Morphological characterization of cacao cultivars in different socio-ecological settings of Ghana

Master's thesis in the scientific programme (Sustainable International Agriculture) at the Georg-August-Universität Göttingen, Faculty of Agricultural Sciences and the University of Kassel, Faculty of Organic Agriculture

by

Esther Asare, 21800762

Ghana

Supervisor: Dr. Martin Wiehle, Organic Plant Production and Agroecosystems Research in the Tropics and Subtropics, Steinstraße 19, 37213 Witzenhausen

Co-supervisor: Prof. Dr. Andreas Bürkert Organic Plant Production and Agroecosystems Research in the Tropics and Subtropics, Steinstraße 19, 37213 Witzenhausen

Date of submission: 15 July 2022

Contents

List of figures

List of tables

Abstract

Knowledge of Theobroma cacao morphological variability provides the foundation for the utilization and conservation of genetic resources in Ghana. This study concentrated on cacao varieties, varietal richness and diversity maintained on farmers` fields. The objectives of the study were (1) to assess varietal richness per farm and the level of homozygosity of cacao cultivars on farmers' fields through morphological analysis and farm management. (2) To identify existing cacao cultivars in the agroforestry system and compare them between organic and conventional farms. Ten agro-morphological characteristics were studied in 6 cacao cultivars. 387 individuals were surveyed in farmers´ fields belonging to Amelonado, Angoleta, Calabacillo, Cundeamor, Criollo and Pentagona cacao cultivars. Quantitative pod and beans traits included diameter at breast height, pod length and width, fresh pod weight, and bean fresh weight. Additional qualitative traits sampled were pod basal constriction, pod apex form, ripened and unripened pod color, and cotyledon color among others. Analysis of variance was used to determine the variations among cultivars. The dendrogram was generated to show the cluster relationship among cultivars. Simpson`s diversity index, varietal richness and Pielou`s evenness was computed under the IITA, organic and non-IITA cacao management systems in Ghana. From this study, Amazonian Forastero (Amelonado, Angoleta, Calabacillo, Cundeamor) and Criollo contributed to 99.7% of all cultivars studied. Cacao tree density ranged from 1,072 to 2,592 trees per hectare in conventional farms whilst organic farms recorded 736 to 2,240 trees per hectare. The diameter at breast height recorded ranged from 3.38 cm to 30.02 cm depending on the age of the cacao tree. There was no difference between leaf shapes found. Cacao cultivars differed significantly in terms of these quantitative traits; pod wall thickness, the weight of fresh beans, number of beans and beans width have each $P < 0.001$ as well as beans thickness ($P < 0.01$), while pod weight and beans length were not significantly different. In conclusion, a comprehensive understanding of the morphological relationship among the cultivars studied alongside information on allelic richness could be valuable in selecting core parents and for future breeding purposes.

Keywords: *Theobroma cacao*, cultivar, morphology, characterization, Amelonado, Angoleta, Calabacillo, Cundeamor, Criollo, Pentagona.

Acknowledgement

I would like to express my sincere gratitude to Prof. A. Bürkert and Dr. M. Wiehle my master thesis supervisors who gave me the golden opportunity to do this wonderful cocoa research. Secondly, I would like to thank Dr. C. Hülsebusch and ATSAF team for the funding. Thirdly, I would also like to thank Dr. R. Asare, IITA Graduate Training program, NORAD CocoaSoils project for the financial and technical support, Mr. M. Dalaa, Ms. A. Tettey, Deogratias, field technicians and the farmers who supported in finalizing this research work within the time frame.

1. Introduction

Cacao (*Theobroma cacao* L.) (2n = 2x = 20) is a humid tropical perennial tree crop cultivated in regions of high annual rainfall (Ofori et al., 2016). It is extensively established that Latin America is the epic hub of the diversity of *Theobroma cacao* (Motamayor et al., 2008), where it grows between 10 to 20 degrees north and south of the equator, identified as the "Cacao Belt". The cacao tree can grow as tall as 12 m and is known as an understory rainforest tree crop which is sensitive to drought (Carr & Lockwood, 2011). The tree is mostly grown in the shades of other trees in hot and moist conditions. In most cacao vegetations, the trees cannot survive long periods of drought conditions without a drastic decline in vegetation and reproductive growth. Cacao is normally grown for its pods, from which the beans are extracted and used for making chocolates, cosmetics, and other confections (ICCO, 2014). Cacao beans have vital components due to the presence of procyanidin, polyphenols and antioxidants (Toro-Uribe, López-Giraldo & Decker, 2018). *Theobroma cacao* is morphologically and genetically differentiated according to the pods` appearance, bean yields, color and thickness of cacao beans, characteristics of flavor and its resistance to pests and diseases (Afoakwa et al., 2008; Afoakwa, 2010; Adeyeye, et al., 2010; Everaert et al., 2017). The cacao pods can grow up to 30 cm, brownish-yellow to purple color. It contains 20-40 sweet-sour pulp beans which are pink in color (Cacaoweb, 2020). Hence, these pod and tree characteristics are often used in cacao morphological characterization.

Cacao bean quality is made up of several components such as flavor volatiles, nutritional composition, polyphenolic content, and fermentative quality. The most important components are the flavor volatiles of the beans as this affects cacao bean acceptability. The unique flavor of different cacao varieties depends on the genetic setup, geomorphology, soil conditions, and climatic conditions such as the amount and timing of sunlight and rainfall, ripening conditions, and time of harvesting (Kongor, 2016). Ghana is the second biggest cacao producer after Ivory Coast in the world (ICCO, 2019; Vigneri & Kolavalli, 2018). On the world market, Ghana's cacao is considered the “golden standard” due to its premium quality (Läderach et al., 2013).

1.1 Annual cacao production

Annual global demand for cacao production is between 2 to 3%, of which West Africa contributes approximately 70% of the global supply. On the contrary, West African cacao-producing countries such as Ghana are confronted with a 2% decline due to unsustainable farm management practices, poor varietal quality, over-ageing of tree plantations, pests and disease, and low and untimely application of fertilizer. This caused a decline in Ghana`s cacao production from 811,700 tonnes in 2019 to 800,000 tonnes in 2020 (FAOSTAT, 2022). There is a need for sustainable cacao production and intensification using premium cultivars to increase the supply volume in Ghana since the cacao sector is critical for the survival of millions of small-scale farmers' households whose livelihood depends on cacao and the global cacao products lovers. Hence, the sustainability of cacao production can improve the quantity and quality of the cacao beans produced and the standard of living of farmers.

1.2 General characterization of cacao cultivars

Cheesman (1944), classified cacao cultivars as Criollo (Central and South America) and Forastero (Amazonia). He later subclassified Forastero as, Angoleta, Cundeamor, Amelonado, and Calabacillo. Pentagona and his new cultivar Trinitario were further added to improve his cacao classification (Hunter, 1990; Bartley 2005).

In recent times, Motamayor et al. (2008) suggested 10 genetic classes which are: Marañon, Guiana, Contanama, Curaray, Nanay, Iquitos, Nacional, Purús, Criollo and Amelonado. Over the years, many genetically assorted accessions have been sampled in the epicentre of cacao diversity and distributed to the innumerable cacao-growing regions via transitional quarantine and gene banks. Globally, the most common cacao cultivars are the Forastero (the bulk, from upper and lower Amazon region, and Guianese), Criollo (fine flavor), Trinitario (Forastero * Criollo), and Nacional (Motamayor et al., 2008). Forastero, native to the Amazon basin, comprises 95% of the world's production of cacao and is commonly referred to as “bulk cacao” in trade. Forastero cacao is predominantly cultivated in West Africa particularly, Ghana. The beans are flat, astringent, and purple (more rarely ivory or pale) due to the presence of anthocyanins. The bean aroma is weak. Forastero cacao trees are very productive and are moderately resistant to pests and diseases (Ofori et al., 2016; Ha et al., 2016). Based on the findings of Ortiz de Bertorelli, Graziani de Fariñas, and Gervaise (2009), Forastero beans have superior pH after fermentation and drying as contrasted with Criollo beans. Thus, chocolate produced from the Forastero beans is less bitter, less astringent, and less acidic than chocolate produced from either Criollo or Trinitario beans. Criollo is the originally cultivated cacao, indigenous to Northern, Central and South America. The criollo-flavored beans are white to ivory or have a very pale purple color, due to an anthocyanin inhibitor gene. Their low yields and susceptibility to many diseases make them rare to cultivate (Ofori et al., 2016; Ha et al., 2016). The Trinitario type derived in Trinidad and includes all the results of recombination and natural hybridization of the Forastero and Criollo populations. Trinitario has a strong aroma from Criollo with its disease resistance and high yield from Forastero. The beans are variable in color, although rarely white, and the trees show susceptibility to pests and diseases intermediate to Forastero and Criollo populations. The Trinitario cultivar is known to have strong basic chocolate characters and some typical winery types of aromas that are not found in other varieties (Afoakwa et al., 2008). Both Trinitario and Criollo varieties produce the "fine" cacaos, whose share in the total world production is below 5% (ICCO, 2015b). These cacao cultivars are used to make high-quality dark chocolate. Nacional cacao is considered a rare fine cultivar which produces well-renowned Arriba beans with unique floral and spicy flavor.

1.3 The history of cacao cultivars in Ghana

In 1878, a Ghanaian agriculturalist and trader Tetteh Quarshie, was the first to introduce the traditional West African Amelonado cacao cultivar, known as "Tetteh Quarshie", to Ghana after his return from Fernando P`oo (currently Bioko in Equatorial Guinea). These uniform beans were planted in the Akwapim Mountains of the Eastern Region of Ghana. Under sound agronomic management practices, the Amelonado cultivar began to bear pods 5 years after field planting with yields ranging from 500 to 1,000 kg ha^{-1} (Abdul-Karimu, Adomako & Adu-Ampomah, 2006). In 1887, Governor Griffiths, Governor of Ghana (formerly Gold Coast) and missionaries introduced Amelonado and Trinitario which were successfully cultivated in the Aburi Botanic Gardens (Edwin & Masters, 2005). The Amelonado and Trinitario genotypes introduced were susceptible to the cacao swollen shoot virus (CSSV). The upper Amazon accession collected by F.J. Pound in 1937-1943 consisted of mainly four populations namely Nanay, Parinari, Iquitos Mixed Calabacillo and Scavina (Pound, 1938). Progenies of the upper Amazon cacao cultivars have unique traits such as high vigor, early pod bearing and high yielding (Thresh et al., 1988). Under good agronomic conditions, the upper Amazon cacao progenies start bearing pods in the third year with an approximate yield of 2,000 kg ha^{-1} (Toxopeus, 1964). These genotypes dominated farms till the commencement of Trinidad (commonly known as T) clones official research in 1943/44. Posnette (1943) collected Amelonados and Trinitarios samples from the Aburi Garden and established them at the West African Cocoa Research Institute (WACRI) in Tafo, Ghana. The first generation of Posnette cacao selections became the parents of the first bi-parental crosses called the ´series II hybrid´ developed by WACRI (Rogers & Knight, 1953; McKelvie, 1956). From 1946 to 1971, numerous clones were introduced belonging to the sub-group of the Marañon (Criollo, Trinitario, Nacional, Parinari, Nanay, Iquitos) and Scavina genotypes from the sub-group of the Contanama (Ofori et al., 2016) to widen the genetic foundation for breeding purposes of cacao in Ghana. The new introductions of cacao accessions are different from the older introductions in terms of pod and beans characteristics, the number of beans per pod, beans weight, percentage shell and fat content of dried seeds (Adomako & Adu-Ampomah, 2003). After 2004, several other cacao accessions from diverse genotypes were introduced that are characterized by improved yield, pests, and cacao swollen shoot disease resilience. Other traditional Trinitario cultivars embrace a wide array of hybrids with Criollo and Amazonian Forastero as its progenitors (Bekele et al., 2020) and the Forastero cacao group are commonly found in cacao agroforestry systems (Peprah, 2019). In recent times, more than 1000 germplasm accessions have been preserved at the Cocoa Research Institute of Ghana (CRIG) (Padi et al., 2015).

1.3 Problem statement

Cacao production is key to the development of Ghana’s economy and the livelihood of small-scale, resource-poor farmers. Most of the study areas are classified as semi-deciduous forests with unproductive cacao farming practices (MoFA, 2020). Many cacao farmers in Ghana experience lower yields because of overaged plant stands of low varietal richness and diversity with poor-yielding cultivars. These farming practices drive farmers to invade forests to expand farms` production. Some environmental threats associated with cacao production are deforestation, poor land and resource management, loss of biodiversity, and loss of soil fertility through the depletion of soil carbon stock and organic matter. Characterization of cacao cultivars employing morphological traits is a vital constituent in the identification of dominant and resilient cultivars and may support Spatio-temporal and system-specific adaptation to suit diverse cacao agroecological zones adapted to changing climatic conditions (Abdulai et al., 2018; Bunn et al., 2019; Oyekale, 2020), and may thus significantly contribute to the understanding of the factors linked to sustainable cacao in Ghana (Kongor et al., 2016). Lastly, this is essential for screening the yield, pests, and disease resilience germplasm and thus for future breeding purposes (Ofori et al., 2016).

1.4 General objective

The study seeks to contribute to the scientific knowledge of *Theobroma cacao* L. populations in the cacao systems of Ghana and its implications for the management of cacao plantations in Ghana. It also explores the role of organic certification in the maintenance of the diversity of cacao stands. The main objective of this study is to examine the elucidated patterns of association among different cacao accessions based on morphological characteristics.

1.4.1 Specific objectives

▪ To assess varietal richness per farm and level of homozygosity of cacao cultivars on farmers' fields through morphological analysis and farm management.

▪ To identify existing cacao cultivars in the agroforestry system and compare them between organic and conventional farms

2. Materials and methods

This study was conducted under the Organic Plant Production and Agroecosystems Research in the Tropics and Subtropics at the University of Kassel in partnership with the Cacao Soils program by IITA-CGIAR. The Cacao Soils program focuses on Integrated Soil Fertility Management (ISFM), good agronomic practices, and Integrated Pests Management (IPM).

2.1 Study areas

Field data were collected between November 2020 to March 2021, in forty villages across the Eastern, Central, Western, Brong Ahafo and Ashanti regions. These regions are well-known as the cacao belt of Ghana. The locations of the farms are in the forest zones of Ghana with suitable climatic conditions for cacao development.

Three different management systems were classified: IITA conventional (on-farm experimental fields), organic (Yayra Glover farmers), and non-IITA conventional.

In total 120 farms were surveyed. The non-IITA forty conventional and forty organic farmers` study area, Suhum Municipal, is situated in the south-central part of the Eastern Region, Ghana.

Suhum, the capital of the municipality, is 60 km northwest of Accra. It covers a land area of about 400 km^2. The municipal´s annual rainfall varies between 1270 mm and 1651 mm. The study areas have a tropical climate with temperatures ranging from 24 to 29 degrees Celsius. Relative humidity fluctuates between 87% and 91% during the rainy season and ranges between 48% and 52% during the dry season. The soil is deeply well-drained, has a good water-retaining capacity and is well aerated (MoFA, 2020). The area is covered with loamy or clayey loam soil with a pH ranging from 5 – 8. The study areas are the Nsuta, Kuano, Adimediem, Okanta, Oboadaka and Sowakey communities. These communities are selected because of their long cacao production history with about 40% of farmers cultivating cacao on 8,720 ha of land to sustain their livelihoods (Dormon et al., 2004).

Furthermore, forty conventional cacao farmers were randomly selected from the IITA-CGIAR cacao farmers list across the major cacao belt of Ghana. The climatic conditions of these regions are shown in *Figure 1* below.

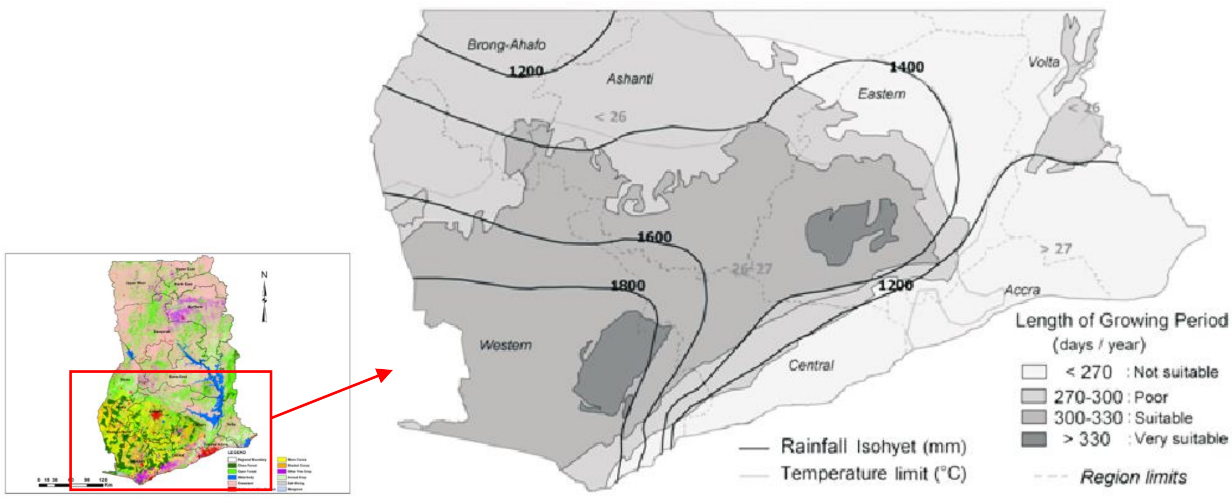

Figure 1: Climatic conditions of cacao zones of Ghana *Adapted from Snoeck et al. (2010)*

2.2 Sampling method

The on-site farm sampling was carried out using the 25 m x 25 m quadrat method by randomly selecting trees from the core of the farm to reduce cacao diversity disturbances since trees are mostly planted at stake in irregular rows. Depending on the varietal diversity, the selection of trees was done by randomly sampling every fifth tree within a quadrat (Nadege et al., 2020; Oderinde & Afolayan, 2021). Two to six trees per farm were randomly selected depending on the varietal variation within the farm. Individual varieties were sampled until a new variety was considered to have different distinguishable morphological traits resulting in a total of 389 individuals. The morphological characteristics were based on the following traits: tree architecture, fruit shape, number of pods per tree, pod surface texture, pod color of unripened pods, pod color of ripened pods, pod length and width, pod thickness and color of flush leaves (Phillips-Mora et al., 2013 & Bekele et al., 2020) (*Table 1*). Diameter at breast height for cacao trees at 0.3 m and shade tree species at 1.3 m above ground (with measuring tape) were measured. Depending on the availability of ripened cacao pods on the trees, a total of 83 pods were harvested for further seed analysis. Three leave samples per tree were collected, stored in tea filter bags, and dried at room temperature. The leaf samples were transported to Germany for further genetic analysis to ascertain the genotypes of cacao cultivars in Ghana. The locations of the farms were determined with a hand-held GPS device, Garmin-eTrex 30, horizontal accuracy ± 2 m, GARMIN® Ltd., Southampton, UK. A questionnaire was developed with Kobocollect software to collect data on-farm management practices.

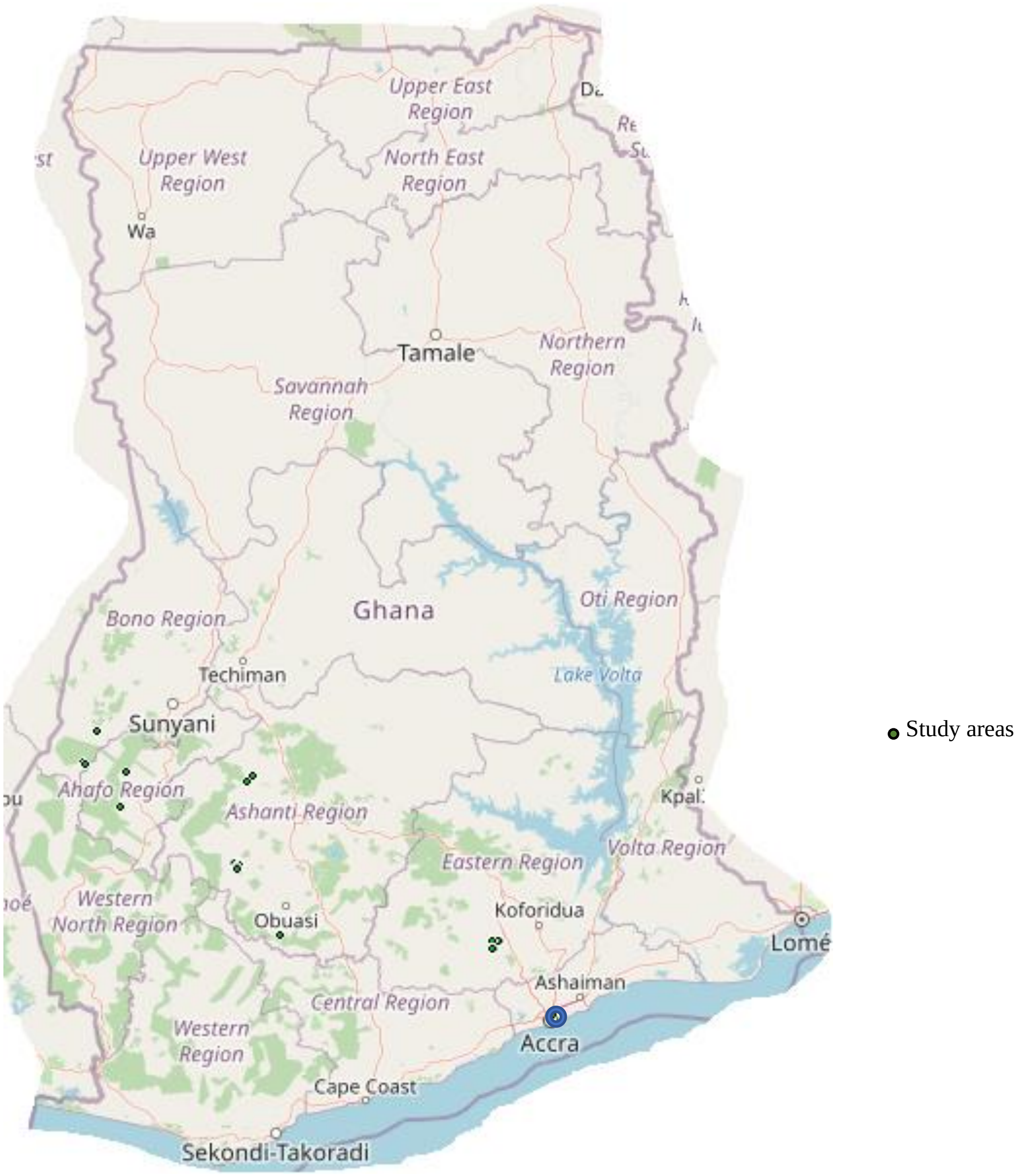

Figure 2:Map of the study area

2.3 Cacao morphological description

Twenty-three morphological parameters were used to describe the different cacao varieties identified on the farms (*Table 1*). These morphological parameters were selected according to research work done by Phillips-Mora et al. (2013) and Bekele et al. (2020). Qualitative and quantitative morphological parameters were used as stated in *Table 1*. The cacao varieties sampled were characterized as Amelonado and Calabacillo (oval shape, smooth skin, dark purple cotyledon), Angoleta (elongated shape, rough, narrow neck, dark purple cotyledon, shape in bottom), Criollo and Cundeamor (elongated shape, rough, dark purple cotyledon) fruit shapes *(Figure 4)*.

Table 1: Morphological parameters used in the present study to characterize cacao trees, leaves and pods in 2021 in Ghana

Cacao morphological descriptor			
Tree architecture	1 = erect	2 = intermediate	3 = pendulous
Tree vigor	1 = weak	2 = intermediate	3 = vigorous
Leaves base	1 = acute 4 = cordate	2 = obtuse 5 = cuneiform	3 = rounded
Leaves apex	1 = acute	2 = acuminate short	3 = acuminate long
Leaves petiole	1 = non-visible pulvinus	2 = visible pulvinus	
Flush leaf color	1 = pale red with green 4 = red with intense brown	2 = light greenish-brown	3 = intense pink
Leaf shape	1 = elliptic 4 = aristate	2 = obtuse 5 = others	3 = ovate
Shape of cocoa pods	1 = Cundeamor 4 = Pentagona	2 = Angoleta 5 = Amelonado	3 = Criollo 6 = Calabacillo
Pod base constriction	0= absent 7= strong	3= slight	5= intermediate
Pod apex form	1 = attenuate 4 = rounded 7 = mamillate	2 = acute 5 = indented	3 = obtuse 6 = caudate
Color of unripe pod surface	1 = green 2 = purple with eventual presence of green 3 = pale green with very soft red tones 4 = green with purple 5 = light green and whitish furrows 6 = dark purple 7 = purple with light green		
Color of ripe pod surface	1 = orange with yellow sectors 2 = yellow with orange and eventually red flecks 3 = yellow 4 = orange with yellow		
Pod rugosity	0 = absent 7 = intense	3 = slight	5 = intermediate
Average pod length per tree (cm)	[n=3 per tree]		
Average pod width per tree (cm)	[n=3 per tree]		
Fruit wall thickness at ridge (mm)			
Fresh beans weight (g)			
Number of beans			
Beans length (mm)	[n=5 beans per pod]		
Beans diameter (mm)	[n=5 beans per pod]		
Beans thickness (mm)	[n=5 beans per pod]		
Beans shape	1= oblong 4= irregular	2= elliptic	3= oval
Beans shape in cross-section	1= flattened	2= intermediate	3= rounded

Figure 3: Morphological descriptors of the pod: A. fruit length (cm) B. pod base constriction C. pod width (cm) D. pod apex form

a

b

Angoleta (a, b)

Criollo

Calabacillo Amelonado Cundeamor

Figure 4: Different cacao varieties in Ghana

2.4 Statistical analysis

R software was used for the statistical analysis. To assess the phenotypic variation among the cultivars, the coefficient of variation (CV%) was computed for the quantitative traits. Analysis of variance (ANOVA) was computed for each of the quantitative variables to determine whether there is a mean difference across the cacao cultivars. The `multcompView` package was utilized to compute Tukey's multiple comparison technique for the separation of means if significant differences exist between cocoa cultivars (Hsu, 1996). In addition, a correlation matrix for standardization of the variables was used to compute the principal components (Bekele et al., 2020). The `vegan` package based on the Simpson index and Pielou`s evenness was used in computing the varietal alpha (α) diversity, abundance, and richness among the different management systems (Dixon, 2003). Additionally, `ggdendro` package was used to show the hierarchical cluster relationship among the different cultivars.

4. Results

Pentagona, one of the six cultivars studied is the wild type. Furthermore, Amelonado and Criollo were found to dominate most cacao farms in Ghana. Amelonado and Criollo form 30.8% of each of the total individuals surveyed. In total, 389 individuals were characterized in terms of 10 morphological traits presented in *Table 2*. To elaborate, the coefficient of variation (%) was highest for the number of pods followed by fresh beans weight, number of beans per pod, fresh pod weight, pod length, pod thickness, pod width, beans thickness, beans length and beans width. Besides, the ratio of pod length to pod width was highest for Angoleta followed by Criollo, Pentagona, Cundeamor, Amelonado and Calabacillo which serve as potential indicators of yield. Pod wall thickness, an indicator of potential adaptation to abiotic stress was highest for Cundeamor followed by Angoleta, Criollo, Pentagona, Amelonado and Calabacillo. Beans weight, as a potential indicator of superior yield, was highest for Angoleta followed by Cundeamor, Criollo, Amelonado and Calabacillo.

Phylogenetic relations among the individuals distributed among species richness, diversity and evenness measures varied among the management systems. Alpha (α) diversity was higher among all the cultivars studied under the different management systems. IITA farms had the highest α-diversity followed by non-IITA farms and organic farms (*Table 3*). The hierarchical clustering methods condensed the individual cacao hierarchy by representing the dissimilarities (*Figure 5*). Cundeamor and Criollo were most similar in terms of pod length.

Table 2: Descriptive statistics of cacao cultivars found in Ghana

Morphological parameters	Cundeamor (n=58)	Angoleta (n=84)	Criollo (n=120)	Pentagona (n=1)	Amelonado (n=120)	Calabacillo (n=6)
Mean number of pods	10.1±8.2	12.2±6.8	10±8.3	26±0	14.3±9.7	13.5±6.7
CV (%) number of pods	81.5	56.1	82.6	*	68.3	49.3
Mean pod length (L) (cm)	18.9±2.8	20.5±9.3	18.3±3.1	13.5±0	17.7±2.7	14.9±1.6
CV (%) pod length	15	45.2	16.8	*	15.3	10.5
Mean pod width (W) (cm)	12.1±2.3	11.2±1.8	10.9±1.9	7.8±0	11.9±2.1	11.6±2.1
CV (%) pod width	19.2	16.5	17.8	*	17.6	17.9
L/W relationship (cm)	1.6	1.8	1.7	1.7	1.5	1.3
Mean fresh pod weight (g)	600±35.3	525±318.2	523.3±191.5	0	507.5±217.7	202.1±93
CV (%) fresh pod weight	5.89	60.6	36.6	0	42.9	46
Mean pod thickness (cm)	13.6±5.3	12.1±3.1	11.8±3.4	0	9.7±2.9	9.1±3.3
CV (%) pod thickness	39	26	29	0	30	36
Mean number of beans per pod	42.5±12	46.2±9.9	47.1±7.5	0	44±10.8	17.5±11.2
CV (%) number of beans per pod	28.2	21.4	15.9	0	24.5	64
Mean fresh beans weight (g)	150±70.7	169.6±69.9	128.7±48.3	0	121.3±44.2	41.7±30.3
CV (%) fresh beans weight	47.1	41.2	37.5	0	36.4	72.7
Mean beans length (mm)	22.4±3.9	25±4.2	23.2±3.8	0	21.7±3.4	24.2±3.6
CV (%) beans length	17.2	16.7	16.2	0	15.5	15
Mean beans width (mm)	13±1.9	13.5±1.9	12.9±1.8	0	12.8±1.8	13.2±1.8
CV (%) beans width	14.3	14.2	13.8	0	14	13.5
Mean beans thickness (mm)	7.7±1.3	8.1±1.3	7.4±1.3	0	7.3±1.2	6.7±1.3
CV (%) beans thickness	17.2	16.6	17.5	0	15.7	19.1

*There is no value associated with the coefficient of variation (CV) (%) where * is given since there was only one observation for Pentagona and standard deviation (SD)(±).*

Table 3: Simpson`s diversity index and Pielou`s evenness under `the cacao different management systems in Ghana

Management system	Varietal richness	Simpson`s diversity index	Pielou`s evenness
Organic	4	0.750	0.999
IITA conventional	6	0.832	0.997
Non-IITA conventional	5	0.793	0.989

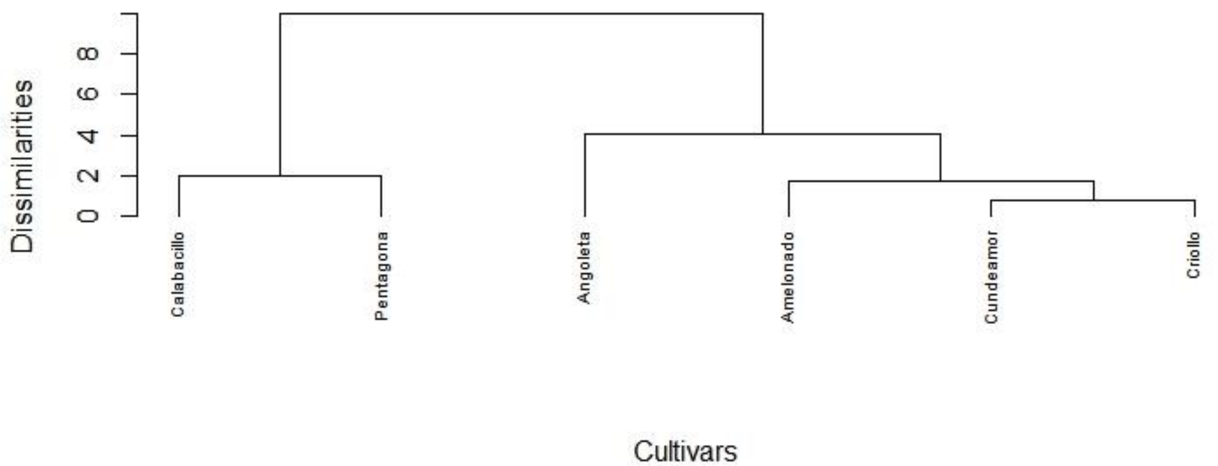

Figure 5: Cluster dendrogram showing the dissimilarities among cultivars

There was no difference between leaf shapes found. Cultivars with “a” or “ab” had no significant difference in terms of pod length and width. On the contrary, cultivars with only “b” were significantly different from group “a” in terms of pod length and width (*Figure 6, Figure 7).* Cacao cultivars differed significantly in terms of these quantitative traits; pod wall thickness, the weight of fresh beans, number of beans and beans width have each $P < 0.001$ as well as beans thickness ($P < 0.01$), while pod weight and beans length were not significantly different (*Figure 8*). Criollo, Cundeamor and Amelonado have all unripened pod color ranging from green to dark purple. Calabacillo on the other hand had only green to green with whitish furrow unripened pod color (*Figure 9*). All cultivars except Pentagona had yellow ripened pods. Conversely, Pentagona had only yellow with orange and the eventual presence of red flecks when ripened. Likewise, orange with yellow ripened pod color is commonly found among Angoleta, Criollo and Amelonado (*Figure 10*). Subsequently, Criollo has all four types of pod basal constriction followed by Cundeamor, Angoleta and Amelonado. Whereas Calabacillo has two types and Pentagona has only strong pod basal constriction (*Figure 11*). Amelonado has the highest number of the different types of pod apex forms followed by Cundeamor, Criollo, Angoleta, Calabacillo and Pentagona (*Figure 12*). Complementary to this, intermediate pod rugosity dominated among Cundeamor, Angoleta, Criollo, Pentagona and Amelonado (*Figure 13*).

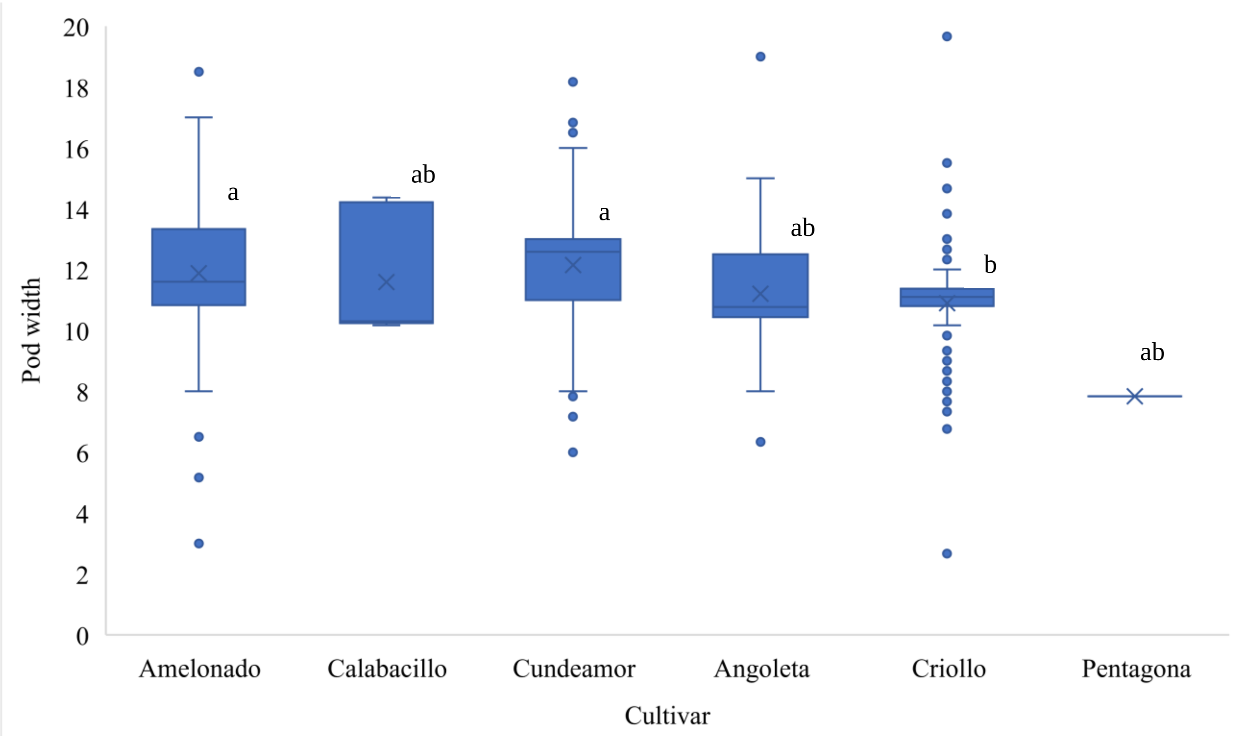

Figure 6: Boxplot of pod width of cacao cultivars with Tukey`s HSD compact letters

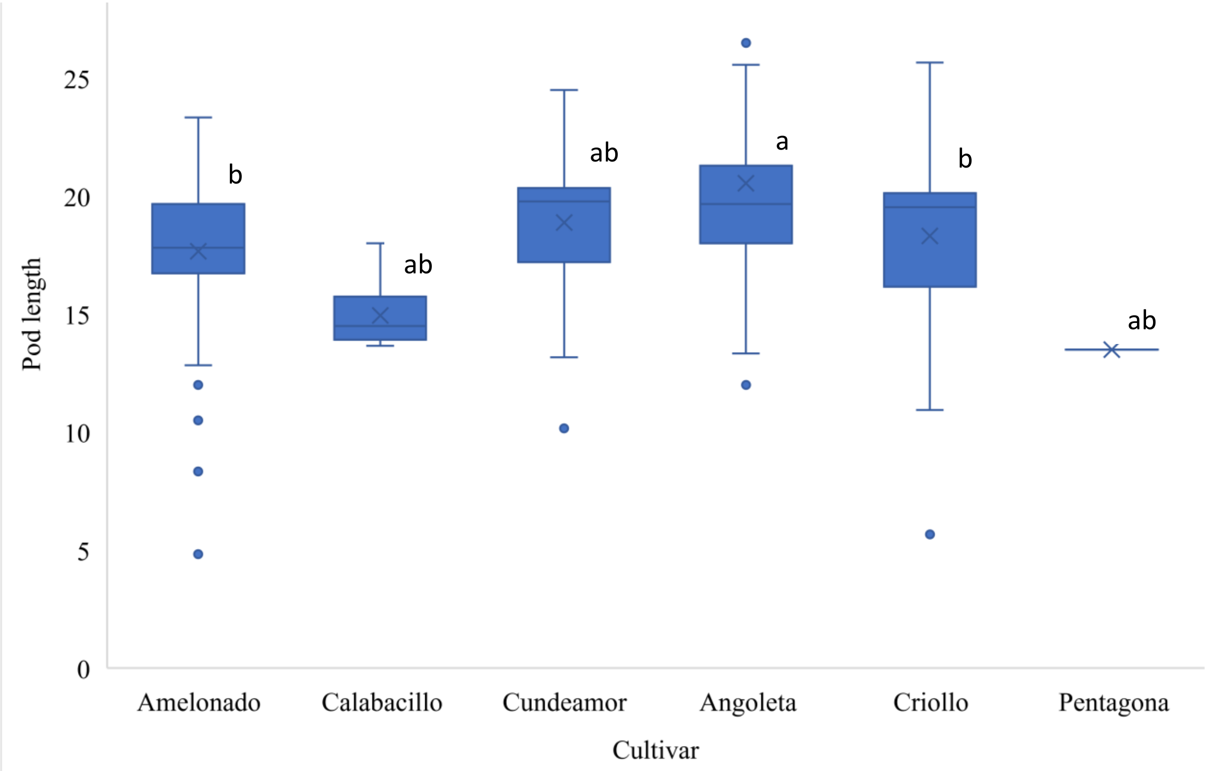

Figure 7: Boxplot of pod length of cacao cultivars with Tukey`s HSD compact letters

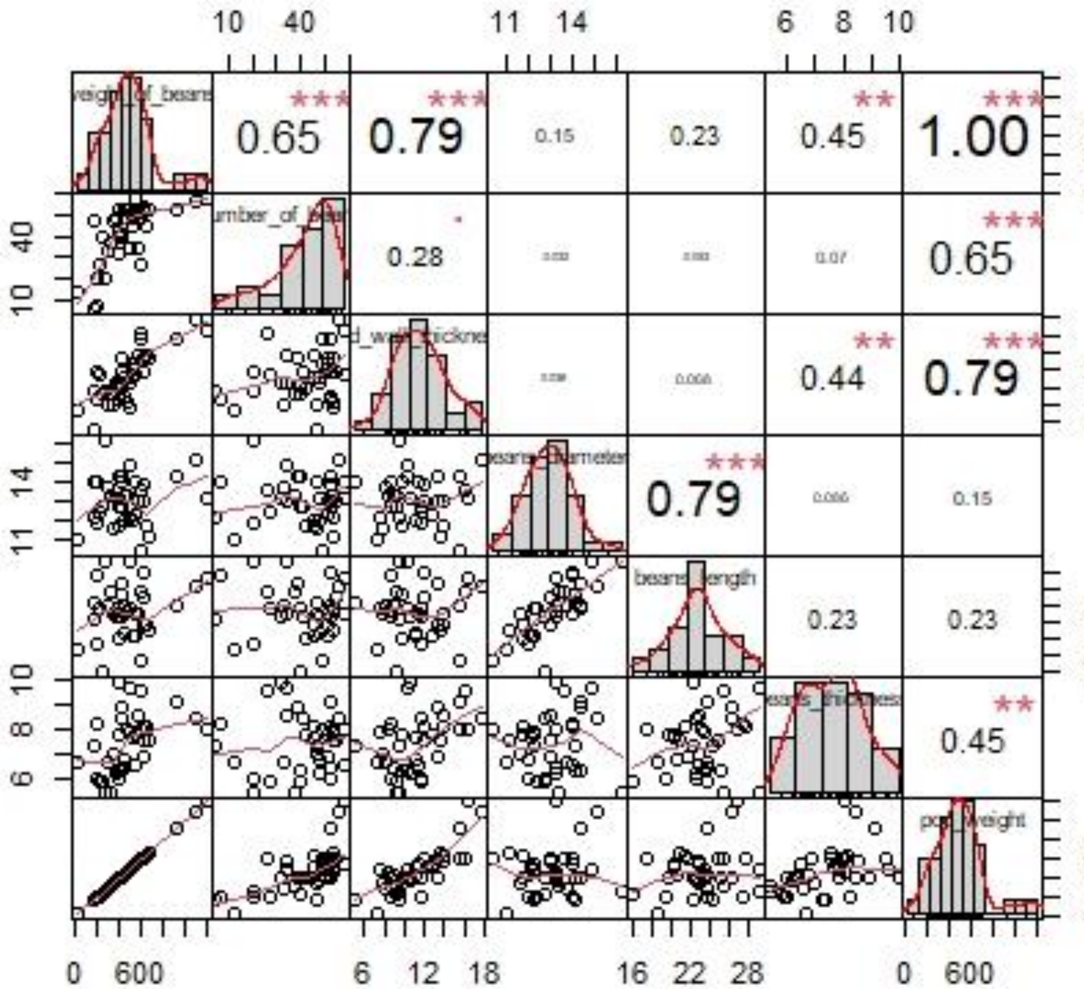

Figure 8: Correlation plot of pod and beans parameters showing the pod weight, pod wall thickness, pod length, number of beans, beans length, beans thickness at P < 0.001 and P < 0.01

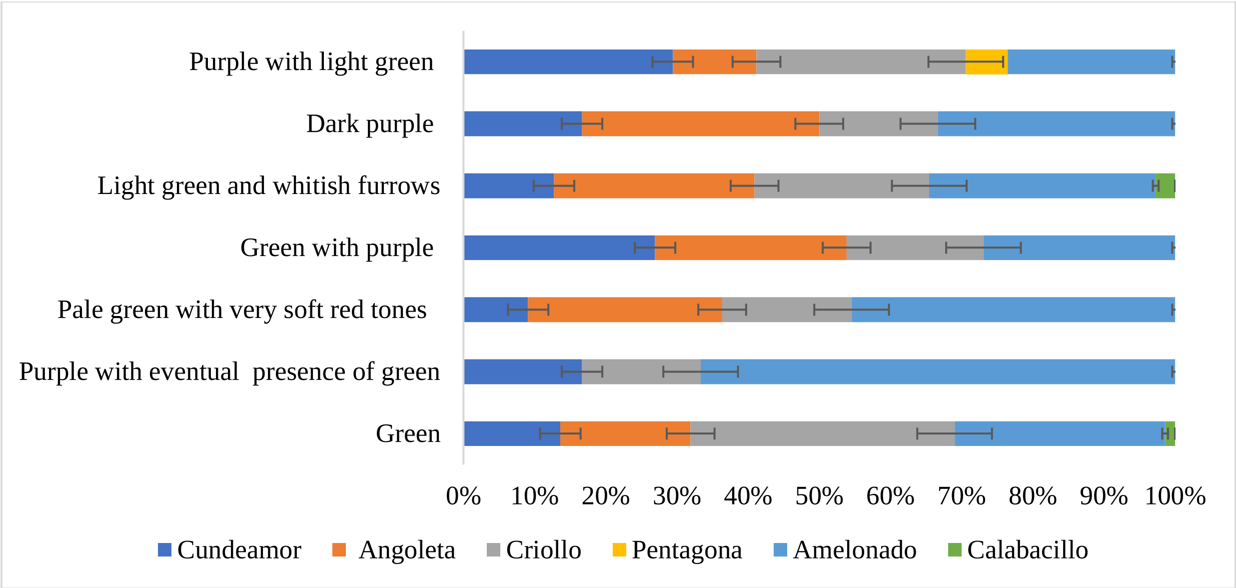

Figure 9: Unripened pod color of the different cacao cultivars observed on farmers` field

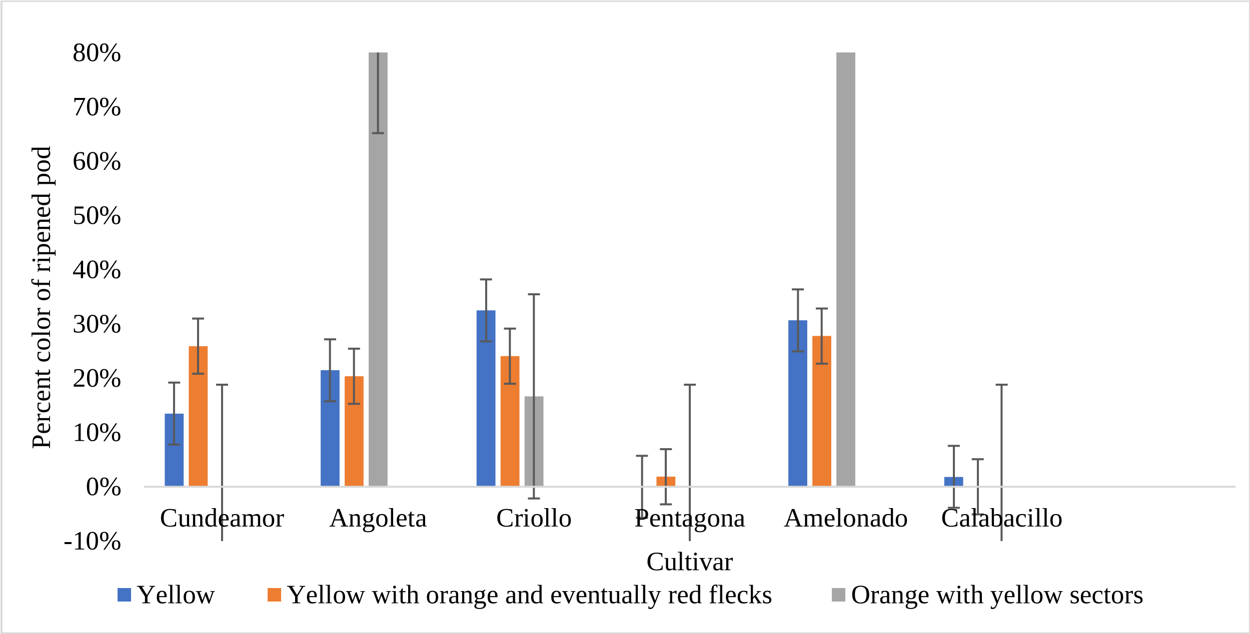

Figure 10: Color of the ripened pod of cacao cultivars surveyed on farmers` farm in Ghana

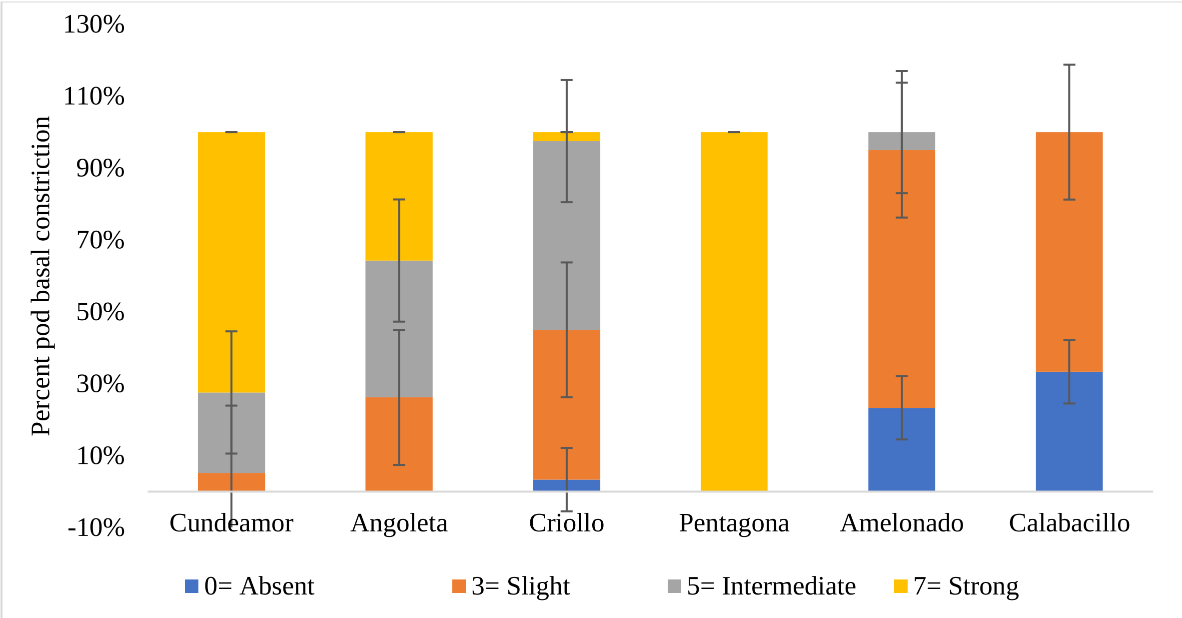

Figure 11: Percent pod basal constriction of the different cacao cultivars in Ghana

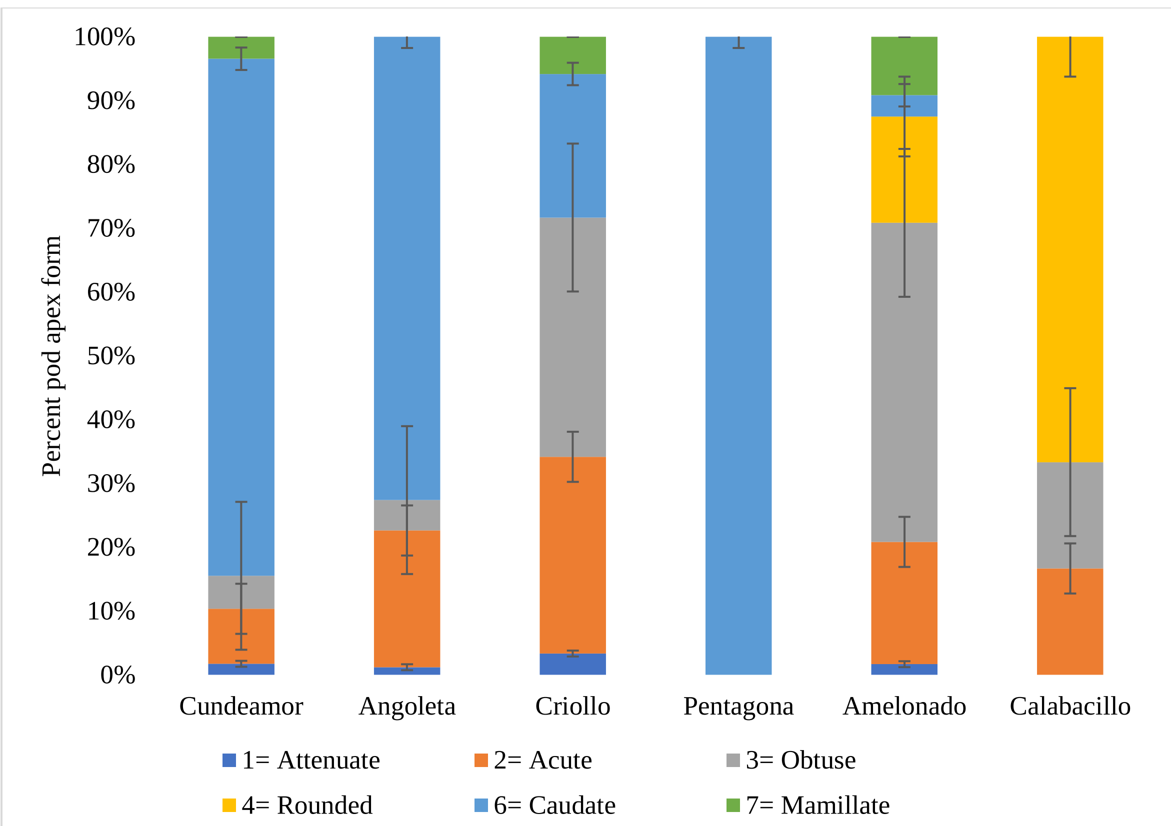

Figure 12: Percent of different pod apex forms of the six cacao cultivars observed on-farm

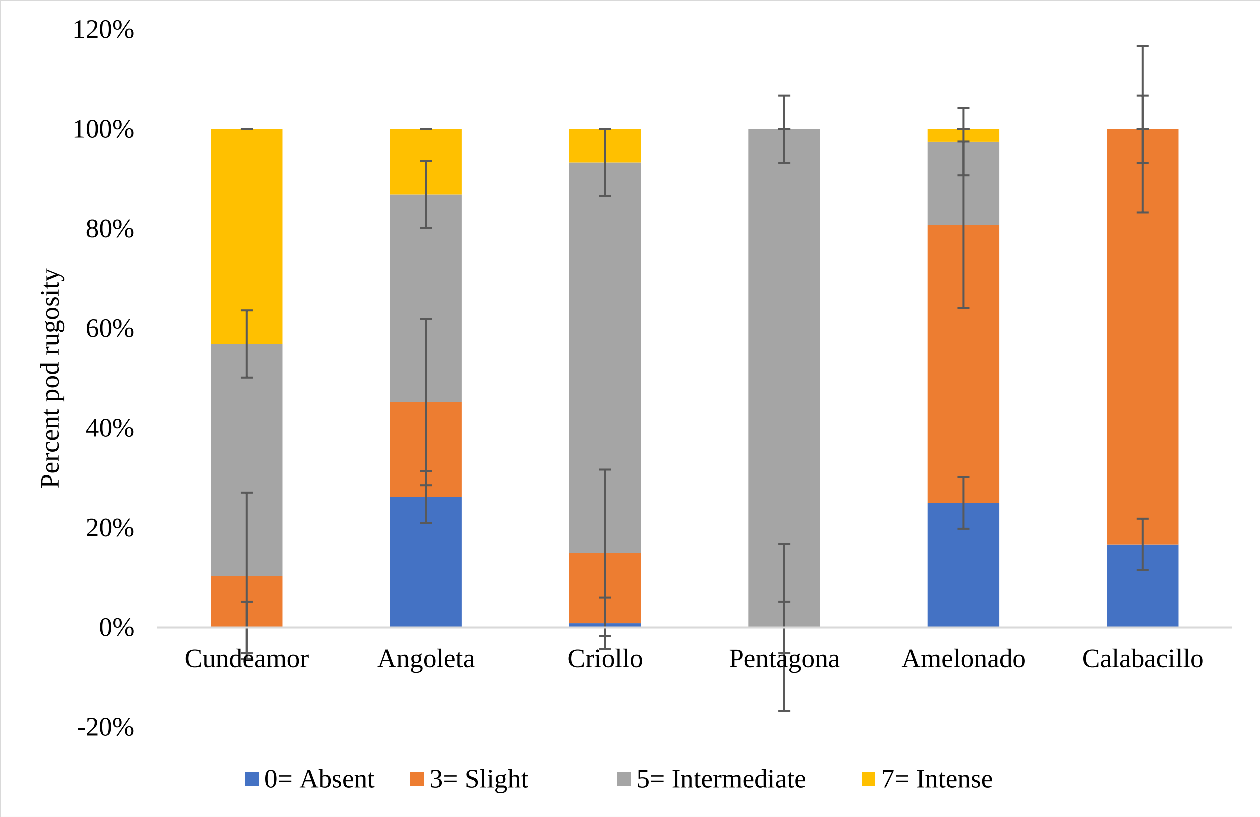

Figure 13: Percent count pod rugosity with the standard error bars of the six cacao cultivars in Ghana

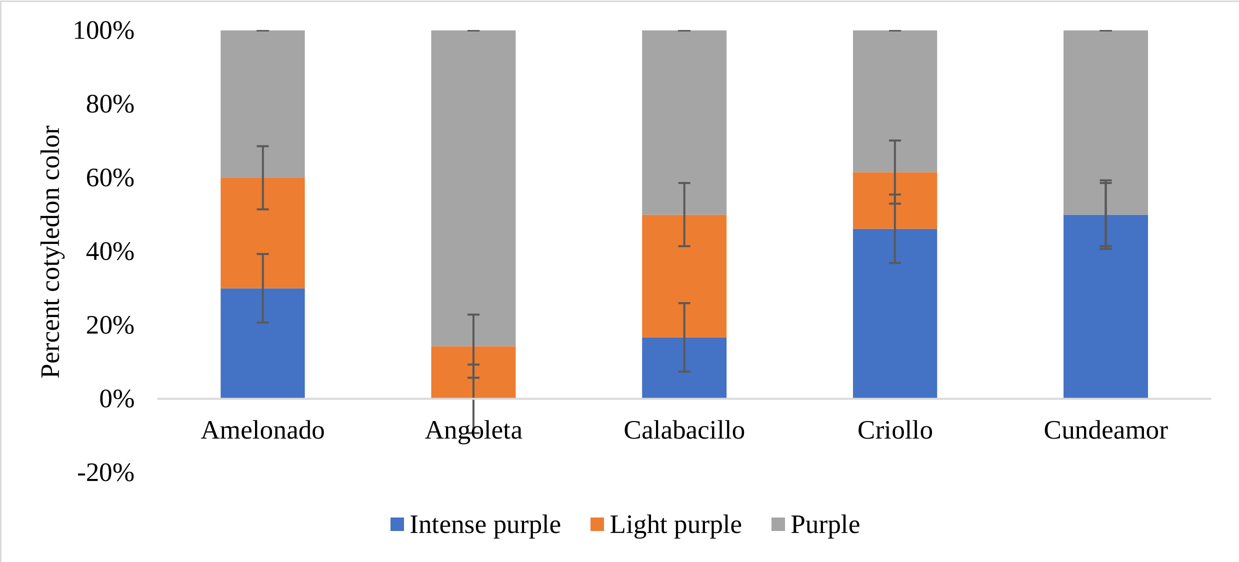

Figure 14: Beans cotyledon color from intense purple, light purple to purple of the cacao cultivars collected on-farm, Ghana

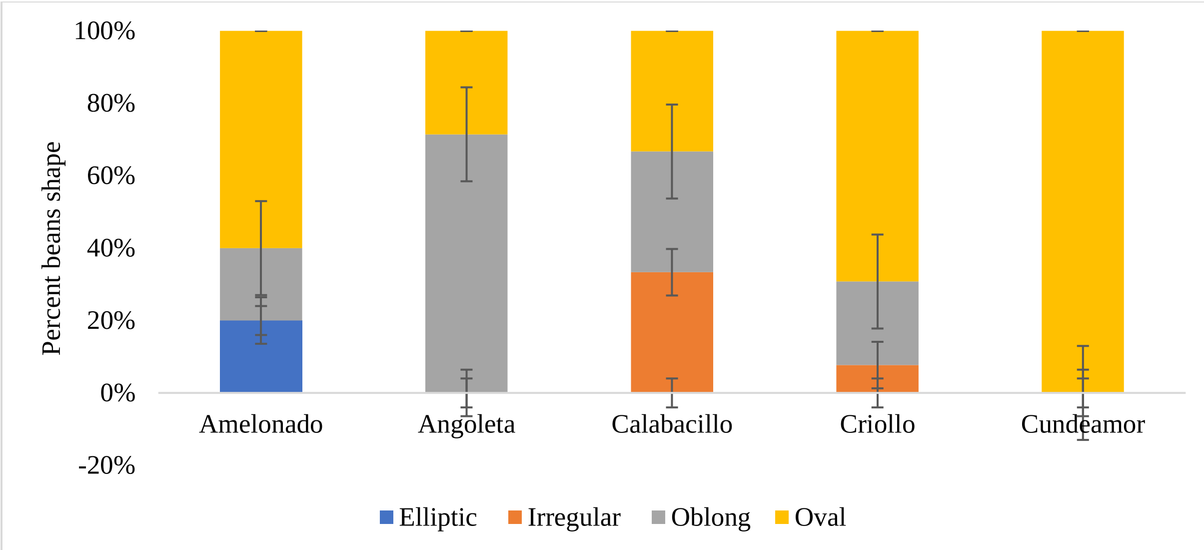

Figure 15: Percent beans shape and standard error bars of the cacao varieties observed on farmers` farm

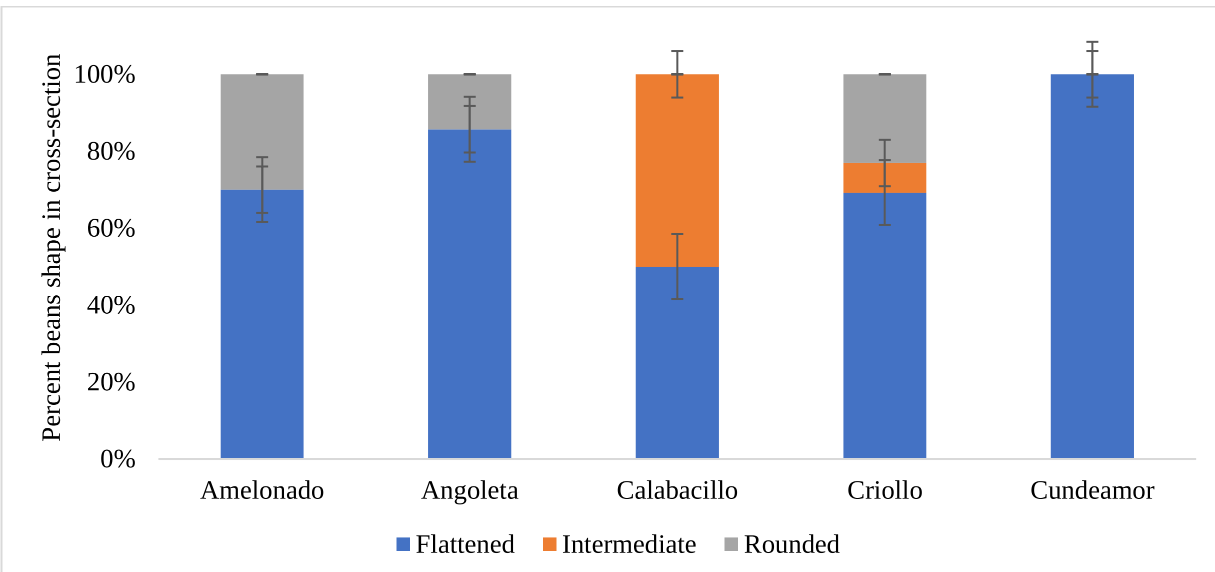

Figure 15: Percent beans shape in cross-section ranging from flattened, intermediate to rounded

5. Discussion

Morpho-agronomic characterization of cacao pods, seeds and flowers have been used by other authors to establish the relationship among genotypes. Other diversity research focused on field genebank accessions (Engels, 1986; Bekele & Bekele, 1996; Lachenaud, Bonnot & Oliver, 1999; Lachenaud & Oliver, 2005). However, this study concentrated on cacao varieties, varietal richness and diversity maintained on farmers` fields.

All quantitative and qualitative traits studied were useful in the characterization of cacao. The analysis of variation, pod, and bean characteristics indicated significant morphological diversity and richness in cacao varieties in farmers` fields. Pod length and width, pod color, pod shape, pod apex form, pod basal constriction, and beans weight were the most meaningful traits accounting for the variation among the cultivars and economic value. Furthermore, these traits explained more than 50% of all morphological variations studied comparably to the findings of Bekele et al. (2020).

Significant variation of pod and beans of all cultivars studied indicated the significance of on-farm collections as a precious pool of genetic diversity. The enormous cacao variations studied showed a high level of heterogeneity in the genetic resources preserved on-farm. Several traits are of economic importance and have been used as selection criteria by farmers in selecting parent materials for raising seedlings in new plantings and farm expansion.

From this study, Amazonian Forastero (Amelonado, Angoleta, Calabacillo, Cundeamor) and Criollo contributed to 99.7% of all cultivars studied. This explained that most of the cacao accessions in Ghana were derived from the Amazonian Forastero origin. This observation could confirm that Amazonia Forastero provides more than 95% of global cacao output (Aikpokpodion, 2010). Additionally, Amazonian Forastero consisted of a significant amount of morphological variation with mostly yellow ripened pods, flattened beans shape in cross-section, oval beans shape and intense purple to purple cotyledon color. The predominance of Cundeamor (14.9%) characterized by slight to strong basal pod constriction (100%), intermediate to intense pod rugosity (89.7%) and obtuse to attenuate pod apex forms (89.7%) indicated that the Upper Amazonia Forastero (UAF) ´Parinari´ and `Nanay` populations had a significant impact on cacao establishment in Ghana. This finding also corresponds to studies done by Aikpokpodion (2010) in Nigeria. Furthermore, Angoleta is characterized by its long pods with slight to strong basal constriction (100%) and is considered one of the best cultivars due to its pod thickness and a high number of beans as similarly reported by Ha et al. (2016) in Vietnam. Cultivars with the highest diversity in terms of unripened pod color are Cundeamor, Amelonado and Criollo. However, Criollo is characterized by intense purple to purple cotyledon color, oval bean shape (69.2%) and rounded bean shape in cross-section (69.2%), this finding was also reported by (Umaharan, 2018). Amelonado, Angoleta, Cundeamor, Pentagona and Calabacillo tend to be more vigorous than Criollo. This could explain why Criollo has a rich aroma, is low-yielding, less vigorous and highly susceptible to biotic and abiotic stress. On the other hand, Forasteros have weak beans aroma, are high-yielding and resistant to pests and diseases. From the study, farmers preferred selecting Upper Amazonian Forasteros and red-podded `Trinitarios` trees as parent materials due to their good tree vigor allowing for easier field establishment and high yielding ability. Calabacillo is characterized by its smooth small pod whilst Pentagona (alligator cacao) has a purple-light green unripened pod (Umaharan, 2018; Ha et al., 2016).

The existing field structure of cacao varieties reflected a swing from earlier years when uniform `Amelonado` cacao was chiefly grown. This could have resulted from the wide distribution of Upper Amazonian cacao cultivars to farmers via seed gardens. An observation by Bartley (2005), revealed that the introduction of planting materials from other sources into the original population could lead to crossbreeding between them. Consequently, recombination amongst the hybrids could result in genotypes which are genetically diverse from the parent accessions but conceivably have morphological traits of the parents (F1).

All farms were managed under an agroforestry system with different beneficial food crops and shade trees such as *Musa spp., Swietenia macrophylla, Terminalia superba, Entandrophragma cylindricum, Terminalia ivorensis, Chloroplora excelsa, Citrus sinensis.* According to the farmers, shade trees protected the cacao trees against adverse weather conditions. However, trees were pruned regularly to allow the interception of sunlight. According to the study, 53.7% of all individual trees surveyed aged less than or equal to 15 years (young cacao system). Traditionally, farmers selected seeds (52.8%) from the best-performing trees as the source of planting material. Moreover, direct seed planting (planting at stake) accounted for 81.5% of the planting materials used. The diameter at breast height recorded ranged from 3.38 cm to 30.02 cm depending on the age of the cacao tree. Cacao tree density ranged from 1,072 to 2,592 trees per hectare in conventional farms whilst organic farms recorded 736 to 2,240 trees per hectare. On the contrary, shade trees were higher in organic farms, with 6 to 82 trees per farm as compared to 3 to 25 trees per farm for conventional farms. This could explain why organic farms have lower cacao tree density.

IITA conventional farms were selected from agro-ecological Zones (AEZ) with existing cacao plantations aged from 8 to 22 years old and at an active economic production stage with good agronomic practices such as regular pruning, fertilization, and weeding. Besides, these farms were selected from areas with acrisols. IITA farms were managed by these cacao marketing and cooperatives; OLAM, Rockwind/TransRoyal, Kuapa kokoo and Mondelez. IITA farms had the highest varietal diversity because these farms are widely spread across all the cacao growing zones in Ghana. Organic farms are managed by the Yayra Glover cooperative and marketing company. These farms are only concentrated in a small zone in Suhum Municipality in the Eastern of Ghana. Furthermore, these farmers practiced good agronomic practices and used only PhytoGreen NPK organic fertilizer and AgroPy 5EW organic insecticide. The non-IITA conventional farms were partly managed by the Mondelez company, and the rest were self-managed. The farms were spread out in the Suhum Municipality, Eastern Region, Ghana. These farms are fertilized with conventional agrochemicals commonly found on the Ghanaian market such as SIDALCO NPK fertilizer.

6. Conclusion

Significant morphological diversity and evenness exist in Ghana`s cacao farms. The pod shape and color are the most significant morphological characteristics to distinguish individual cacao cultivars. Six cacao cultivars namely, Angoleta, Amelonado, Calabacillo, Criollo, Cundeamor, and Pentagona were discovered. Further field surveys of wild cacao discovery should be continued to better understand the traditional cacao richness and diversity in Ghana. These findings could serve as fundamental for genetic diversity research. It could provide useful evidence for future cacao breeding programs and expedite the design of strategies to successfully manage cacao's genetic resources. A comprehensive understanding of the morphological relationship among the cultivars studied alongside information on allelic richness could be valuable in selecting core parents. Depending on the breeding objectives, different cultivars with desirable traits could be utilized. Specifically, for yield, disease resistance, pod, and beans characteristics suitable to farmers and cacao product manufacturers, Angoleta, Cundeamor, Criollo and Amelonado could be selected.

7. References

Abdulai, I., Jassogne, L., Graefe, S., Asare, R., Van Asten, P., Läderach, P., & Vaast, P. (2018). Characterization of cocoa production, income diversification and shade tree management along a climate gradient in Ghana. *PloS one*, *13*(4).

Abdul-Karimu, A., Adomako, B., & Adu-Ampomah, Y. (2006). Cocoa introductions into Ghana. *Ghana Journal of Agricultural Science*, *39*(2), 227-xx.

Adeyeye, I. E., Akinyeye, O. R., Ogunlade, I., Olaofe, O., & Boluwade, O. J. (2010). Effect of farm and industrial processing on the amino acid profile of cocoa beans. *Food Chemistry*, *118*, 357–363.

Adomako, B., & Adu-Ampomah, Y. (2003, October). Assessment of the yield of individual cacao trees in four field trials. In *Proceedings of the International Workshop on Cocoa Breeding for Improved Production Systems* (Vol. 19, pp. 41-49). Accra: Cocoa Research Institute of Ghana; COPAL.

Afoakwa, E. O., & Paterson, A. (2010). Cocoa fermentation: Chocolate flavor quality. Encyclopedia of biotechnology in agriculture and food. U.K: *Taylor & Francis Publishing Inc*, 457–468.

Afoakwa, E. O., Paterson, A., Fowler, M., & Ryan, A. (2008). Flavor formation and character in cocoa and chocolate: A critical review. Critical Reviews in Food Science and Nutrition, 48, 840–857.

Aikpokpodion, P. O. (2010). Variation in agro-morphological characteristics of cacao, *Theobroma cacao* L., in farmers' fields in Nigeria. *New Zealand Journal of Crop and Horticultural Science*, *38*(2), 157-170.

Bartley, B. G. (2005). The genetic diversity of cacao and its utilization. Cabi, pp. 98-310.

Bekele, F. L. & Bekele, I. (1996). A sampling of the phenetic diversity of cacao in the international cocoa gene bank of Trinidad. *Crop Science /36*: 57_64.

Bekele, F. L., Bidaisee, G. G., Singh, H., & Saravanakumar, D. (2020). Morphological characterization and evaluation of cacao (*Theobroma cacao* L.) in Trinidad to facilitate utilization of Trinitario cacao globally. *Genetic Resources and Crop Evolution*, *67*(3), 621-643.

Bunn, C., Läderach, P., Quaye, A., Muilerman, S., Noponen, M. R., & Lundy, M. (2019). Recommendation domains to scale out climate change adaptation in cocoa production in Ghana. *Climate Services, 16*, 100123.

Cacaoweb (2020). About the Cacao Tree. Retrieved from http://www.cacaoweb.net/cacao-tree.html.

Carr, M. K. V., & Lockwood, G. (2011). The water relations and irrigation requirements of cocoa (*Theobroma cacao* L.): a review. *Experimental Agriculture*, *47(4),* 653-676.

Cheesman, E. E. (1944). Notes on the nomenclature, classification, and possible relationships of cocoa populations. *Trop. Agric.* (Trinidad) 27:144-159.

Dormon, E. V., Van Huis, A., Leeuwis, C., Obeng-Ofori, D., & Sakyi-Dawson, O. (2004). Causes of low productivity of cocoa in Ghana: farmers' perspectives and insights from research and the socio-political establishment. *NJAS-Wageningen journal of life sciences*, 52(3-4), 237-259.

Dixon, P. (2003). Vegan, a package of R functions for community ecology. J. Veg. Sci. 14 (6), 927–930. Retrieved from https://onlinelibrary.wiley.com/doi/10.1111/j.1654-1103.2003.tb02228.x

Edwin, J., & Masters, W. A. (2005). Genetic improvement and cocoa yields in Ghana. *Experimental Agriculture*, *41*(4), 491-503.

Engels, J. M. M. (1986). The identification of cacao cultivars. Acta Horticultura /182: 195_202.

Everaert, H., Rottiers, H., Pham, P. H. D., Ha, L. T. V., Nguyen, T. P. D., Tran, P. D., ... & Messens, K. (2017). Molecular characterization of Vietnamese cocoa genotypes (*Theobroma cacao* L.) using microsatellite markers. *Tree genetics & genomes, 13(5),* 99.

FAOSTAT (2022) (Food and Agriculture Organization Statistics). Retrieved from https://www.fao.org/faostat/en/#data/QCL.

Ha, L. T. V., Hang, P. T., Everaert, H., Rottiers, H., Anh, L. P. T., Dung, T. N., ... & Messens, K. (2016). Characterization of leaf, flower, and pod morphology among Vietnamese cocoa varieties (*Theobroma cacao* L.). *Pakistan Journal Botany*, *48*(6), 2375-2383.

Hsu, J. C. (1996) Multiple comparisons, theory, and methods. Chapman & Hall, New York.

Hunter, J. R. (1990). The status of cacao (*Theobroma cacao*, Sterculiaceae) in the western hemisphere. *Economic Botany*, *44*(4), 425-439.

ICCO (International Cocoa Organization) Economic Committee (2014). *The cocoa market situation, Fourth meeting report, EC/4/2, London*, pp 1-14.

ICCO (International Cocoa Organization) (2015b). Fine or flavor cocoa. Retrieved from http://www.icco.org/about-cocoa/fine-or-flavour-cocoa.html.

ICCO (International Cocoa Organization) (2019). *ICCO Quarterly Bulletin of Cocoa Statistics, 45/4*, Cocoa year 2018/19.

Kongor, J. E., Hinneh, M., Van de Walle, D., Afoakwa, E. O., Boeckx, P., & Dewettinck, K. (2016). Factors influencing quality variation in cocoa (*Theobroma cacao)* bean flavor profile—A review. *Food Research International*, *82,* 44-52.

Lachenaud, P. H., Bonnot, F. & Oliver, G. (1999). Use of floral descriptors to study variability in wild cocoa trees (Theobroma cacao L.) in French Guiana. *Genetic Resources and Crop Evolution* /46: 491_500.

Lachenaud, P. H. & Oliver, G. (2005). Variability and selection for morphological bean traits in wild cacao trees (*Theobroma cacao* L.) from French Guiana. *Genetic Resources and Crop Evolution* /52(3): 225_231.

Läderach, P., Martinez-Valle, A., Schroth, G., & Castro, N. (2013). Predicting the future climatic suitability for cocoa farming of the world's leading producer countries, Ghana, and Côte d'Ivoire*. Climatic Change*, *119(3-4*), 841-854.

McKelvie, A. D. (1956). W.A.C.R.1. breeding material. *Proceedings of the Cacao Breeding Conference 1956:* 10:13. West African Cocoa Research Institute.

Ministry of Food and Agriculture (MoFA) (2020). Suhum Municipal Assembly. Retrieved from https://mofa.gov.gh/site/sports/district-directorates/eastern-region/209-suhum-municipal-assembly.

Motamayor, J. C., Lachenaud, P., Da Silva e Mota, J. W., Loor, R., Kuhn, D. N., Brown, J. S., & Schnell, R. J. (2008). Geographic and genetic population differentiation of the Amazonian chocolate tree (*Theobroma cacao* L). *PloS one*, *3*(10), e3311.

Nadege, M. T., Claude, S., Roger, K. B. L. P., Cedric, C. D., Ntsomboh, N. G., Yonkeu, N. A. F., ... & Louis, Z. (2020). Ecological and Economic Potentials of Cocoa Agroforestry Systems in the Center Region of Cameroon. *American Journal of Agriculture and Forestry, 8*(5), 214-222.

Ofori, A., Padi, F. K., Ansah, F. O., Akpertey, A., & Anim-Kwapong, G. J. (2016). Genetic variation for vigour and yield of cocoa (*Theobroma cacao* L.) clones in Ghana. *Scientia Horticulturae*, *213*, 287-293.

Oderinde, F. O., & Afolayan, O. S. (2021). Evaluation of the Capacity of Agroforestry of Cocoa Trees in Atmospheric Carbon Dioxide Reduction. *Journal of Applied Sciences and Environmental Management*, *25*(7), 1231-1237.

Ortiz de Bertorelli, L., Graziani de Fariñas, L., & Rovedas, G. (2009). Influence of several factors on characteristics of the fermented and sun-dried cocoa bean1. *Tropical Agronomy*, *59*(2), 119-127.

Oyekale, A. S. (2020). Dataset on cocoa production and climate change adaptation strategies in Ahafo Ano North District, Ghana. *Data in brief*, *29*, 105275.

Padi, F. K., Ofori, A., Takrama, J., Djan, E., Opoku, S. Y., Dadzie, A. M., ... & Zhang, D. (2015). The impact of SNP fingerprinting and parentage analysis on the effectiveness of variety recommendations in cacao. *Tree Genetics & Genomes*, *11*(3), 1-14.

Peprah, K. (2019). Cocoa Plant, People and Profit in Ghana. In *Theobroma Cacao-Deploying Science for Sustainability of Global Cocoa Economy*. IntechOpen.

Phillips-Mora, W., Arciniegas-Leal, A., Mata-Quirós, A., Motamayor-Arias, J. C. (2013). *Catalogue of Cacao Clones: Selected by CATIE for Commercial Plantings*. CATIE.

Posnette, A. F. (1943). Cacao selection on the Gold Coast. *Trop. Agric., Trin*. 20, 149-155.

Pound, F. J. (1938). Cacao and Witchbroom Disease (*Marasmius pemiciosus*) of South America with Notes on Other Species of *Theobroma*. Yuilles Printerie, Trinidad and Tobago.

Rogers, H. H. and Knight, R. (1953). Plant breeding at W.A.C.R.1. *Report of the Cocoa Conference held at Grosvenor House London,* 15-17 September 1953: 64:67. The Cocoa Chocolate and Confectionery Alliance Ltd.

Snoeck, D., Afrifa, A. A., Ofori-Frimpong, K., Boateng, E., & Abekoe, M. K. (2010). Mapping fertilizer recommendations for cocoa production in Ghana using soil diagnostic and GIS tools. *West African Journal of Applied Ecology*, *17*, 97-107.

Thresh, J. M., Owusu, G. K., Boamah, A. & Lockwood, G. (1988). Ghanaian cocoa varieties and swollen shoot virus. *Crop Prot.* 7, 209-231.

Toro-Uribe, S., López-Giraldo, L. J., & Decker, E. A. (2018). Relationship between the physiochemical properties of cocoa procyanidins and their ability to inhibit lipid oxidation in liposomes. *Journal of Agricultural and Food Chemistry*, *66*(17), 4490-4502.

Toxopeus, H. (1964). F3 Amazon cocoa. *Ann. Rep. West African Cocoa Res. Inst.* 1963-1964, 13-23.

Umaharan, P. (2018). *Achieving sustainable cultivation of cocoa*. Burleigh Dodds Science Publishing.

Vigneri, M., & Kolavalli, S. (2018). Growth through pricing policy: The case of cocoa in Ghana. *Background paper for UNCTAD-FAO Commodities and Development Report*. Pp 1-6. Retrieved from http://www.fao.org/3/I8329EN/i8329en.pdf.