MORPHOLOGICAL AND GENETIC CHARACTERIZATION OF ELITE COCOA *(Theobroma cacao)* TREES IN THE SOLOMON ISLANDS: A STUDY IN MAKIRA ISLAND IN MAKIRA ULAWA PROVINCE

by
Elison Toramo

A thesis submitted in fulfilment of the
requirements for the degree of
Masters of Agriculture

School of Agriculture and Food Technology
Faculty of Business and Economics
The University of the South Pacific

July 2018

Declaration

Statement by Author

I declare that this thesis is a report of research work I did and has not been submitted in any form for another degree at any other Universities. The reference list acknowledges other authors work used in this thesis.

Signature: Date:

Name: Elison Toramo

Student ID No: S02005648

Statement by Supervisors

The research in this thesis was performed under my supervision and to my knowledge is the sole work of Mr Elison Toramo.

Signature:

Name: Dr. Nandakumar Desai

Designation: Principal Supervisor

Signature:

Name: Mr Faumuina Falaniko Amosa

Designation: Co- Supervisor

Dedication

Glory to Jesus, great things he has done. For in him we live and move and have our being (Acts 17:28)

Acknowledgement

First of all, I wish to thank the almighty Jesus Christ for his faithfulness in giving me the strength, wisdom and knowledge to complete my studies. I also wish to thank my immediate family (My wife Louicy Toramo and children; Erastus Toramo, Godfrey Toramo and Lois Toramo) for their great support and prayers.

I wish to extend my sincere gratitude to the following;

- The principal supervisor Dr. Nandakumar Desai and the Co-supervisor Mr. Falaniko Amosa at the University of the South Pacific, Alafua campus, Samoa, for their guidance and support during the course of the study.
- Associate Professor Siaka Diarra for his continuous encouragements and advises during challenging times while carrying out the study.
- The ACIAR project leader Yan Diczbalis of Department of Agriculture Forest and Fisheries of Queensland for his support from the development of my research proposal and throughout the implementation of the study.
- Natalie Dillon of the Department of Agriculture Forest and Fisheries for providing the silica gel bags used for collecting leaf bud samples and carrying out DNA extraction on the dried leaf buds. In addition to that, I wish to thank her for the output of Principal coordinates for the genotypes.
- The Agriculture Chief Field Officer Mr. Alphonses Punia of Makira Ulawa Province for his assistance in arrangements to carry out the research in his province of responsibility.
- The Agriculture Senior Field officer for East Bauro of Makira Ulawa Province, Mr. Sam Carlos for his assistance during data collection. Your engagement to travel from one cocoa field to another during the entire data collection contributed very much to the completion of data collection in the study.
- The farmers in wards 10, 11, 8 and 7 in Bauro and Arosi for availing their cocoa fields and trees to collect data for the study. And also for their enormous support in moving from field to field during data collection, without their support, the study would not have eventuated smoothly.

- I would also like to extend my sincere thanks to the Australian Centre for International Agricultural Research and the University of the South Pacific (ACIAR/USP) scholarship donors for funding me to undertake studies for the Masters in Agriculture at USP, Alafua Campus, Western Samoa.
- Finally, I wish to thank Taniela Kepa and all my colleagues not mentioned who assisted. Thank you very, very much.

Abstract

Characterization by morphology and genetics in *Theobroma cacao* is essentially important to determine the type of cocoa population grown by farmers in a particular geographical location. In this study, 40 cocoa accessions from forty cocoa fields in wards 10, 11, 8 and 7 in Makira Island in Makira Ulawa Province of the Solomon Islands were characterized by 18 quantitative and qualitative morphological traits and DNA analysis from collected dried young flushing leaf buds. The Ministry of agriculture and Livestock (2013) in the SI stated that the cocoa planting material in the SI is highly variable.

Significant difference between the pod external thickness, pod internal thickness and cotyledon length were determined by the performance of ANOVA at ward level. Principal component analysis (PCA) was performed to identify the traits responsible for variation among the cocoa accessions; the first three principal components explain 46.2 percent of the total variation. A dendrogram with 3 clusters based on unweighted pair group mean with arithmetic average (UPGMA) was created. Grater cotyledon length and cotyledon width were found in cluster 1 while red pods were found in both cluster 1 and 2. A single accession in cluster 3 was found to have the longest pod. The correlation matrix indicated that all negative correlations were not significant. On the other hand significant correlation was realized in some positive correlations.

The frequency distribution of qualitative traits showed 90 percent of the pod surface texture to be smooth and slightly rough, 97 percent of the pod surface shape as slightly furrowed and medium furrowed, 85 percent of Amelonado shape was observed for the pod shape. The 3 main traits in the pod apex shape was mammelate, obtuse and rounded accounted for 73 percent, 63 percent of the accessions had no anthocyanin on the mature pod ridge. In measuring the strength of the accessions to withstand *Phytophthora palmivora*, 72 percent of the accessions were resistant; likewise, 72 percent of the accessions were vigorous.

In this study, the Shannon Weaver Diversity Index (SWDI) showed high level of phenotypic diversity within the population, except for the qualitative traits including reaction to *Phytophthora palmivora,* tree vigour, pod shape and pod surface shape.

The study found phenotypic and genetic markers to correspond that the cocoa population in Makira Island consists mainly of Amelonado type of cocoa. Genetic tests also confirmed that the red podded cocoa trees, which were traditionally identified as a pod colour of Trinitario or Criollo is of Amelonado. Furthermore, the results from genetic tests also found that 42.5 percent of the accessions were Amelonado with genes of Criollo, IMC, Parinari, Nacional and Nanay. Moreover, a single cocoa accession was discovered as totally different from Amelonado, it was found with genes from IMC and Parinari. Therefore, the major findings in this study concluded that the cocoa population in Makira Island consisted mainly of Amelonado type of cocoa with minimum genetic variability. And so, information generated from this study will serve as the base for future breeding programs to improve genetic material of cocoa in the Solomon Islands.

Abbreviations

ANOVA: Analysis of Variance

CATIE: Centro Agronómico Tropical de Investigación y Enseñanza

DNA: Dioxyrhybonucleicacid

ICCO: International Cocoa Organization

ICS1: Imperial college selection 1

IMC: Iquitos Mixed Calabacillo

NA32: Nanay 32

NA33: Nanay 33

PHAMA: Pacific Horticultural and Agricultural Marketing Access

PNG: Papua New Guinea

PCA: Principal Component Analysis

PCa: Principal Coordinate analysis

SCA 6: Scavina 6

SCA 12: Scavina 12

SI: Solomon Islands

SWDI: Shannon weaver diversity index

WCF: World Cocoa Foundation

Table of Contents

List of Tables

List of Figures

Chapter 1: General Introduction

1.1 The Origin of Cocoa (*Theobroma cacao* L)

Theobroma cacao L. is a diploid fruit tree species (2n = 20) (Aikpokpodion, 2012) originated from South America to the east of Andes (International Cocoa Organization, 2013). Initially, *Theobroma cacao* L. was identified as the Botanical family of Sterculiaceae, but later re-classified as the Malvaceae family (Aikpokpodion, 2012). Although the knowledge of this origin is widely published, the actual birth place of *T. cacao* L. is uncertain (International Cocoa Organization, 2013). Scientific claims for the home of cocoa include several areas in Central and South America. These areas include: the upper Amazon region, the upper Orinoco region of northeast Colombia and northwest Venezuela, the Andean foothills of northwest Colombia and Central America, from southern Mexico to Guatemala (International Cocoa Organization, 2013). Cocoa grow in hot rainy and tropical climate and remains in lush vegetation which provides shade. Although Cacao is a native tree species to the regions of Central and South America, it is grown largely in West and Central Africa (Aikpokpodion, 2012). In 2016/17, Africa produced 75.8 percent, Latin America 16.1 percent and Asia 8.1 percent of production (International cocoa organization, 2017).

1.2 Economic Importance of cocoa globally

Cocoa beans provide the main raw material in the manufacture of chocolate, confectioneries and some cosmetic products (Aikpokpodion, 2012). It generates export revenues, income for the growers, and provides employment for the countries which are producers.

Gayi and Tsowou (2012) wrote that chocolate manufacturing contributed significantly to global economy. Historically, the chocolate confectionary industry surpassed $100 billion in value for the first time in 2011. Côte d'Ivoire as the world's top- producing country recorded exports valued at more than $1.2 billion; Indonesia recorded $840.00

million as the second highest producer, and $650.00 million from Ghana the third highest producer.

Further to that, Gayi and Tsowou (2012) reported, in Europe and the United States that the cocoa industry has been serving as economical engines. The Netherlands is the leading importer which imports approximately $2 billion worth of cocoa beans annually, the United States imports an estimated $1.2 billion worth of cocoa beans, having approximately 650 companies which employ nearly 70,000 Americans. In Europe more than 2,000 companies are participating in chocolate manufacturing and confectionary products industry. In such a scenario, cocoa beans travel a long way from the grower to the manufacturer.

All the stake holders involving in cocoa benefited economically since cocoa production process is complex including farmers, buyers, shipping organizations, processors, chocolatiers and distributors.

Houston and Wyer (2012), Gayi and Tsowou, (2012), and the World Cocoa foundation (2018) reported that cocoa provides livelihood for about 40 to 50 million people around the world. The authors further stated that unlike other industrialized agribusiness, the vast majority of cocoa is produced by family run small farms that have limited access to resources and organized market. Small holder farmers accounts for 80 to 90 percent of global production and the number of cocoa growers range between 5 to 6 million (Samuel and Tsowou, 2016 and World Cocoa Foundation, 2018) out of which 90 percent of global production come from growers in areas less than 5 hectares (F.A.O, 2001).

1.3 Cocoa growing regions in the world

Cocoa growing regions in the world are grouped based on global production of dry cocoa beans. The main cocoa producing countries in Africa are; Coted’Ivoire (43 percent of global production), Ghana (14 percent), Nigeria (6 percent) and Cameroon (5 percent) followed by Togo, Gabon, Sao Tome, Equatorial Guniea, Sierra Leone, Congo and Liberia (Aikpokpodion 2012). In Asia, cocoa is grown in Indonesia and Malaysia and in the Americas it is grown largely in Brazil, Ecuador, and Columbia (World cocoa foundation, 2014).

1.4 Overview of cocoa growing in the Pacific

In the Pacific region, cocoa is grown in Samoa, Fiji, Vanuatu, Papua New Guinea and the Solomon Islands. It is an important cash crop (Pacific Horticultural and Agricultural Market Access Program, 2016) in those countries and a significant source of livelihood income for thousands of families. Certified organic cocoa in the Pacific Island countries is produced in Samoa, Vanuatu and Papua New Guinea. Currently, the market for cocoa has a trend that it is under supplied (Bell, 2009). It was reported that Vanuatu and Samoa have the oldest cocoa industries in the South Pacific (McGregor, Watos and Tora, 2009).

1.4.1 Cocoa growing in Samoa

Initially, cocoa was an important crop in Samoa which contributed to the economy of Samoa from export of dry cocoa beans. In 1962, cocoa production went to a peak of 5,000 tons of dry cocoa beans, but exports experienced a progressive long term decline of the industry after the impacts of cyclones where there were no replanting's, because of that the industry was brought to its knees (Pacific Horticultural and Agricultural Market Access Program, 2016). Other factors which reduced production (Malua, n.d) attributed to the decline in world market price, segmentation of the government estates and private, inefficiencies in the industry and natural disasters. Cocoa production is now recovering and it is mainly used for local market with the popular product known as koko Samoa. Samoa produce fine flavored, high quality cocoa (Malua, n.d and Pacific Horticultural and Agricultural Market Access Program, 2016). The Trinitario and Criollo types of cocoa grown in Samoa attracted chocolate manufacturers.

1.4.2 Cocoa growing in Vanuatu

The cocoa industry in Vanuatu was established in the late 19th century, where it was planted as a plantation crop on Santo Island. There was rapid growth in the commodity in the 20th century while coffee was facing disease problems, high labour cost and low prices (McGregor, Watas and Tora, 2009). These authors reported that 2,700 hectares were planted by the year 1921 with exports exceeded 1,000 tones.

Cocoa plantings increased to 4,500 hectares in 1930's and in 1935 production reached 2,700 tons from some 60 commercial plantations in operation. This production records was the highest achievement in the history of cocoa industry in Vanuatu. Production continued to decline over the next 30 years. In 2007, the agriculture census recorded 8,484 households engaging in growing cocoa with a total area of 22,153 hectares earning a total of 58.9 million vatu. This gave an average of 12.225 vatu per household (Pacific Agribusiness Research & Development Initiative, 2012).

In 2008, the export of cocoa from Vanuatu was recorded at 1,058 tones (McGregor, Watas and Tora, 2009). Initiation of cocoa as a small holder crop came about in 1950's and in 1960 small holder production surpassed that of plantation. In the years 2009-2011 Vanuatu exported an average of 1360 tons of cocoa (Pacific Agribusiness Research & Development Initiative, 2012).

1.4.3 Cocoa growing in Fiji

Cocoa was first introduced in Fiji in 1880 by the British. The varieties which were initially brought in to Fiji were Trinitario type from Trinidad to Ceylon (Sri Lanka) via Singapore to Fiji. Currently, cocoa production in Fiji is very insignificant compared to its Pacific Island neighbors (Dillon *et al*., 2014). Efforts were made to establish cocoa as a smallholder crop in the rural areas in Fiji in 1960 partly to provide a source of income to reduce urban drift. In 1987 cocoa production was recorded at 468 tons of dry beans and production reduced and continued in small quantities in the years following.

Although an estimation of 5,000 hectares of abundant cocoa may have survived, markets were lost because of lack of quality, labeling and inconsistent supply. In addition to that, the same author reported that a mini bean-to-bar facility is set up in Nadi. Establishment of the facility brings prospects for future growth of a boutique chocolate industry to supply the demands of the tourist industry. However, the supply of quality beans is unreliable.

1.4.4 Cocoa growing in Papua New Guinea

The history of cocoa in Papua New Guinea (PNG) could be traced back in 1900's when the labourers from New Guinea were taken to work in the plantations in Samoa by the Germans. During this period, mobility between New Guinea and Samoa was routine since the communication and transport systems between the two countries were well established. Hence, labourers were transported back and forth between Samoa and New Guinea. It is likely that cocoa planting materials were transported during these trips (Cocoa board of Papua New Guinea, n.d). In 1932 a subsequent importation of cocoa materials into PNG came from Java. Those planting materials were seed of the upper Amazonian material including Na32, Pa 35 crosses and a wide range of crosses within the Nanay, Parinari, Scavina and IAC groups. These forms the bases of the parents of the present hybrids in PNG. Other initial planting materials include open pollinated seed from ICS clones from Trinidad, Amelonado seed of West Africa from Malaya. In 1980s, ICS clones and Amelonado materials from Malaysia came into the South Pacific via Solomon Islands. The Cocoa Board of Papua New Guinea (2017) published in the special report that cocoa is now among the three top export crops of PNG earning an average annual revenue of K300 million from 36,000 tons of processed cocoa beans.

1.4.5 Cocoa Growing in the Solomon Islands

1.4.5.1 Importance of cocoa in Solomon Islands Economy

The bulk of the population of Solomon Islands depends on agriculture, fishing, and forestry for at least part of its livelihood. For most of the manufactured goods and petroleum products the country depends on imports. In 2016 the Gross Domestic Product (GDP) of Solomon Islands stood at US$1.198 billion (Central Intelligence Agency, 2017).

Coconuts, palm kernels, cocoa, rice, fruit, cattle, fish and timber are the major agricultural activities. Cocoa is mostly grown by small holder farmers in the provinces except Renbel province, therefore, it is observed to be the second most important cash crop in the Solomon Islands (Hivu, 2013). The same author wrote that initial export of first cocoa beans from the Solomon Islands was made in 1960 in very small quantities. Performance of this lifeline crop under the circumstances greatly influences the economy of the Solomon Island through the export of dry cocoa beans. In the year 2015, Cocoa alone contributed $90.80 million Solomon Islands dollars to the economy of the Solomon Islands (Statistical Bulletin, 2015). The crop provides employment for men and women who carry out different activities at growing and post-harvest processing stages of the crop. During the first cocoa festival in the Solomon Islands held in 2016, both men and women participated. A female participant got the first award for producing the best quality cocoa beans (Ripin, 2016). In the country, more than 24,000 small holders are engaged in cocoa farming. In terms of human resource engaged in cocoa, production and processing the total labor amounts to 133,000 family members and these amounts to 26 per cent of the total population of the Solomon Islands. Thus, cocoa in the Solomon Islands assumes the status of a life-line crop since, income generated from cocoa production goes back in to rural communities (Vadnjal and Pelomo, 2014).

1.4.5.2 Initial entry of cocoa planting material into the Solomon Islands

The introduction of cocoa into Papua New Guinea paves the way for first cocoa planting materials to be imported into the Solomon Islands. Initial importation of cocoa planting materials into Solomon Islands was reported in 1951. Those importations include Trinitario seeds which were taken from Kerevat in PNG. Those seeds were distributed directly to the farmers. During this same period a further selection of Trinitario seeds known as the Auki selection (AS 1-38) was imported for observations from Kerevat. In 1960 a seed garden was established at Aimela with the imported Amelonado seeds from Sabah, was then North Borneo (Friend, 1970). The author further reported that bud wood of Trintario clones ICS1 and ICS6 were also imported in which ICS1 survived. ICS1 is self –compatible (Johnson, Bekele and Schnell, 2004) hence, it may have its strains existing in the farmer's fields because Self-Compatible (SC) cocoa clones were found to have significance to obtain and maintain their genetic purity (Jean-Claude, *et al*., 2017). And also, bud wood of the upper Amazon Forasteros; SCA6 and SCA12 were imported where SCA6 survived. Cuttings of the upper Amazon clone Na32 were also imported from Fiji. Moreover, in 1960 another set of Amelonado importation was made from Fiji and Kerevat in PNG (Hivu, 2013). A statement in the publication on proposals on processing and marketing of cocoa in the British Solomon Islands (1961) mentioned that almost all the cocoa seeds planted in the Protectorate were hybrids of Criollo type.

Prio to 1950's a Trinitario type known as Santa Cruz with seeds obtained from two cocoa trees at Santa Cruz were selected and planted in Aimela and Auki for observations. Over the period, observations made on this Santa Cruz Trinitario type of cocoa showed that, Santa Cruz Trinitario type was far out yielding as compared to Amelonado. Due to the many constraints, the evaluation of different types of cocoa did not move forward. Among the constraints, the cocoa plots were very small (0.31 and 0.04 acres) and the bean size of Santa Cruz Trinitario was extremely small (Friend, 1970). By year 1964 the cocoa population of Solomon Islands consisted of the planting materials listed in Table 1.

Table 1 Summary of Solomon Islands cocoa population in 1964

Year	Type of cocoa
1964	1. Farmers Trinitario ex Kerevat 1951-1958 (unrecorded) 2. Government cocoa- Trinitario ex Kerevat (recorded) 3. Cocoa introduced prior to 1951 (origin unknown, in most cases and unrecorded, widely scattered). 4. Clones of ICS1 , SCA6, NA32 5. F1 , open pollinated progeny of Na32 6. F1, open pollinated progeny of Santa Cruz 7. Amelonado from N. Borneo (Sabah) (recorded)
1970	The first hybrid seed (Trinitario Amazon Forastero was produced)

Friend (1970)

After these importations of cocoa planting material, Amelonado was identified as the variety suitable for the Solomon Islands climatic conditions (Hivu, 2013).

1.4.5.3 Entry of cocoa into Makira Ulawa Province

The history of cocoa in Makira Ulawa province is quite recent compared to Malaita and Guadalcanal provinces. Cocoa was introduced in Makira through the establishment of 1.2 hectares of cocoa at Kaunasugu agriculture training center with seeds from Tenavatu farm also known as Black post (Kaihou 2017, personal communication, 28 July). The 1.2 hectares of cocoa at Kaunasugu was established for distributing planting seeds to farmers who were interested to grow cocoa. Cocoa seeds for planting were Amelonado types harvested from Amelonado trees planted at black post by the Ministry of Agriculture and Livestock formally known as the Ministry of Agriculture and Lands. Under the supervision of the Ministry of Agriculture and Livestock, with financial assistance from the Development bank of Solomon Islands (DBSI), cocoa planting materials in seed form were distributed to the cocoa farmers. Beside this mode of seed distribution, farmers also obtained seeds directly from Black post as well as from other farmers within Makira Ulawa province and other cocoa growing provinces.

1.5 Problem statement

Although cocoa grown by the farmers over the last decade is categorized as Amelonado, the Ministry of Agriculture and Livestock (2013) and Konam (2015) reported that the cocoa genetic material used throughout Solomon Islands is highly variable. Therefore, seedlings were likely to be produced from open pollinated sources (Ministry of Agriculture and Livestock, 2013).

Due to the variability in the planting materials, the cocoa industry is classified to be of bulk type and therefore does not have an influence in fetching special prices. To narrow down the variability in the cocoa beans quality, agriculture extension workers are educating the cocoa growers to establish the crop by grafting trees (Konam, 2015). Part of this diverse genetic variability could be mainly attributed to the initial entry of cocoa planting material of different types from different sources into the Solomon Island. Therefore, based on the existing variability, there is a need to analyze this situation systematically to align the cocoa genetic resources, production and postharvest systems of Solomon Islands to the market opportunities (Pacific Agribusiness Research & Development Initiative, 2012.).

1.6 Objectives

The main aim of this research was to study the morphological characteristics and determine the genotypes of the elite cocoa trees (*Theobroma cacao* L.) in the cocoa fields in Makira Island. It is assumed that, the identified elite cocoa trees could be used to produce large scale cocoa planting material to be distributed to farmers to establish cocoa fields with improved planting materials. Hence, Solomon Islands will produce high quality cocoa beans which in turn fetch better prices in the market. The specific objectives of the study are to:

(i) Identify the morphological characteristics of Elite cocoa trees in Makira Island in Makira Ulawa province of the Solomon Islands (SI).

(ii) Assess the phenotypic diversity of Elite cocoa trees in Makira Island in Makira Ulawa province in the Solomon Islands

(iii) Determine the genotypes of the Elite cocoa trees in Makira Island in Makira Ulawa Province in the Solomon Islands

1.8 Organization of the thesis

The thesis comprises of six chapters, beginning with chapter one as the general introduction which covers the origin of Cocoa; economic importance of cocoa globally; cocoa growing regions in the world; overview of cocoa growing in the Pacific (Samoa; Vanuatu; Fiji; PNG); cocoa growing in the SI; initial entry of cocoa planting materials into the SI; importance of cocoa to Solomon Islands economy; entry of cocoa into Makira Ulawa province; objectives; significance of the study and organization of the thesis. Chapter two includes the review of literature under the subtitles including the botany of cocoa; cocoa varieties; characterization of morphological traits; pod shape and bean cotyledon; DNA tests; concept of genetic diversity; genetic markers; molecular makers; DNA test on Pacific cocoa. In chapter three is the description of the materials and method employed to carry out the research. The chapter begins with the location of the study area with a map; reasons for selecting the site; ethical considerations; method of selecting elite cocoa trees; plant materials; data collection; DNA extraction; data analysis and DNA analysis. The forth chapter delivers the results on the outcome after the implementation of field research activities outlined in chapter three. Discussions on the results in chapter four are presented in chapter five. In chapter six, conclusions are made with some recommendations based on the findings in this study.

Chapter 2: Review of Literature

2.1 Botany of cocoa

Cocoa (*Theobroma cacao* L) is an understory tree which originally designated a member of the Sterculiaceae family. *Theobroma cacao* was recently re-classified into the Malvaceae plant family (Aikpokpodian, 2012). Propagation of cocoa is common by seeds as well as cuttings or clones. Young shoots of cocoa has colors range from lightest green to red shades. Immature cocoa pods exhibit green and red colors. The green color turns yellow when it is mature and red may turn orange or reddish orange when mature. A cocoa pod contains 20 to 70 seeds also known as cocoa beans embedded in white pulp (Callebaut, 2016). Cocoa seeds are raised in the nursery and seedlings are usually transplanted at three months. Seedling plants grow up to 1 to 1.5 meters to form the jorquette. Well managed cocoa trees are usually pruned to a height of 3.5 to 4 meters (Solomon Islands cocoa book, 2010) while trees growing under poor pruning management may grow up to 12.5 meters (Obando, 2009). In the Amazon forest, cocoa trees grow up to 20 meters (Department of Agriculture and Fisheries, 2015). The root system of cocoa has a tap root which grows down to 2 meters long and the feeder lateral roots which occupy a depth of 20 centimeters from the ground surface. The same author wrote that roots spread out at 5 to 6 meters forming a dense mat.

2.2 Cocoa varieties

Traditionally, cocoa varieties were categorized into three broad types viz, Criollo, Forastero and Trinitario throughout the world (Callebaut, 2016). They are classified based on fruit (pod) and seed (bean) traits. Criollo is originated from Central, South America and the Caribbean Islands. It makes up 5 percent of the world's production because it is difficult to grow and is vulnerable to environmental threats. The color of the beans has a white to pale pink and tastes very delicate. It is considered as the prince of cocoas because it is the finest of chocolates. Criollo trees are said to be low in yield and highly susceptible to *Phytophthora palmivora* (Aikpokpodian, 2012). Now days, Criollo can only be found in Central America, Venezuela, Madagascar, Sri Lanka and Samoa (Aprotosoaie, Luca and Miron, 2016). It's morphology is highly diverse (Charrier *et a.*, 2001) with elongated pod like cundeamor type with warty pod surface, acuminated points, smooth or rough surface and the unripe pods are red or green with a low vigor type of tree (Afoakwa, 2014).

Forastero is native to the Amazon basin which constitutes 80 percent of the world's cocoa. Forastero is a popular cocoa because it is hardier and less susceptible to diseases. It has small, flat and purple colored beans where it gives chocolate its full bodied flavor. Amelonado is a subspecies of Forastero which is grown extensively while Trinitario is a natural hybrid class of cocoa originally from Trinidad. As reported by Zhang and Motilal (2016), Trinitario is a product of hybridization and recombination. Cocoa populations cultivated from products of recombination usually have greater genetic diversity and may result in larger numbers of individual genotypes than the parental population (Bartley, 2005; Aikpokpodion, 2010). Trinitario consists of the best characteristics from Criollo and Forastero including the hardiness and exhibits high yield character of Forastero and the refined taste of Criollo. The quality varies from average to superior with predominantly fine flavored cocoa. Initially, Trinitario had 100 clones known as the Imperial College Selections (ICS). In 2001, only 57 ICS clones survived at San Juan Estate in Trinidad (Johnson, Bekele and Schnell, 2004). Although Trinitario is said to be of Trinidad origin, Mortilal and Sreenivasan (2012) stated that Trinitario cacao can also be a natural hybrid between Forastero and Criollo that arose in other different countries. Nevertheless, these two authors reiterated that the Trinitario from Trinidad is mostly preferred for its flavor.

Beside the three common cocoa varieties, a primitive cultivated population of *Theobroma cacao* known as Nacional variety is found as native in Ecuador (Loor *et al*., 2009). In addition, (Aprotosoaie *et al*., 2016) supported that statement and added that Nacional type of variety is known for its fine flavor. It has large pale purple beans producing Arriba flavor with aromatic, floral spicy and green notes.

Studies by Motamayar *et al.* (2008) suggested following their genotyping of the South American cocoa populations that, there are 10 genetic groups of cocoa. Those genotypes include Marañon, Curaray, Criollo, Iquitos, Nanay, Contamana, Amelonado, Purús, Nacional and Guiana. Cocoa and the resultant chocolate flavor are linked to tree genetics.

In Bahia, (Santos *et al*., 2015) spontaneous mutation occurred in their cocoa population generating varieties such as Almeda and catongo. Marahao and Catongo are spontaneous mutants of Comum cacao producing white beans. The parent tree of Catongo was found in an Amelonado population and therefore it is described as Amelonado white bean (Wood and Lass, 1985). Those two varieties are currently sold to fine cocoa market reported to have less astringent and more flavorful chocolate (Santos *et al*., 2015).

2.3 Characterization of morphological traits

In describing the physical properties of Mexican Criollo cocoa during post-harvest processing, Garcia- Alamilla *et al.* (2012) discussed that the features of pods and beans were used to identify the types of Criollo cocoa grown in Mexico. These features consist of physical characters and textural characters. Physical characters include pod length, pod diameter, pod weight (wet weight), internal and external thickness, and number of beans per pod and bean weight. Numerical figures were used to measure these features. In textural characterization, the features were described including color, roughness, shape, base, apex, and ridges. The study mentioned that cacao pod and bean characteristics are indicators of quality, productivity and production. Aikpokpodion (2010) reported that the morphological traits of cocoa mentioned above are very important to measure variability in the genotypes that exists. Correct characterization of morphological traits is important to align the traits with the genotype. Knowledge of the variability in morphological diversity portrays a phenotypic variation that is in the field and it is useful for conservation and utilization of useful variation. A study by Aikpokpodian (2010) showed that classification in the International cocoa gene bank in Trinidad (ICG,T) based on morphological variation was congruent with the traditional classification of cocoa types. Morpho-Agronomic characteristics of pods, seeds and flowers were used to evaluate relationships among cocoa genotypes. Morphological descriptors are useful (Ballesteros, Logos and Ferny, 2015) for selecting the best accessions for breeding programs. The author published that other studies on morphological diversity involving flowers, fruits and leaves of accessions from cocoa germplasm revealed the two morphological groups; Criollo/Trinitario and Forastero with variation between the groups due to the genetic mixtures among the genotypes.

2.3.1 Pod shape and Bean Cotyledon

Ha *et al.* (2016) stated that there are four types of shapes in cocoa pods which are distinguishable in the world; they are Amelonado, Calabacillo, Angoleta and Cundeamor. Fruits with ovoid shape, smooth skin, and dark purple cotyledon and without a prominent point are said to be Amelonado. Cundeamor are used for elongated cylindrical fruits with a pronounced bottleneck, dark purple cotyledon and a sharp point. Round fruits with no point are described as Calabacillo and Angoleta are specified as long fruits without a bottle neck and pointed ends. Pod shape of Amelonado and Calabacillo are attributed to fruits of Forastero while Angoleta and Cundeamor are attributed to Criollo types of pods. Generally, the phenotypic traits of cocoa pods plays an important (Ballesteros, Logos and Ferny, 2015) role in defining the types. High variation could be seen on the pods and beans. It is understood that cocoa beans can be used as a prime indicator of a quality in a certain cocoa genotype. As indicated by Wood and Las (1985) white to pale purple cotyledons are features of the finest cocoa variety while bulk varieties have generally dark purple colors.

Callebaut (2016) mentioned that, white to pale pink colored cotyledons are attributed to Criollo types of cocoa. In addition, Afoakwa (2014) and Garcia-Alamilla *et al.* (2012) wrote that white cotyledon is a feature of Criollo type, dark purple to Amelonado and Trinitario with white to deep purple. Furthermore, Manjit and Surinder (2013) wrote that variability of cotyledon color can be white, pink, purple or mottled.

2.4 DNA test

2.4.1 Concept of genetic diversity

Genetic diversity in a species is considered as fundamentally important (Aikpokpodian, 2012) for its continuity to survive since it provides the crop species with the ability to adapt to prevailing biotic and abiotic conditions and enables change in the genetic composition to coop with changes in the environment. Knowledge about the level of genetic diversity in a species is the pre-requisite in studying the survival of a particular species.

2.4.2 Genetic Markers

Genetic markers are inherited variations (Aikpokpodian, 2012) used to understand genetic events in a population of interest. Genetic markers are described as DNA sequence with a known location on a chromosome that can be used to identify individuals or species. Genes or other DNA variations are useful to explain observed genetic events in a population of interest. The majority of genetic markers are variations in DNA at sites that may or may not be part of a functional gene. Transmissions of genetic markers to offspring follow Mendelian rules of inheritance. The properties of genetic markers can be categorized into three main properties; it must be Locus-specific, polymorphic in the population that is under studies and it must be easily genotyped. Aikpokpodian (2012) wrote that the two measures of the quality of a genetic marker include; it's heterozygosity in the population of interest and the polymorphism information content of a molecular marker.

The author further explained that there are three groups of genetic markers used in population genetics; these include phenotypic or morphological markers, biochemical markers or isozymes and molecular markers utilizing variation at Nuclear DNA level. There is an observed variation in phenotypic makers since they are explained by Mandels law of inheritance. Examples of phenotypic markers are; colour variation, growth habit and fruit shape. Phenotypic makers are used to clearly determine population structure (Aikpokpodian, 2010). Efombagn *et al.* (2009) wrote that morphological markers could enable the structuring of the diversity of different populations in germplasm collections in research stations. On the other hand (Aikpokpodian, 2010) wrote that several factors have limit the usefulness of morphological markers inclusive of environmental influences, limited morphological variation among cultivars and restriction of some useful characters such as flowering or fruit ripening and limitation to only one locus.

Allozymes are allelic variants of enzymes encoded by structure genes. Enzymes are proteins consisting of Amino acids. Variations of alleles can be detected by gel-electrophoresis. The invention of molecular markers has revolutionized (Joshi, Ranjekar and Gupta, 1999) the entire scenario of entire biological science.

DNA markers enabled complete sampling of the genome and help to overcome the limitations of morphological markers and Isozyme markers (Aikpokpodian, 2012). The application of DNA markers in identifying cultivars, control of seed purity of hybrids and checking the relationship between cultivars has seen as great success.

2.4.3 Molecular markers

There are three major groups of allelic variations within a genome of the same species. The differences are distinguished according to the number of tandem repeats at a particular locus. These major allelic variations are; single sequence repeats (SSR), segmental insertions/deletions (InDels) and single nucleotide polymorphisms (SNPs) (Mammadov *et al*., 2012). Detection and tracking of these variations are made possible by genetic tools known as molecular markers which have been developed by researchers. Molecular markers are used in molecular biology and biotechnology to identify a particular sequence of DNA in a pool of unknown DNA.

These molecular markers include restriction fragment length polymorphism (RFLP) as low throughput hybridization based, random amplified fragment length of polymorphic

DNA (RAPD) as medium throughput and high throughput as single nucleotide polymorphism (SNPs) maker system.

SNPs were first discovered in humans and said to be the most abundant forms of genetic variation among individuals of the same species. And also SNPs are proved to be universal. Moreover, Mammadov *et al.* (2012) reiterated that SNP's are less polymorphic than SSR markers because they are biallelic. However, they are abundant, ubiquitous, and amenable to high- and ultra-high-throughput automation, hence they can compensate this drawback of being less polymorphic.

2.4.4 DNA test on Pacific Cocoa

The use of SNP's in the Pacific region was done with cocoa accession collections by an ACIAR project Number "PC/2014/032" (Dillon,

, 2014). The project used 96 SNP markers to analyze cocoa accessions collected from Vanuatu, Fiji, Samoa and partial part of Guadalcanal in the SI (Dillon *et al.*, 2014). DNeasy® Plant extraction was used to extract DNA according to the manufacturer's instructions. The concentration and integrity of DNA was assessed by 1 percent (w/v) agarosed/TBE gel electrophoresis. NanoDrop spectrophotometer was used to determine the concentration of DNA, thereafter; extracted DNA was stored at 4^{o}c and later diluted to a final concentration of 10ng/µl before proceeding to SNP analysis (Dillon, *et al.*, 2014). A circular dendrogram was used to illustrate the relationship between the accessions collected. The results indicated that some accessions from Vanuatu were closely related to Criollo clone CATIE reference 12 while some are related to a node branching from CATIE Criollo clones. These accessions are related to Criollo clones than the rest of the accessions. It was evident that accessions from Fiji were not identified correctly so a difficulty will exist when it comes to selecting the correct planting material for farmers. Samoa has a unique result with its accessions. Comparisons made with the accessions from the International Coco Gene bank (ICG), Trinidad and the collection from the tropical Agricultural Research and High education (CATIE), Costa Rica, these accessions grouped into two main clusters with very few of the reference accessions included in these two groupings. Suggestions were made (Dillon, *et al*., 2014) that these field accessions are grafted and grown on a site for preservation for use by the future generation. A scoping study in 2008 (Dillon, *et al*., 2014) indicated that Trinitario type dominate the gene pool in Savaii. Illustrations from the dendrogram on the results of the accessions from the Solomon Islands showed that all the accessions were not closely related to CATIE Criollo reference clones but the majority sat close to Amelonado although informal reports indicated that the elite accessions were taken from Criollo and Trinitario lines.

Chapter 3: Materials and Method

3.1 Location of study

The study was conducted in Makira Island formerly known as Santa Cristobal in Makira Ulawa Province of the Solomon Islands. Makira Island is located at 10.60^{o}10^{o} South and 161.85^{o} East (Island directory, 1998). The average rainfall received in Makiara Island amounts to 3600mm to 4000mm with no dry season. In the southeastern part of the Island, higher rainfall is in the month from May to Occtober while in the western part of Makira Island, it was recorded from Novermber to April. On the other hand, in the mountainous regions of the southeast, the rainfall per annum was recorded at 8000 mm (Allen *et al*., 2006).

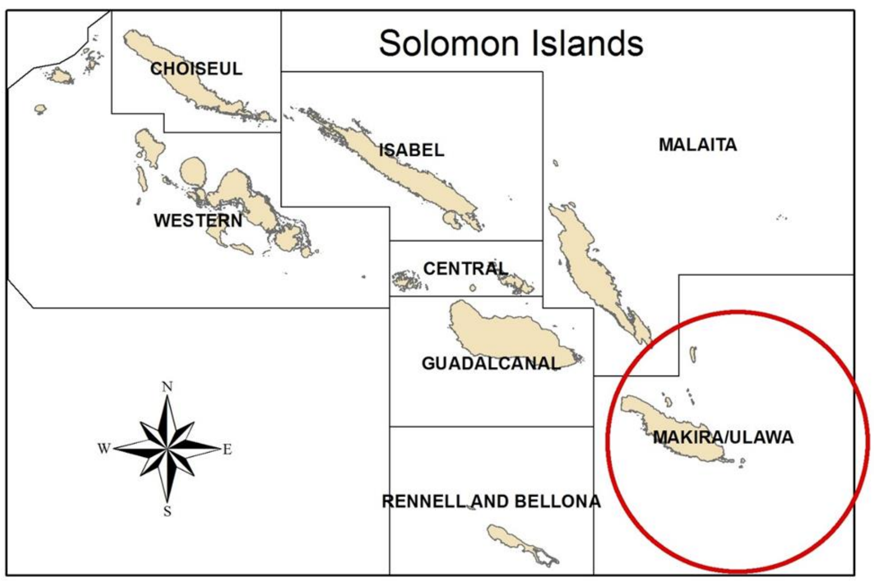


Figure 1 Location of Makiara/Ulawa province in the Solomon Islands in red circle (Source: GIS Unit, Ministry of Agriculture and Livestock, 2018).

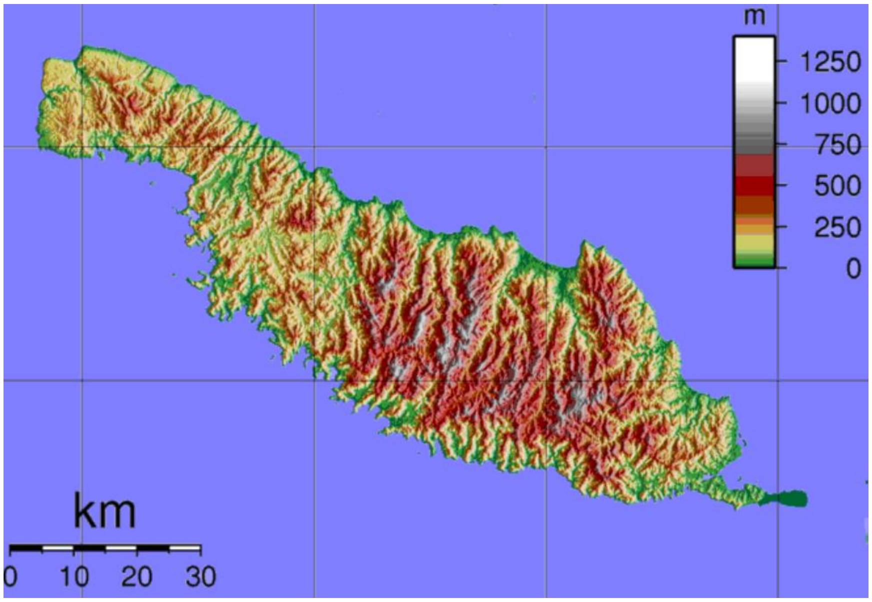


Figure 2 Topographical map of Makira Island (Adopted from: Fasi, 2009)

3.2 Reasons for site selection

Solomon Islands have a total of nine provinces. A province refers to the main Islands excluding Honiara to administer the provincial Government (The Provincial Government Act, 1981). In this study a multi stage sampling procedure was followed to get reliable data, hence, Makira Ulawa was selected at the initial stage. Makira Ulawa province holds the third position in cocoa production in the Solomon Islands. Based on the highest cocoa population criteria, central Makira and West Makira constituencies were selected out of the four constituencies for the study. A constituency covers a group of people who are represented in parliament by someone who is elected by the people who are living in a particular geographical location. Therefore, wards 10 and 11 were identified in central Makira where data collection begun and followed by wards 8 and 7 in West Makira constituency. A ward is established in the province to define a group of people to elect members for the provincial assembly in each province (The Provincial government act, 1981)

Figure 3 Map of Makiara Island showing wards and the geographical locations of the elite cocoa trees in green triangles. (Source: GIS Unit, Ministry of Agriculture and Livestock, 2018).

3.3 Ethical Considerations

Initial discussions on the research were held with the Agriculture Chief Field Officer (ACFO) of Makira Ulawa Province during his visit to Honiara prior to writing the research proposal. Considering the academic merits of the study, a research permit (Appendix 2) and a business license (Appendix 3) were obtained from the Ministry of Education and Human Resources Development and Makira Provincial Assembly respectively to carry out the research in the Province. Prior to data collection in the cocoa fields, a series of discussions were held with the involvement of the Agriculture Extension Officer assigned to assist in the data collection including the participating farmers at each site. Discussions were held at Togori in ward 10, Ngarianagahuto in 11, Asimanioha in ward 8 and Heuru in ward 7. The discussions led to a participatory team work among the farmers where selected elite cocoa trees were identified in the cocoa fields. A cocoa field consists of an area grown with 200 to 2000 cocoa trees.

Figure 4 Participating farmers during the first meeting at a farmer's field east of Togori village in ward 10.

3.4 Method of selecting elite cocoa trees

Identification and selection of elite cocoa trees were done through discussions with the farmers who owned the cocoa fields. These marked elite cocoa trees were favored and identified by the farmers as their high productive trees. The age of the elite cocoa trees ranged between 7 to 25 years which may have phenotypic characters of Amelonado or Trinitario. Identification of elite cocoa trees were done by employing the elite cocoa tree marking protocol adopted and modified from the Department of Agriculture, Fisheries and Forest (DAFF) in Queensland, Australia.

3.5 Plant Materials

In total, the study collected morphological data from forty (40) cocoa accessions from forty elite cocoa trees from 40 cocoa fields where data collection started in wards 10, 11, 8 and 7 according to the geographical location of the wards located near the provincial head quarter, Kirakira. In this order, plant materials and geographical positions of the trees were listed in Appendix 1 while details of the pod morphological characteristics are listed in Appendix 10. The accession number consisted of the first figure representing the order of numbers of the accessions, the letters are initials of the farmers' names who own the elite cocoa trees and the last figure is the number of the elite cocoa tree in the particular cocoa field.

3.6 Data collection

Data on morphological traits were recorded in two categories including 18 quantitative and Qualitative traits. The quantitative traits were recorded with a caliper, horse brand wooden ruler, tape measure and the weight of pods and beans were recorded by an Abron digital electronic balance powered by two double 'A' batteries. Both the quantitative and qualitative traits were photographed by a Panasonic FT6 Lumix digital camera. In addition, young flushing leaves, at least the first three to four leaves, were collected and placed in silica gel plastic bags. The flushing leaves were sent to the Department of Agriculture, Fisheries and Forestry in Queensland, Australia, where extraction of DNA was done. Collection of data on qualitative traits was based on the parameters in appendices 4, 5, 6, 7, 8 and 9. In recording cocoa bean data, a fine cut was made along the pod with a sharp bush knife to expose the cotyledon color. In the samples where further observations of the Cotyledon colors were required, all the beans in a pod were cut symmetrically with a small sharp kitchen knife to enable total color observations. During this procedure, a laminated gray card was used as a background where pods and bean cotyledons were placed for color observations. In addition, the length and width of the cotyledons were also measured with a wooden horse brand ruler. All sampled cocoa trees were tagged with metal sheets for possible follow up for multiplication of the trees. At the end of the procedure, longitude and latitude coordinates were recorded by a garmin etrex® 10. Quantitative and qualitative traits observed for this study are listed in Table 2 and 3.

Table 2 Quantitative morphological traits

Variable	Character
1.	Pod length
2.	Pod weight (wet weight)
3.	Pod circumference
4.	Pod external thickness
5.	Pod internal thickness
6.	Number of beans per pod
7.	Weight of beans per pod
8.	Cotyledon length
9.	Cotyledon width

Adopted from Garcia-Alamilla *et al.* (2012); Aikpokpodion (2010)

Table 3 Qualitative morphological descriptors

Variable	Character	Modality
1.	Pod surface texture	1. Smooth 2. Slightly rough 3. Rough 4. Rough and warty
2.	Pod surface shape	1. Un-furrowed 2. Slightly furrowed 3. Medium furrowed 4. Deep furrows
3.	Pod shape	1. Amelonado 2.Cundeamor 3. Angoleta 4.Calabacillo
4.	Pod apex shape	1. Attenuate 2. Acute 3. Obtuse 4. Rounded 5. Mammelate 6. Indented
5.	Pod neck	0. Absent 1. Slight 2. Intermediate 3. Strong 4. Wide shoulder
6.	Cotyledon color	1. Dark purple 2.Medium purple 3. Light purple 4. Light pink
7.	Mature pod ridge colour	0. Absent 1. Present 2. Red pods
8.	Reaction to black pod	1. Susceptible 2. Moderately susceptible 3. Moderately resistant 4. Resistant
9.	Tree vigor	1. Weak 2.Intermediate 3. Vigorous

Adopted from Ballesteros, Logos and Ferny (2015); Aikpokpodion (2010)

Part of the procedure followed is shown in figures 5, 6, 7, 8, 9 and 10.

Figure 5. Measuring the pod

Figure 6. Weighing of cocoa

circumferenc

pods

Figure 7. Weighing of cocoa beans

Figure 8. Measuring the length of cotyledon

Figure 9. Tagging of elite trees

Figure 10. Collection of cocoa accessions for DNA test.

3.7 DNA Extraction

DNA was extracted from the dried cocoa flushing leaf buds stored in silica gel bags. The procedure used for DNA extraction was based on the manufacturer's instructions DNeasy® plant extraction kits (Qiagen GmbH, Germany). In addition to that, assessment was done on the DNA concentration and integrity by 1 percent (w/v) agarose/TBE gel electrophoresis. The level of concentration of DNA was then determined by NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA).

Following that, extracted DNA was stored at 4^oC and diluted to a final concentration of 10ng/µl prio to SNP analysis.

3.8 Data analysis

Descriptive statistics for all traits were obtained and all the nine quantitative variables analyzed showed normal distribution. The following analyses were performed with the Statistical tool for agriculture research (STAR) version 2.0.1 (2014). ANOVA was performed at the probability level of 0.05 to determine the significant difference between the quantitative traits at ward level. All the 18 traits went through Principal Component Analysis (PCA) to show the traits with high variability, responsible for variation between the accessions. Eigen values ≥ 0.95 were selected to define variations among the morphological traits. Pearson correlation was carried out to be able to identify the significance of correlations among the traits at the probability level of 0.01. All the data were standardized with STAR while cluster analysis was performed based on unweighted pair group method with the arithmetic mean (UPGMA) to group the accessions with similarities together. Clustering was performed with Euclidean distance method based on the average clustering method where three clusters were performed at the cophenetic correlation of 0.724. In tabulating the frequencies of the qualitative traits, excel was used with the countif function. Further to the frequency Table 7, phenotypic diversity of qualitative traits was analyzed with Shannon-Weaver Diversity Index (SWDI) with the formula;

$$H = \sum_{i=1}^{s} - (P_i * \ln P_i)$$

Where, H = the Shannon diversity index, Pi = Fraction of the entire population made up of traits i (proportion of a trait i relative to total number of traits present, not encountered) and S = Numbers of traits encountered (Beals, Gross and Harrell, 2000).

Allelic polymorphism was analyzed by Principal coordinate analysis to determine the relationship of the accessions with the reference clones from the CATIE clones.

3.9 DNA Analysis

DNA Analysis of the cocoa accessions was done by using ninety six-makers. The markers were selected from 1560 candidate; SNPs developed from cDNA sequences form a wide range of cocoa organs (Dillon *et al*., 2014). The author continued to mention that based on the level of polymorphism and their distribution across the 10 chromosomes in cocoa, selection was done. None of the 96 markers used in his study were linked to traits. Genotyping with the use of SNP was performed at the United States Department of Agriculture (USDA) Agricultural Research Centre (ARS), Maryland, USA, using a Fluidigm EP1 system (San Francisco, CA, USA). Analysis to get the Principal coordinates of the genotypes was performed at the Queensland Department of Agriculture, Fisheries and Forest (Dillon *et al*., 2014).

Chapter 4: Results

4.1 Variation of morphological traits.

At ward level, the analysis of variance (Table 4) showed significant difference ($P<0.05$) in the pod external thickness, pod internal thickness and cotyledon length. Pod external thickness was greater in ward 7 than wards 8 and 11, while there was no significant difference between ward 7 and 10; Ward 7 also has greater pod internal thickness than wards 8, 10 and 11. The cotyledon length was greater in wards 7, 8 and 11 than ward 10 (Table 4).No significant difference ($P>0.05$) was found in the other quantitative traits including pod length, pod weight, pod circumference, number of beans per pod, weight of beans per and the cotyledon width among the four wards (Table 4).

Table 4 Analysis of quantitative traits of forty cocoa accessions from farmer's fields in 4 wards in Makira Island

Characters	**Ward 7**	**Ward 8**	**Ward 10**	**Ward 11**	CV %	**SEM**	**ANOVA Pr>F-value**
	Mean	Mean	Mean	Mean			
Pod length (cm)	15.5	16.9	14.6	15.8	12.8	0.9	ns
Pod weight (g)	454.3	519	460.3	505.5	21.9	47.4	ns
Pod circumference (cm)	26.5	28.1	26.7	27.9	6.5	0.8	ns
Pod external thickness (mm)	12.9a	9.8b	10.9ab	10.5b	19.5	1	*
Pod internal thickness (mm)	9.3a	5.6c	7.3b	6.7bc	21.6	1	*
Number of beans per pod	42.9	40.3	41.6	39.4	9.4	1.7	ns
Weight of beans per pod (g)	111.4	113.3	116.6	127.2	14.4	7.5	ns
Cotyledon length (cm)	2.41a	2.48a	2.16b	2.42a	9.5	0.1	*
Cotyledon width (cm)	1.2	1.3	1.1	1.3	12.9	0.1	ns

SEM: Standard error of mean; *p*- level:* =*p*<0.05*;* ns= not significant respectively.

4.2 Principal component analysis

The principal component analysis (PCA) of the 18 morphological traits showed the first 3 Principal components accounted for 46.2 percent of the total variation among cocoa accessions (Table 5). Principal component (PC) axes 1 consisted mainly of quantitative traits including pod weight, pod length, pod circumference, cotyledon width and weight of beans per pod accounting for 23.1 percent of the total variation. Qualitative traits contributed to the variations in PC 2 and PC3 accounting for 12.2 percent and 10.9 percent respectively. PC axes 2 consisted of traits including the cotyledon color, pod ridge color, pod surface texture and pod surface shape and PC 3 comprised of the pod neck, pod surface shape, pod surface texture and pod ridge color. In the fourth PC axes, pod internal thickness, pod neck and cotyledon length made up to 8.1 percent of the total variation. The fifth PC axes accounted for 7.4 percent of the total variation where pod shape, tree vigor, reaction to black pod and the number of beans per pod were the main contributors. Principal component axes number 6 was found to have 6.4 percent of the total variation which composed of pod apex shape, pod external thickness and pod length (Table 5). The 7^{th} PC axes consisted of pod shape, tree vigor, and cotyledon width and pod internal thickness contributing 6.3 percent of the total variation. In the 8^{th} PC axes, reaction to black pod, pod external thickness, pod apex shape and mature pod ridge colour (anthocyanin) accounted for 5.3 percent of the total variation. Ultimately, the entire 8 PC's accounted for 79.7 percent of the total variation among the accessions in Makira province (Appendix 12).

Table 5 Principal component analysis of eighteen traits of forty cocoa accessions in Makira Island

	PC1	PC2	PC3
Eigen Values	4.16	2.20	1.97
Total Variation (%)	23.1	12.2	10.9
Cumulative (%)	23.1	35.3	46.2

Character	PC1	PC2	PC3
Pod length (cm)	0.3510	-0.0724	0.2665
Pod circumference (cm)	0.3887	-0.0728	-0.1969
Pod weight (g)	0.4355	-0.1844	0.0370
Pod external thickness (mm)	0.1773	-0.1876	0.0916
Pod internal thickness (mm)	-0.0207	-0.0130	0.1350
Number of beans per pod	-0.1958	-0.2878	0.0739
Weight of beans per pod (g)	0.3102	-0.1817	0.1199
Cotyledon length (cm)	0.3630	-0.1111	-0.0371
Cotyledon width (cm)	0.3452	0.1111	-0.1215
Pod surface texture	0.1322	0.4208	0.4081
Pod surface shape	0.0675	0.3750	0.4209
Pod shape	0.0065	0.0246	-0.2154
Pod apex shape	-0.0938	-0.2031	-0.0497
Pod neck	-0.0411	-0.0574	0.5012
Cotyledon color	-0.0161	0.5086	-0.1938
Mature Pod ridge colour (anthocyanin)	0.1436	0.3797	-0.3291
Reaction to black pod	-0.1892	-0.1173	0.0104
Tree vigor	-0.1717	0.0121	0.1910

4.3 Correlation analysis

The correlation matrix (Table 6) showed significant relationships (**) at the probability level of 0.01 between the pod length and the pod circumference, pod weight and the cotyledon length. Significant relationship also existed between the pod circumference and the pod weight, weight of beans per pod, cotyledon length and the cotyledon width. In addition, there was significant relationship between the pod weight and the weight of beans per pod and the cotyledon length and width. Moreover, significant relationships were also observed between the weight of beans per pod and also between the cotyledon length and the cotyledon width. All other relationships were seen to be nonsignificant (ns) (Table 6).

Table 6 Correlation matrix of quantitative traits of forty cacao accessions in Makira

	Pod length	Pod circumference	Pod weight	Pod external thickness	Pod internal thickness	No of beans per pod	Weight of beans per pod	Cotyledon length	Cotyledon width
Pod length	1.0000								
Pod circumference	0.4384**	1.0000							
Pod weight	0.7406**	0.7721**	1.0000						
Pod external thickness	0.1828ns	0.1813ns	0.3829ns	1.0000					
Pod internal thickness	0.1295ns	-0.0728ns	-0.0295ns	0.0038ns	1.0000				
Number of beans per pod	-0.2347ns	-0.3100ns	-0.2334ns	0.0142ns	-0.0965ns	1.0000			
Weight of beans per pod	0.3987ns	0.4041**	0.6328**	0.2831ns	0.0554 ns	0.1016ns	1.0000		
Cotyledon length	0.4525**	0.6387**	0.5862**	0.1935ns	-0.1818ns	-0.2250ns	0.4045**	1.0000	
Cotyledon width	0.2655ns	0.4749**	0.4429**	0.2276ns	-0.0893ns	-0.3704ns	0.3872ns	0.6711**	1.0000

***p*-level**=*p*<0.01; ns=not significant respectively** .

4.4 Cluster Analysis

Cluster analysis showed 3 clusters (Figure 11). Long and wide cotyledons, Amelonado shaped pods, light purple to light pink cotyledons, red podded trees; rough pods, strong pod neck, obtuse pod apex shape and trees with vigorous growth and intermediate pod neck were found in cluster 1. The first cluster made up 92.5 percent of the total number of accessions. Cluster 2 made up of 5 percent of the total number of accessions with traits including least pod weight, least pod external thickness, and least cotyledon length. Cluster 3 constituted only 2.5 percent of the accessions; this cluster represents one single accession which has a long pod, large pod circumference, and high number of beans per pod, large pod internal and external thickness.

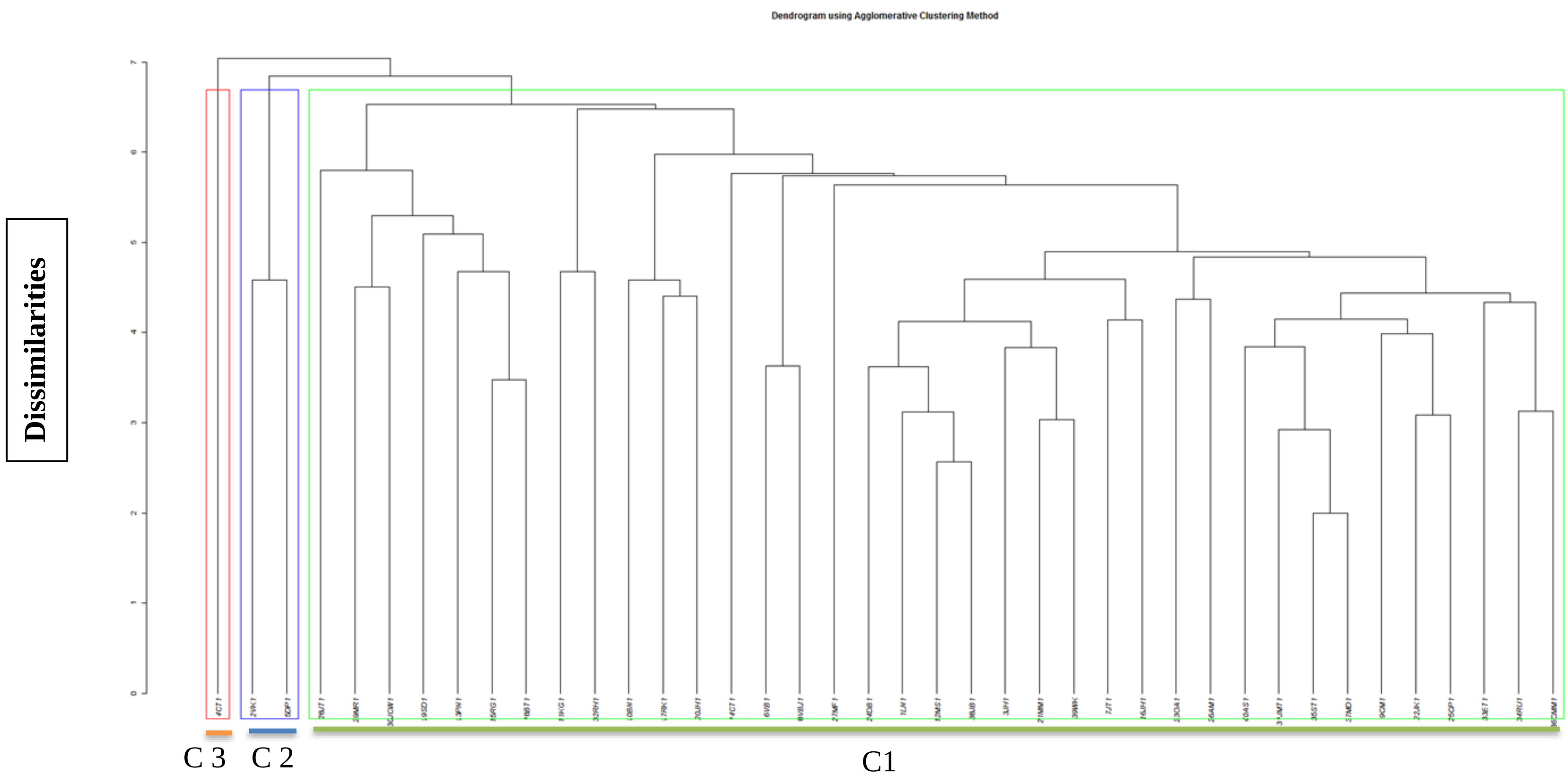


Figure11. Dendrogram showing three clusters of 18 traits of cocoa accessions in Makira

C1-Cluster 1, C2-Cluster 2, C3- Cluster 3

4.5 Frequency distribution of qualitative traits

The pod surface texture had two major contributing traits viz, smooth and slightly rough and for the pod surface shape, slightly furrowed and medium furrows were major traits with deep furrows as minor (Table 7). Indications from the pod shape showed Amelonado shape as the major type, followed by Calabacillo, Cundeamor and Angoleta. The pod apex shape was observed with Mammelate as the major trait, followed by acute, obtuse and rounded. Concerning the pod neck, more pods were found with no neck, followed with slight neck, intermediate neck and strong neck. In this study, dark purple cotyledons were found to be the most frequent, next, with light purple, medium purple and light pink. The absence of anthocyanin on mature ridged pods was observed to be more, followed by the red pods and the mature pods with anthocyanin on their ridges. Accessions which were resistant to Black pod were more than those moderately resistant. Similarly, vigorous trees made up the majority of the accessions (Table 7).

Table 7 Frequency distribution of nine qualitative traits in farmers cocoa fields in Makira

Character	State	Frequency	Percentage
Pod surface texture	1- Smooth	51	46
	2- Slightly rough	48	44
	3- Rough	11	10
Pod surface shape	1- Un furrowed	0	0
	2- Slightly furrowed	79	72
	3- Medium furrowed	28	25
	4- Deep furrows	3	3
Pod shape	1- Amelonado	93	85
	2- Cundeamor	4	4
	3- Angoleta	3	3
	4- Calabacillo	10	9
Pod apex shape	1- Attenuate	2	2
	2- Acute	28	25
	3- Obtuse	22	20
	4- Rounded	10	9
	5- Mammelate	48	44
	6- indented	0	0
Pod Neck	0- Absent	46	42
	1- Slight neck	41	37
	2- Intermediate	13	12
	3- Strong	10	9
Cotyledon color	1- Dark purple	51	46
	2- Medium purple	24	22
	3- Light purple	30	27
	4. Light pink	5	5
Mature pod ridge color (Anthocyanin)	0-Absent	70	63
	1-Present	14	13
	2- Red pods	26	24
Reaction to black pod	1- Susceptible	0	0
	2-Moderately susceptible	0	0
	3-Moderately resistance	11	28
	4-Resistance	29	72
Tree Vigor	1-Weak	0	0
	2-Intermediate	11	28
	3-Vigorous	29	72

4.6 Forty Cocoa Genotypes from Makira Island determined by Single Nucleotide Polymorphism (SNPs) Markers

The cocoa accessions from Makira Island were analyzed with SNP makers and were compared with the reference genotypes from CATIE. In red, the majority of the accessions from Makira, SI were observed to fall in the Amelonado group with purple triangles (Figure 12). Some of the accessions leaned towards Trinitario, while others leaned towards the Parinari group. Additionally, 1 accession fell in the IMC, Parinari and Nanay groups.

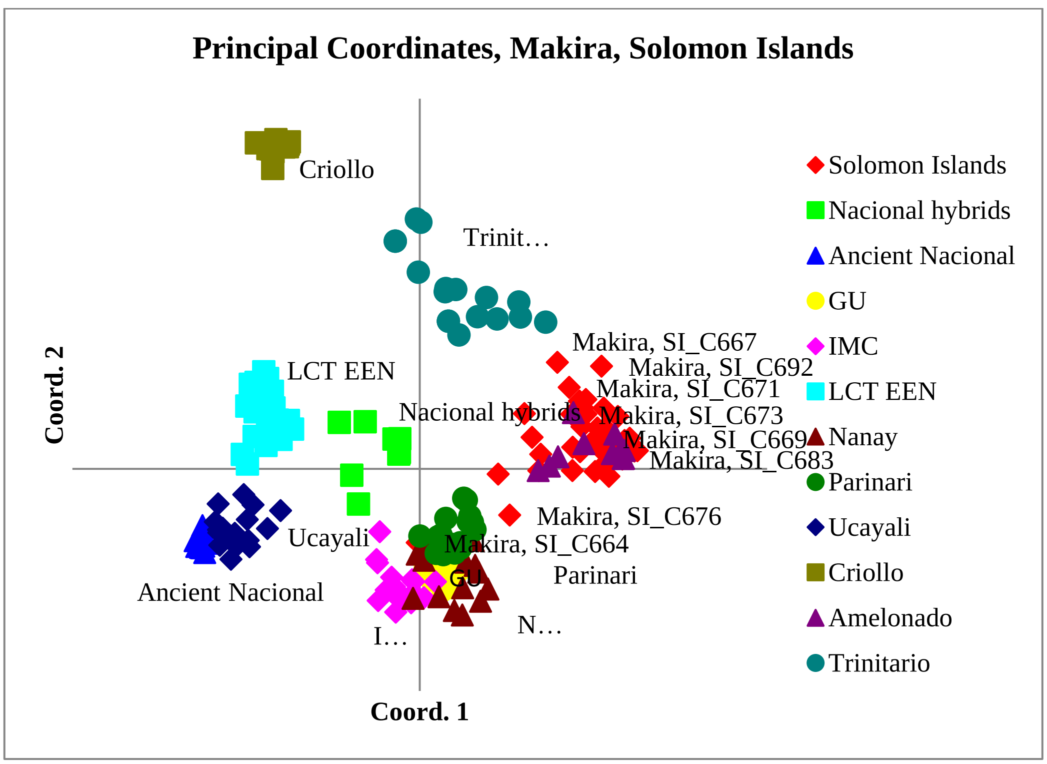


Figure 12 Principal coordinate Analysis of the cocoa genotypes from Makira in the Solomon Islands.

4.7 Cocoa accessions found with major contributing genes from Criollo, Parinari, IMC, Nanay and Nacional genotypes with Amelonado

As indicated in the Principal coordinates (Figure 12), the majority of cocoa accessions studied in this research were pooled in the Amelonado group. They carried more than 90 percent of genes from Amelonado. Nevertheless, 17 cocoa accessions were identified with DNA strains from other cocoa genotypes beside Amelonado (Table 8). These genotypes include Criollo, Parinari, IMC, Nanay and Nacional with the weightings from 10.3 to 53.5 percent. Detailed genotypic weightings are tabulated in appendix 13. Field accession numbers; 1LN1, 6VB1, 8VBJ1, 12MS1, 15RG1, 27MF1, 30JCW1 were found with genes of Amelonado and Criollo. Interestingly, accession number; 16JH1 expressed genes from Amelonado, Criollo and Nacional. Other accessions found with genes from Amelonado and Nacional included 11KG1 and 26AM1. Genes from Amelonado and Nanay were found in accessions 2VK1 and 3JH1 while 13PN1 and 18BT1 were observed with DNA strains of Amelonado, Parinari and IMC. In the other accessions including 22JK1, DNA strains of Amelonado and Parinari were present, while 32RH1 carried genes from Amelonado and IMC. The only accession found to carry the majority of genes from IMC and Parinari was 4CT1. This accession totally grouped away from the Amelonado group (Table 8).

.

Table 8 Cocoa accessions having more than 10 percent of gene heritage from Criollo, Parinari, IMC, Nanay and Nacional.

Accession Name	Field accession number	Amelonado	Criollo	Parinari	IMC	Nanay	Nacional
C663	1LN1	87.2	11.2	0.1	0.1	0.2	0.1
C664	4CT1	4.7	0.2	21.9	53.5	0.9	0.8
C665	3JH1	78.9	0	0.1	0.1	20.5	0
C666	2VK1	53.7	7.1	0.5	0.5	36.3	0.6
C667	6VB1	78.3	20.8	0.1	0.1	0.1	0.1
C671	8VBJ1	83.8	13.4	0.3	0.2	0.3	0.7
C673	11KG1	83.4	1.1	0.4	0.1	0.1	12.7
C674	12MS1	84.5	10.6	1.8	0.7	0.9	0.5
C676	13PN1	49.4	1.5	9.5	26.2	3.4	1.6
C678	15RG1	87.8	10.3	0.2	0.1	0.2	0.3
C679	16JH1	68.8	13.1	0.5	0.3	2.1	10.9
C680	18BT1	44.5	6.3	17.7	13.2	8.3	2.7
C684	22JK1	64.0	0.1	32.9	0.1	0.5	0.1
C688	26AM1	75.1	0.3	0.7	3.1	0.4	15.1
C689	27MF1	80.3	18.2	0.2	0.3	0.4	0.1
C692	30JCW1	87.0	12.5	0.1	0.1	0.1	0
C694	32RH1	77.2	0.2	0.1	21.4	0.3	0.3

Chapter 5: Discussion

5.1 Morphological variation of Quantitative traits

Evaluation of morphological traits was observed to have variations among genotypes in this study. Mean statistics of the number of beans per pod and the cotyledon length were observed to be 41.1 and 24mm just above the mean number of beans per pod of 40.5 and cotyledon length of 23.8 mm respectively for farm accessions in Cameroon. On the other hand, the mean cotyledon width was observed to be a little lower with 12mm than those of the farm accessions in Cameroon with 13.1 mm (Efombagn *et al.*, 2009). The variations, in pod external thickness, pod internal thickness and cotyledon length in this study could be due to the impact of the environment as suggested by Bartley (2005); Efombagn, *et al.* (2009) and Ballesteros Logos and Ferny (2015).

The PC analysis identified all quantitative traits including pod length, pod circumference, pod weight, pod external thickness, pod internal thickness, number of beans per pod, weight of beans per pod, cotyledon length and cotyledon width (Appendix 12) to be essential for differentiating accessions similar to the findings of Bekele, Butler and Bidaisee (2008); Efombagn *et al.* (2009); Aikpokpodian (2010) and Ballesteros; Logos and Ferny (2015). The total variation of the 8 PC's (Appendix 12) suggested homogeneity among the cocoa accessions as discussed by Ballesteros, Logos and Ferny (2015). On the other hand, such a dispersion of variation calls for the utilization of all the recommended morphological traits (Aikpokpodion, 2010). However, leaves were not used in this study because they are said to be non-discriminative as reported by Efombagn *et al.* (2009).

This study found a significant positive relationship between the cotyledon length and width ($r=0.6711$, $p<0.01$), similar to the findings by Aikpokpodion (2010) with the $r= 0.522$ at the probability level $=p<0.001$, this relationship was indicated in PC axes 1. Bekele, Butler and Bidaisee (2008) and Efombagn *et al.* (2009) also reported the same trend on the seed length and width. An increase in the seed length will result in the increase in the seed width resulting in large cocoa bean sizes. Apparently, the number of beans per pod indicated a non-significant relationship ($r= 0.1016$, $p<0.01$) with the weight of beans per pod. This result contradicted with the findings of Aikpokpodion in 2010 where the number of beans per pod had a significant relationship with the weight of beans per pod at $r = -0.131$, $p<0.0001$. Similarly, Iwaro *et al.* (2003) found a weak significant relationship between the number of beans per pod and the weight of beans per pod at $r = – 0.19$, $p \leq 0.001$. The dissimilarity in these findings could be due to the low number of samples in this study.

Cluster 1 has the cocoa accessions with agronomic traits such as long and wide cotyledon and red pods. This cluster has the highest number of the cocoa accessions among the groups with Amelonado type of pod shape. The cocoa accession in cluster 3 has a traditional history that the genotype was taken directly into Makira Island recently from Panama by someone from Makira Island who was a sailor. The farmers who grow the genotype revealed that it has an estimated pod index of 20; it is labeled as 4CT1 in appendix 10.

The presence of red podded cocoa trees could indicate some self-compatible (Johnson, Bekele and Schnell, 2004) DNA strains of ICS 1 introduced in to the SI (Friend, 1970). Wood and Lass (1985) and Aikpokpodian (2010) wrote that red podded trees have traits of Trinitario and Criollo types of cocoa. Leonard Nahu (1LN1; Appendix 10) a farmer who applied Integrated Pest and Disease Management (IPDM) pruning method revealed that red podded trees produce high yield with no significant occurrence of *Phytophthora palmivora*. As such, the red podded cocoa trees are considered to be high yielding as reported by Aikpokpodian (2010). In addition, James Clayton Watoto (30JCW1, Appendix 10) mentioned that a spacing of 4mx4m was ideal for the red podded trees to overcome *Phytophthora palmivora*.

Bartly (2005) wrote that variation of the phenotypes expressed the resultant actions of the different alleles that occur in the genes which controls the specific characteristics and the total number of the alleles which make up the plant genotype.

5.2 Morphological Variation of Qualitative traits

The pod surface texture had two most frquent observed traits viz, smooth and slightly rough. Those two traits are dominant in the Amelonado type of cocoa. Dominance of slight furrowed and medium furrows in the pod surface shape were traits also present in Amelonado as discribed by (Bartley, 2005; Wood and Lass, 1985 ; Aikpokpodion, 2010 ; Ha *et al*., 2016). When the percentage of Amelonado pod shape was added to that of callabacillo pod shape, they indicate a very strong trait of Amelonado type of cocoa in the accessions.

In this study, the percentage weightings of mammelate , obtuse and rounded types of pod apex shape also showed that Amelonado was dominant in the cocoa accessions studied.

A shift of 51 percent from Amelonado was expressed in the pod neck. Forty one percent was found to be with no pod neck which could be a trait of SCA6 since it has no pod neck (Bekele, Butler and Bidaisee, 2008) and 9 percent was observed with strong neck of Criollo type. This observation confirmed the statements by Friend (1970) and Proposals on processing and marketing of cocoa in the British Solomon Islands (1961) that Criollo hybrids were recommended and was already grown by farmers in their fields before Amelonado was imported for trials.

Four types of cotyledon colours were observed among the cocoa accessions in this study. They were, dark purple (Figure 13), medium purple (Figure 14), light purple (Figure 15) and light pink (Figure 16). Dark purple is attributed to Amelonado (Wood and Lass, 1985; Bartley, 2005). In this study dark purple and medium purple are categorized as dark purple on the bases that the two colours were almost similar. On the other hand, the existence of light purple and light pink showed traits of Trinitario strains in the cocoa population in Makira.

Figure 13. Dark purple cotyledon

Figure 14. Medium purple cotyledon

Figure 15. Light purple cotyledon

Figure 16. Light pink cotyledon

The weightings by percentage in accession vigorousity correspond to the strength to resist *Phytophthora palmivora* (Table 7). Strong tree vigor and resistance to *P. palmivora* are traits found in Amelonado. Hence, Amelonado variety have desired traits for adoptability which is used widely for hybridization with other varieties especially, Criollo type (Zhang and Motial, 2016).

5.3 Estimation of Phenotypic Diversity

An estimation of phenotypic diversity (Table 9) showed a high level of diversity in the qualitative traits among the accessions. Those traits were found useful to conduct studies on phenotypes in cocoa as suggested by Efombagn *et al.* (2009) and Bekele, Butler and Bidaisee (2008). In this study, phenotypic diversity in pod shape was low compared to other characters due to the high percentage of Amelonado type of pod shape (Table 7). Similarly, the pod surface shape, reaction to black pod and tree vigour also showed low level of phenotypic diversity compared to other characters since they were mostly showing Amelonado traits (Table 7). High values of “H” indicated high level of phenotypic diversity while low values showed low level of phenotypic diversity. Therefore, high phenotypic diversity among the accessions in this research portrayed intra- population phenotypic diversity (Table 9).

Table 9: SWDI (H’) Values for qualitative traits of cocoa accessions in Makira

Character	H’ values
Pod surface texture	0.968582575
Pod surface shape	0.69837765
Pod shape	0.591055433
Pod apex shape	1.292872885
Pod Neck	1.216350099
Cotyledon colour	1.046845745
Anthocyanin on mature pod ridge	0.86923611
Reaction to black pod	0.584174571
Tree vigour	0.576334128

5.4 Genotypes of the Elite cocoa trees

Majority of the studied cocoa genotypes fall in the Amelonado group; thus, coincide with the report (Kaihou 2017, personal communication, 28 July) that farmers obtain seeds for planting from the seed distribution plot established at Kaunasugu Agriculture training center in 1977. In addition, the findings are similar with the report by Dillon *et al.* (2014) that coco accessions from Guadalcanal were also found to be Amelonado types. These findings, confirmed that seeds from black post were used by farmers on Guadalcanal as well as Kaunasugu Agriculture training center for seed production in Makira Ulawa province. On the other hand, the findings also indicate that some farmers obtain seeds for planting from other sources as discussed in the section on the entry of cocoa into Makira.

Specification of the genotypes discussed in this section is presented in Table 8. The Principal Coordinate Analysis of the genotypes revealed an introduction of a new cocoa genotype into Makira Province. The new genotype fell in the IMC and Parinari group. It was identified as 4CT1 9 (Appendix 10), with high proportion of IMC and Parinari and it is described and singled out in cluster 3. In addition, two accessions viz, 13PN1 and 18BT1 were also identified close to the Parinari group (Figure 12). They possessed high proportions of IMC and Parinari second to Amelonado. In other findings, 11KG1 and 26 AM1 carried genes from Nacional type of cocoa which is a type of cocoa with fine flavor found only in Ecuador. An unpopular phenotype of light pink (Figure 16) was found in the cotyledon colour of 40 beans in one pod of 11KG1. This finding seemed to contradict the description by Aprotosoaie *et al.* (2016) that cocoa beans of Nacional variety has pale purple colour. Accessions 16JH1 showed proportions of interest including Criollo and Nacional. Apart from accession number 4CT1, the entry of DNA strains from IMC, Parinari and Nacional in the cocoa population in Makira was not indicated in the history of cocoa of the Solomon Islands.

However, Bartley (2005) wrote in his explanation on the influence of ecological conditions on cocoa that, new populations may express part of the diversity that could have been existed in the older populations. Additionally, he mentioned that variability might be resulted from simultaneous consequences of mutation and recombination. As such, individual trees of younger populations may express characteristics that are not present in the parent populations according to the ecological conditions specific to each habitat.

Accessions indicated close to the Trinitario pool in the Principal coordinates were further analyzed and they were found with DNA strains of Criollo (Table 8). Those accessions include 1LN1, 6VB1, 8VBJ1, 12MS1, 15RG1 and 27MF1. In the section on the entry of cocoa into the Solomon Islands, Criollo hybrids were already grown by farmers (Proposals on processing and marketing of cocoa in the British Solomon Islands, 1961) therefore the occurrence of Criollo strains in the cocoa population is obvious. According to Mortilal and Sreenivasan (2012) Trinitario can be a cross between Criollo and Forastero types in any location. Concerning the accessions which have Criollo DNA strains, all the pod morphology has features which are very much like ICS1 except 8VBJ1.

Chapter 6: Conclusion and Recommendations

6.1 Conclusion

The study found that there was phenotypic variation in the quantitative and qualitative traits among the 40 elite cocoa accessions in the cocoa population in Makira Island of Makira Ulawa province of the Solomon Islands. Phenotypic markers showed that the morphology of the pod surface texture, pod surface shape, pod shape, pod apex shape, cotyledon colour and tree vigor with the ability of the tree to withstand *Phytophthora palmivora* were observed to show traits of Amelonado as prominent. In addition, the genotypes confirmed the cocoa population to be Amelonado. Hence, both the phenotypic markers and genetic markers showed that the cocoa population in Makiara Province of the Solomon Islands consists mainly of Amelonado type of cocoa. Even the red podded trees generally known as phenotypes of Trinitario or Criollo were Amelonado by genetics. In addition, the study found a new cocoa accession totally different from Amelonado carrying genes from IMC and Parinari.

Existence of the DNA strains of Criollo, IMC, Parinari and Nanay among the accessions in this study indicated that DNA strains from the various types of cocoa planting materials imported into the Solomon Islands still exist in the cocoa trees grown in the farmer's fields. An increase in the DNA strains of the aforementioned genotypes will ultimately contribute to elevate the level of genetic diversity in the cocoa population in the Solomon Islands. Moreover, an increase in the genes of Criollo and Nacional in the cocoa population with the recommended post-harvest practices will improve the flavor of cocoa in the Solomon Islands. Furthermore, increase in the DNA strains of Criollo and Nacional paves the way to develop a fine cocoa population.

Informal discussions with the participating farmers found that farmers did not have a fair knowledge about the impact of having different cocoa genotypes on the flavor of cocoa beans.

6.2 Recommendations

Theobroma cacao was introduced in to the SI more than fifty years ago and it is assumed to be a popular crop in the SI. However, based on the findings from this study, some important recommendations are suggested as follows;

Further studies such as this is required for other regions of Guadalcanal and Malaita provinces to determine the types of elite cocoa trees grown by farmers and also to have concrete background knowledge of the cocoa population in the Solomon Islands. Such information will be used to prepare plans on how to improve the current cocoa population in relation to tree genetics and flavor. In addition, an organoleptic study of the Solomon Islands type of Amelonado cocoa is also paramount and timely to determine the type of cocoa flavor because environment has an impact on cocoa flavor.

Qualitative traits such as light purple and light pink cotyledon are attributed to fine cocoa varieties. As such, it is important that the elite cocoa trees with those traits are given more attention for further studies to determine the stability of those traits.

It is important for the Agriculture Extension division to assist the farmers who own the trees with strains of Criollo and Nacional to multiply the trees by cloning to increase the number of those trees in their fields for further observations on the type of flavor those cocoa beans may produce.

Establishment of bulking centers in Makira and other cocoa growing provinces under the care of the Agriculture extension and research division of Ministry of Agriculture and Livestock (MAL) is significantly important to collect and establish identified quality genotypes from farmers' fields.

The dominance of Amelonado in the cocoa population showed that the flavor of cocoa in Makira will mainly be bulk. Therefore, it is recommended for MAL to introduce some clones of fine varieties with clear passport data to develop a fine flavor cocoa population in the Solomon Islands.

Considering the economic importance of the crop to the SI economy and the rural livelihood it provides, it is recommended that MAL should establish a cocoa division to carry out further research in all aspects of the crop.

References

Afoakwa, O.E., 2014. Cocoa *Production and Processing Technology*. [e- book] Boca Raton: CRC Press, Taylor& Francis group. Available at: Google Books https://books.google.ws/books?hl=en&lr=&id=6JOlAgAAQBAJ&oi=fnd&pg=PP1&dq=cocoa+production+and+processing+Technology [Accessed: 16 December 2017]

Anglia Ruskin University, 2015. *Guide to Harvard referencing.* 5th ed. [pdf] Available at: https://www.international-macro.economics.unimainz.de/Dateien/Harvard_referencing_2015.pdf [Accessed: 22 June 2016]

Aikpokpodion, O. P., 2010. Variation in agro-morphological characteristics of cacao, Theobroma cacao L., in farmers' fields in Nigeria, *New Zealand Journal of Crop and Horticultural Science*. [pdf] 38(2), p. 157-170. Available at: http://www.tandfonline.com/doi/full/10.1080/0028825X.2010.488786 [Accessed: 27 February 2017]

Aikpokpodian, O. P., 2012. Defining genetic diversity in chocolate tree. Theobroma cacao L grown in West and Central Africa. In: Caliskan M (ed) .*Genetic diversity in plants.* ISBN: 978-953-51-0185-7, Intech, Chapter 10. [pdf] Available from: http://cdn.intechopen.com/pdfs/31478.pdf [Accessed: 30 June 2017]

Allen, M.G., Bourke, R.M., Evans, B.R., Iramu, E., Maemauri, R. K., Mullen, B.F., Pollard, A.A, Wairiu, M., Watoto, C. and Zotalis, S., 2006. *Solomon Islands Smallholder Agriculture Study*. *Common wealth of Australia, 4 Provincial Reports.* [pdf] .Available at: http://repository.usp.ac.fj/9646/1/solomon_study_vol4.pdf [Accessed: 3 January 2018]

Aprotosoaie, C. A., Luca, V,S. and Miron, A., 2016. *Flavor Chemistry of Cocoa and Cocoa Products- An Overview*,[online] 15(1) p 73-91Wiley Online Library. Available at: http://onlinelibrary.wiley.com/doi/10.1111/1541-4337.12180/full [Accessed: 9 June 2017]

Ballesteros, P. W., Logos, C. and Ferny. H., 2015. Morphological characterization of elite cacao trees (Theobroma cacao L.) in Tumaco, Nariño, *Colombia, Revista Colombiana de Ciencias Horticolas.* [pdf] 9(2), p. 313-328. University of Nariño. Available at: http://www.scielo.org.co/pdf/rcch/v9n2/v9n2a11.pdf [Accessed: 2 February 2017]

Bartley, B.G.D., 2005. *Genetic Diversity of Cacao and its Utilization*. [e-book] CABI. Available at https://ebookcentral.proquest.com/lib/uspfj-ebooks/reader.action?docID=295077&ppg=39 [Accessed: 15 December 2017]

Beals, M. L., Gross, L. and Harrell, S., 2000. *Diversity indices: Shannon's H and E*, [online] Available at http://www.tiem.utk.edu/~gross/bioed/bealsmodules/shannonDI.html [Accessed: 7 May 2017]

Bekele F.L., Butler, D. R. and Bidaisee, G.G., 2008. Upper Amazon Forastero cacao (Theobroma cacao L.) 1: An assessment of phenotypic relationships in the International Cocoa Genebank, Trinidad. *Trop. Agric. (Trinidad,*. [online] 85 (1) Cocoa Research Unit, The University of The West Indies, St. Augustine, Trinidad and Tobago; Available at: https://www.academia.edu/login?post_login_redirect_url=https%3A%2F%2Fwww.academia.edu%2Ft%2FgnVMy-L7Mq1R2-bdEizE%2F2567154%2FUpper_Amazon_Forastero_cacao_Theobroma_cacao_L._1_An_assessment_of_phenotypic_relationships_in_the_International_Cocoa_Genebank_Trinidad [Accessed: 30 January 2018]

Bell, F.W., 2009. A desk study on Organic Agriculture and Fair Trade in Pacific Island Countries. *Natural Resources Management and Environment Department, Food and Agriculture Organization of the United Nations*. [pdf] Available at: http://www.fao.org/3/a-ak356e.pdf [Accessed: 3 June 2017]

Callebaut, B., 2016. *Theobroma Cacao the food of the gods*, [online] Available at: https://www.barry-callebaut.com/chocophilia/theobroma-cacao-food-gods.[Accessed: 14 September 2016]

Central Intelligence Agency., 2017. *The World Fact Book*, [online]Available at: https://www.cia.gov/library/publications/the-world-factbook/geos/bp.html [Accessed: 7 June 2017]

Charrier, A., Jacquot, M., Hamon, S. and Nicolas, D., 2001. *Tropical Plant Breeding*. [e-book] Enfield: Science publishers, Inc : Available at: Google books https://books.google.ws/books?id=lBTgyfZ_Q0C&pg=PR3&source=gbs_selected_pages&cad=2#v=onepage&q&f=false [Accessed: 23 June 2017]

Cocoa Board of Papua New Guinea., n.d. *Cocoa history in Papua New Guinea*, [online] Available at: http://www.cocoaboard.org.pg/cocoa-in-papua-new-guinea/ [Accessed: 26 March 2017]

Cocoa Board of Papua New Guinea., 2017. *Cocoa Board special report.* Cocoa Board of PNG. [pdf] Available at: http://www.cocoaboard.org.pg/wp-content/uploads/2017/02/Cocoa-Board-Final-Draft2.pdf [Accessed: 4 June 2017]

Dalas, G., 2013. Principal Component Analysis 4 Dummies: *Eigenvectors, Eigenvalues and Dimension Reduction*, [online]. Available at https://georgemdallas.wordpress.com/2013/10/30/principal-component-analys [Accessed:28 October 2017]

Department of Agriculture and Fisheries., 2015. *Growing cocoa*. Queensland Government, [online] Available at: https://www.daf.qld.gov.au/about-us/the-organisation [Accessed: 15 November 2017]

Dillon, N., Hucks, L., Diczbalis, Y. and Kete, T., 2014. *Evaluation of molecular marker technology for identification of elite cocoa germplasm in the South Pacific*. PC/2014/032, ACIAR, GPO Box 1571, Canberra ACT 2601, Australia

Efombagn, M. I. B, O., Sounigo, O., Nyassé,S., Manzanares- Dauleux, M. and Eskes, A. B., 2009. Phenotypic variation of cacao (Theobroma cacao L.) on farms and in the gene bank in Cameroon. *Journal of Plant Breeding and Crop Science.* [pdf] 1(6). pp. 258-264 Institute of Agricultural Research for Development (IRAD), Yaoundé, Cameroon. Available at: http://www.academicjournals.org/article/article1379429075_Efombagn%20et%20al.pdf [Accessed: 18 January 2018]

FAO., 2001. *Cocoa*, [online] available at: http://www.fao.org/docs/eims/upload/216251/Infosheet_Cocoa.pdf [Accessed: 13 March 2018]

Fasi, J., 2009. Quantifying the dominance of little fire ant (*Wasmannia auropunctata*) and its effects on crops in the Solomon Island. *A thesis submitted in fulfillment of the requirement for the Degree of Masters of Science in Biology*. School of Biology and chemical sciences. Laucala campus: University of the South Pacific, [online] Available at: http://digilib.library.usp.ac.fj/cgi-bin/library.cgi?e=d-01000-00---off-0usplibr1--00-1----0-10-0 [Accessed: 23 December 2017]

Friend D., 1970. *A report on cocoa growing in the British Solomon Islands Protectorate*. Department of Agriculture, Government printer. British Solomon Islands Protectorate

Garcia-Alamilla, G. P., González-Lauck, V.W., De La Cruz-Lázaro, E., Lagunes-Gálvez, L.M. and García- Alamilla, R., 2012. Description and physical properties of Mexican Criollo cacao during post-harvest processing. *Revista Iberoamericana de Tecnología Postcosecha.* [pdf] 13(1) 58-65.Available through: http://www.redalyc.org/pdf/813/81324433008.pdf. [Accessed:14 February 2017]

Gayi, S. K. and Tsowou, k., 2012. Cocoa industry: *Integrating small farmers into the global value chain United Nations Conference on trade and development.* [pdf] Available at: http://unctad.org/en/PublicationsLibrary/suc2015d4_en.pdf [Accessed: 12 March 2018]

Ha, L., Thai Hang, P., Everaerti, H., Rottiers, H., Tunganh, L., Nhandung, T., Ducphuoc, P., Thanh Toan, H., Dewettinck, K. and Messens, K., 2016. Characterization of Leaf, Flower and Pod Morphology among Vietnamese Cocoa Varieties (Theobroma cacao). *Pakistan Journal of Botany*,[online] Available at: https://www.researchgate.net/publication/312040661 [Accessed: 14th February 2016]

Hivu, D. O., 2013. A Case Study in the Solomon Islands on the Impact of Small Holder Cocoa Production on Rural Livelihoods. *A Thesis Presented in Partial Fulfillment for the Degree of Master of AgricScience. Palmerstone North: Massey University*, [online] Available at:http://mro.massey.ac.nz/bitstream/handle/10179/5981/02_whole.pdf?sequence=2&isAllowed=y-[Accessed: 22, June, 2016]

Houston, H. and Wyer, T., 2012. Why Sustainable Cocoa Farming Matters for Rural Development. *Center for Strategic & International Studies*, [online] Available at: https://www.csis.org/analysis/why-sustainable-cocoa-farming-matters-rural-development

ICCO (International cocoa organization)., 2013. *What is the origin of Cocoa tree?* [online] Available at: https://www.icco.org/faq/51-cocoa-trees/114-what-is-the-origin-of-the-cocoa-tree.html [Accessed: 29 April 2018]

ICCO., 2017. *ICCO Quarterly Bulletin of Cocoa Statistics*, Vol. XLIII, No. 3, Cocoa year 2016/17, [online] Available at: https://www.icco.org/about-us/international-cocoa-agreements/cat_view/30-related-documents/46-statistics-production.html [Accessed: 29 April 2017]

Island Directory., 1998. *Islands of Solomon Islands,* [Online] Available at: http://islands.unep.ch/CLU.htm [Accessed: 21 December 2017].

Iwaro, A. D., Bekele, F. L. and Butler, D.R., 2003. Evaluation and utilisation of cacao (*Theobroma cacao* L.) germplasm at the International Cocoa Genebank, Trinidad. *Cocoa Research Unit*, *Euphytica* 130: 207–221. [pdf] The University of The West Indies, St. Augustine, Trinidad and Tobago, West Indies. Kluwer Academic Publishers. https://eurekamag.com/ftext.php?pdf=003757318 [Accessed: 13 April 2018]

Jean-Claude, N.'Zi., Kahia J., Diby, L . and Kouamé, C. 1., 2017. Compatibility of Ten Elite Cocoa (Theobroma cacao L.) Clones. *Horticulturae, MDPI*. [pdf] Available at: http://www.mdpi.com/2311-7524/3/3/45/pdf [Accessed: 15 April 2018]

Johnson, E., Bekele F. and Schnell, R., 2004. *Field Guide to the ICS Clones of Trinitario.*[e-book] CABI. Available at: Google books https://books.google.com.fj/books?id=KCEOAQAAIAAJ&pg=PT8&lpg=PT8&dq=Field+Guide+to+the+ICS+Clones+of+Trinidad&source=bl&ots=N3Afyb0bg-&sig=UI_BVh-Ykz8hAI8o1heoOnrjDBk&hl=en&sa=X&ved=0ahUKEwii7rjbr6TaAhWBfrwKHcztDuMQ6AEIODAD#v=onepage&q=Field%20Guide%20to%20the%20ICS%20Clones%20of%20Trinidad&f=false [Accessed: 6 April 2018]

Joshi, P. S., Ranjekar, K. P. and Gupta, S. V., 1999. *Molecular markers in plant genome analysis*, Plant Molecular Biology Group, Division of Biochemical Sciences, National Chemical Laboratory, Pune 411 008, [online] Available at: http://www.iisc.ernet.in/currsci/jul25/articles15.htm [Accessed: 8 July 2017]

Konam, J., 2015. Cocoa initiatives research & Development initiatives in Solomon Islands. *End of Mission Report*. Ministry of Agriculture and Livestock, Government of Solomon Islands. Honiara. Solomon Islands

Loor, R.G., Risterucci, A.M., Courtois, B., Fouet, O., M. Jeanneau, M., Rosenquist, E., Amores, F., Vasco, A., Medina, M., C. and Lanaud, C., 2009. Tracing the native ancestors of the modern Theobroma cacao L. population in Ecuador. *Tree Genetics & Genomes* (5) 421–433,[online] Available at: https://www.researchgate.net/signup.SignUp.html?ev=su_exitPopup [Accessed: 3 May 2018]

Lund, A. and Lund, M., 2013. Pearson Product-Moment Correlation. *Laerd Statistics*, [online] Available at : https://statistics.laerd.com/aboutus.php [Accessed: 4 December 2017]

Malua, B.M., n.d. The Pacific Islands: Case study *KVA Consultant limited.* [pdf] Available at: http://unctad.org/en/docs/ditctncd20031p5_en.pdf [Accessed: 1 June 2017]

Mammadov, J., Aggarwal R., Buyyarapu R. and Kumpatla, S., 2012. SNP Markers And their Impact on Plant Breeding. *International Journal of Plant Genomics*, Vol2012, ID728398, Hindawi publishing corporation, [Online] Available from: https://www.hindawi.com/journals/ijpg/2012/728398/ [Accessed: 15 March 2017

Manjit, S. K. and Surinder, S. B., 2013. Combating Climate Change: *An Agricultural Perspective.* [e-book] CRC Press. Available at: Google books https://books.google.com.fj/books?id=S8XMBQAAQBAJ&dq=white+cocoa+cotyledon &source=gbs_navlinks_s [Accessed: 16 December 2017]

McGregor, A., Watas, C. W. and Tora, L., 2009. The Vanuatu Organic Cocoa Growers Association: *A case study of Agriculture for Growth in the Pacific*. [pdf] Food Agriculture Organization.Availableat:http://www.fao.org/docrep/013/am013e/am013e00.pdf [Accessed: 3 June 2017]

Microbeatic., 2013. *Diversity Index: Shannon Index/Shannon-Weaver Index (H),* [online]. Available at https://microbeatic.wordpress.com/2012/01/26/diversity-index-shannon-indexshannon-weaver-index-h/#comments [Accessed: 18 February 2018]

Ministry of Agriculture and Livestock., 2013. New national strategy to benefit 20,000 households. *Solomon times*. 26 April, [Online] Available at: http://www.solomontimes.com/news/new-national-cocoa-strategy-to-benefit-20000-households/7624 [Accessed: 19 September 2016]

Minitab, 18., 2017.*Interpret all statistics and graphs for Principal Components Analysis,* [online]. Available at: https://support.minitab.com/enus/minitab/18/help-and-how-to/modeling-statistics/multivariate/how-to/principal-components/interpret-the-results/all-statistics-and-graphs/ [Accessed: 27 November 2017]

Motamayor, J.C., Lachenaud, P., da Silva e Mota, J.W, Loor, R., Kuhn, D.N. and Brown, J.S., 2008. Geographic and genetic population differentiation of the Amazonian chocolate tree (Theobroma cacao). *PLoS one* 3(10), [online] Available from: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0003311 [Accessed: 18th February 2017]

Motilal, L. A. and Sreenivasan, T. N., 2012. Revisiting 1727: Crop Failure Leads to the Birth of Trinitario. *Cacao Journal of Crop Improvement*. [pdf] 26 (5), p. 599–626. Available at: http://www.tandfonline.com/doi/pdf/10.1080/15427528.2012.663734?needAccess=true [Accessed: 1 April]

NCSS Statistical Software., nd. *Hierarchical Clustering / Dendrograms*. Ch 445, p 445-1 -445-15. [pdf] Available at: https://ncss-wpengine.netdna-ssl.com/wp-content/themes/ncss/pdf/Procedures/NCSS/Hierarchical_Clustering-Dendrograms.pdf [Accessed: 16 February 2018]

Obando, A. M. E , 2009. Genetic characterization of Theobroma cacao L in Nicaragua. *A thesis Submitted in partial fulfillment of the Requirements of Helsinki University for a Master's Degree* . University of Helsinki. [pdf] Available at https://helda.helsinki.fi/bitstream/handle/10138/14806/ErwinAragonThesiscacao.pdf?sequence=1 [Accessed:15/12/17]

Pacific Agribusiness Research & Development Initiative., 2012.
Cocoa value chain review. *Australian Centre for International Agricultural research.* [pdf] The University of Adelaide. Available at: https://www.adelaide.edu.au/global-food/documents/pardi-cocoa-chain-review-oct-2012.pdf [Accessed: 6 June 2017]

Pacific Horticultural and Agricultural Market Access Program., 2016. *Pacific Cocoa Export Industry Overview*. [pdf] AECOM Services. Available at: http://phama.com.au/wp-content/uploads/2016/05/Cocoa_FS_Overview6.pdf [Accessed: 11 June 2017]

Penkova, T. G., 2017. Principal component analysis and cluster analysis for evaluating the natural and anthropogenic territory safety. *International Conference on Knowledge Based and Intelligent Information and Engineering. Systems*, KES2017, 6-8 September 2017, Marseille, France. Procedia Computer Science (112) 2017, p. 99-108, [online] Available at: https://www.sciencedirect.com/science/article/pii/S1877050917315363 [Accessed: 25 March 2018]

Proposals on the processing and Marketing of cocoa in the British Solomon Islands., 1961. *British Solomon Islands Protectorate*

Ripin, S., 2016. Empowering women in Solomon Islands cocoa industry, *Aecom impact blog*.[blog] 4 August . Available at: http://blogs.aecom.com/empowering-women-in-solomon-islands-cocoa-industry/ [Accessed:6 February 2017]

Samuel, K. G and Tsowou, K., 2016. Cocoa industry: Integrating small farmers into the global value chain. *United Nations conference on trade and development*. [pdf] Available at: http://unctad.org/en/PublicationsLibrary/suc2015d4_en.pdf [Accessed: 12 March 2018]

Santos, E.S.L., Cerqueira-Silva, C.B.M., Mori. G.M., Ahnert, D., Mello, D.L.N., Pires, J.L, Corrêa R.X., de Souza, A P., 2015. Genetic Structure and Molecular Diversity of Cacao Plants Established as Local Varieties for More than Two Centuries. *The Genetic History of Cacao Plantations in Bahia, Brazil. PLoS ONE* 10(12): e0145276, [online] Available at: https://doi.org/10.1371/journal.pone.0145276 [Accessed: 1 February 2018]

Statistical Bulletin., 2015. *Commodities Export Marketing Authority*, Honiara, Solomon Islands

Solomon Islands cocoa book., 2010. *Solomon Islands livelihood improvement project*. Solomon Islands, [online] Available at : http://www.pacificfarmers.com/solomon-islands-cocoa-book/ [Accessed: 10 June 2017]

STAR, version 2.0.1 2014. Biometrics and Breeding Informatics, PBGB Division, *International Rice Research Institute*, Los Baños, Laguna.

The Provincial Government Act., 1981. C 118. *National Parliament of Solomon Islands,* [Online] Available at: http://www.paclii.org/sb/legis/consol_act/pga253 [Accessed: 4 April 2018]

Tibshirani, R., 2013. Clustering 3: *Hierarchical clustering (continued); choosing the number of clusters, Data Mining*: 36-462/36-662. [pdf] Available at: http://www.stat.cmu.edu/~ryantibs/datamining/lectures/06-clus3.pdf [Accessed: 16 February 2018]

Vadnjal, D. and Pelomo, M., 2014. Solomon Islands cocoa value chain analysis, *World Bank*, *Australian Department of Foreign Affairs and Trade*, Mondelez International. [pdf] Available at https://openknowledge.worldbank.org/bitstream/handle/10986/17802/860440WP0P0892 0ain0Analysis000FINAL.pdf?sequence=1&isAllowed=y

Wood, R. A. G. and Lass, A.R., 1985. *Cocoa.* [e-book] John Wiley & Sons: Available through: ProQuest E-book Central https://ebookcentral.proquest.com/lib/uspfj-ebooks/reader.action?ppg=1&docID=470294&tm=1521168781338. [Accessed:16 March 2018]

World cocoa Foundation., 2014. World cocoa market update. *World cocoa Foundation.* [pdf] Available at: http://www.worldcocoafoundation.org/wp-content/uploads/Cocoa-Market-Update-as-of-4-1-2014.pdf [Accessed: 15 February 2017]

World Cocoa Foundation., 2018. *Knowledge center*, [Online] Available at: http://www.worldcocoafoundation.org/category/program-region/worldwide/ [Accessed: 12 March 2018]

Zhang, D. and Motilal, L., 2016. Origin, Dispersal, and Current Global Distribution of Cacao Genetic Diversity. In: Bailey, B.A and Meinhardt, L .W (eds). 2016. *Cocoa diseases. A history of old and new encounters* DOI 10.1007/978-3-319-24789-2_1 Springer International Publishing Switzerland,[online] Available from: http://www.springer.com/cda/content/document/cda.../9783319247878-c1.pdf?SGWID [Accessed: 14 December 2017]

Appendices

Appendix 1: Accession number, field location and geographical location of the elite cocoa trees

Accession Number	Field Location	Ward	Longitude S	Latitude E
1LN1	Togori	10	$10^{o}27.674$	$161^{o}56.299$
2VK1	Tawaitara	10	$10^{o}27.330$	$161^{o}56.330$
3JH1	Tawaitara	10	$10^{o}27.631$	$161^{o}56.363$
4CT1	Pawa	10	$10^{o}27.693$	$161^{o}56.428$
5DP1	Pawa	10	$10^{o}27.509$	$161^{o}56.314$
6VB1	Pawa	10	$10^{o}27.581$	$161^{o}56.258$
7JT1	Togori	10	$10^{o}27.370$	$161^{o}56.020$
8VBJ1	Togori	10	$10^{o}27.431$	$161^{o}56.003$
9CM1	Pawa	10	$10^{o}27.588$	$161^{o}56.288$
10BN1	Maona	10	$10^{o}27.580$	$161^{o}56.472$
11KG1	Maniahu	11	$10^{o}30.063$	$161^{o}52.552$
12MS1	Ngarianagahuto	11	$10^{o}30.524$	$161^{o}57.449$
13PN1	Maniahu	11	$10^{o}30.215$	$161^{o}57.756$
14CT1	Toratataka	11	$10^{o}30.195$	$161^{o}50.037$
15RG1	Ngarianagahuto	11	$10^{o}30.688$	$161^{o}57.766$
16JH1	Ngarianagahuto	11	$10^{o}30.702$	$161^{o}57.672$
17RK1	Ngarianagahuto	11	$10^{o}30.447$	$161^{o}57.787$
18BT1	Barabaraora	11	$10^{o}29.615$	$161^{o}50.118$
19SD1	Maopa	11	$10^{o}29.996$	$161^{o}57.858$
20JH1	Maopa	11	$10^{o}29.957$	$161^{o}57.803$
21MM1	Asimanioha	8	$10^{o}15.092$	$161^{o}29.231$
22JK1	Asimanioha	8	$10^{o}14.880$	$161^{o}28.033$
23OA1	Asimanioha	8	$10^{o}14.866$	$161^{o}.27.997$
24DB1	Asimanioha	8	$10^{o}14.893$	$161^{o}27.881$
25CP1	Asimanioha	8	$10^{o}15.037$	$161^{o}27.931$

26AM1	Asimanioha	8	10^{o}14.863	161^{o}27.536
27MF1	Asimanioha	8	10^{o}14.875	161^{o}29.355
28JT1	Boroni	8	10^{o}16.111	161^{o}30.025
29MR1	Boroni	8	10^{o}16.102	161^{o}30.023
30JCW1	Boroni	8	10^{o}16.380	161^{o}.29.605
31JMT1	Heuru	7	10^{o}13.778	161^{o}25.720
32RH1	Heuru	7	10^{o}13.731	161^{o}25.763
33ET1	Heuru	7	10^{o}13.601	161^{o}21.868
34RU1	Heuru	7	10^{o}13.550	161^{o}25.869
35ST1	Heuru	7	10^{o}13.572	161^{o}25.917
36CM1	Heuru	7	10^{o}13.412	161^{o}25.929
37MD1	Heuru	7	10^{o}13.084	161^{o}25.695
38JB1	Heraniau	7	10^{o}13.145	161^{o}25.562
39WK1	Tawaniau	7	10^{o}13.233	161^{o}26.476
40AS1	Tawaniau	7	10^{o}13.376	161^{o}26.564

Appendix 2: Research permit

THE RESEARCH ACT 1982
(No. 9 of 1982)

RESEARCH PERMIT

Permission is hereby given to:

1. Name (s): Elison Toramo
2. Country: Solomon Islands
3. Research subject areas: The main aim of the study is to identify elite cocoa trees (Theobroma Cacao) in the cocoa orchards in Makira Province.
4. Ward (s): Central Makira and West Makira
5. Province: Makira
6. Conditions:

   a. To undertake research only in subject areas specified in 3 above.
   b. To undertake research only in the ward (s) and Province (s) specified in 4 and 5 above.
   c. To observe with respect at all times local customs and the way of life of people in the area in which the research is carried out.
   d. Not to take part at any time in any political or missionary activities or local disputes.
   e. To leave four (4) copies of your final research report in English with the Solomon islands Government Ministry responsible for research at your own expense.
   f. A research fee of SBD500.00 must be paid in full or the Research Permit will be cancelled. (See sec. 3 subject 7 of the Research Act).
   g. This permit is valid until **October 2017** provided all conditions are adhered to.
   h. No live species of plants and animals to be taken out of the country without approval from relevant authorities.
   i. A failure to observe the above conditions will result in automatic cancellation of this permit and the forfeit of your deposit.

MINISTER
MINISTRY OF EDUCATION, HUMAN RESOURCES DEVELOPMENT

Signed: ……………………………………………………
Minister of Education and Human Resources Development

Date: 13/7/17

Appendix 3: Business License

NO: MUP 968

MAKIRA ULAWA PROVINCIAL GOVERNMENT

MAKIRA ULAWA PROVINCIAL ASSEMBLY

BUSINESS LICENCE CERTIFICATE

(BUSINESS LICENCE ORDINANCE 2006)

Type of Licence RESEARCH-COCOA GENETIC RESOURCES

Pursuant to Section 4 of the Makira Ulawa Province Business Licence

ELISON TORAMO

Has been granted permission to operate the business of:

RESEARCH-COCOA GENETIC RESOURCES

This Licence shall be valid until: *31 MARCH 2018*

Provincial Principal Accountant

Dated this 25TH *day* SEPTEMBER *2017*

Secretary:

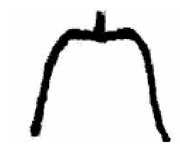
Department of Trade, Commerce & Industry

Authorized Officer

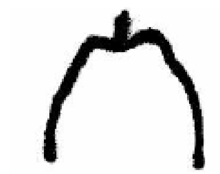
Stamp of Issuing Office Date: 25.9.17 Initial:	Stamp of Issuing Office Date:............... Initial:...........	Stamp of Issuing Office Date:............... Initial:...........	Stamp of Issuing office Date Initial:...........	Stamp of Issuing Office Date:............... Initial:...........

Appendix 4: Pod neck (Bottleneck or basal constriction)

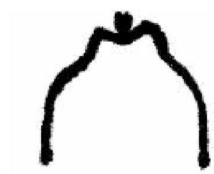
0. Absent



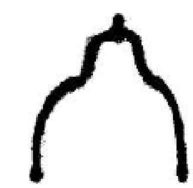
1. Slight



2. Intermediate



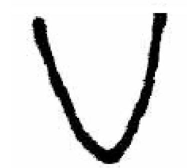
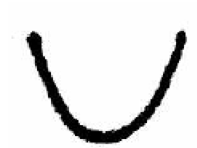
3. Strong



4. Wide shoulder

Adapted from Dillon *et al.* (2014)

Appendix 5: Pod apex shape

1. Attenuate	
2. Acute	
3. Obtuse	
4. Rounded	
5. Mammelate	
6. Indented	

Modified from Dillon *et al.* (2014)

Appendix 6: Pod surface texture

1. Smooth	
2. Slightly rough	
3. Rough	
4. Rough and warty	

Adapted from Dillon *et al.* (2014)

Appendix 7: Pod surface shape

1. Unfurrowed	-
2. Slight furrowed	
3. Medium furrows	
4. Deep furrows	

Adapted from Dillon *et al.* (2014)

Appendix 8: Pod shape

1. Amelonado	
2. Cundeamor	
3. Angoleta	
4. Calabacillo	

Appendix 9: Color of cotyledon

1. Dark purple	
2. Medium purple	
3. Light Purple	
4. Light pink	

Appendix 10. Pod morphological characteristics and accession Numbers of forty cocoa accessions in Makira Province .

Ward 10

1LN1, Togori village, Tree No 1 pod No. 1, 2 and 3

2VK1, Tawaitara Village, Tree1 /pod 1 and 2

3JH1, Tawaitara village, Tree 1, pod 1 and 2

4CT1, Pawa village, Tree 1 Pod 1 and 2

5DP1, Pawa village, Tree 1, Pod 1 and 2

6VB1, Pawa, Tree 1, pod 1,2 and 3

7JT1, Togori village, Tree 1, pod 1,2 and3

8VBJ1, Togori village, Tree 1, pod 1and 2

9CM1, Pawa village, Tree 1, pod 1,2 and3

10BN1, Maona village, Tree 1, Pod 1 and 2

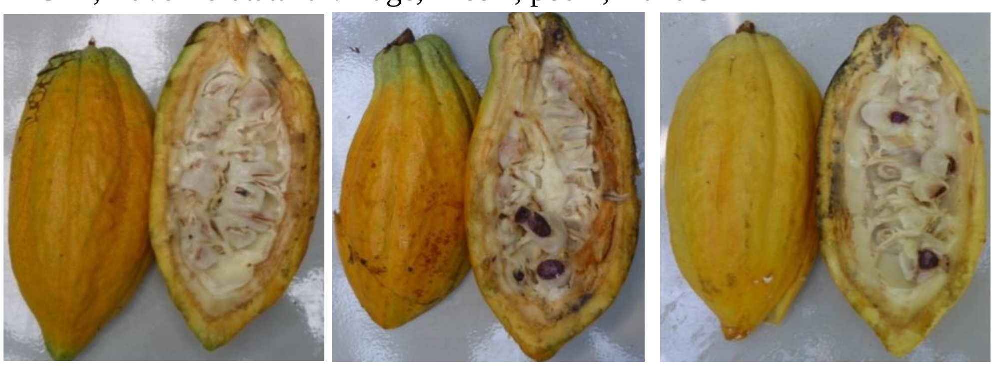
Ward 11

11KG1, Maniuhu village,Tree 1, pod 1 and 2

12MS1, Ngarianagahuto village Tree 1, pod 1,2 and 3

13PN1, Maniuhu village Tree 1, pod 1, 2 and 3

14CT1, Ravo Toratataka village, Tree 1, pod 1, 2 and 3



15RG1, Ngarianagahuto village, Tree 1, pod 1 and 2

16JH1, Ngarianagahuto village, Tree 1, pod 1, 2 and 3

17RK1, Ngarianagahuto village, Tree 1, pod 1 and 2

18BT1, Barabaraora village , Tree 1. pod 1, 2 and 3

19SD1, Maopa village, Tree 1, pod 1, 2 and 3

20HK1, Maopa village, Tree 1, pod 1, 2 and 3

Ward 8

21MM1, Asimanioha village, Tree 1, pod 1, 2 and 3

22JK1, Asimanioha village, Tree 1, pod 1, 2 and 3

23OA1, Asimanioha village Tree 1, pod 1, 2 and 3

24DB1, Asimanioha village, Tree 1, pod 1, 2 and 3

25CP1, Asimanioha village, Tree 1, pod 1, 2 and 3

26AM1, Asimanioha village, Tree 1, pod 1, 2 and 3

27MF1, Boroni village, Tree 1, pod 1, 2and 3

28JT1, Boroni river basin, Tree 1, pod 1, 2 and 3

29MR1 Boroni river basin, Tree 1, pod 1, 2 and 3

30JCW1 ,Boroni river basin, Tree 1, pod 1, 2 and 3

Ward 7

31JMT1 ,Heuru village, Tree 1, pod 1, 2 and 3

32RH1,Heuru village, Tree 1, pod 1, 2 and 3

33ET1 ,Heuru village, Tree 1, pod 1, 2 and 3

34RU1 ,Heuru village, Tree 1, pod 1, 2 and 3

35ST1 ,Heuru village, Tree 1, pod 1, 2 and 3

36CM1 ,Heuru village, Tree 1, pod 1, 2 and 3

37MD1, Heraniau village, Tree 1, pod 1, 2 and 3

38JB1, Manua'a village, Tree 1, pod 1, 2 and 3

39WK1,Tawaniau village, Tree 1, pod 1, 2 and 3

40AS1,Tawaniau vilage.Tree 1, pod 1 and 2

Appendix 11: List of participating farmers in the study

Accession Number	Name of Farmer	Field Location	Ward	Constituency
1LN1	Leonard Nahu	Togori	10	Central Makira
2VK1	Victor Kohaia	Tawaitara	10	Central Makira
3JH1	James Haga	Tawaitara	10	Central Makira
4CT1	Cicilia Tagua	Pawa	10	Central Makira
5DP1	Dicksn Pinihimana	Pawa	10	Central Makira
6VB1	Vincent Baugi	Pawa	10	Central Makira
7JT1	John Tauasi	Togori	10	Central Makira
8VBJ1	Vincent Bauguhu	Togori	10	Central Makira
9CM1	Cain Muri	Pawa	10	Central Makira
10BN1	Ben Nanau	Maona	10	Central Makira
11KG1	Kemuel Gapu	Maniahu	11	Central Makira
12MS1	Michael Salau	Ngarianagahuto	11	Central Makira
13PN1	Patrick Nasi	Maniahu	11	Central Makira
14CT1	CalistoTautaumae	Toratataka	11	Central Makira
15RG1	Rex Gamagi	Ngarianagahuto	11	Central Makira
16JH1	Japhet Haga	Ngarianagahuto	11	Central Makira
17RK1	Robert Kangia	Ngarianagahuto	11	Central Makira
18BT1	Bernard Tau	Barabaraora	11	Central Makira
19SD1	Simon Dick	Maopa	11	Central Makira
20JH1	Jacob Hakumae	Maopa	11	Central Makira
21MM1	Michael Mae	Asimanioha	8	West Makira
22JK1	Jonathan Kere	Asimanioha	8	West Makira
23OA1	Oddie Aeei	Asimanioha	8	West Makira
24DB1	David Bua	Asimanioha	8	West Makira
25CP1	Chris Poleka	Asimanioha	8	West Makira
26AM1	Alick Mae	Asimanioha	8	West Makira
27MF1	Mathew Faramo	Asimanioha	8	West Makira
28JT1	John Taro	Boroni	8	West Makira
29MR1	Mary Rau	Boroni	8	West Makira
30JCW1	James Clayton Watoto	Boroni	8	West Makira
31JMT1	Joan Mary Taro	Heuru	7	West Makira
32RH1	Rose Hura	Heuru	7	West Makira
33ET1	Earnest Tarobae	Heuru	7	West Makira
34RU1	Rex Uma	Heuru	7	West Makira
35ST1	Smith Taki	Heuru	7	West Makira

36CM1	Chris Maesu	Heuru	7	West Makira
37MD1	Michael Dodo	Heuru	7	West Makira
38JB1	Jack Boo	Heraniau	7	West Makira
39WK1	Wilson Kipa	Tawaniau	7	West Makira
40AS1	Albert Subu	Tawaniau	7	West Makira

Appendix 12: Principal component analysis of cocoa accessions in Makira Province

	PC 1	PC 2	PC3	PC4	PC5	PC6	PC7	PC 8
Eigen Values	4.16	2.20	1.97	1.45	1.34	1.16	1.13	0.95
Total Variation (%)	23.1	12.2	10.9	8.1	7.4	6.4	6.3	5.3
Cumulative (%)	23.1	35.3	46.2	54.3	61.7	68.1	74.4	79.7

Character	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC 8
Pod length	0.3510	-0.0724	0.2665	-0.1519	0.0007	-0.3196	0.0807	-0.0490
Pod circumference	0.3887	-0.0728	-0.1969	0.0316	0.2671	-0.0096	-0.1228	-0.0791
Pod weight	0.4355	-0.1844	0.0370	-0.0308	0.1231	-0.0803	-0.1045	-0.1533
Pod external thickness	0.1773	-0.1876	0.0916	-0.0047	-0.1888	0.3848	-0.0473	-0.3604
Pod internal thickness	-0.0207	-0.0130	0.1350	-0.5879	0.2597	0.1726	0.3983	-0.1194
Number of beans per pod	-0.1958	-0.2878	0.0739	0.1272	-0.3428	0.0481	-0.1121	-0.2952
Weight of beans per pod	0.3102	-0.1817	0.1199	0.0197	-0.1759	0.1743	0.0310	-0.2273
Cotyledon length	0.3630	-0.1111	-0.0371	0.3352	0.0790	0.1177	0.1065	0.2624
Cotyledon width	0.3452	0.1111	-0.1215	0.2015	-0.0740	0.1866	0.3669	0.2211
Pod surface texture	0.1322	0.4208	0.4081	-0.1517	0.0760	0.2143	-0.1430	-0.0705
Pod surface shape	0.0675	0.3750	0.4209	0.0099	-0.0293	0.1134	-0.3187	-0.0320
Pod shape	0.0065	0.0246	-0.2154	0.0324	0.4626	0.2227	-0.5660	0.1230
Pod apex shape	-0.0938	-0.2031	-0.0497	-0.1494	-0.2192	0.6453	-0.0706	0.3213
Pod neck	-0.0411	-0.0574	0.5012	0.4212	-0.1071	-0.0704	-0.0337	0.2342
Cotyledon color	-0.0161	0.5086	-0.1938	0.1944	-0.1070	0.0455	0.2090	-0.1092
Mature Pod ridge colour (anthocyanin)	0.1436	0.3797	-0.3291	0.0610	-0.2644	0.1120	-0.0341	-0.3072
Reaction to black pod	-0.1892	-0.1173	0.0104	0.3419	0.3151	0.0574	0.0198	-0.5273

Appendix 13: Detailed gene heritage of forty cocoa accessions from other cocoa genotypes in percentage

Accession Name	Accession Number	Amelonado	Criollo	Parinari	IMC	Nanay	Nacional	Scavina	LCT EEN	Guiana	Purus
C663	1LN1	87.2	11.2	0.1	0.1	0.2	0.1	0.1	0.8	0.2	0.1
C664	4CT1	4.7	0.2	21.9	53.5	0.9	0.8	8.7	0.3	1.8	7.3
C665	3JH1	78.9	0	0.1	0.1	20.5	0	0.1	0	0.1	0.1
C666	2VK1	53.7	7.1	0.5	0.5	36.3	0.6	0.2	0.5	0.3	0.3
C667	6VB1	78.3	20.8	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1
C668	9CM1	99.6	0	0.1	0.1	0.1	0	0	0	0.1	0
C669	5DP1	88.3	3.6	1.2	0.7	0.5	2.1	0.4	1.9	1.1	0.2
C670	7JT1	92.2	4.2	0.2	0.2	1.2	0.3	0.1	1.1	0.3	0.2
C671	8VBJ1	83.8	13.4	0.3	0.2	0.3	0.7	0.2	0.6	0.2	0.3
C672	10BN1	99.6	0	0.1	0.1	0.1	0	0	0	0.1	0
C673	11KG1	83.4	1.1	0.4	0.1	0.1	12.7	0.2	1.6	0.2	0.1
C674	12MS1	84.5	10.6	1.8	0.7	0.9	0.5	0.4	0.1	0.2	0.4
C675	17RK1	90.8	7.3	0.5	0.2	0.3	0.3	0.1	0.1	0.2	0.2
C676	13PN1	49.4	1.5	9.5	26.2	3.4	1.6	1.5	1.3	1.8	3.8
C677	14CT1	86.7	7.5	1.4	1.2	1.5	0.2	0.4	0	0.1	0.8
C678	15RG1	87.8	10.3	0.2	0.1	0.2	0.3	0.3	0.4	0.2	0.2
C679	16JH1	68.8	13.1	0.5	0.3	2.1	10.9	0.6	1.2	0.4	2.0
C680	18BT1	44.5	6.3	17.7	13.2	8.3	2.7	2.5	2.2	1.0	1.5
C681	19SD1	91.1	0.1	0.4	0.5	0.3	5.2	1.2	0.4	0.2	0.6
C682	20JH1	88.6	5.8	0.1	0.5	1.0	0.3	1.3	0.1	0.1	2.2
C683	21MM1	98.3	0.1	0.1	0.7	0.4	0.1	0	0.2	0.1	0.1
C684	22JK1	64.0	0.1	32.9	0.1	0.5	0.1	0.2	0.1	1.6	0.4
C685	23OA1	86.5	0.1	9.1	0.3	0.2	0.2	0.3	0.1	2.1	1.2
C686	24DB1	91.9	5.1	0.7	0.1	0.2	0.1	0.4	0.1	0.4	1.0
C687	25CP1	99.6	0	0.1	0.1	0.1	0	0	0	0.1	0
C688	26AM1	75.1	0.3	0.7	3.1	0.4	15.1	1.4	2.8	0.2	0.9

		Amelonado	Criollo	Parinari	IMC	Nanay	Nacional	Scavina	LCT EEN	Guiana	Purus
C689	27MF1	80.3	18.2	0.2	0.3	0.4	0.1	0.1	0.1	0.3	0.1
C690	28JT1	93.9	5.4	0.1	0.1	0.1	0	0	0.1	0.2	0.1
C691	29MR1	97.1	0.9	0.4	0.1	0.2	0	0	1.0	0.1	0.1
C692	30JCW1	87.0	12.5	0.1	0.1	0.1	0	0	0.1	0.1	0.1
C693	31JMT1	99.6	0	0.1	0.1	0.1	0	0	0	0.1	0
C694	32RH1	77.2	0.2	0.1	21.4	0.3	0.3	0.1	0.2	0.2	0.2
C695	33ET1	82.9	2.6	1.2	3.0	1.0	7.5	0.4	0.8	0.3	0.3
C696	34RU1	84.5	3.1	1.5	5.5	1.4	0.6	0.9	0.4	1.1	1.0
C697	35ST1	99.6	0	0.1	0.1	0.1	0	0	0	0.1	0
C698	36CM1	84.8	8.9	1.3	0.6	0.6	0.4	0.3	1.1	1.7	0.3
C699	37MD1	99.6	0	0.1	0.1	0.1	0	0	0	0.1	0
C700	38JB1	99.6	0	0.1	0.1	0.1	0	0	0	0.1	0
C701	39WK1	67.9	7.9	1.3	2.7	3.4	6.8	2.0	5.7	2.0	0.5
C702	40AS1	90.3	0.2	6.5	0.3	0.2	0.4	0.8	0.2	0.5	0.4