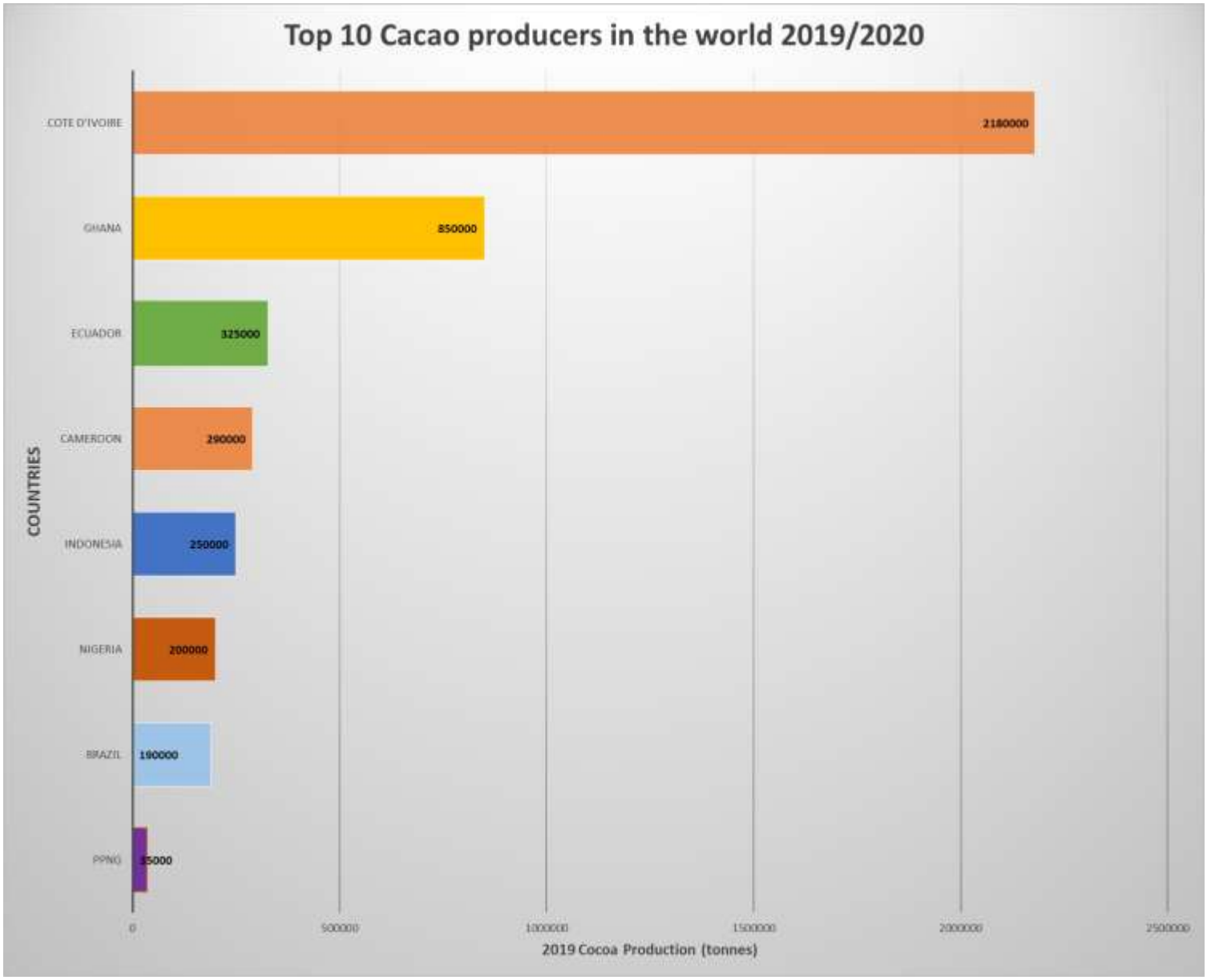


Figure 2. List of cocoa producing countries 2019 (Source: Chocolate Phayanak, Phayanak.com).

Annex 2. Standard Operating Procedures Cacao Quarantine Procedures

Standard Operating Procedures

Cacao Quarantine Greenhouses
USDA-ARS
Subtropical Horticulture Research Station
Miami, FL 33158

Prepared May 2, 2015

Major Revisions: November 14, 2015

Mission SHRS

I. The mission of the Subtropical Horticulture Research Station (SHRS) is to support the agricultural industries in the southern areas of the United States by providing environmentally sound research on: (1) the genetics of tropical and subtropical fruit, cacao and ornamental crops; (2) the interdiction and control/eradication of exotic plant insect pests; and (3) developing a potting mix from construction debris, biosolids, and organic composts with improved water holding and ion exchange capacities to reduce the quantity and improve the quality of wastewater from nursery irrigation operations.

SHRS's main goal for cacao research is to follow a preemptive breeding strategy, based on genomic selection for disease resistance, yield and quality traits, to select new cultivars with resistance to witches' broom (WB), frosty pod (FP), and black pot (BP). Combining genomic approaches with evaluation data, will enable the identification of a large suite of genetic markers that will be used to accelerate the breeding of superior varieties with disease and pest resistances, tolerance to environmental stresses, improved tree architecture for clonally-propagated trees, and the quality attributes required by the confectionary industry.

II. Cacao Importance to US Agriculture

Cocoa beans, the seed of *Theobroma cacao,* are an important cash crop in the tropics of Central and South America, Asia, and Africa. The commercial cultivars grown in these areas are derived principally from ten diverse germplasm groups known as Maranon, Curaray, Criollo, Iquitos, Nanay, Contamana,Amelonado, Purus, Nacional and Guiana (Motamayor, et al., 2008). These groups differ greatly in flavor, yield, and susceptibility to diseases and insect pests.

Cacao cultivation and production of cocoa for the American chocolate industry is a multibillion-dollar effort centered largely in Africa, Asia, and South America. The U.S. chocolate industry alone generated $19 .5 billion in sales of chocolate products in 2011 (Lindell, 2012). Significant amounts of U.S. produced agricultural commodities including milk, sugar, almonds, peanuts, and com syrup sweeteners are used in the chocolate and confectionery industry. For the long-term

well-being of the industry, there is a need to foster international collaboration among producers and develop superior trees that can resist disease and still produce high quality cocoa beans.

III. Background SHRS Cacao Quarantine Operations

In 1955, the U.S. Department of Agriculture initiated a program for the quarantine and distribution of cacao cultivars as a service to the cacao research centers located in the tropics (Fisher et al., 1960; 1967). This activity was divided between two locations with the SHRS serving as the quarantine facility and the Tropical Agriculture Research Station (TARS), Mayaguez, Puerto Rico, serving as the official USDA germplasm repository for cacao germplasm. This arrangement still continues.

The primary goal of the quarantine program at the SHRS is to develop a research approach to solving disease and production problems in cacao. In addition, the USDA-ARS and Mars Inc. are coordinating an international cacao genetics project that provides information into national and regional breeding programs to increase the efficiency of developing superior varieties for the cacao farmers worldwide. All introductions of cacao accessions adhere to safe movement guidelines of cacao germplasm (End, et. al.,2014).

The USDA cacao germplasm collection at TARS is unique in that many of the serious insect and disease pests of concern for cacao are not present in Puerto Rico. Black pod, caused by a worldwide distributed soil-borne and pod-infecting Oomycete *(Phytopthora palmivora),* is the only disease of consequence. To avoid the introduction of devastating diseases, all germplasm introductions into the USDA cacao collection at TARS follow guidelines and quarantine procedures (End, et al., 2014). Scionwood received at TARS is quarantined at the Intermediate Cocoa Quarantine Center at the University of Reading (ICQC,R), England and/or the USDA-ARS-SHRS. Worldwide distributions of cacao material from this collection also follow strict phytosanitary protocols.

IV. Summary of Pest Risks

Cacao production is plagued by very serious losses globally from pests and diseases which can sometimes reduce yield by as much as 80 percent. Black pod (BP), witches' broom (WB), Cacao Swollen Shoot Virus (CSSV), frosty pod (FP), Vascular Streak Dieback (VSD), cocoa pod borer (CPB) and Mirids have caused a decrease in worldwide production. In the Americas the cacao industry has undergone a severe production decline due to losses principally to BP, WB, and FP. If this trend were to continue it would greatly increase bean prices and have a negative impact on the confectionery industry. Breeding cacao with inherent host-plant resistance is an optimal solution to the preceding challenges. Furthermore, cacao with improved productivity, tolerance to environmental stresses, improved tree architecture, and superior product quality are needed in support of research that will lead to higher cacao productivity.

V. Standard Operating Procedures (SOP's)

1. Purpose of SOP's

The purpose of the standard operating procedures is to document procedures and protocols to monitor introduced cacao germplasm for infectious diseases and ensuring that if plants are identified with quarantined pests, these are handled and discarded properly and according to

established procedures. All introductions of cacao accessions adhere to safe movement guidelines of cacao germplasm (End, et al., 2014).

2. Pests and Diseases of Concern of Cacao

The most relevant diseases of concern in cacao as well as their geographical spread are described below:

Cacao necrosis virus (CNV): genus Nepovirus (Ghana, Nigeria); *Cacao swollen shoot virus* (CSSV): genus *Badnavirus* (Benin, Cote d'Ivoire, Ghana, Liberia, Nigeria, Sierra Leone, Togo, Sri Lanka); Cacao yellow mosaic virus (CYMV): genus *Tymovirus* (Sierra Leone); Witches' broom disease *(Moniliophthora perniciosa)* [Brazil (Bahia, Espirito Santo, Amazonian regions), Bolivia, Colombia, Ecuador, French Guiana, Grenada, Guyana, Panama, Peru, St. Lucia, St. Vincent, Surinam, Trinidad and Tobago, Venezuela]; *Moniliophthora* pod rot (frosty pod rot or moniliasis disease) (Colombia and Ecuador on both sides of the Andes, western Venezuela, Peru, Panama, Costa Rica, Nicaragua, Honduras, Guatemala, Belize and Mexico, El Salvador, Bolivia); *Phytophthora megakarya* (West and Central Africa); *Trachysphaerafructigena* (Africa, Brazil); Vascular streak die-back *(Ceratobasidium theobromae)* [Most cacao-growing areas in South and South East Asia: PNG, (islands of New Guinea, New Britain, New Ireland), Hainan Island (China), Kerala State (India), West Malaysia and Sabah, Indonesia, Thailand, Myanmar, Vietnam and the southern Philippines]; Mirids: 56 species of Miridae: species of *Mona/onion; Distantiella theobroma* (All cacao-growing regions except Caribbean); Cocoa pod borer *(Conopomorpha cramerella)* (Southeast Asia including Malaysia, Indonesia, the Philippines and Papua New Guinea).

3. General Recommendations

The following general recommendations (End, et al., 2014) will be followed for any movement of cacao germplasm from any cacao producing region to SHRS:

a) All imported plant material should be free of soil, other foreign matter or debris, other prohibited plants and pests, noxious weed seeds, and living organisms such as pathogens, parasitic plants, snails and mites.
b) Germplasm should be obtained from the safest source possible, e.g. from a pathogen-tested
c) intermediate quarantine collection.
d) Shipping of whole pods from countries where cacao is grown commercially or where diseases listed in Section V.2 exist is **NOT** recommended.
e) When transferring material as seed, a sterile inorganic packing material such as vermiculite or perlite is preferable to an organic material such as sawdust. Used packaging material should be incinerated or autoclaved prior to disposal.
f) Region to region and final transfer of budwood to USDA germplasm collection should take place via a quarantine center.
g) Budwood for international exchange should be treated with an appropriate fungicide/ pesticide mixture in cases where this is specified on the import certificate of the recipient country.
h) After grafting the budwood at SHRS, any waste plant material should be incinerated or autoclaved prior to disposal.
i) The transfer of germplasm should take place in consultation with the relevant plant health authorities in both the importing and exporting countries. Movement of germplasm must follow F AO guidelines (End, et al., 2014)

j) Any material being transferred internationally must be accompanied by a phytosanitary certificate.

4. Specific Requirements for SHRS Quarantine Operations

a) The person identified in the USDA-APHIS-PPQ Departmental Permit (Controlled Import Permit to Import Restricted or Not Authorized Plant Material) will be considered the Permit Holder and ultimately responsible for adherence to the recommendations listed herein. However, any collaborating scientific personnel or person with delegated authorities by the Permit Holder is expected to abide by the same recommendations.

b) The introduction of all plant materials from foreign nations by ARS personnel or collaborators must adhere to international, Federal, state, county laws, regulations and treaties applicable to importing plant materials, including phytosanitary regulations, and those covering access and benefit-sharing arrangements. The relevant Agricultural Research Service germplasm acquisition, quarantine, and plant genetic resource management policies must also be followed.

c) The Permit Holder or designated person shall be responsible for the operation of the facility including:
   - care of material,
   - checking of material,
   - notifying the Supervisor of any detected disease symptoms,
   - undertaking any required treatment,
   - destruction of infected/infested material through autoclave (in consultation with Supervisor and
   - authorized APHIS-PPQ plant pathologist if a prohibited disease is found,
   - meeting all security requirements,
   - keeping and maintaining all required records,
   - ensuring all personnel entering the facility understand and abide by the quarantine
   - requirements.

d) The following records will be kept in a designated ARS laboratory notebook by the Permit Holder or designated person:
   - the phytosanitary certificate (photocopy acceptable),
   - arrival date in the quarantine (starting quarantine period),
   - any treatment undertaken upon arrival,
   - Passport information,
   - dates of planting or potting quarantine material,
   - room or bench location of material during quarantine,
   - visual observations of any abnormal growth or symptoms and date,
   - date of end of quarantine period,
   - date of removal from quarantined greenhouse.

e) All introduced germplasm into quarantine is logged and accessions are given inventory/tracking numbers assigned by the Permit Holder or designated person. All germplasm received is documented as completely as possible based upon the information available. Minimum passport information should include:
   - **Collector:** Full name and affiliation;
   - **Date collected:** Year, Month, day;
   - **Location:** Country, region, state, province, city, town, latitude/longitude, elevation;

- **Name:** accession or cultivar or other identifiers;
- **Status:** breeding line, cultivar, wild;
- **Traits:** resistant, productive, tall;
- **Pedigree:** if known (or specific genetic population to which it belongs, if the sample in question has been adequately genotyped);
- **Genotype:** if genotyped, then provide profile to include in GRIN as reference;
- **Evaluation:** provide any evaluation available;
- **Form:** scionwood, seed, cuttings;
- **Taxonomy:** Usually *T. cacao*, but could be other species or subspecies;
- **Collection source:** field trial, market, riparian environment. If accession was donated by someone (e.g., Institute), describe.
- Other germplasm acquisition documentation/authorization, e.g., access and benefit-sharing
  certification from the relevant authority that some nations might require for germplasm export.
- Any available information on pathogen status.

Documentation is maintained in the SI-IRS databases. Appropriate portions of the documentation are uploaded into the Germplasm Resources Information Network (GRIN).

f) Introduced cacao germplasm will be kept in quarantine for two years and observed for symptoms of diseases discussed in Section 2 above. At the end of this period, the Permit Holder or designated person will make arrangements to assay plants for *cacao swollen shoot virus* (CSSV). Symptoms of this disease are highly variable and depend on the virus strain and the stage of infection. No universal molecular technique is currently available and for this reason visual indexing is still recommended. It is important to note that infection with CSSV may be latent for up to 20 months and hence the duration of the quarantine period. Indexing for CSSV after the 2-year quarantine period will be done by grafting accessions onto Amelonado (TARS-16542) rootstocks (Figures l, 2). This variety has shown to be very sensitive to CSSV and upon infection will show a characteristic red vein banding of the young leaves, yellow vein banding, interveinal flecking and mottling of mature leaves, vein clearing on leaves and stem swellings.

g) Each quarantined accession will be grafted onto at least three Amelonado rootstocks. Two months after 'bud break' of grafted accessions, the Permit Holder or designated person will make

h) arrangements with an APHIS-PPQ plant pathologist to inspect materials for signs of CSSV on the Amelonado rootstock. At least one graft should "take" in order for an accession to be declared **CSSV-free.** ln the event of presence of symptoms, an APHIS-PPQ plant pathologist will be called immediately and will make a final decision on steps to take for disease confirmation or disposition, most probably by autoclaving.

i) Upon release by APHIS plant pathologist, quarantined accessions will be moved immediately from the quarantine greenhouse to greenhouses 2 or 3 or as appropriate to other greenhouses. The Permit Holder will ensure that this move is carried out on a timely basis.

j) The Permit Holder will ensure that quarantined accessions are not grown in the quarantine greenhouse for a period longer than two (2) years.

k) During the quarantine period all vegetative material from quarantined accessions will be disposed of by autoclaving. Removal of this material from the greenhouse to the autoclave should be carried out using heavy-duty polyethylene bags (Figure 3).

l) Any equipment (e.g. sprayers, trash cans, etc.) removed from the quarantined greenhouse must be first washed or sprayed with a 10% bleach solution before exiting the greenhouse.
m) The Permit Holder in collaboration with a SHRS entomologist will monitor insects in the greenhouse. Presence of disease-transmitting insects (e.g. aphids, mealybugs, etc.) will be dealt with immediately through the application and rotation of systemic and contact insecticides and insecticidal oils.
    a. Upon release from quarantine, the Permit Holder or delegated person will contact the cacao curator in Mayaguez, Puerto Rico to make arrangements to ship relevant accessions for permanent establishment in the USDA/A RS NPGS cacao germplam collection.

Literature cited

End MJ, Daymond AJ, Hadley P, editors 2014 Technical guidelines for the safe movement of cacao germplasm. Revised from the FAO/lPGRI Technical Guidelines No. 20 (Second Update, August 2014).

Global Cacao Genetic Resources Network (CacaoNet), Bioversity International, Montpellier, France.

Fisher HH, Haun JR, Ackerman WL 1960 Cacao seedling production and distribution through plant quarantine. Cacao 5:1-8.

Lindell C 2012 Report: $19.5 billion in U.S. chocolate sales-And 20% growth in organic. 3 May 2015. <http://www.candyindustry.com/articles/8521 5-report---1 9-S-bi llion-in-chocolate-sales-and-20--growthin-organic.>

Motamayor JC, Lachenaud P, da Silva E, Mota JW, Loor R, Kuhn DN, Brown JS, Schnell RJ 2008 Geographic and Genetic Population Differentiation of the Amazonian Chocolate Tree *(Theobroma cacao* L). PLoS One 3:e33 l l .

Prepared by Ricardo Goenaga, PhD., Acting Research Leader, USDA-ARS, Miami, FL. Special thanks to Dr. Peter Bretting, National Program Leader, USDA-ARS, Beltsville. MD; Dr. Gary Kinard, Research Leader, USDA-ARS, Beltsville, MD; Dr. Robert Krueger, Horticulturist, USDA-ARS, Riverside, CA and; Dr. Weston Msikita, Plant Pathologist, USDA-AP HIS, Miami, FL.for useful comments and suggestions.

Annex 3

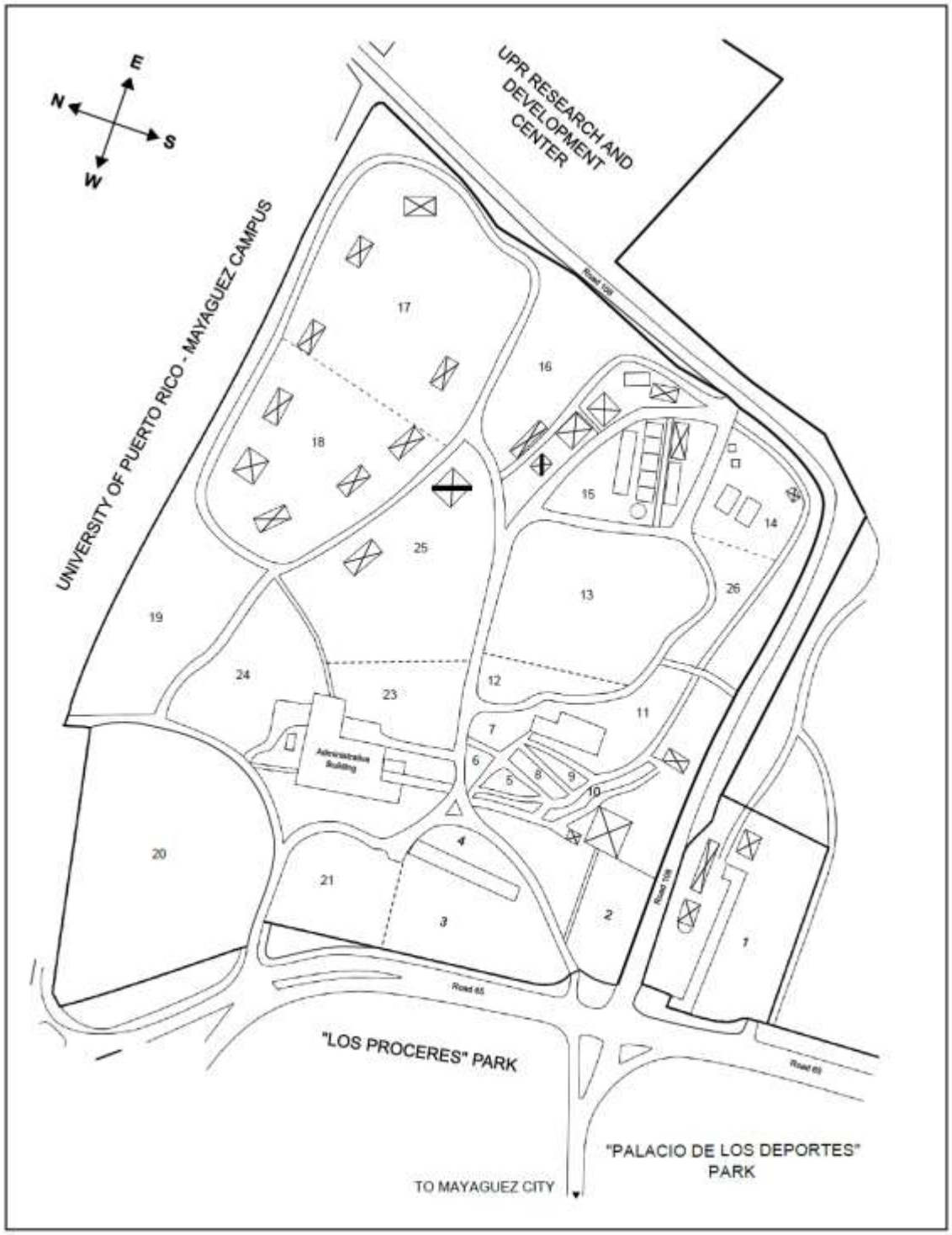
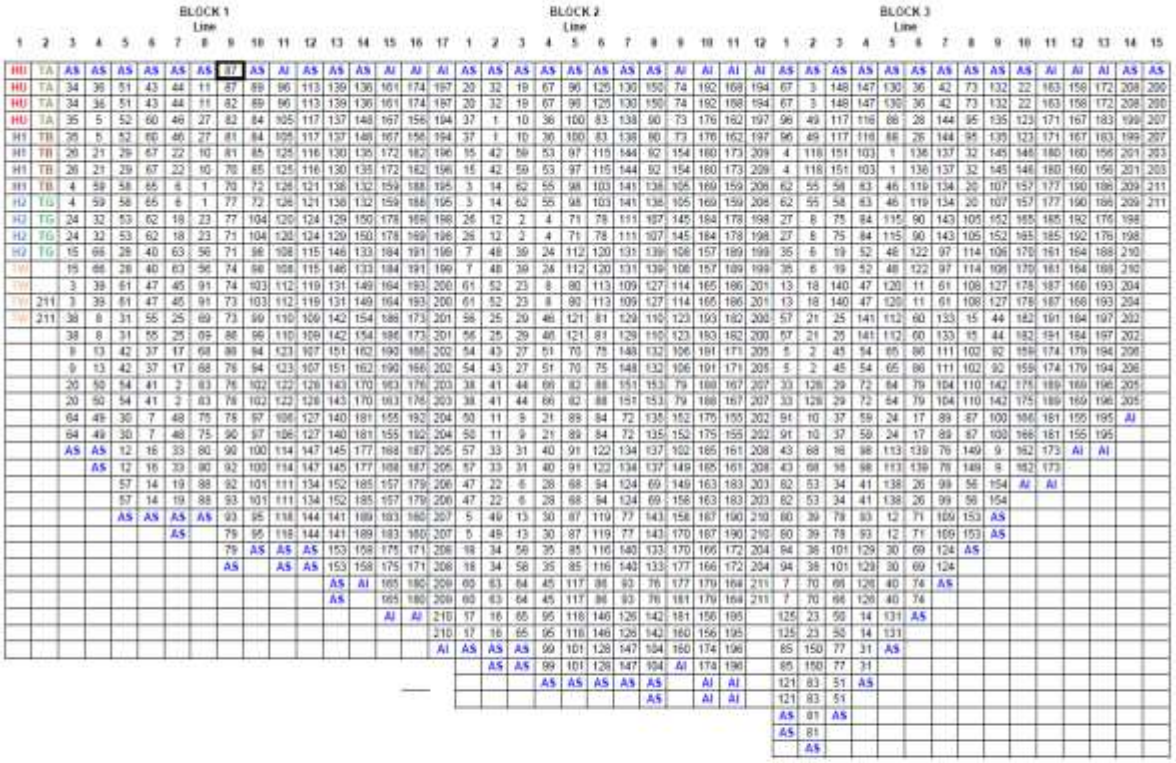


Figure 1. Map of TARS facilities at Mayaguez, PR.

Annex 4. Cacao collection Field layout.



Annex 5

Table 1. Distribution of cacao materials from TARS between 2013 and 2018.

Year	No. orders distributed
2013	152
2014	178
2015	281
2016	346
2017	169
2018	108

Annex 6. Website Links to other cacao genetic resources

- CATIE
- Cocoa Coconut Institute of Papua New Guinea (CCI)
- CEPLAC
- CIRAD (France)
- CNRA (Cote d'Ivoire)
- Cocoa Producers Alliance (COPAL)
- Cocoa Research Institute of Ghana (CRIG)
- Cocoa Research Unit (University of west indies, Trinidad and Tobago)
- CRIN (Nigeria)
- IITA (Nigeria)
- INGENIC
- INIAP (Ecuador)
- IRAD (Cameroon)
- Malaysian Cocoa Board (MCB)
- Reading University International Cacao Germplasm Database (ICGD)
- Sustainable Tree Crop Program (STCP)
- World Cocoa Foundation (WCF)
- Summary production data for Cocoa beans (Source of original data: FAOSTAT**)**

Annex 7. Publications related to Cacao Research at USDA

1. Borrone JW, Kuhn, DN, and Schnell R J 2004. Isolation, characterization, and development of WKRY genes as useful genetic markers in *Theobroma cacao*. Theor. Appl. Genet. 109: 495-507.
2. Boza, E. J., Irish, B M, Meerow, AW, Tondo, C. L., Rodríguez, O. A., Ventura-López, M., Gómez, J. A., Moore, J. M, Zhang, D., Motamayor, J. C., and Schnell, R. J. 2013. Genetic diversity, conservation, and utilization of *Theobroma cacao* L.: Genetic resources in the Dominican Republic. Genet. Res. Crop Ev. 60: 605-619.
3. Cosme S, Cuevas, Zhang, HD, Oleksyk, T, and B.M. Irish BM, 2016 SNP genotyping of naturalized cacao (*Theobroma cacao* L.) in Puerto Rico. Tree Genet. Genomes. 12:88
4. Goenaga RJ, Guiltinan M, Maximova S, Seguine E, Irizarry H 2015 Yield performance and bean quality traits of cacao propagated by grafting and somatic embryo-derived cuttings. HortScience. 50:358-362.
5. Irish BM, Goenaga R, Park S, and Kang S 2006 First report of *Phytophthora palmivora*, causal agent of black pod, on cacao (*Theobroma cacao* L.) in Puerto Rico. Plant Dis 91:1051.
6. Irish, B.M., Goenaga, R., Zhang, D., Schnell, R., Motamayor, J.C. and Brown, S. 2010. Microsatellite fingerprinting of the USDA-ARS-Tropical Agriculture Research Station cacao (*Theobroma cacao*) germplasm collection Crop. Sci. 50:656-667.
7. Irizarry, H., and Goenaga, R. 2000. Clonal selection in cacao based on early yield performance of grafted trees. J. Agric. Univ. P. R. 84: 153-163.
8. Irizarry, H., and Rivera, E. 1998. Early yield of five cacao families at three locations in Puerto Rico. J. Agric. Univ. P. R. 82: 163-171.
9. Kuhn, D.N, Figueira, A., Lopes, U., Motamayor, J.C., Meerow, A.W., Cariaga, K. 2010. Evaluating *Theobroma grandiflorum* for comparative genomic studies with *Theobroma cacao*. Tree Gen. Genomes. 6:783–92.
10. Kuhn, D. N., Livingstone, D., Main, D., Zheng, P., Saski, C., Feltus, F. A., Mockaitis, K., Farmer, A. D., May, G. D., Schnell, R. J., and Motamayor, J. C. 2012. Identification and mapping of conserved ortholog set (COS) II sequences of cacao and their conversion to SNP markers for marker-assisted selection in *Theobroma cacao* and comparative genomics studies. Tree Genet. Genomes. 8: 97-111.
11. Livingstone, D., Royaert, S.,Stack, C., Mockaitis,K.,May, G. Farmer, A.,Christopher Saski, C., Schnell, R., Kuhn, D., Motamayor, J. C. 2015 Making a chocolate chip: development and evaluation of a 6K SNP array for *Theobroma cacao*. DNA Res 22: 279-291. doi: 10.1093/dnares/dsv009.
12. Motamayor, J. C., Lachenaud, P., Wallace da Silva e Mota, J., Loor, R., Kuhn, D. N., Brown, J. S., and Schnell, R. J. 2008. Geographic and genetic population differentiation of the Amazonian chocolate tree (*Theobroma cacao* L). PLoS ONE 3:1-8.
13. Motamayor, J. C., Risterucci, A. M., Heath, M., Lanaud, C. 2003. Cacao domestication II: progenitor germplasm of the Trinitario cacao cultivar. Heredity. 91: 322-330.
14. Motamayor, J. C., Risterucci, A. M., Lopez, P. A., Ortiz, C. F., Moreno, A., and Lanaud, D. 2002. Cacao domestication I: the origin of the cacao cultivated by the Mayas. Heredity. 89: 380-386.

15. Royaert, S., Phillips-Mora, W., Arciniegas-Leal, A. M., Cariaga K., Brown, J. S., Kuhn, D. N., Schnell, R. J., and Motamayor, J. C. 2011. Identification of marker-trait associations for self-compatibility in a segregating mapping population of *Theobroma cacao* L. Tree Genet. Genomes. 7: 1159-1168.
16. Schnell, R. J., Brown, J. S., Kuhn, D. N., Cervantes-Martinez, C., Borrone, J. W., Olano, C. T., Phillips, W., Johnson, E., Monteverde-Penso, E. J., Motamayor, J. C., Amores, F., Lopes, U., Litz, R. E., and Scorza, R. 2007. Current challenges of tropical tree crop improvement: integrating genomics into an applied cacao breeding program. Acta Hort. 738: 129-144.
17. Schnell, R. J., Kuhn, D. N., Brown, J. S., Olano, C. T., Phillips-Mora, W., Amores, F. M., and Motamayor, J. C. 2007. Development of a marker assisted selection program for cacao. Phytopathology. 97: 1664-1669.
18. Schnell, R. J., Olano, C. T., Brown, J. S., Meerow, A. W., Cervantes-Martinez, C., Nagani, C., and Motamayor, J. C. 2005. Retrospective determination of the parental population of superior cacao (*Theobroma cacao* L.) seedlings and association of microsatellite alleles with productivity. J. Am. Soc. Hortic. Sci. 130: 181-190.

Annex 8. List of ACRONYMS

Agricultural Research Service (ARS)
Amplified Fragment Length Polymorphism (AFLP)
Animal and Plant Health Inspection Service (APHIS)
Black Pod Rot (BPR)
Cacao Genome Database (CGD)
Cacao Swollen Shoot Virus (CSSV)
Centro Agronomico Tropical de Investigacion y Ensenanza (CATIE)
Cocoa Research Unit (CRU)
Coffee and Cocoa Research Institute (ICCRI)
Convention on Biological Diversity (*CBD*)
Crop Germplasm Committee (CGC)
Crop Vulnerability Statement (CVS)
Deoxyribonucleic Acid (DNA)
El Nino Southern Oscillation (ENSO)
Food and Agriculture Organization Corporate Statistical Database (FAOSTAT)
Frosty Pod Rot (FPR)
Genetic Modified Organism (GMO's)
Genetic Resources Information Network (GRIN)
Germplasm Resources Information Network (GRIN)
Global Network on Cacao Genetic Resources Conservation and Use (CACAO NET)
Imperial College Selections (ICS)
Instituto Nacional de Investigaciones Agropecuarias (INIAP)
Integrated Pest Management (IPM)
Intermediate Cocoa Quarantine Center at the University of Reading (ICQC, R)
International Cocoa Organisation (ICCO)
Malaysian Cocoa Board (MCB)
Miami (MIA)
National Agricultural Research System (NARS)
National Clonal Germplasm Repository (NCGR)
National Plant Germplasm System (*NPGS*)
Natural Resources Conservation Service (NRCS)
Pacific Basin Agricultural Research Center National Clonal Germplasm Repository (PBARC
Pacific Center for Genetic Resources Preservation (PCGRP)
Papua New Guinea Cocoa and Coconut Institute (CCI)
Plant Introduction number (PI)
Plant Protection and Quarantine (PPQ)
Quantitative Trait Locus (QTL)
Quick Response code (QR2)
Scavina (SCA)
Single Nucleotide Polymorphisms (SNPs)

Single Sequence Repeats (*SSRs*)
Standard Operating Procedures (SOP)
Subtropical Horticultural Research Station (*SHRS*)
Sustainable Perennial Crops Laboratory (SPCL)
The French Agricultural Research Centre for International Development (CIRAD)
The International *Cocoa* Quarantine Centre at the University of Reading (*ICQC*, R)
Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA)
Trinidad Select Hybrids (TSH)
United States Department of Agriculture (USDA)
United States Department of Agriculture - Foreign Agricultural Service (USDA-FAS)
University of Puerto Rico (UPR)
US Dollar (USD)
Vascular Streak Dieback (VSD)
West African Research Institute (WARI)
Witches' Broom (WB)