

CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

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AgriSciences**

Genetic diversity of cacao (*Theobroma cacao*)

BACHELOR'S THESIS

Prague 2020

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Declaration

I hereby declare that I have done this thesis entitled Genetic diversity of cacao (*Theobroma cacao*) independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague 2020

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Jakub Samek

Acknowledgements

I would like to thank my supervisor Ing. Marie Kalousová who had that unbelievable patience with my work process and helped me to finish this paper.

Abstract

The rainforests of South America are home to *Theobroma cacao*, L. It is an evergreen plant, 4-8 m tall, belonging to the *Malvaceae* family, which is grown in the shade. Theobroma means in Greek "food of the gods" derived "*Theos*" - god and "*Broma*" - food. The varieties are divided into Criollo, Forastero, and Trinitario. Cacao produces beans that are full of seeds and wrapped in a sweet wrapper. Chocolate is then made from the seeds by further processing. Cacao is therefore one of the important economic crops. Cacao, like any crop, faced various diseases, pests, and external influences. Cacao production suffered considerable damage in the early 19th century due to various fungal diseases decimating entire plantations. Modern methods of protecting genetic resources have now been used to stop the genetic erosion that intensive agricultural production has brought. Thanks to this, the methods of microsatellites were used, which helped to trace the original wild generations of cacao and to divide them into ten groups. This allowed them to be compared to the cultivars used in production with the potential to create a new hybrid individual with better traits for better traits and resistance.

Key words: genetic diversity, germplasm conservation, *Theobroma cacao*, L.

Abstrakt

Deštné pralesy Jižní Ameriky jsou domovem *Theobroma cacao*, L. Jedná se o stále zelenou rostlinu, 4-8 m vysokou, patřící do rodiny Malvaceae pěstovanou ve stínu. *Theobroma* znamená v řečtině "jídlo bohů" odvozeno "*Theos*" - bůh a "*Broma*" - jídlo. Odrůdy se dělí na Criollo, Forastero a Trinitario. Kakao produkuje boby, které jsou plné semen a obalené ve sladkém obalu. Ze semen se poté dalším zpracováním vyrábí čokoláda. Kakao je tudíž jednou z důležitých ekonomických plodin, jako každá plodina čelilo různým nemocem, škůdcům a vnějším vlivům. Produkce kakaava utrpěla značné škody na začátku 19. století díky různým houbovým nemocem decimující celé plantáže. Nyní jsou využívány moderní metody ochrany genetických zdrojů pro zastavení genetické eroze, které intenzivní zemědělská produkce přinesla. V důsledku toho byly využity metody mikrosatelitů a ty pomohly vystopovat původní divoké generace kakaava a nově je rozdělit to deseti skupin. Toto umožnilo je porovnat s kultivary použitými v produkci s potenciálem pro vytvoření nového hybridního jedince s lepšími vlastnostmi a vyšší odolností.

Klíčová slova: genetická diverzita, ochrana genetických zdrojů *Theobroma cacao*, L.

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1. Introduction

Theobroma cacao, L. originates in the tropics of South America, with the centre of origin in the Upper Amazon (Zhang et al. 2006). The vast majority, up to 90%, of cacao is grown by many smallholder farmers for which the income from the production is essential in the production regions including Latin America, West Africa, and Indonesia (Rice & Greenberg 2000). The species *Theobroma cacao*, L. includes a large variety of highly variable populations. Those populations can all be crossed within each one another (Bartley 2005). However, Cacao, as well as other crops, is suffering from genetic erosion.

Since the 1900s the genetic erosion became a large global problem. Over 75% of plant genetic diversity has been lost since local farmers started using high-yielding varieties that are genetically uniform instead of the local traditional varieties (FAO 2010). This step leads to lowering the agrobiodiversity which can effect sustainable yield, adaptability of the crops for climate and environmental changes, their resistance to diseases and pests, and general food security.

In the case of *T. cacao*, L. the risk of genetic erosion was upscaling due to many factors like deforestation, disease pressure, or replacement of primitive cultivars with modern ones with yield. Nowadays, there is a demand for more resistant hybrid cultivars from breeding programmes with the achievement in increasing the genetic diversity (Eskes & Efron 2006).

There is a high level of native intraspecific variations which are often mixed with introduced germplasm of the modern varieties when the crops are cultivated in the place of their origin. In recent years the South American region experienced the germplasm introduction from exotic varieties from other global cacao production regions with reduced diversity (Zhang et al. 2012). In-situ conservation is recognised as an important step for planning necessary on-farm conservations and rehabilitations as a strategy for crop genetic resources (Zhang et al. 2012).

2. Aims of the Thesis

The general aim of the thesis was to compose an in-depth literature review of the genetic diversity of cacao in the main cacao producing regions in the world. The specific objectives were to evaluate the state of the effectivity of germplasm conservation and to review the potential of utilization of cacao genetic resources in overcoming the main issues that global cacao production is facing.

3. Methodology

Present knowledge about the genetic diversity of cacao (*Theobroma cacao*, L.) will be reviewed by description and comparison of the plants. The main methods for assessment of genetic diversity will be summarized and the state of utilization of existing cacao gene pool in cacao producing countries will be analysed and compared to the germplasm available in wild populations. As a resource for the information in the review will be used scientific databases such as Scopus (<https://www.scopus.com>), Web of Science (<https://www.webofknowledge.com>), etc., scientific journals, and specialized books. The key topic in searched articles will be cacao genetic diversity and its impacts.

4. Literature Review

4.1. Cacao (*Theobroma cacao*, L.)

Cacao (*Theobroma cacao*, L.) is an evergreen 4-8 m tall tree belonging to family Malvaceae and the genus *Theobroma*, which in Greek means "food of gods." The genus *Theobroma* also includes 25 other species; however, they do not have the same economical potential. All these species occur almost exclusively in Latin America (Pospíšil & Hrachová 1989).

4.2. Botany

The root system is formed by main and lateral roots. The main root can reach up to 2 m depth after 10 years of growth which helps to secure the tree. Side roots are located in the upper 20 cm of the soil where they can grow up to 6 m length (Valíček & et al. 2002).

The trunk is woody with a diameter of around 30 cm. The colour of the bark ranges from light grey up to darker brown. After 18 months of age, the growing process is interrupted, the terminal bud is replaced by three to five axillary buds. The crown is formed by horizontal branches with flowers and alternate leaves and vertical branches with opposite leaves and no flowers. Leaves on the vertical branches are unlobed, up to 50 cm long and 5–10 cm broad, and have a petiole of 7-9 cm of length. The alternate leaves on the horizontal branches are smaller and shorter (Valíček & et al. 2002).

The flowers are relatively small and grow directly on the trunk and older branches. They are from white to pink, with sepals, five petals, five stamens, and one pistil. The flowers are hermaphrodites. It is an outcrossing species due to the action of self-incompatibility mechanisms in wild individuals, while the cultivated ones are generally self-compatible (Motamayor et al. 2008). While many of the world's flowers are pollinated by bees (*Hymenoptera*) or butterflies/moths (*Lepidoptera*), cacao flowers are pollinated by tiny flies, *Forcipomyia* midges in the subfamily *Forcipomyiinae*. Using the

natural pollinator *Forcipomyia* midges for *T. cacao* was shown to have more fruit production than using artificial pollinators (Forbes & Northfield 2017).

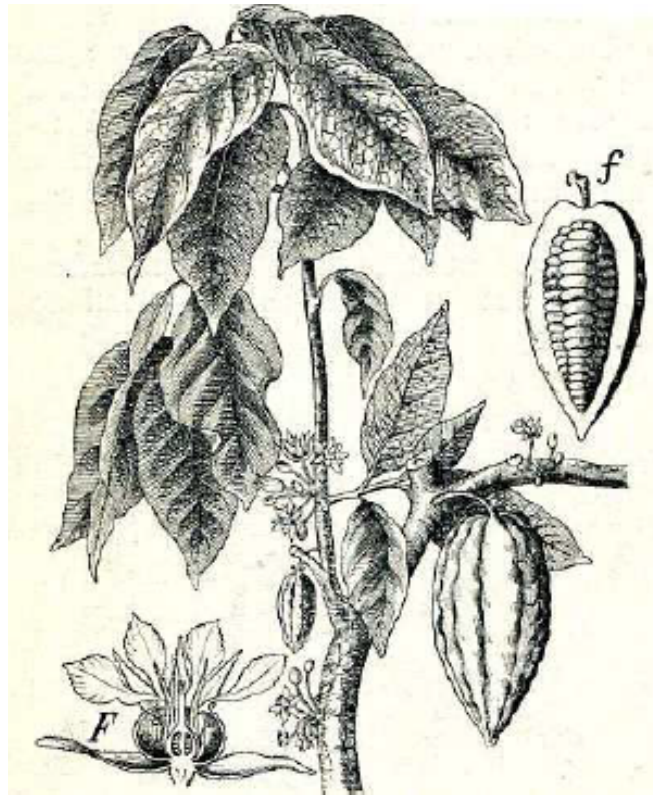


Figure 1. *Theobroma cacao*, L. (Polivka 1908)

The fruit is ovoid shape drupe 15-30 cm long and 8-10 cm wide. The fruit, commonly known as a pod, contains the seeds embedded in a mucilaginous pulp with sweet and sour taste notes. Each pod contains 20-60 seeds arranged in 5 rows. The fruit colour varies from white to deep purple and weighs about 500 g when harvested (Wood & Lass 2001).

4.3. Ecology

Cacao is grown in areas where the temperature is between a maximum of 30-32°C and a minimum of 18-21 °C, where growth stops. The natural habitat for cacao is the lower floor of the evergreen rainforest, where a minimum of sunlight penetrates and there is almost no air movement. Cacao tree freely occurs in well-watered and well-drained locations such as near rivers on alluvial soils (Wood & Lass 2001).

The hot and moist climate is suitable for cacao growth. In countries without a dry season, cacao will grow faster than in countries where growth can be stopped by low temperatures or drought in some months of the year (Wood & Lass 2001).

In the north of the Amazon River, where *T. cacao* originates the annual rainfall is over 2,000 mm and is evenly distributed. If the precipitation is less than 1,250 mm, the evaporation from the soil may exceed the precipitation, so irrigation should be considered when planting. Annual rainfall above 2,500 mm can lead to higher cases of fungal diseases. High precipitation results in heavy soil leaching and, in general, less fertile soils would be expected under heavy rain conditions (Wood & Lass 2001).

Due to the lack of light on the lower floor of the rainforest, the cacao tree has traditionally been grown in the shade. It originates from the Amazon rainforest, which gave the plants natural shade. Shading is therefore necessary for the first few years of cacao growth. During the first stages of growth, cacao plants need shading that transmits 20-50% of light. Fully developed trees can provide shade themselves, so shading needs to be reduced so that 70% of the light is released (Wood & Lass 2001).

Growing cacao at higher altitudes is affected by temperature. Most cacao growing areas are below 300 masl but can be found at higher altitudes as well. One of the most extensive cacao areas is in eastern Cameroon, which lies at about 600 masl, but cacao can grow in much higher altitudes. In Colombia, cacao cultivation can be found in 900 masl in the Cauca valley. In other parts of Colombia, the cacao has been successfully grown at altitudes up to 1,400 masl (Wood & Lass 2001).

Cacao is very sensitive to water shortages and therefore needs soil containing coarse particles with adequate amounts of nutrients to a depth of 1.5 m to enable aeration, which will allow the development of a good root system. Sufficient water retention properties, as well as good drainage, are essential for cacao to withstand wetting. The ideal soil pH for cacao is between 5-8 with an optimal value of 6.5. Cacao must be grown in soil rich in organic matter. Organic matter should be present in about 3.5% of the top 15 cm of soil. Chemical properties of the soil are the most important since most absorbing roots are in the top of the soil (Wood & Lass 2001)

4.4. Production

Cacao (*Theobroma cacao*, L.) is grown for commercial purposes in the moist, tropical "cacao bean belt," 20° above and below the equator (Fulton 1989). Africa leads the production by 70% and the biggest producers are Côte d'Ivoire, Ghana, and Indonesia as shown in Table 1. The world average yield is 443 kg. ha⁻¹ and the total production is 5,252,377 (FAO 2018). The yield is expected to be growing by 2.3% a year in the African region and by 2,7% a year in Far East Asian region but the increase in production in Latin America is expected to grow slower due to the previous production loss caused by witches' broom disease which forced smallholder farmers to orient to alternative crop production (FAO 2003).

Table 1. Largest producers of cacao beans (FAO 2018)

Rank	Country	Production (1000 tons)	Yield (kg. ha ⁻¹)
1.	Côte d'Ivoire	1,963	489
2.	Ghana	947	529
3.	Indonesia	593	353
4.	Nigeria	332	281
5.	Cameroon	307	409
6.	Brazil	239	414
7.	Ecuador	235	468
8.	Peru	134	839
9.	Dominican Republic	85	555
10.	Colombia	52	362

Source: FAOSTAT

Although cacao production in African countries is large, cacao processing is more common in developed countries. In countries such as the Netherlands, Belgium, and the USA cacao has traditionally been processed. Recently, however, Indonesia, Malaysia, and Côte d'Ivoire have joined these traditional countries as shown in Table 2.

Table 2. Cacao Beans and products processing countries (FAO 2018)

Country	Processing (1000 tons)
1. Indonesia	858
2. Netherlands	699
3. United States of America	448
4. Germany	423
5. Brazil	241
6. France	209
7. Belgium	163
8. Malaysia	156
9. Côte d'Ivoire	141
10. Italy	131

Source: FAOSTAT

4.5. Cultivation

Cacao's natural environment is under the canopy of large tropical forest trees. When Cacao is cultivated, it is mostly grown under shade. Planting under thinned forests might appear to be the best way and in West Africa, this method is used almost exclusively. The method is cheap and simple and relatively quick, but it has considerable disadvantages. The forest trees may not provide the right type of shade (Wood & Lass 2001).

The shade is needed to obtain adequate nutrients and moisture throughout the year. Shading is needed for mature cacao as well as for young cacao to ensure the right form of growth. The amount of light falling on a young tree will influence the growth, low light

intensities with heavy shade leading to long internodes and few side branches. With the high light intensities and with little shade the opposite effect will occur which leads to bushy growth. Too much light is, therefore, undesirable as it will delay the time when, at normal spacings, a canopy will be formed. The early formation of a canopy is necessary to reduce weed growth and to allow the shade to be reduced to promote flowering and cropping (Wood & Lass 2001). Shade trees also serve to lessen the winds with the effect of protection from windborne spores of fungal diseases. Depending upon the species involved, shade trees can also fix atmospheric nitrogen and hold it within the soil layer. (Rice & Greenberg 2000). One study has shown that shade also promotes greater long-term production of older cacao plants with low levels of fertilization. (Ahenkorah et al. 1974). Lee and Garot (1972) claimed that cacao can be planted without shade. This was based on experience with planting eight-month-old seedlings in the field following clear-felling in Malaysia. Despite the lack of shade, losses of cacao seedlings during the six months after planting were less than 2 percent.

Another factor besides the protection from pests and diseases to be considered is protection from wind which can cause mechanical damage by rupture at the pulvinus, the swelling at the base of the leaf. Even low wind speeds of 3-4 m per sec can cause considerable damage if they are persistent (Wood & Lass 2001).

Propagation is mostly made by rooted cuttings or by buddings, but both methods suffer from the disadvantage that the planting material, for cuttings or budwood, is more freely available from fan shoots than from chupons. Fan shoot material has a spreading bushy growth which requires pruning. On the other hand, chupon material will develop like a seedling but usually, there is only a small quantity available. Clonal propagation is often done to preserve the unique characteristics of selected genotypes (Wood & Lass 2001).

The use of fertilizers is difficult because of its effectiveness. The effectiveness of fertilizers depends on many factors such as the type of fertilizer used, the time and method of application, the type of soil, and the crop system (Hamzat et al. 2006). Another source of nutrients is from the leaf litter from trees. The leaf litter provides mulch and supply of organic matter for the soil which, in turn, can increase aeration, infiltration, and drainage, as well as result in a slow and steady release of nutrients into the soil (Rice & Greenberg

2000). However, it has been reported that nitrogen-fixing leguminous trees can enrich soils in cacao agroforest with 70 kg/ha nitrogen (Bos et al. 2007).

Weeding should be carried out on young stands 4 to 5 times a year. The roots are usually in the upper soil layer, so weeds must be removed carefully to avoid damaging them (Hamzat et al. 2006). Pruning is also an important technique. There are two types of pruning: formative and managing pruning. Formative is advantageous to form a crown in trees originating from cuttings. Lateral branches inclined to the ground should be cut off. The managing pruning is used to remove old and dry branches, parasitic and epiphytic parts. This pruning can increase yield (Wood & Lass 2001).

Harvesting is done by hand with machetes or knives in the regular range of 10-15 days. To maintain the quality, it is important to harvest only ripe pods, which can be recognized by changing the color from green to yellow or from red to orange, and thanks to the fact that they emit a duty sound when tapped. To ensure the production of quality beans, only mature and ripe pods that are not diseased or damaged must be harvested. Harvesting is done twice a month for the main crop season which is from August to January and at least once a month for the light crop season from February to May (Hamzat et al. 2006).

4.6. Uses

The main parts that are processed from the cacao tree are the seeds of the fruit, which are a source of commercial cacao, chocolate, and cacao butter. For the seeds to be processed, they must be fermented. Fermentation takes place to remove the rest of the seed pulp residues covering the seeds, which will decompose within 24-36 hours due to microbial activities, to kill unwanted germs, and to create the chemical compounds associated with desirable chocolate flavour. (Wood & Lass 2001).

Next, the seeds are roasted, crushed, and finally ground to obtain a powdery mass from which fat (cacao butter) is extracted. Cacao butter is used in confectionery and the manufacture of tobacco, soap, and cosmetics (Lim 1993).

In the preparation of chocolate, the cacao mass is mixed with sugar, flavour, and extra cacao butter. Although the production of chocolate is by far the most important way of processing cacao, this ingredient can be used in other ways such as Dutch-process

chocolate which is a basic staple ingredient. It is used as an ingredient in various products and at the same time cacao is the basis of instant hot chocolate. Hot chocolate is the forerunner of solid chocolate, so it is no wonder that has been gaining great popularity over the centuries. As a result, the variety of ingredients that are added to liquid chocolate is perhaps even greater than in the case of solid chocolate (Pospíšil & Hrachová 1989).

The other parts of the fruit such as seed pulp are consumed locally as fruit and find use in products such as yogurts, juices, ice cream and also can be used to prepare jams, liqueurs, vinegar, or a drink from roasted seed peels (Polívka 1908). The pods can be fed to animals, and the pruned wood of older trees is used to produce charcoal (Lim 1993).

4.7. Pests and diseases

Cacao as a suitable crop for small farms as well as large plantations and can suffer from a variety of diseases. The major diseases are fungal. Fulton (1989) refers to those as a “trilogy of crippling fungal diseases: black pod (*Phytophthora spp.*), Monilia pod rot (*Moniliophthora roreri*), and witches'-broom (*Crinipellis perniciosa*)”.

Attack by disease or pests may result in a direct loss of crop. Wood and Lass (2001) state that to counter disease it is first necessary to identify the cause, then to assess the damage likely to be caused; possible control measures should then be examined. In certain locations, several cacao diseases can be controlled economically. Control can be achieved by simple field sanitation which might involve a change in the level of shade or improved drainage, though in other cases spraying may be necessary. Some diseases such as the virulent forms of the virus are very difficult to control, and the diseased trees have to be cut down, but even then it is difficult to eradicate the disease (Wood & Lass 2001).

4.7.1. Phytophthora pod rot (often called ‘black pod’)

World losses due to black pod have been estimated recently as 450,000 t a year, probably accounting for 20 to 25% of the expected crop and making it the biggest constraint to production. However, the genus *Phytophthora* is now known to have its complex on each continent which can induce black pod symptoms in cacao (Evans 2007).

The initial symptoms are the appearance of a small translucent spot on the pod surface appearing about two days after infection under conditions of high humidity. The spot darkens rapidly turning the whole surface of the pod black within 14 days ending in rotting or necrosis of the pod. This pathogen can also infect other parts such as cushions, leave, shoot, seedlings, and roots. The spores can survive and perhaps multiply in the soil (Wood & Lass 2001).

The incidence of black pod peaks at harvest. In drought years, when infection levels are low, growers and spray masters become lax with their programs. Then when climatic conditions are conducive and disease levels skyrocket, they apply excessive amounts of fungicides at short intervals (Fulton 1989). Chemical control of pod rot by spraying with copper fungicide is a well-established control method, but it is expensive and not completely effective. Cultural techniques such as shade reduction, regular harvesting, and frequent weed control may reduce infection, but some losses from *Phytophthora* pod rot are almost inevitable if the area is subject to long periods of high humidity. In such areas, it is not unusual to see a tree with every pod attacked by the disease if no control measures are adopted. The long-term solution must lie in breeding for resistance or tolerance to the disease and in obtaining a better understanding of the methods of spread (Wood & Lass 2001). Yet another control strategy that has yet to be fully tested is the manipulation of potential arthropod vectors, notably tent-building ants, which have been associated with disease foci in the cacao canopy. Removal of black pods from the soil surface would be a simple strategy to reduce inoculum spread by ants, as well as by flying vectors (Evans 2007).

4.7.2. Witches' broom (*Crinipellis perniciosa*)

Witches-broom is endemic to the area where cacao thrives and can cause pod losses of 50% which is a factor limiting cacao production in several Latin American countries (Fulton 1989). However, losses have now been put at 250,000 t per year (Evans 2007). Ploetz (2007) tells us that Witches' broom can cause significant losses as it decimated production in Brazil, by the 50% pod losses within 6 years in R ondonia in the 1970s and then in Bahia in the early 1990s by 60% reduction in 5 years. Elsewhere in

South America and the Caribbean, losses were reported to range from 30 to 90% (Ploetz 2007).

The pathogen (*Crinipellis carniciosa*) causes the hypertrophic growth of buds to give the characteristic witches' broom from which the name is derived but also causes pod infections which can lead to a very high percentage of pod loss. The infection attacks all actively growing aerial meristematic tissue, resulting in hypertrophy. The 'fan brooms' produced on the fan branches, which may be terminal when the leading bud is attacked or lateral when an axillary bud is infected, are the most common. Flower cushion infection causes cushion brooms or strawberry-shaped pods. Early infection of the pods can destroy undeveloped beans and infection on the developed pods can result in some part of beans to be unusable. After 5-6 weeks the infected plant parts become necrotic. The conducive environmental conditions emerge usually 4-6 weeks from the onset of a wet period. Infection is caused by basidiospores, which are killed by exposure to sunlight and desiccation. The fungus can infect seeds, giving rise to infected seedlings (Wood & Lass 2001).

4.7.3. *Moniliophthora roreri* (frosty pod rot)

Frosty pod rot caused by *Moniliophthora roreri* is most alarming. Although it is currently responsible for less damage than the other mentioned diseases due to its absence in the major producing countries (Ploetz 2007). Conversely, the invasive frosty pod rot quickly overtook Witches' broom (*Crinipellis perniciosa*) as the principal constraint to production in the Peruvian region. Frosty pod rot represents one of the major global threats to the cultivation of *T. cacao*, perhaps reflecting the analogous situation of rubber and its coevolved pathogen, *Microcyclus ulei* which inhibited natural rubber production on a commercial scale in central and south America in the past century (Evans 2007).

The fungus only invades green pod tissues between the parenchyma cells of the cortex and produces conidia on the surface and within the host tissue (Wood & Lass 2001). In the first 90 days of development, pods are at high risk of infection. The incubation period is from 40 to 60 days depending on the age of the pod. The first notable symptoms are conspicuous swellings or areas of premature ripening on pod surfaces (Fulton 1989).

The key to effective control of frosty pod rot is to eliminate the primary inoculum sources which are the mummified hanging pods since they are at risk of infection for 90 days (Fulton 1989). Efforts to manage the disease must be in improving the quality of the planting material (Evans 2007).

4.7.4. Pests

The Mirids (capsids) are the most important and widely represented pests of cacao worldwide. In South America, the *Monalonion* species are present. In West and Central Africa, it is *Sahlbergella singularis* the pest occurring from Sierra Leone to the Congo, while *Distantiella theobroma* is especially active from the Ivory Coast to western Nigeria. These mirids have been a major problem in cacao production for over half a century reducing yield by as much as 75% (Wood & Lass 2001).

Cacao mirids perforate the surface of cacao stems, branches, and pods with the addition of killing the penetrated host cells and producing necrotic lesions. Pests usually appear in areas of the trees which are exposed to sunlight. Pesticides are still the main protection. An alternative to the purely chemical way is to attempt to lower pest numbers by the combined use of biological control and appropriate cultural methods such as shade and canopy management (Wood & Lass 2001).

4.8. Biological diversity

Cacao is native to the South American rainforests. It was cultivated by the Mayan civilization in Central America more than 2,600 years ago. As wild populations were not found in Central America, the cacao tree was likely introduced there (Arcimovičová & Valíček 1999). The location of the greatest diversity of wild cacao is in the Amazonian region. During the period of its domestication cacao was exposed to different destinations, which helped the worldwide spread of cacao cultivation before 1950. The cultivation helped to form traditional groups described as Criollo, Forastero, and Trinitario (Loor et al. (2009).

The spread of cacao trees began to occur only thanks to Europeans. At the turn of the 16th and 17th centuries, the Spaniards began to cultivate cacao in their American colonies. Venezuela soon became the largest cacao grower, holding its position until the

nineteenth century. Other major exporters included Trinidad, Colombia, and Haiti. Gradually, cacao production began to appear in Asia. It appeared on the Indonesian island of Sulawesi as early as 1560, and in 1670 it was planted in the Philippines. From there, cultivation spread throughout South and Southeast Asia. To India and Sri Lanka, the cacao tree came in 1798. The cacao tree was planted on the island of Bioko in 1822, from where it gradually began to spread to mainland Africa which is today's largest cacao producer. Towards the end of the nineteenth century, the popularity of chocolate in Europe increased rapidly, which, of course, raised the demand for cacao. The European countries used their African colonies, where the climate was suitable and the colonies were closer than America or Southeast Asia, where cacao was already grown. Africa then quickly became a large growing area (Arcimovičová & Valíček 1999).

The *Criollos* were probably domesticated by the Mayas. Archaeological evidence shows that *Criollo* cacao beans have been cultivated and used in food for more than 2,600 years (Motamayor et al. 2002). *Criollo* can now be subdivided into two geographical groups: Central American *Criollos* and South American *Criollos* (Wood & Lass 2001). Wood and Gass (2001) tell us that the latter group was introduced into the north-eastern part of South America by monks of the Capuchin order. *Criollos* dominated the market until about the middle of the eighteenth century and accounted for most of the exports to Europe. Nowadays *Criollo* is highly prized on the market and has rare, less bitter, and more aromatic flavour profile than other types. Only 5-10% of chocolate is made from *Criollo* (Rusconi & Conti 2010). The higher quality of *Criollo* comes with less vigour and lower yields than *Forastero* cacao (Cheesman 1944).

Forastero trees are harder than *Criollo* trees and produce cheaper cacao beans. Due to their properties, they are used for 80% of world chocolate production (Rusconi & Conti 2010). The *Forastero* group is made up of very diverse populations originating in different areas: Upper Amazon, Lower Amazon, Orinoco, and the Guianas. *Forastero* trees can be differentiated by pod shape. One of them is *Amelonado* type which is characterised by a melon shape of the fruit which can be found in Brazil, the Orinoco basin, and the Guyanas (Motamayor et al. 2003).

Trinitario has been slowly replacing *Criollo* and *Forastero* since the 19th century. *Trinitario* was described as a hybrid between *Criollo* and *Forastero* originating in Trinidad. The hybridization between the former two genetic groups started after

importing them to Trinidad when the local *Criollo* plantations were decimated by the natural disaster (Motamayor et al. 2003). *Trinitario* was more productive and resistant to diseases compared to *Criollo* and *Amelonado* (Cheesman 1944).

The three main types or groups of populations of *T. cacao* are described in Table 3.

Table 3. Cacao pod description

	<i>Criollo</i>	<i>Forastero</i>	<i>Trinitario</i>
<i>Pod Husk</i>			
Texture	Soft	Hard	Mostly hard
Colour	Red occurs	Green	Variable
<i>Beans</i>			
Average no. per pod	20-30	30 or more	30 or more
Colour of cotyledons	White, ivory or very pale purple	Pale to deep purple	Variable, white beans rarely occur

Source: Wood and Lass (2001)

5. Genetic diversity

Each species is adapted to certain environmental conditions to survive in competition with other species (Chloupek 2008). Genetic diversity and its distribution are determined by micro-evolutionary processes, such as the demographic history of populations, natural selection, or gene flow (Carvalho et al. 2019). However, diversity between species is not the final degree of diversity that we can observe, it can go much deeper. Each species consists of more or less separate populations (i.e., groups of individuals occurring at a single site at a particular time that may interbreed) (Primack et al. 2011). These populations live in various localities that are part of the range of the species. Each population can thus occur in a different habitat and be adapted to its environment (Chloupek 2008). Differences between species, between populations, but also within populations - these are the levels at which genetic diversity can be studied.

Low genetic diversity reduces survival ability and fitness by providing only a limited amount of genetic material for selection, thus decreasing the ability to adapt to any changes. With sudden and strong changes, the population can then become more endangered. It is clear that if more such events occur, the probability of species extinction increases (Frankham et al. 2002). When mankind initially started farming, selective breeding was used to pass on desirable traits of the crops while excluding the undesirable ones. Selective breeding leads to monocultures. Almost no genetic diversity makes crops extremely susceptible to widespread disease. Bacteria morph and change constantly. When a disease-causing bacterium changes to attack a specific genetic variation, it can easily wipe out the vast majority of the species. If the genetic variation that the bacterium is best at attacking happens to be that which humans have selectively bred for monoculture, the entire crop can be wiped out (Bartley 2005). Genetic diversity is therefore essential for adaptation to changes in the external environment - it reflects the evolutionary potential of the entire populations and from a long-term perspective (maintaining evolutionary potential) and from a short-term perspective (maintaining reproductive fitness), genetic diversity is a primary topic of conservation genetics (Frankham et al. 2002).

Cheesman (1944) points out that the greatest variation of *T. cacao* known is found at the headwaters of the Amazon River near Iquitos, Peru. However, the diversity in the areas of the *T. cacao* domestication is lower than the diversity in the wild, meaning that only a small part of the wild gene pool was used for cultivation purposes. The vast majority of cacao is based around tree traditional cultivars and only a few contain hybrids cultivars developed by breeding in Africa or South East Asia (Eskes & Efron 2006). Since the cultivars are furthering away from their wild ancestors this may lead to reducing their resistance to diseases and viruses (Loor Solorzano et al. 2012).

Due to the decreasing of the cacao's genetic diversity during the past century, several expeditions have been made to the Peruvian Amazon which is known for its large number of diverse cacao populations (Bartley 2005). Nowadays, a minority of germplasm is maintained as *ex-situ* collections in gene banks all over the globe. Two main collections are stored in International Cacao Genebank, Trinidad (Cacao Research Unit, The University of the West Indies) with more than 2,800 accessions and CATIE (Centro

Agronómico Tropical de Investigación y Enseñanza - Tropical Agricultural Research and Higher Education), Costa Rica with about 1,200 accessions (Motilal & Butler 2003).

Zhang et al. (2006) found evidence for the distance isolation hypothesis in cacao populations on a regional scale in Peru and Bolivia. This provided pattern was related to the limited flow of short-distance genes in cacao telling us that self-pollination could be more frequent than in natural populations (Zhang et al. 2012). This shows the adaptability of *T. cacao* to the environmental changes. It is important because it can provide information that may lead to the recognition of areas ideal for the future germplasm material collection for *ex-situ* conservation for use in breeding programs (Lachenaud & Zhang 2008). The understanding of what is currently conserved in existing *ex-situ* collections is crucial. The main goal is to avoid duplications. Furthermore, *ex-situ* conservation needs to be complemented by *in-situ* and on-farm conservation of diverse wild and cultivated populations. Such populations are exposed to various influences such as evolutionary stress, human-mediated selection, which allows the continuous adaptation to the environment (Thomas et al. 2012).

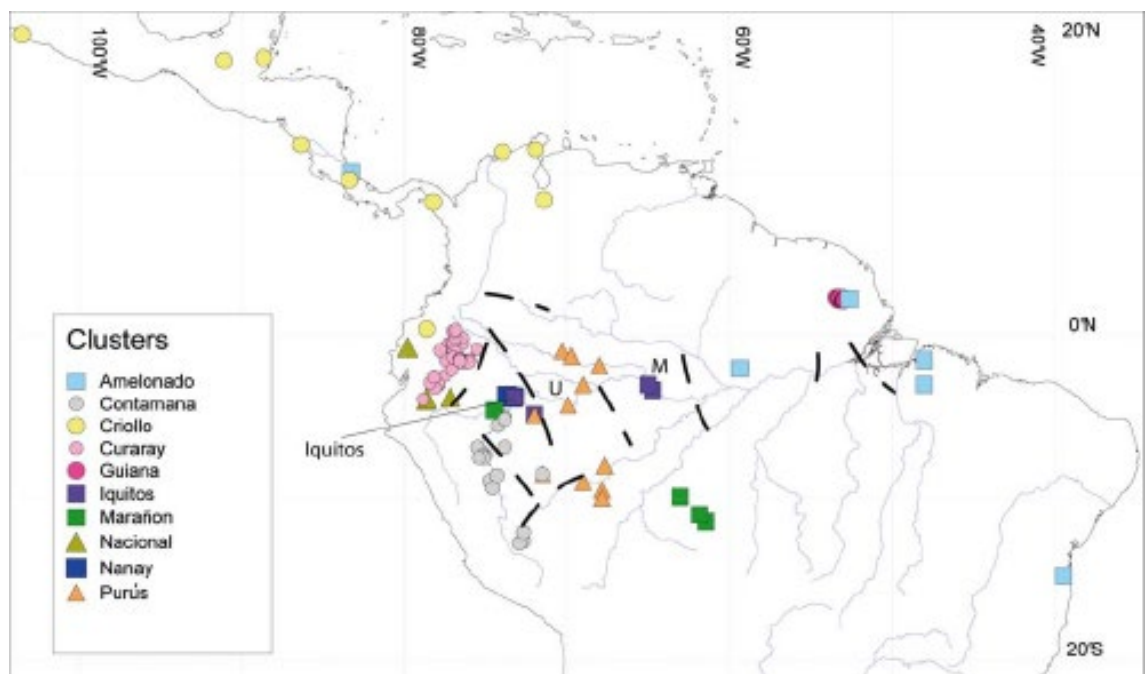
Molecular markers have been successfully applied in cultivar identification, controlling seed purity of hybrids, and checking the genetic relatedness between cultivars. Some of the detection techniques are Random Amplified Polymorphic DNA (RAPD). A study using this method in cacao was reported by Wilde et al. (1992) which used RAPD to study relationships among cacao groups. Their work was soon followed by Russel et al. (1993) who also used RAPD to assess available diversity among Peruvian Forasteros and Ecuadorian Nacional to distinguish them since it is difficult to do so by morphological descriptors. Lerceteau et al. (1997) analysed the genetic diversity in *T. cacao* clones using forty-three genomic probes using both RADP and RFLP (Restriction Fragment Length Polymorphisms). They found that within the group genetic diversity was nearly identical between Forastero, Trinitario, and Criollo (Aikpokpodion 2012); (Zhang et al. 2006). The RFLP method is generally found to be moderately polymorphic and can be applied in comparisons ranging from the individual level to closely related species. RFLPs have been used by Motamayor and Lannaud (2002) to determine the genetic diversity of cacao populations. Nonetheless, the previously mentioned methods were later replaced by microsatellites, which proved to be more useful in cacao fingerprinting for decomposing

of the genetic structure as well as identifying mislabelled accessions in germplasm banks (Aikpokpodion 2012).

Microsatellites, also known as SSRs (Simple Sequence Repeats) are molecular marker loci consisting of repetitive DNA of two to six bases short nucleotide sequences. Microsatellites are the international standard for defining genetic identity and have been used in the study of genetic diversity of cacao (Aikpokpodion 2012) (Zhang et al. 2006).

Motamayor et al. (2008) proposed a new classification after genotyping over one thousand cacao samples with 106 microsatellite markers resulting in the new classification. Instead of the traditional three groups Criollo, Forastero, and Trinitario they proposed ten new groups that have been named according to their geographic origin or by dividing cacao germplasm by the cluster. These groups are Amelonado, Criollo, Nacional, Contamana, Curaray, Guiana, Iquitos, Marañon, Nanay, and Purús.

Figure 2. Localization of the origin of individuals analysed; colours indicate the inferred genetic cluster to which they belong (Motamayor et al. 2008)



The regions with the highest diversity in *T. cacao* species are the upstream areas of the western Amazon, and smaller parts of Central America (Thomas et al. 2012).

Motamayor and Lanaud (2002) by observing the upper Amazon *Forastero* group assumed that these high values (8.69 alleles per locus) are indicative for wild populations, or at least populations that are most closely related to the wild populations. The present

results also give support to previous findings that the highest number of private alleles are typically observed in the Upper Amazon region of Peru and Brazil (Thomas et al. 2012). Based on the values of the genetic parameters obtained for the different clusters the closest relatives to original wild cacao populations are cluster Nacional, Contamana, and Purus (Motamayor et al. 2008).

Zhang et al. (2006) and Zhang et al. (2009) call attention to high levels of locally common alleles which are interesting because it can demonstrate the isolation of the populations which may lead to natural selection and local adaptation of genotypes if environmental conditions differ from other areas (Thomas et al. 2012).

Other newer methods based on NGS (Next-generation sequencing) are methods for sequencing genomes at high speed and at low cost providing data that are available to improve genome assemblies. Current NGS platforms offer the ability to produce long reads and positional information using mate-pair templates of large insert-size libraries which can cover many repetitive or low complexity elements in the assembly process. Combined with a precise gap closing procedure, this allows a significant increase in the size of contiguous genomic sequences with a reduction in the number of scaffolds and no discernible misassemblies (Argout et al. 2017).

Argout et al. (2017) showed that the power of genotyping by sequencing methods is in producing a high number of SNP (Single nucleotide polymorphism) molecular markers which leads to an increase in the proportion of the assembly anchored to chromosomes. Better chromosome-scale assembly of the Criollo genome could help to identify syntenic blocks shared between the *T. cacao* genome and related species. This could enable researchers to identify genes, regulatory elements, non-coding RNAs, and conserved sequences of unknown function involved in *T. cacao* agronomic traits in combination with further genetic studies and comparative genomics studies (Argout et al. 2017).

Lachenaud et al. (2018) collected in the forests of south-eastern French Guiana a core collection of 41 clones selected from genetic material. The collection was established after the sequencing of 181 clones. The new core collection, accounting for 100 % of the known diversity of the Guianan wild cacao trees, is an appropriate instrument for germplasm management. The generation could be planted in a small plot at the Perennial Crops Biological Resource Centre (CRB-PPG) at Sinnamary in French Guiana, which

will help to simplify part of the cacao tree collection arrangements in the future. Under these conditions, it will also be easier to assess these original genetic resources. Exporting the core collection from French Guiana could enable the identification of new sources of resistance to some major diseases that do not currently exist there, such as Frosty Pod Rot. This could be useful for the international cacao community (Lachenaud et al. 2018).

Ricaño-Rodríguez et al. (2019) using a method of GBS (genotyping-by-sequencing) for the characterization of biobanks in Mexico have identified Single nucleotide polymorphisms (SNPs) in *T. cacao*. The samples were collected at traditional agroforestry plots located in the municipalities of Cardenas, Huimanguillo, Comalcalco, Paraiso, Jalpa de Mendez, and Cunduacan, Tabasco, as well as Ixtacomitan and Pichucalco, Chiapas, Mexico from May till June 2018. By the subsequent filtering of crude sequences, genomic assembly, identification of SNPs, taxonomic molecular characterization, and characterization of coding regions as well as minimum evolution of protein transcripts were performed. The results where samples of *T. cacao* showed different SNPs percentages (2–11%) and the molecular evolution analyses suggested similar maximum compound probabilities respect to their phylogeny. The GBS method allowed to identify SNPs in cacao which assisted in the characterization of reduced genomes determining the structural and transcriptional correlation between the samples and the reference genome of cacao Criollo (Ricaño-Rodríguez et al. 2019).

The fact that cacao trees in general show low neutral genetic diversity does not necessarily mean that they cannot contain interesting functional or adaptive qualities. The best examples are the Nacional and Criollo cultivars which have the lowest genetic diversity partly caused by the consequence of human-induced bottlenecks. This reduction of the size of the gene pool led to the development of these cultivars by selection and domestication processes, whereby the frequency of favourable traits was culturally enhanced resulting in the finest quality chocolate known today (Thomas et al. 2012).

In the comparison, Boza et al. (2013) presented allelic diversity in the Dominican Republic germplasm collection (8.36 alleles per locus) which is comparable to those identified by Irish et al. (2010) in Puerto Rico's collection by USDA-ARS TARS (8.80 alleles per locus) and the allelic diversity recently identified in a young cacao production area in Nicaragua (7.73 alleles per locus) (Trognitz et al. 2011). The allelic diversity observed is also similar to that reported for 'Cacao Nacional Boliviano' from the

Amazonia regions of La Paz and Beni, Bolivia (7.30 alleles per locus) reported by Zhang et al. (2012) and Ghanaian cacao collections (7.50 alleles per locus). In contrast, allelic diversity is greater in the collection of CATIE (14.2 alleles per locus) which contains a larger number of accessions from a much broader geographic area (Zhang et al. 2009). Collections in Cameroon (9.41 alleles per locus) (Efombagn et al. 2008) and West Africa (12.5 alleles per locus), (Aikpokpodion et al. 2010) are also highly diverse. On the other hand, Loor et al. (2009) reported a low amount of genetic diversity in accessions collected along the Pacific coast of Ecuador (4.22 alleles per locus). This report has a similar number of alleles per locus for a 'Refractario' population in Ecuador but different for the total number of alleles (4.20 alleles per locus) (Lachenaud & Zhang 2008). Low allelic diversity was also reported for Peruvian collections from the Ucayali (5.7 alleles per locus) and even lower from Huallaga (3.68 alleles per locus) valleys (Zhang et al. 2006). Sereno et al. (2006) described similar low genetic diversity values (4.45 alleles per locus) in areas collected from 19 Amazon River basins. This result is likely to be related to physical isolation and fewer admixtures among wild populations (Boza et al. 2013).

Aikpokpodion (2012) determined that genetic diversity was introduced by the use of *T. cacao* clones and in the germplasm accessions which were used to cultivate hybrids with improved abilities. Hybrids were later distributed to farmers across the sub-region In West and Central Africa. Microsatellite markers (SSR) were used to assess genetic diversity in these cases. In addition to the microsatellite studies, some studies were also carried out to determine variation in morphological characteristics of *T. cacao* germplasm in farmers and gene bank collections (Aikpokpodion 2012).

The results of these studies mention the presence of appreciable genetic diversity in farmer-grown cacao. This indicated that there is enough resilience in the West and Central African cacao that will possibly be able to withstand any major disease outbreak, which may be a serious threat to the cacao industry in the future (Aikpokpodion 2012).

Some private alleles presented in farmer's populations could allow selecting advantageous recombinants in the field conditions that have shown greater adaptation. These recombinants possibly accumulate genes for resistance to diseases such as Phytophthora pod rot, mirid attack, or abiotic stress. The limited gene flow observed in Cameroon, Côte d'Ivoire, Ghana, and Nigeria may lie in inefficient seed delivery systems that do not extend the advantages of researched varieties to farmers. It is possible to

establish a functional and reliable seed supply system that allows farmers easy access to better quality planting materials (Aikpokpodion 2012).

6. Discussion

Theobroma cacao, L. is usually grown under the canopy of the original forest, which is considered the most environmentally sounding production form. Although shade-grown cacao results in some lower biodiversity, on the other hand, shade systems are benefiting from overall higher biodiversity than full-sun systems. Shading lowers the levels of pesticide use and may also contribute to higher levels of biodiversity, and this increase in biodiversity is associated with better pest control and pollination and more efficient nutrient cycling. On the other hand, full-sun cacao provides very little benefit in terms of biodiversity conservation (Franzen & Borgerhoff Mulder 2007).

All the SSR markers used by Motamayor et al. (Motamayor & Lanaud 2002), were used to show the distribution to new groups and to measure the genetic diversity. Low numbers of alleles per locus causing the low diversity are in countries of origin of *T. Cacao* in South America such as in Ecuador, Peru, or the area of Amazonian River Basin due to physical isolation according to Boza et al. (2013). On the other hand, higher numbers of alleles per locus are reported from regions where new cultivars of *T. Cacao* were introduced after programs for germplasm collection which supports the highest number of alleles per locus (14.2 alleles per locus) from CATIE (Zhang et al. 2009).

The genetic diversity of cacao is being lost at the expense of higher producing varieties. On the fields, the original varieties are mostly cut down and used as a stock for grafting high yields varieties and with only a few older trees remaining. Original varieties are better adapted to local conditions and soil and are used to make the base for the higher producing imported varieties. This is done because grafting is faster than propagation by seeds or cuttings. This method largely contributes to the loss of genetic diversity and loss of properties unique to the original varieties. This lack of structure in the population caused by previously mentioned methods can be explained by strong human intervention, as the population is no longer natural and made up mostly of individual clones that are influencing each other (Argout et al. 2011). On the other hand, there has been no major advantage in developing pests and disease-resistant varieties in the past decades (Zhang et al. 2009). In cases where some resistant varieties exist they have not been adopted and utilized by the major cacao-growing region. The adoption of resistant cultivars is replaced by the wide use of pesticides carrying the possible risk to human health as well as the

environment. The risk could be prevented by use agroforestry that favour antagonism or microclimate conditions that reduce the incidence of pests and diseases (Bos et al. 2007). The research of Argout et al. (2011) which has highlighted the close evolutionary relationship of the *T. cacao* genome with the results representing a new and simple model to study the evolutionary processes, gene function, genetics and biochemistry of tree fruit crops and in future can help to discover and understand the new varieties and to genetically diversify the cacao production by gaining new information using NGS-based research methods such as WGS (Whole genome sequencing).

Now the trend shows that it is important to diversify the cacao production to be more resistant to the environmental changes and diseases by introducing resistant germplasm to contribute to the sustainability of cacao production. The improved material already exists in germplasm banks in major cacao producing countries such as CATIE, but it has been made more available to farmers. To increase the genetic diversity by long-term germplasm improvement in cacao-producing regions. Methods such as hybridization of exotic germplasm, which allows accumulation of resistance genes/alleles and to reorient the new breeding strategies to breed disease-resistant and shade tolerant varieties instead of breeding the high-yielding varieties (Duguma et al. 2001).

The new methods of germplasm conservation and the right techniques of growing and producing can fulfill the trend for more ecological and sustainable production. The possible potential in leading to positive financial income to the smallholder farmers. With the use of the NGS-based methods which lowering the price of the research and overall cost of development of new varieties limitation could be the slow introduction of the new cultivars. Rice and Greenberg (2000) suggested that farmers who grow cacao according to specific criteria aimed at increasing biodiversity benefits could receive not only higher prices for their product but also access to credit and extensions services if environmental funds were made available for such programs. Increases in the price of labour make full-sun cacao cheaper to produce than shade-grown cacao due to the higher productivity and the replacement of labour with chemical inputs which is important because it could from the long-term perspective accelerate the introduction of the new cultivars.

7. Conclusions

The work evaluated the genetic diversity of cacao in the main producing countries. Cacao as such has its origin in South America, where its wild variants can still be found. Today, they can be used to collect germplasm material to genetically investigate the traits of wild individuals and to create new hybrid offspring that are more resistant to the change of natural conditions or diseases and pests. However, wild variants not only serve as a source of genetic diversity. Cultivars from other regions, such as Central Africa or Southeast Asia, can be used as another source of properties. Where, thanks to different influences, individuals were able to acquire suitable traits that they could overcome new obstacles that cacao production will face in the future.

All these new germplasm materials should, therefore, be stored and propagated in genetic banks or other facilities for their later use. With that said, the new hybrid cultivars need to get to small cacao producers, who make up the majority of producers, so that the population of *Theobroma cacao*, L. can respond more flexibly to possible threats in the environment or disease in the future.

8. References

Ahenkorah Y, Akrofi G, Adri A. 1974. The end of the first cocoa shade and manurial experiment at the Cocoa Research Institute of Ghana. *Journal of Horticultural Science* **49**:43-51. Taylor & Francis.

Aikpokpodion P. 2012. Defining Genetic Diversity in the Chocolate Tree, *Theobroma cacao* L. Grown in West and Central Africa. Page 10 in M. Caliskan, editor. *Genetic Diversity in Plants* edition. IntechOpen, Rijeka.

Aikpokpodion P, Kolesnikova-Allen M, Adetimirin V, Gultinan M, Eskes A, Motamayor J, Schnell R. 2010. Population Structure and Molecular Characterization of Nigerian Field Genebank Collections of Cacao, *Theobroma cacao* L. *Silvae Genetica* **vol. 59**:273-285.

Arcimovičová J, Valíček P. 1999. *Čokoláda pokrm bohů* edition.. Start, Benešov.

Argout X, Martin G, Droc G, Fouet O, Labadie K, Rivals E, Aury J, Lanaud C. 2017. The cacao Criollo genome v2.0: an improved version of the genome for genetic and functional genomic studies. *BMC Genomics* **vol. 18**. Available from <http://bmcbgenomics.biomedcentral.com/articles/10.1186/s12864-017-4120-9>.

Argout X et al. 2011. The genome of *Theobroma cacao*. *Nature Genetics* **vol. 43**:101-108.

Bartley B. 2005. The genetic diversity of cacao and its utilization. Page 341 in *The genetic diversity of cacao and its utilization* 1st edition. CABI, Wallingford.

Bos M, Steffan-Dewenter I, Tschardt T. 2007. Shade tree management affects fruit abortion, insect pests and pathogens of cacao. *Agriculture, Ecosystems & Environment* **120**:201-205.

Boza E et al. 2013. Genetic diversity, conservation, and utilization of *Theobroma cacao* L: genetic resources in the Dominican Republic. *Genetic Resources and Crop Evolution* **vol. 60**:605-619.

Carvalho Y, Vitorino L, Souza U, Bessa L. 2019. Recent Trends in Research on the Genetic Diversity of Plants: Implications for Conservation. *Diversity* **vol. 11**:21.

Duguma B, Gockowski J, Bakala J. 2001. Smallholder Cacao (*Theobroma cacao* Linn.) cultivation in agroforestry systems of West and Central Africa: challenges and opportunities: challenges and opportunities. *Agroforestry Systems* **51**:177-188.

Efombagn I, Motamayor J, Sounigo O, Eskes A, Nyassé S, Cilas C, Schnell R, Manzanares-Dauleux M, Kolesnikova-Allen M. 2008. Genetic diversity and structure of farm and GenBank accessions of cacao (*Theobroma cacao* L.) in Cameroon revealed by microsatellite markers. *Tree Genetics & Genomes* **vol. 4**:821-831.

Efombagn M, Sounigo O, Nyassé S, Manzanares-Dauleux M, Eskes A. 2009. Phenotypic variation of cacao (*Theobroma cacao* L.) on farms and in the gene bank in Cameroon. *Journal of Plant Breeding and Crop Science* **1**:258-64.

Eskes A, Efron Y. 2006. Global approaches to cocoa germplasm utilization and conservation.: Final report of the CFC/ICCO/IPGRI project on “Cocoa Germplasm Utilization and Conservation: a Global Approach” (1998–2004) edition. CFC Technical Paper.

Evans H. 2007. Cacao diseases—the trilogy revisited. *Phytopathology* **97**:1640-1643.

FAO. 2003. Medium-term prospects for agricultural Commodities: PROJECTIONS TO THE YEAR 2010 edition. FAO COMMODITIES AND TRADE TECHNICAL PAPER, Rome.

FAO. 2010. The Second Report on the State of the World’s Plant Genetic Resources for Food and Agriculture:398pp.

FAO. 2018.. Page in Food and Agriculture Organization of the United Nations (FAO) edition. FAOSTAT-Crops. Rome, Italy. (accessed 2020-05-05).

Forbes S, Northfield T. 2017. Increased pollinator habitat enhances cacao fruit set and predator conservation. *Ecological Applications* **vol. 27**:887-899.

Frankham R, Briscoe D, Ballou J. 2002. Introduction to Conservation Genetics, 4 edition. Cambridge University Press, United Kingdom.

Franzen M, Borgerhoff Mulder M. 2007. Ecological, economic and social perspectives on cocoa production worldwide. *Biodiversity and Conservation* **vol. 16**:3835-3849.

Fulton R. 1989. The cacao disease trilogy: black pod, monilia pod rot, and witches'-broom: The cacao disease trilogy: black pod, monilia pod rot, and witches'-broom. *Plant Disease (EUA)* **73**:601-603.

Hamzat R, Olaiya A, Sanusi R, Adedeji A. 2006. State of cocoa growing, quality and research in Nigeria: Need for intervention edition. DGCC, Lagos.

Cheesman E. 1944. Notes on the nomenclature, classification and possible relationships of cocoa populations. *Tropical Agricultur* 21:144-159.

Chloupek O. 2008. Genetická diverzita, šlechtění a semenářství Vyd. 3., upr. 2.. . Academia, Praha.

Irish B, Goenaga R, Zhang D, Schnell R, Brown J, Motamayor J. 2010. Microsatellite Fingerprinting of the USDA-ARS Tropical Agriculture Research Station Cacao (*Theobroma cacao* L.) Germplasm Collection. *Crop Science* **vol. 50**:656-667.

Lachenaud P, Clément D, Argout X, Scalabrin S, Doaré F. 2018. The Guiana cacao genetic group (*Theobroma cacao* L.): a new core collection in French Guiana. *Botany Letters* **vol. 165**:248-254. Available from <https://www.tandfonline.com/doi/full/10.1080/23818107.2018.1465466>.

Lachenaud P, Zhang D. 2008. Genetic diversity and population structure in wild stands of cacao trees (*Theobroma cacao* L.) in French Guiana. *Annals of Forest Science* **vol. 65**:310p7.

Lee A, Garot A. 1972. Cocoa Planting without Shade in Pahang, West Malaysia. Incorporated Society of Planters: 25–27 November 1971:112-115. Kuala Lumpur, Malaysia.

Lerceteau E, Robert T, Pétiard V, Cruzillat D. 1997. Evaluation of the extent of genetic variability among *Theobroma cacao* accessions using RAPD and RFLP markers. *Theoretical and Applied Genetics* **vol. 95**:10-19.

Lim K. 1993. Quality of cocoa wood charcoal as a function of carbonisation temperature. *Biomass and Bioenergy* **vol. 4**:301-302.

Loor Solorzano R, Fouet O, Lemainque A, Pavék S, Boccara M, Argout X, Amores F, Courtois B, Risterucci A, Lanaud C. 2012. Insight into the Wild Origin,

Migration and Domestication History of the Fine Flavour Nacional *Theobroma cacao* L. Variety from Ecuador. *PLOS ONE* 7:11. Public Library of Science.

Loor R, Risterucci A, Courtois B, Fouet O, Jeanneau M, Rosenquist E, Amores F, Vasco A, Medina M, Lanaud C. 2009. Tracing the native ancestors of the modern *Theobroma cacao* L. population in Ecuador. *Tree Genet Genomes* 5:421-433.

Motamayor J, Lachenaud P, da Silva e Mota J, Loor R, Kuhn D, Brown J, Schnell J, Borevitz R, editor. 2008. Geographic and Genetic Population Differentiation of the Amazonian Chocolate Tree (*Theobroma cacao* L). *PLoS ONE*: e3311 3:8. *PLoS ONE*.

Motamayor J, Lanaud C. 2002. Molecular Analysis of the Origin and Domestication of *Theobroma cacao* L. Engels JMM, Ramanatha Rao V, Brown AHD and Jackson MT (Eds.) *Managing Plant Genetic Diversity*:77-87. CABI Publishing, Wallingford, UK.

Motamayor J, Risterucci A, Heath M, Lanaud C. 2003. Cacao domestication II: progenitor germplasm of the Trinitario cacao cultivar. *Heredity*:322-330. Nature Publishing Group.

Motamayor J, Risterucci A, Lopez P, Ortiz C, Moreno A, Lanaud C. 2002. Cacao domestication I: the origin of the cacao cultivated by the Mayas. *Heredity*:380-386. Nature Publishing Group.

Motilal L, Butler D. 2003. Verification of identities in global cacao germplasm collections. *Genetic Resources and Crop Evolution* vol. 50:799-807.

Opoku S, Bhattacharjee R, Kolesnikova-Allen M, Motamayor J, Schnell R, Ingelbrecht I, Enu-Kwesi L, Adu-Ampomah Y. 2007. Genetic Diversity in Cocoa (*Theobroma cacao* L.) Germplasm Collection from Ghana. *Journal of Crop Improvement* 20.

Ploetz R. 2007. Cacao diseases: important threats to chocolate production worldwide. *Phytopathology* 97:1634-1639.

Pokou N, N’Goran J, Lachenaud P, Eskes A, Montamayor J, Schnell R, Kolesnikova-Allen M, Clément D, Sangaré A. 2009. Recurrent selection of cocoa populations in Côte d’Ivoire: comparative genetic diversity between the first and second cycles. *Plant Breeding* vol. 128:514-520.

Polívka F. 1908. Užitkové a paměťhodné rostliny cizích zemí edition.. Volvox Globator, Praha.

Pospíšil F, Hrachová B. 1989. Užitkové rostliny jižních zemí. Page 157 in Užitkové rostliny jižních zemí edition.. Academia, Praha.

Primack R, Kindlmann P, Jersáková J. 2011. Úvod do biologie ochrany přírody edition.. Portál, Praha.

Ricaño-Rodríguez J, Hipólito-Romero E, Ramos-Prado J, Cocolletzi-Vásquez E. 2019. Genotipado por secuenciación de variedades tradicionales de *Theobroma cacao* (Malvaceae) del Estado de Tabasco, México. *Botanical Sciences* **vol. 97**:381-397. Available from <http://www.botanicalsciences.com.mx/index.php/botanicalSciences/article/view/2258>.

Rice R, Greenberg R. 2000. Cacao Cultivation and the Conservation of Biological Diversity. *AMBIO: A Journal of the Human Environment* **vol. 29**:167-173.

Rusconi M, Conti A. 2010. *Theobroma cacao* L., the Food of the Gods: A scientific approach beyond myths and claims. *Pharmacological Research* **vol. 61**:5-13.

Russell J, Hosein F, Johnson E, Waugh R, Powell W. 1993. Genetic differentiation of cocoa (*Theobroma cacao* L.) populations revealed by RAPD analysis. *Molecular Ecology* **vol. 2**:89-97.

Sereno M, Albuquerque P, Vencovsky R, Figueira A. 2006. Genetic Diversity and Natural Population Structure of Cacao (*Theobroma cacao* L.) from the Brazilian Amazon Evaluated by Microsatellite Markers. *Conservation Genetics* **vol. 7**:13-24.

Thomas E, van Zonneveld M, Loo J, Hodgkin T, Galluzzi G, van Etten J, Fuller D. 2012. Present Spatial Diversity Patterns of *Theobroma cacao* L. in the Neotropics Reflect Genetic Differentiation in Pleistocene Refugia Followed by Human-Influenced Dispersal. *PLoS ONE* **7**:17.

Trognitz B, Scheldeman X, Hansel-Hohl K, Kuant A, Grebe H, Hermann M, Mailund T. 2011. Genetic Population Structure of Cacao Plantings within a Young Production Area in Nicaragua. *PLoS ONE* **vol. 6**.

Valíček P, et al. . 2002. Užitkové rostliny tropů a subtropů, 2 edition.. Academia, Prague.

Wilde J, Waugh R, Powell W. 1992. Genetic fingerprinting of Theobroma clones using randomly amplified polymorphic DNA markers. *Theoretical and Applied Genetics* **83-83**:871-877.

Wood G, Lass R. 2001. *Cocoa*, 4 edition. John Wiley & Sons, Incorporated.

Zhang D, Boccara M, Motilal L, Mischke S, Johnson E, Butler D, Bailey B, Meinhardt L. 2009. Molecular characterization of an earliest cacao (*Theobroma cacao* L.) collection from Upper Amazon using microsatellite DNA markers. *Tree Genetics & Genomes* **5**:595-607.

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

Zhang D, Arevalo-Gardini E, Mischke S, Zúñiga-Cernandes L, Baretto-Chavez A, Aguila J. 2006. Genetic Diversity and Structure of Managed and Semi-natural Populations of Cocoa (*Theobroma cacao*) in the Huallaga and Ucayali Valleys of Peru. *Annals of Botany* **vol. 98**:647-655.

