CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

Diversity of crop wild relatives of cacao (*Theobroma cacao* L.) and their use in the crop breeding: a literature survey

BACHELOR'S THESIS

Prague 2022

Author: Šárka Svobodová

Supervisor: doc. Ing. Zbyněk Polesný, Ph.D.

Declaration

I hereby declare that I have done this thesis entitled "Diversity of crop wild relatives of cacao (*Theobroma cacao* L.) and their use in the crop breeding: a literature survey" 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 2022

....

Šárka Svobodová

Acknowledgements

I would like to give my warmest thanks to my supervisor, doc. Ing. Zbyněk Polesný, Ph.D. (Department of Crop Sciences and Agroforestry, Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague) for his advice, and guidance and patience, which made this thesis possible.

I would also like to express my gratefulness to my friends and colleagues from the Faculty of Tropical AgriSciences for their suggestions and comments.

Finally, my deepest appreciation goes to my family for the emotional and financial support they gave me throughout my studies.

Abstract

Theobroma cacao L. is a crop species under the family *Malvaceae* subf. *Sterculioideae* and the genus *Theobroma* L. consisting of 22 species. *Theobroma cacao* is used mainly to produce chocolate (processed material) and cocoa (raw material), but it offers many other uses. It is used, for example, in cosmetics and has many medicinal properties. The crop faces biotic and abiotic stresses, including several diseases and pests attacks that can damage the cacao trees, resulting in significant economic losses for producers. Wild relatives of *Theobroma cacao* as well as clones which are the result of intentional breeding programmes showed some resistant traits against diseases, pests, and climatic stressors. For this purpose, it is essential to value them as genetic resources and use their properties in further research in cacao breeding. There are 21 crop wild relatives of *Theobroma cacao* in the genus *Theobroma*, however, the genus *Herrania* Goudot, consisting of 17 species, is closely related. Even though crop wild relatives of cacao are not suitable for commercial cultivation like *Theobroma cacao*, breeding programmes that have been done in the past, showed that cross-pollination of some species is possible. For that reason, they should be included in breeding programmes to cultivate better performing varieties of the cacao tree.

Keywords: biodiversity, cacao, *Herrania*, hybridization, germplasm, conservation, plant genetic resources

Contents

List of tables

List of figures

List of the abbreviations used in the thesis

CWR - crop wild relatives

CSSV - cacao swollen shoot virus

IUCN - International Union for Conservation of Nature

1. Introduction

Theobroma L. is a small genus in the *Malvaceae* family and has been classified into six sections (*Andropetalum, Glossopetalum, Oreanthes, Rhytidocarpus, Telmatocarpus, and Theobroma*) based on morphological traits and highly supported by molecular evidence (Santos et al. 2012). *Theobroma cacao* L. is a neotropical plant that grows naturally in the tropical lowland rainforests that stretch from the Amazon basin to Southern Mexico (18°N to 15°S) (Bhattacharjee 2018). Cacao is an excellent crop for both small-scale farmers and plantations, especially with the introduction of hybrids with higher yield. Cacao is threatened by various diseases and pests, as well as climate change, including changes in precipitation, humidity and the temperature. The usual methods of controlling diseases are fungicides and pesticides but they are not very efficient. They are often expensive and require reapplication (Fulton 1989). For this reason it is important to focus on cacao crop wild relatives and include them in breeding programmes. Studies show that hybrids between the species of *Theobroma* L. and *Herrania* Goudot are possible and some of them even showed resistance to diseases and pests (Silva et al. 2004). For this reason, it is very important to have cacao wild relatives in germplasm collections but it is necessary to keep them *in-situ* conditions, because the seeds lose the ability to germinate after a short period of time because of their sensitivity to temperature and humidity fluctuations (de Souza et al. 2018).

2. Aims of the Thesis

This bachelor's thesis aimed to review the literature that has been published on cacao tree and wild relatives of this crop. The thesis provides a review of cacao as a crop in terms of production issues and associated breeding goals/aims, and related use of germplasm of cacao crop wild relatives. The thesis identifies and characterizes species considered as cacao crop wild relatives, which were so far identified within two genera, i.e. *Theobroma* and *Herrania*. For each species, the common name(s), taxonomy, geographical origin and distribution, key interspecific morphological characters, ethnobotanical information, and breeding potential was summarized in this review. The thesis results can form a good basis for further research on cacao breeding.

3. Methodology

A qualitative systematic literature review was performed using an electronic search of Scopus, ScienceDirect, SpringerLink, Web of Science, JSTORE, Google Scholar, and a manual search of relevant books. Primary search terms used were “Theobroma”, “crop wild relatives”, “breeding”, “hybrids”, “germplasm”, “biodiversity” and “ethnobotany”. No restriction on the year of the publication was applied, but publications that were older than 2005 were used rarely. The accepted scientific names of investigated species were verified according to The World Flora Online (www.worldfloraonline.org) and results were crosschecked with Plants of the World Online (powo.science.kew.org), International Plant Names Index (www.ipni.org), and Tropicos (www.tropicos.org) databases. Further searches were undertaken using the names of each species with the focus on ethnobotany and breeding programmes as well as breeding potential.

4. Literature Review

4.1. Cacao tree botany and morphological description

Theobroma cacao L. is the most economically significant species in the genus *Theobroma* L., and it is grown in humid tropics in South and Central America, West Africa, and Malaysia. The cacao tree originates in the upper Amazon basin (Motamayor et al. 2008). During their time the local Mayas, Aztecs, and Pipil-Nicaraos cultivated the cacao tree and spread it to many parts of the world. The locals roasted and ground the cacao seeds to make an energy drink known as "xocoatl", which the Spanish carried to Europe and named "chocolate" and mixed it with sugar and milk, which gained popularity all around the world (Malhotra 2017). Smallholder farmers produce more than 80 % of cocoa globally, giving employment in many rural areas (Curry et al. 2007). Smallholder cacao is farmed mostly beneath shade trees, either intercropped or in a semi-natural agroforestry system. Farmers have been choosing cacao genotypes for years based on disease tolerance and cacao bean quality. Cacao has historically been divided into two primary categories Criollo and Forastero based on physical characteristics and geographical origins, with a third group, Trinitario, recognizing hybrids between Criollo and Forastero genotypes (Acebo-Guerrero et al. 2012).

4.1.1. Taxonomy of *Theobroma cacao* L.

The first to identify cacao and make a citation in botanical literature was Charles de *l'*Ecluse in 1605 in his *"Exoticorum Libri Decem"* under the name *Cacao fructus* but it refers only to the fruit and gives a basic illustration of cacao seeds. In 1737 Charles Linné introduced cacao in Polyadelphia pentandria as *Theobroma* – with the meaning *"The food for the Gods"* (Cuatrecasas 1964). *Theobroma* is a genus that belongs to the family *Malvaceae* and the tribe *Byttnerieae* or *Theobromeae* (Bhattacharjee 2018) and consists of 22 species that are mainly found in the understory of the Amazonian rainforest. *Theobroma* species have been divided into six sections (*Andropetalum, Glossopetalum, Oreanthes, Rhytidocarpus, Telmatocarpus,* and *Theobroma*) which have been made to identify the species based on morphological features but have been supported by molecular analysis as well (Santos et al. 2012).

4.1.2. Morphology of *Theobroma cacao* L.

4.1.2.1. The root and trunk

The root system is made up of one tap root whose length as well as form differs depending on the composition and roughness of the soil. The tap root may grow up to 2 meters in depth with sufficient drainage. Secondary roots are crucial for the nourishment of the tree, and the majority of them (70-90 %) are found in the first 30 cm of soil (de Souza et al. 2018). The morphology of the root system is affected by the method of cultivation. When the cacao tree is grown in an agroforestry system, the roots are competing with other trees for water, nutrients, and space in the soil (Rajab et al. 2018). The tree may grow to a height of 5-8 meters and a crown diameter of 4-6 meters. Due to competition for light with other species, it can attain a height of up to 20 meters in rainforest conditions. The stem grows upright, and after two years, the trunk reaches 1-1.5 meters in height. Following that, the first crowns form, consisting of three to five primary branches that proliferate into lateral and subsidiary branches. The cacao tree has a smooth trunk in its early years but when older, it becomes coarse and rugged as a result of the growth of flower cushions (de Souza et al. 2018).

4.1.2.2. Leaves

The leaves have an oblong and acuminate shape with the absence of hairs. There is a strong rib in the centre of the leaf. The colour of the leaves can differ depending on the cultivar or variety of the tree but they range in colour from green, which may or may not have a rosy undertone, to violet when immature (Figure 1b), depending on the content of anthocyanin in the cells. When the leaves mature, the rosy pigmentation disappears and they turn from pale to dark green and become rigid (Figure 1c) (de Souza et al. 2018).

Figure 1: Leaves of *Theobroma cacao***.** A) *Theobroma cacao* leaves (photo: Marina Chang 2021), B) Leaves of a matured tree (photo: Jesus Sanchez 2021), C) Leaf in detail (photo: Felipe Gómez Villota 2019)

4.1.2.3. Flowers

Cacao flowers emerge from buds that grow in the armpits of old leaves and appear in flowery cushions on the trunk or woody branches. This type of flowering is called cauliflory (Figure 2a). The blooms are hermaphroditic, with five sepals, five petals, five staminodes, five stamens, and a single pistil with five ovules in the ovary (de Souza et al. 2018). The sepals of each flower begin to split in the late afternoon, and the flower is fully open by the start of the following day, a period during which the anthers release pollen and the stigma is receptive (Almeida & Valle 2007). Because of its structural properties, cacao blossoms can only be pollinated by insects. A tiny group of insects belonging to the *Ceratopogonidae* family, genus *Forcipomya*, are the primary pollinators of cacao. The cacao tree has two flowering periods in the Amazon Region: a minor one that happens at the end of the dry season and the beginning of the rainy season, and a major one that comes at the end of the dry season and the beginning of the rainy season. A mature cacao tree may produce over 100,000 blossoms each year, but only approximately 0.1 % (this could be due to the lack of pollinators in the area as well as a low rate of auto pollination) of them mature into fruits. Within 48 hours, if the flower remains unpollinated, it dies and falls off the tree. However, the flowers that have been pollinated remain attached to the peduncle and form into a fruit (de Souza et al. 2018).

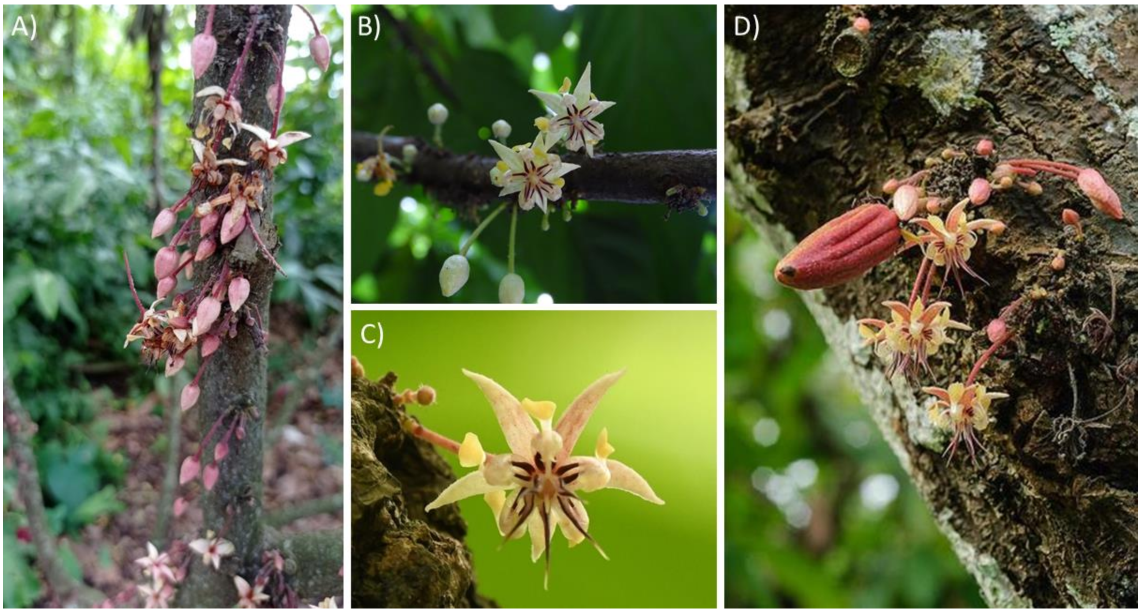

Figure 2: Flowers of *Theobroma cacao*. A) Cauliflory (photo: Carmen Amelia 2021), B) Flowers (photo: E. V. Restrepo 2019), C) Flower in detail (photo: Nuwan Chathuranga 2021), D) Fruit developing from the flower (photo: Chien Lee 2008)

4.1.2.4. Fruit and seeds

The fruit, commonly referred to as a pod, grows from the trunk and branches, this phenomenon is called cauliflory, and each pod contains about 20-40 seeds (also called cacao beans) inside on average (Micheli et al. 2010). The type of the fruit is very unique and it qualifies as *amphisarca* (Stuppy 2014). The fruit has a fleshy pericarp which consists of three distinct parts: the epicarp, which is fleshy and thick, and the outer layer is often pigmented, the mesocarp, which is thin and rigid and the endocarp which is fleshy but not as thick as the epicarp. The appearance of the fruits of cacao wild relatives differs very much (Figure 4). When immature, the fruit is usually green, and when ripe, it is yellow. It can also have a purple to red colour (Figure 3) when developing and bright orange colour when it reaches maturity. The time between pollination and fruit maturity ranges from 140 to 205 days on average. To produce 1 kg of commercial cocoa 15 to 31 fruits are needed on average (de Souza et al. 2018). The seeds are elliptical to ovoid in form and are 2-3 cm in length. They are coated with a white viscous aril, commonly referred to as pulp, with an acidic to sweet flavour. Two cotyledons, varying in colour from white to violet, are present in the embryo. When the fruit has ripened, the seeds are ready to germinate, and in some cases, they can germinate inside the pods. (Cuatrecasas 1964). Cacao seeds are extremely sensitive to temperature fluctuations and die quickly if they become dehydrated (de Souza et al. 2018).

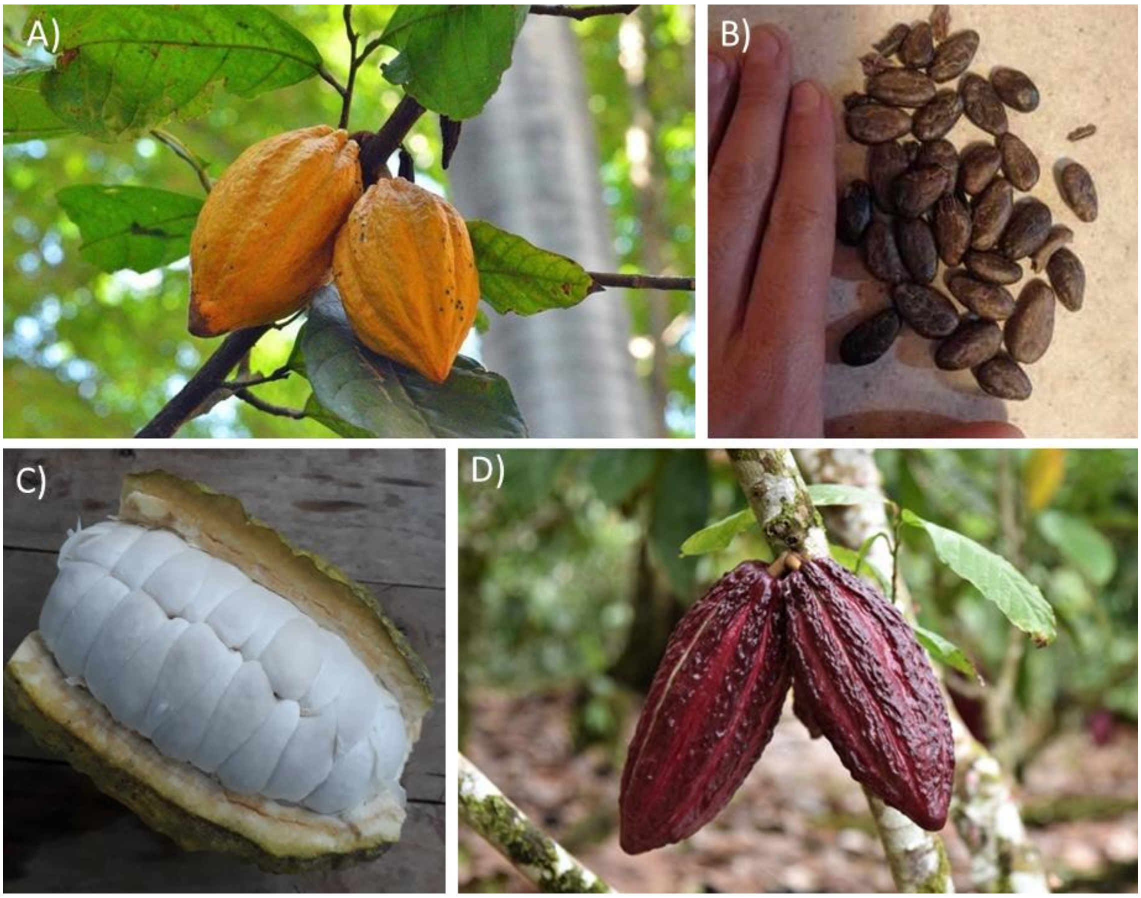

Figure 3: Pods and seeds of *Theobroma cacao*. A) Matured cacao pod (J. C. G. Morales 2014), B) Dried cacao seeds (D. E. V. Castañeda 2020), C) Seeds inside of the husk covered in pulp (Adriana López 2017), D) Matured cacao pods (Karsten Mody 2019)

The right temperature, humidity, and timing are crucial for the germination of the seeds since their germination period lasts only a few weeks (if outside of the pod). An experiment done in the Turrialba district in Costa Rica concluded that exposure of the seed to 2°C for 4 minutes inhibited the germination almost completely (Boroughs & Hunter 1961). This could be why cacao seeds lose the ability to germinate when they are being transported by planes because they require a stable temperature between 20-25°C (Hunter 1959; Boroughs & Hunter 1961).

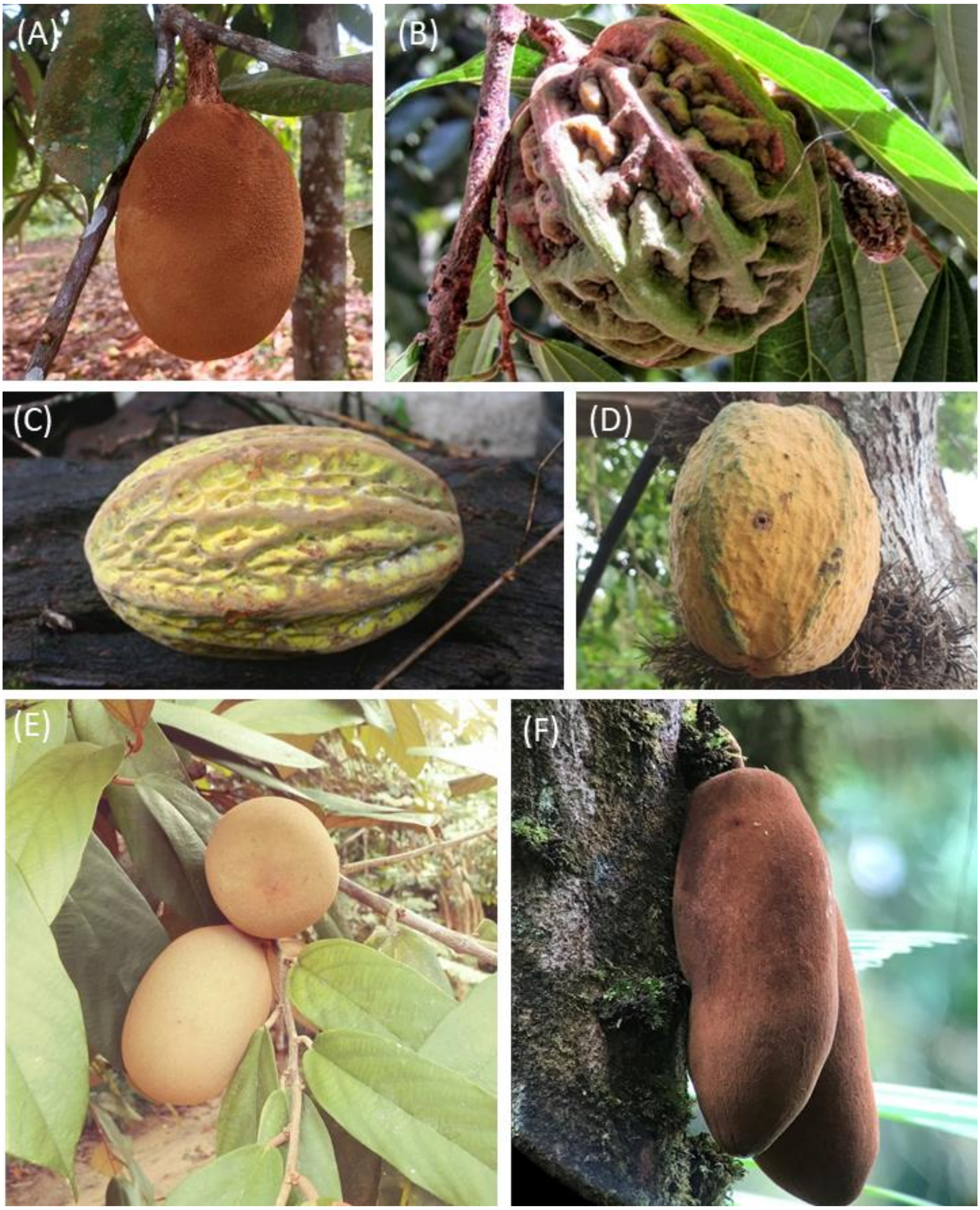

Figure 4: Fruits of selected *Theobroma* species. (A) *T. grandiflorum* (photo: Rusbel Chapalbay 2019), (B) *T. microcarpum* (photo: Mirna Caniso 2019), (C) *T. bicolor* (photo: Sean A. Higgins 2013), (D) *T. speciosum* (photo: Mequiel Z. Ferreira 2019), (E) *T. subincanum* (photo: Miquéias Queiroz 2021), (F) *T. simiarum* (photo: Hugo Lopez 2021)

4.2. Variations in cacao germplasm

Crop diversity is evaluated using a variety of indicators of variability (Table 1), particularly physical features that are crucial for cataloguing and characterizing cacao germplasm (Malhotra 2017). The activity of alleles of genes that occur and determine the particular traits as well as the total of alleles that make up the genotype of the plant, causes variance in phenotypic expressions (Bartley 2005). Biodiversity International has defined cacao's descriptor status. It now includes 60 characteristics such as plant architecture, leaf, flowers, seed qualities, and unique features such as reactivity to biotic and abiotic factors. Genetic variation allows for an extensive range of possible genotypes to be chosen for future breeding (Malhotra 2017).

Table 1: Variability in cacao germplasm (adapted from Malhotra 2017)

Part of the tree	Possible characteristics
Tree architecture	Erect, Intermediate, and pendulous growth habits
Leaves	Shades of green and purple, with or without pulvinus in the petiole
Flowers	Colour, Petal diameter, Pedicel length
Fruit shape	Angoleta - deeply ridged, warty, square at the stalk end
	Cundeamor - bottlenecked angoleta
	Amelonado - smooth, shallow furrows, melon-shaped, blunt end, slight bottleneck
	Calabacillo - small and nearly spherical Oblong / Elliptic / Obovate / Orbicular
Basal constriction	Absent / Slight / Intermediate/ Strong
Apex form	Attenuate / Acute / Obtuse / Rounded / Mammilate
Surface rugosity	Absent / Slight / Intermediate / Intense
Prominence of ridges	Slight / Distinct
Primary furrow depth	Superficial / Intermediate / Deep
Husk hardness	Soft / Intermediate / Hard
Anthocyanin in ripe pods	Absent / Slight / Intermediate / Intense
Pod size	Big / Medium / Small
Beans/seeds	Shape, Colour, Size

4.3. Cacao production and its constraints

Cacao is regarded as an essential crop in various tropical nations because of the additional revenue it offers to many small-scale farmers. It is also linked to the worldwide chocolate and cocoa production chain. South America accounts for 12.49 % of the worldwide cocoa output, and its countries earn $2.4 billion per year from cocoa exports. Brazil is the leading cocoa producer in South America and the sixth most significant producer globally, with an annual output of 235,809 tons. The Brazilian Amazon biome accounts for 52.12 % of Brazilian cocoa output (Igawa et al. 2022).

4.3.1. Cultivation and propagation methods

Cacao is increasingly being grown in unshaded monocultures to boost output, rather than the more typical shaded farming (Rajab et al. 2018). Shade trees may have both harmful and good impacts on the crop. While the competition for light, water, and nutrients above and below ground may reduce resource availability, potentially reducing yield, shade trees can improve nutrient supply by fixing atmospheric nitrogen, and they can improve nutrient cycling by providing additional leaf and root litter (Beer 1987). Shading also reduces the cacao tree’s exposure to excessive light and increased water vapor saturation deficit in the atmosphere (Rajab et al. 2018). Propagation by seed is the most common method. Hand pollination can be used to acquire good planting material from selected parents. These hybrids might have hybrid vigour as well (giving faster growth and earlier bearing). Trees that have been grown from seeds tend to be drought-resistant and may require less pruning as well. They do, however, have a lot of diversity in their features, which is not ideal. Cuttings, grafting, and micropropagation systems are some of the vegetative approaches that may be used to overcome this. Grafting on young seedlings and even old trees is possible. Grafted trees have more open branching structures than hybrid cacao trees and they do not have the straight single trunk that trees from seeds do (Beckett 2009).

4.3.2. Environmental requirements of *Theobroma cacao* L.

Since cacao is native to the South and Central American rainforests, it is well adapted to local conditions. Cacao is grown in low light conditions which are further lowered by clouds during rainy seasons (Willson 1999). The cacao tree usually grows in the shade and thrives in tropical climates with high humidity and rainfall with a range of temperatures. Cacao trees flourish at temperatures ranging from 30-32°C to 18-21°C and have even been successfully grown in places with temperatures as low as 14°C. However, rainfall levels have a greater impact on cacao tree production than any other climatic component. Because trees are sensitive to soil moisture deficit, rainfall should be ample and evenly distributed throughout the year (Ramtahal 2012). A hot, humid environment is also necessary for cacao trees to grow well. Premature leaf fall and eventual tree dieback come from long-term exposure to low humidity and little rainfall, lowering production well below its potential. The cacao tree makes the most of any available light and has generally been planted in the shade. In fact, when cacao trees are small and immature, shade is required (Wood & Lass 2008; Beckett 2009). They are frequently intercropped with banana, citrus, other fruit trees, and wood species such as cedar in local commercial plantations (Ramtahal 2012). Strong winds damage cacao trees, and their impact must be managed by planting trees close by such as walnut, palms, or rubber trees (Ortiz 2016). Cacao thrives in loose, gritty soil because it allows for better drainage and root development. Also, good nutritional levels and organic matter content should be present in the depth of 1.5 meters of the soil. Soils with 50 % sand, 30-40 % clay, and 10-20 % small-sized particles are the best option. Cacao does not tolerate drought and requires constant watering for its roots (if the tree goes more than three months without receiving at least 100 mm of rain, it will die). Furthermore, the tree is susceptible to excessive water and requires good drainage to avoid issues such as fungus. The pH of the soil should range from 4.0 to 7.5 maximum, so it prefers soils that are slightly acidic with a pH of around 6.5 (Ortiz 2016).

4.3.3. Diseases

Cacao is a key source of income for several tropical countries. However, various diseases and pests threaten the crop, which is responsible for 30 % of harvest loss worldwide (Lanaud et al. 2009). Cacao diseases are a significant problem in this crop, not only because of crop losses but also because of the high cost of management methods. Diseases can harm the cacao tree's trunk, branches, leaves, roots, and pods. Oomycetes such as *Phytophthora* spp., ascomycetes such as *Ceratocystis cacaofunesta*, and basidiomycetes such as *Moniliophthora roreri* and *M. perniciosa* are fungi that cause the majority of cacao diseases (Table 2). Only one virus disease (cocoa swollen shoot virus, CSSV) has been identified, and it is only found in West Africa. There are no significant bacterial diseases that impact cacao. Pests such as mirids (*Sahlbergella singularis*) and pod borer (*Conopomorpha crammerella*) attack cacao stems and pods (Micheli et al. 2010). Since 1989, black pod, frosty pod, and witches' broom have been discovered as the three most devastating diseases of *T. cacao* (Fulton 1989).

Table 2: Losses caused by the major cacao diseases in 2001 and 2016 (adapted from Marelli et al. 2019)

Disease	**Annual losses in 2001 (x1,000 metric tons)**	**Annual losses in 2016 (x1,000 metric tons)**
Phytophthora spp.	450	873
Witches' broom (*Moniliophtora perniciosa*)	250	492
Cacao swollen shoot virus	50	96
Vascular streak dieback (*Ceratibasidium theobromae*)	30	61
Frosty pod (*Moniliophtora roreri*)	30	76
Cocoa pod borer (*Conopomorpha cremerella*)	40	81
Total	850	1679

4.3.3.1. The black pod disease (*Phytophthora* spp.)

The cacao black pod disease, so-called black pod rot (Marelli et al. 2019), is prompted by numerous species of the stramenopile *Phytophthora* genus, which has a wide geographical distribution (Acebo-Guerrero et al. 2012). *P. megakarya* and *P. palmivora* seem to be the most prominent cacao diseases in Central and West Africa and most studies focus on these two species (Adomako 2007; Guest 2008). The occurrence of *P. megakarya* has not been recorded in Central nor South America (Davison 1998), but *P. palmivora* appears to have a global range, since it has been found in Africa, Asia, and America (Davison 1998; Guest 2008). The appearance of black pods in a cacao crop is the most visible indicator of *Phytophthora* infection (Hebbar 2007). Though the infection can develop anywhere on the tree, it commonly occurs on the pods that are the closest to the ground or often at the tip or a stem end. The infection looks like chocolate brown lesions that spread and will eventually cover the whole pod. When *Phytophthora* infects the husk, it invades the tissue on the inside of the pod, causing browning and shrivelling of the cacao beans and the pods become black and mummify over time causing them to die and become a source of further spread (Acebo-Guerrero et al. 2012). If the infection appears on a seedling it will compromise the growth. Both *P. megakarya* and *P. palmivora* can infect flower cushions as well as bark which serve as a hiding spot so it is hard to notice for farmers. The spores can come from the soil where it can remain dormant for several years, dead pods, and any infected part of the tree (Appiah et al. 2004). The black pod rot is controlled using a variety of strategies, including chemical compounds (fungicides based on copper), genetically resistant trees, biological control, and phytosanitary measures. The spraying of fungicides is not very efficient, because of the trees height and rainfall washout, which requires reapplication. In response to this issue, injecting the fungicide right into the trunk of the tree becomes a good and cost-efficient way of coping with this disease (Acebo-Guerrero et al. 2012). Although many breeding programmes have aimed for finding genotype resistance to *Phytophthora*, cacao genotypes that are entirely resistant are currently unavailable (Acebo-Guerrero et al. 2012). Several microorganisms have a beneficial effect on the management of *Phytophthora* because they inhibit its growth. Microorganisms that show these characteristics are, *Trichoderma bamatum* and *T. martiale* (Hanada et al. 2009). Actinomycetes are another microbial group that showed potential for controlling this disease because of their antagonistic *in-vitro* effects on *Phytophthora* (Barreto et al. 2008). Then there are some bacteria that have been proven to have a positive outcome, such as *Pseudomonas aeruginosa*, *P. fluorescens* as well as *Azopirillum brasilense* and *Bacillus subtilis* because of their antagonistic effects on black pod rot. The use of antagonistic microorganisms is considered to be one of the safest methods for the environment, if used correctly, and does not belong to the expensive group of management strategies (Acebo-Guerrero et al. 2012).

4.3.3.2. Witches' broom (*Moniliophtora perniciosa*)

The fungus *Moniliophthora perniciosa* causes cacao witches' broom, the second most economically destructive disease that affects the cacao tree. It is only surpassed by black pod rot, which is caused by numerous *Phytophthora* species and is more common (De Souza et al. 2018). South America, Central America, and the Caribbean are the only places where witches' broom occurs (Purdy & Schmidt 1996). Witches' broom does not affect only species under the genus *Theobroma* but many others, including its sister genus *Herrania*. Research has shown a significant genetic diversity of *M. perniciosa,* which is why it has been classified into different biotypes. Only biotype C affects *Theobroma* and *Herrania* (De Souza et al. 2018). Infections of the apical buds of leaf flushes result in developing vegetative terminal brooms, as the fungus infects all meristematic tissues. The size of vegetative brooms is determined by the variety's level of resistance, tree vigour, and fungal genotype. Petioles and *pulvini* may also experience hyperplasia and hypertrophy, in addition to terminal buds. As a result of the loss of apical dominance, lateral budding of vegetative brooms is widespread. Abnormal flowers may be produced as well as deformed pods whose shape resembles a strawberry. Infected pods remain small in size and may show signs of chlorosis, necrotic lesions, and early ripening. The pulp as well as seeds inside the pods become rotten and are not appropriate for harvest. Pods that are older than three months are not at risk, and even if the infection occurs, the seeds are still useful. Disease control includes phytosanitation, chemical, biological control, and genetic resistance of varieties (De Souza et al. 2018).

4.3.3.3. Cacao swollen shoot virus (*Badnavirus*)

The cacao swollen shoot virus (CSSV), which belongs to the genus *Badnavirus*, causes CSSV disease (Lot et al. 1991). Although CSSV occurs only in West Africa (Muller 2016), it is worth mentioning since it has now impacted cacao production in Africa for more than eight decades (Dzahini-Obiatey et al. 2010), especially in Ghana (Muller 2016). CSSV is genetically diverse so it may cause different symptoms depending on the virus strain. Transient red veins and mottling in immature leaves, various shades of chlorosis in adult leaves and pods, root atrophy and stunting, as well as root and stem swellings, are all possible indications. The majority of these symptoms are specific to distinct strains, and the host's genotype determines symptom manifestation. Highly pathogenic strains generate severe leaf chlorosis, which can cause cacao plants to die quickly (Dzahini-Obiatey et al. 2010). The virus is spread by mealybugs (*Pseudococcidae* spp.) in the adult or a nymph stages (Andres et al. 2017). The only treatment known so far is mechanically removing affected trees in a particular area and some healthy trees in close contact as a preventive measure (Thresh & Owusu 1986). Other preventive strategies that have been applied in the past did not show success mainly because of farmer's scepticism or perspective (Andres et al. 2017). Andres et al. (2017) suggest focusing mainly on resistance breeding of cacao varieties.

4.3.3.4. Vascular streak dieback (*Ceratobasidium theobromae*)

Vascular streak dieback was firstly reported in Papua New Guinea in the early 1960s (Guest & Keane 2018). The first symptom visible and also one that is most characteristic of vascular streak dieback is the chlorosis of one leaf that is further spread to all of the leaves. Within a few days after the chlorosis spreads drastically among the leaves, the xylem is affected, and swollen lenticels on the stem due to basidiocarps of *Ceratobasidium theobromae* are formed. The infection is fungal, and when affected leaves fall to the ground, spores are formed and then spread through the air by wind (Guest & Keane 2018). Vascular streak dieback is usually controlled by regular pruning and cutting of the affected parts of the tree (Beckett 2009).

4.3.3.5. Frosty pod (*Moniliophtora roreri*)

In terms of worldwide crop losses, frosty pod rot of cacao is a minor fungus disease caused by *Moniliophtora roreri*, responsible for an estimated 30 thousand tonnes of cocoa output loss, or less than 4 % of overall crop losses (Bowers et al. 2001). It is thought that frosty pod evolved on *Theobroma gileri* Cuatrec., which is endemic to Ecuador, but the disease turned into an invasive one and has spread throughout Peru, Bolivia, Brazil, Costa Rica, Nicaragua, Honduras, Guatemala, and local plantations are facing a significant threat, and major losses (Evans et al. 2003). It spreads by spores which are carried by the wind. The usual control includes sanitation and pruning of the affected parts (Beckett 2009).

4.3.4. Pests

4.3.4.1. Cacao pod borer (*Conopomorpha cremerella*)

Cacao pod borer (*Conopomorpha cremerella*) is a moth of the family *Gracillariidae,* and cacao is the principal host of this pest, which is capable of causing great yield and financial losses. (Teh et al. 2006). While pesticides can reduce the damage, there is a fear that their effectiveness will decline with time, and they are not the safest option for the environment or the consumers (Lee Chin Tui 1996). Adult female moths lay on average 60-150 eggs on rough-surfaced pods, and their life cycle is about 22-33 days long. When the larvae get inside the pod, they feed on the seeds that have not matured yet. This causes the seeds to clump together and stops the development process, and when the infestation is severe, the whole pod will stop developing (Teh et al. 2006). Pods that have a smooth texture on the outside are much less attacked than rough pods. The reason is that eggs are protected from being washed off by rain, and they stay on the surface for longer. Forastero varieties are less vulnerable than Criollo varieties, although the thickness of the pod was not recognized to be a factor (Wessel 1983). A study done by Teh (2006) compared pod resistance and susceptibility for CPB between 8 clones (AMAZI5-15, BAL244, BRS5, IMC23, KKM22, PA13, PBC123, and TAP1-2) of cacao. They made their results based on the number of entry and exit holes on pods made by CPB. Clone PBC123 had the lowest number of exit holes, and the clone BAL244, which is of a Scavina origin with soft pods, had the highest number of exit holes. BR25, which had the softest pods, had the lowest number of entry holes. Their data suggests that in the matter of the weight of the beans per pod, PBC123 was the most resistant to infestation, and KKM22 showed the highest reduction in the weight of seeds. There is evidence that an important factor in resistance against CPB is the hardness of the sclerotic layer of the pod wall (Teh et al. 2006). The critical point is that some clones may be less susceptible or even resistant, but their yield is not very high compared to other variables that may not be as resistant but provides a much higher yield. In one large-scale testing, BR25 yielded 132 % PBC123, while the second and third most resistant clones, IMC23 and AMAZ15-15, yielded just 59 % and 33 % of BR25 (Teh et al. 2006).

4.3.4.2. The brown cacao mirid (*Sahlberhgella singularis*)

In Ivory Coast, *Sahlbergella singularis* is the most devastating cacao insect pest. Other cocoa-producing countries, such as Ghana, Nigeria, and Cameroon, are also plagued by these insects (N'Guessan et al. 2008). Except for the leaves and roots, mirids consume every plant part. Adult and juvenile stages both cause harm by puncturing vegetative or fruiting parts of the plant. Saliva is injected into the wound while feeding, which causes the disintegration of organic tissues, most likely due to the activity of enzymes (Williams 1953). Mechanical trauma and saliva's impact are sufficient to kill early shoots (N'Guessan et al. 2008).

4.3.5. Climate change

Evidence shows that cacao is vulnerable to a dry environment, which reduces crop production. Future climate change seems to be a warmer and drier environment in the Amazon basin (Igawa et al. 2022). The global average temperature has risen by roughly 0.85°C between 1880 and 2012 and is expected to rise by another 1.4-3.1°C by the end of this century. Rising temperatures, changes in precipitation patterns, and increased frequency of major droughts and floods are anticipated to reduce agricultural yields and threaten farmer's livelihood worldwide (Stocker et al. 2013). Climate change breeding tolerance is one of the potential climate change strategies. Cacao's genetic diversity is an essential resource for developing climate change tolerant cultivars. Numerous studies, for example, have found substantial diversity among cacao genotypes in response to extreme temperatures, water shortages, and flooding (Ceccarelli et al. 2021).

4.4. Cacao breeding objectives

Among the literature published about cacao breeding strategies, the primary goals for breeding are resistance to diseases and pests and, secondly, a higher yield of better quality cacao seeds. Cacao is an evergreen tree with distinct plant morphology and fruit features and high sensitivity to climatic change and growth conditions, necessitating long-term and dynamic breeding efforts. Cacao breeding programmes started in 1930 by Cocoa Research Unit, and in 2008 Mondelez International started funding cocoa research done by Tamil Nadu Agricultural University (Malhotra 2017).

Table 3: Breeding criteria and traits involved (adapted from Malhotra 2017)

Yield	**Quality**
Vigour of young trees • Relationship between yield and vigour of adult trees Interaction with a planting density Ability to adapt in the field Productivity of young trees (earliness) Productivity of adult trees No. of pods per tree Bean wt. per pod (pod index: no. of pods needed for 1 kg of dry beans)	Physical criteria: • Dry bean size (1 gram and above), • Shell percentage (10-15%), • Nib recovery (85-90%), • Butter content (>50%) • Hardness, colour of cotyledons Organoleptic characters: • Flavour, viscosity, taste (bitter, astringent)
Resistance to diseases	**Resistance to insects**
Intrinsic resistance: • resistance to infection, colonization or pathogen multiplication, grading of damage levels Escape: • period of fruiting or vegetative growth, duration of pod ripening, low productivity	Attractiveness Screening: • Grading with the level of infection, husk characters Intrinsic resistance (antibiosis), Antixenosis

Like other perennial crops, cacao's genetic development is extremely slow. A single selection cycle sometimes takes more than a decade, and it is frequently necessary to complete two or more cycles before releasing a new variety. Even for annual crops, it is expected that developing a new variety will take 10 to 20 years. The essential criteria (Table 3) in cacao and evaluated when the plants reach maturity, and several years of data are necessary to make valid conclusions (Phillips-Mora et al. 2012).

4.5. CWR as a solution to overcome cacao production constraints

In general, a crop wild relative is a plant that is more or less closely related to a crop and may contribute genetic material to it but has not been domesticated like the crop species. Crop wild relatives are also likely to be direct ancestors of the crop or its progenitors (Heywood et al. 2007). CWR can differ in the level of relatedness because some species are related more than others so for this case (Harlan & de Wet 1971) gene pool concept is used which separates close relatives that exist in the primary gene pool and more distant relatives that exist in the secondary gene pool (Heywood et al. 2007). Crop wild relatives are becoming an increasingly relevant topic because of their high potential in many sectors of agriculture and research as well as their increased risk of extinction. Rapid biodiversity loss induced by population increase, industrialization, and the expansion of agriculture itself in the second half of the 20th century endangered the existence of many crop wild relatives (Heywood et al. 2007). Due to these problems wild relatives are facing, there is a need for conservation eighter *in-situ* or *ex-situ*, both of which have their benefits. Cultivated crops are threatened by diseases, drought, pests, and many others, so finding genes in crop wild relatives who have built a resistance to these threats may be the key to developing successful hybrids which can be used in further breeding programs. Cacao seeds are recalcitrant, meaning that they do not survive drying and freezing during *ex-situ* conservation, which makes the standard *ex-situ* genetic resource conservation difficult. As a result, germplasm collection, either *in-situ* or *ex-situ*, is required for the long-term conservation of *Theobroma* species (Silva et al. 2004).

Table 4: Main distinctive characteristics of Criollo, Forastero, and Trinitario cacao varieties (adapted from Ramtahal 2012)

	Criollo	**Forastero**	**Trinitario**
Pod			
Texture	Soft	Hard	Mostly hard
Colour	Red + yellow	Green	Variable
Bean/seed			
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

The origins of the Criollo variety are set in Central America and are frequently referred to as the “Prince of Cacaos” due to the great quality of its cacao beans, which have a mild flavour and a distinct, appealing scent (Motamayor et al. 2003; Ramtahal 2012). Throughout time, the quality of the aromatic flavour of the Criollo variety decreased, because of many crossbreeding within genotypes, leading to hybrids that are way more susceptible to diseases (Motamayor et al. 2003). The pods are long and thin, with a slender shape. The tree is narrow and brittle, with ridges on the outside (Willson 1999). Unfortunately, they are disease as well as fungi and insect-prone and generate low yields. Therefore they are rarely grown commercially (Beckett 2009). The Amazon area is home to the Forastero group, the most extensively planted cacao type due to its excellent yields and disease resistance. The Amelonado pod, the most widely planted cacao variety of the Forastero group, is smooth, consistent, and wrinkled with a pointy end (Ramtahal 2012). The Forastero seeds are smaller and have less aroma (Willson 1999). The Trinitario group, is native to Trinidad and was created by crossbreeding of Criollo and Forastero groups (Beckett 2009). Unfortunately, hybrids are often not uniform in pods and seed sizes as well as hardiness and production due to the vastly differing features of their mother species. Fermented seeds have a wide range of features, but they all have a nice, fragrant flavour. Trinitario group is particularly well-suited to commercial production and is grown worldwide (Ramtahal 2012). So far, only some of the wild relatives of *T. cacao* are utilized in breeding programmes, specifically *Theobroma Angustifolium* Sessé & Moc, *Theobroma bicolor* Bonpl., *Theobroma grandiflorum* (Willd. ex Spreng.) K. Schum., *Theobroma mammosum* Cuatrec. & J. León, *Theobroma microcarpum* Mart., *Theobroma simiarum* Donn. Sm., *Theobroma speciosum* Willd. Ex Spreng., and *Theobroma subincanum* Mart. Natural hybrids between *Theobroma* species are very rare, however, they have been reported, mainly between the *Glossopetalum* section. Some natural hybrids of *T. grandiflorum* and *T. subincanum* have been identified and are still present in the CEPLAC germplasm collection in Marituba, Pará state in Brazil. There are no natural hybrids between *T. grandiflorum* and *T. cacao* (Jean-Philippe et al. 2017).

4.5.1. Interspecific hybrids and their outcome

The hybrids between *T. grandiflorum* × *T. subincanum* were highly susceptible to witches' broom disease, and their pulp resembles a banana flavour. Their seeds successfully germinated and produced seedlings. Hybrids between *T. grandiflorum* × *T. obovatum* were resistant to witches' broom disease and produced many pods. Their seeds germinated, but the seedlings died after reaching 10 cm in height (Silva et al. 2004). The outcomes of interspecific hybridization can be seen in Table 5.

Table 5: Interspecific hybridizations in the genera *Theobroma* and *Herrania* (adapted from Silva et al. 2004)

Species x Species	***T. angustifolium***	***T. cacao***	***T. obovatum***	***T. simiarum***	***T. speciosum***	***T. subincanum***	***T. sylvestre***
T. mammosum	xxx	x		xxx			
T. angustifolium		x					
T. grandiflorum		xxx	xxxxx			xxxxx	
T. obovatum						xxxx	
T. microcarpum		xx					
T. bicolor					xx		x
T. glaucum							xxx
T. sylvestre					xxxxx		
T. cacao	xx		x	x	x		
H. baleansis		xx					
H. mariae		x					

x = hybrid fruit, xx = seedling hybrid, xxx = adult hybrid, xxxx = adult hybrid with fruit, xxxxx = F2

4.6. Wild relatives of cacao tree

Literature published about the breeding potential of cacao wild relatives is very limited, but it can be deduced from each species' key and unique characteristics. Almost every one of the species from the genus *Theobroma* is being used by the natives to produced cocoa, chocolate, refreshments or they use the wood or the shells of the pods for handicraft. *T. bernoullii, T. gileri, T. glaucum, T. nemorale* and *T. simiarum* are used to make cocoa of good quality (Table 6) (Cuatrecasas 1964).

Species from the genus *Herrania* are considered a sister genus of *Theobroma*. Studies, have shown that hybrids between these two genera are possible so they should be taken into consideration in breeding programmes (Silva et al. 2004). *H. balaensis* is listed as "endangered" on The IUCN Red List of Threatened Species and *H. umbratica* is "critically endangered". The rest of these species are not listed, but that does not mean they are not endangered. Species of *Herrania* are divided into two sections *Herrania* and *Subcymbicalyx* (Table 7).

Table 6: Wild relatives of *Theobroma cacao* L. from the genus *Theobroma* L.

Scientific name	Common name	Section	Native to	Key characteristics	Uses	Breeding potential	References
T. angustifolium Sessé & Moc. ex DC.	Cacao de mico, cacao silvestre, cushta, cacao de la India, Cacao de mico, mamao meco, coca mono, soró	*Glossopetalum*	Costa Rica, Mexico Southeast, Nicaragua, Panamá	8- 26m high, smooth bark, white wood, yellow-orange flowers, fruit brown, rough and hard	Cocoa source locally without commercial value	Possible substitute for cacao of low quality	1,2,5,6,7,8
T. bernouillii Pittier	Chocolate de monte, cacao de monte bravo, cacao de monte	*Oreanthes*	Colombia, Panamá	Asymmetrical, rigid leaves, white seeds, fruit differs between subspecies	None found	Possibly cocoa of high quality	2,5,6,7,8
T. bicolor Bonpl.	Patashte, white cacao	*Rhytidocarpus*	Brazil North, Colombia, Peru, Venezuela	Thin and hairy leaves, flowers on terminal leafy branchlets, fruit large and with strong-woody pericarp, tree 14 m tall, light bark, white wood, flowers small and reddish, easy to recognize from other species	Seeds are mixed with *T. cacao* seeds to obtain bitter chocolate, the pulp is used to make refreshments by locals	Good quality cacao butter, pulp, leaves, and the hull is high in theobromine, the pulp has a high sugar content	1,2, 4,5,6,7,8

Table 6: Continued

Scientific name	Common name	Section	Native to	Key characteristics	Uses	Breeding potential	References
T. canumanensis Pires & Fróes ex Cuatrec.	None found	*Glossopetalum*	Brazil North	Up to 18m high, small flowers, similar to *T. sinuosum*	None found	None found	2,5,6,7,8
T. cirmolinae Cuatrec.	Bacao,cacao de monte, cacao indio	*Glossopetalum*	Colombia	20m high, grey bark, hard reddish wood, yellow flowers, large thick leaves, grows at very high altitudes (1300m)	Chocolate preparation by the natives	Grows at the highest altitudes so it could be used as a grafting base in colder areas	2,5,6,7,8
T. duckei Huber	None found	None found	Brazil Amazonas	None found	None found	None found	5,6,7,8
T. gileri Cuatrec.	Chocolate de monte	*Telmatocarpus*	Colombia, Ecuador	14m high, brown bark, white hard wood, brownish fruit, sweet pulp, grows in heavy rainforests, suffers from *Monilia roreri*	Natives eat the sweet pulp and prepare cocoa of good taste	Tasty cocoa but suffers from *Monilia roreri*	2,5,6,7,8
T. glaucum H. Karst.	Cacao de monte, cacao silvestre, chucú, bicco	*Oreanthes*	Brazil North, Colombia, Ecuador, Peru	8-15m high, reddish bark, white wood, many red flowers, fruits with blue tone, very sweet pulp, grows near rivers	Used to make cocoa by the natives, very similar to *T. cacao*	Cocoa similar to *T. cacao*, very tasty pulp, has many flowers (up to 200)	2,5,6,7,8

Table 6: Continued

Scientific name	Common name	Section	Native to	Key characteristics	Uses	Breeding potential	References
T. grandiflorum (Willd. ex Spreng.) K.Schum.	Cupuassú, cupuacú. Cupú-assú, copuassú, cupai-acú	*Glossopetalum*	Bolivia, Brazil North, Guyana, Honduras, Venezuela	Usually 6-10m high, grey wrinkled bark, small white flowers, about 50 seeds,	Sour pulp is eaten by natives, soft drinks from the pulp	Has a lot of seeds	2,3,5,6,7,8
T. hylaeum Cuatrec.	Chocolate de monte, cacao silvestre	*Glossopetalum*	Colombia, Panamá	Leaves very similar to *T. nemorale* and *T. angustifolium*		None found	2,5,6,7,8
T. mammosum Cuatrec. & J. León	Cacao silvestre	*Andropetalum*	Costa Rica, Nicaragua	Small tree 6-7m high, smooth brown bark, hard white wood, very small red flowers	None found	None found	2,5,6,7,8
T. microcarpum Mart.	Cacauí, cacaurana, cacao rana, cacau bravo, ceeca de urubú, cacao de monte, me-tró-ree-moo-ee, bóo-e	*Telmatocarpus*	Bolivia, Brazil North, Brazil West-Central, Colombia, Venezuela	10-20m high, reddish flowers, fruit with 10 ribs, the pulp is sweet and scentless	None found	None found	2, 5,6,7,8
T. nemorale Cuatrec.	Bacao de monte, chocolate de monte, bacao, bacaíto, cacao de monte, cacaíto de monte	*Glossopetalum*	Colombia	15-20m high, smooth grey bark, orange flowers, smooth fruit, fragile when matured,	Natives prepare chocolate of fairly good quality	Good quality chocolate	2, 5,6,7,8

Table 6: Continued

Scientific name	Common name	Section	Native to	Key characteristics	Uses	Breeding potential	References
T. obovatum Klotzsch ex Bernoulli	Cabeca de urubú, copu-ai, cupu-curúa, cupurana, cacao de macao, urubú-acain, cabeca de Umbú	*Glossopetalum*	Bolivia, Brazil North, Colombia, Guyana, Peru, Venezuela	15m high, very small flowers, small fruit green-yellow when ripe, pericarp covered in hard warts	None found	Rough surface – possible resistance to *Conopomorpha cremerella*	2, 5,6,7,8
T. simiarum Donn. Sm.	Cacao de mico, cacao de mono, teta negra, kráaku, nunísup, uirub, dzung-mang-uá, ku-gín, bik, uir-ub	*Glossopetalum*	Colombia, Costa Rica, Ecuador, Nicaragua, Panamá	20m high, thick trunk in diameter (60cm), flowers purple-red, hairy large leaves, smooth fruit, woody epicarp, aromatic pulp	Chocolate	The seeds are supposed to give cocoa of good quality	2, 5,6,7,8
T. sinuosum Pav. ex Huber	Cacao de monte, pako kakao	*Glossopetalum*	Brazil North, Peru	Smooth, hard, woody fruit,	Pulp is eaten by natives	None found	2, 5,6,7,8
T. speciosum Willd. ex Spreng.	Cacauí, cacauu, cacao-y, cacao-u, cacau, cacaohy, cacaoíllo, cacao-rana, cacao biaro, capuy, cacao do matta, cupurana, cacao azedo, cacao sacha, chocolatillo	*Oreanthes*	Bolivia, Brazil North, Brazil Northeast, Brazil West-Central, Peru, Venezuela	Up to 15 m high tree with branches on the top of a long branchless stem, purplish-red flowers, fruit without ribs, and yellow when ripe	Pulp is eaten by natives, seeds are rarely used to make low quality chocolate	Flowers rich in antioxidants - alternative food source, low-quality chocolate	2, 5,6,7,8

Table 6: Continued

Scientific name	Common name	Section	Native to	Key characteristics	Uses	Breeding potential	References
T. subincanum Mart.	Cupuí, cupuaí, copuí, copuaí, cupuhy, cupuahy, cupuy do Igapó, cupuarana, cupú do matta, cupú-assuy, cupú-assú-rana	*Glossopetalum*	Bolivia, Brazil North, Brazil West-Central, Colombia, Ecuador, French Guiana, Guyana, Suriname, Venezuela	6-20m high, grey smooth bark, small flowers, smooth fruit orange when ripe, pulp sweet and scentless	Rarely used by the natives	Can be used for cocoa production but the quality is lower than in *Theobroma cacao*, a vitamin E source, high % of cycloartenol in the endosperm	2, 5,6,7,8
T. sylvestre Aubl. ex Mart. in Buchner	Cacao azul, cacauí, cacauú, cacao-hú, cacao rana, cacau rana, cacau bravo	*Oreanthes*	Brazil North, Brazil Southeast, Brazil West-Central	12m high, flowers small, brownish-red, fruit hard, smooth, pulp sweet, scentless	None found	None found	2, 5,6,7,8
T. velutinum Benoist	Bouchi-cacao, cacao sauvage, cacao	*Oreanthes*	Brazil North, Brazil Northeast, Brazil West-Central, French Guiana, Suriname	Many flowers, smooth fruit with prominent ribs, 30 seeds per pod, similar flowers to *T. speciosum* but the fruits of both species are very different	Seeds are used to make chocolate, but no information on the quality	None found	2, 5,6,7,8

References: 1 (Sotelo & Alvarez 1991), 2 (Cuatrecasas 1964), 3 (Malhotra 2017), 4 (Ponce-Sánchez et al. 2021), 5 (“Plants of the World Online | Kew Science” 2022), 6 (“International Plant Names Index” 2022), 7 (“International Plant Names Index” 2022), 8 (“Tropicos” 2022)

Table 7: Wild relatives of *Theobroma cacao* L. from the genus *Herrania* Goudot

Scientific name	Synonyms	Section	Native to	Uses	Red list status	References
H. albiflora Goudot	*Herrania albiflora f. titanica* R.E.Schult., *Theobroma albiflorum* (Goudot) De Wild.	*Herrania*	Colombia, Venezuela	Food, medicines	Not listed	1,2,3,4,5
H. balaensis P. Preuss	*Herrania pulcherrima* var. *pacifica* R.E.Schult., *Theobroma baleanse* (H.Preuss) De Wild.	*Subcymbicalyx*	Colombia, Ecuador	Food	**Endangered**	1,2,3,4,5
H. breviligulata R. E. Schult.	None found	*Subcymbicalyx*	Colombia, Ecuador	Food, medicines	Not listed	1,2,3,4,5
H. camargoana R. E. Schult.	*Theobroma camargoanum* (R.E.Schult.) Ducke	*Subcymbicalyx*	Brazil North, Colombia, Venezuela	Food, medicines	Not listed	1,2,3,4,5
H. cuatrecasasiana García-Barr.	None found	*Subcymbicalyx*	Colombia, Ecuador	Food, medicines	Not listed	1,2,3,4,5
H. dugandii García-Barr.	None found	*Subcymbicalyx*	Colombia, Ecuador	Medicines	Not listed	1,2,3,4,5
H. kanukuensis R. E. Schult.	*Theobroma mariae* var. *lobatum* Pulle	*Subcymbicalyx*	Brazil North, French Guiana, Guyana, Suriname	None found	Not listed	1,2,3,4,5
H. kofanorum R. E. Schult.	None found	*Subcymbicalyx*	Colombia, Ecuador	None found	Not listed	1,2,3,4,5

Table 7: Continued

Scientific name	Synonyms	Section	Native to	Uses	Red list status	References
H. laciniifolia Goudot	*Theobroma laciniifolium* (Goudot) De Wild.	*Subcymbicalyx*	Colombia	Medicines	**Least concern**	1,2,3,4,5
H. lemniscata (M. R. Schomb.) R. E. Schult.	*Lightia lemniscata* M.R.Schomb.	*Subcymbicalyx*	Brazil North, Colombia, Guyana, Suriname, Venezuela	Food	Not listed	1,2,3,4,5
H. mariae (Mart.) Goudot	*Abroma mariae* Mart.	*Subcymbicalyx*	Brazil North, Colombia, Ecuador, Peru	Food, medicines	**Least concern**	1,2,3,4,5
H. nitida (Poepp.) R. E. Schult.	*Abroma nitidum* Poepp., *Brotobroma aspera* H. Karst. Triana, *Herrania aspera* (H.Karst. Triana) H.Karst, *Herrania atrorubens* Huber, *Herrania nitida* f. *nitida*, *Herrania nitida* var. *aspera*, *Theobroma aspera*	*Subcymbicalyx*	Brazil North, Colombia, Ecuador, Peru, Venezuela	Food, medicines	Not listed	1,2,3,4,5
H. nycterodendron R. E. Schult.	None found	*Subcymbicalyx*	Bolivia, Brazil North, Colombia, Ecuador, Peru	Food, medicines	**Least concern**	1,2,3,4,5

Table 7: Continued

Scientific name	Synonyms	Section	Native to	Uses	Red list status	References
H. pulcherrima Goudot	*Theobroma pulcherrimum* (Goudot) De Wild.	*Subcymbicalyx*	Colombia, Ecuador, Panamá	Medicines	Not listed	1,2,3,4,5
Herrania purpurea (Pittier) R. E. Schult.	*Theobroma purpureum* Pittier	*Herrania*	Colombia, Costa Rica, Ecuador, Nicaragua, Panamá, Venezuela	Food, materials	**Least concern**	1,2,3,4,5
H. tomentella R. E. Schult.	None found	*Subcymbicalyx*	Colombia	None found	Not listed	1,2,3,4,5
H. umbratica R. E. Schult.	None found	*Herrania*	Colombia	Medicines	**Critically endangered**	1,2,3,4,5

References: 1 ("Plants of the World Online | Kew Science" 2022), 2 ("International Plant Names Index" 2022), 3 ("The WFO Plant List | World Flora Online" 2022), 4 ("Tropicos" 2022), 5 ("The IUCN Red List of Threatened Species" 2022)

4.7. Morphological differences between *Theobroma* and *Herrania*

The genus *Herrania* is closely related to *Theobroma* and consists of 19 species. *Theobroma* and *Herrania* are in the same family *Malvaceae* and the tribe *Byttnerieeae*, which is known for its distinctive floral morphology (Whitlock & Baum 1999). There has been confusion surrounding the genus *Theobroma* and *Herrania* in the past because of the similarity of their fruits. They have been put in the same genera but further research showed that they are different (Table 8) so they have been separated (Cuatrecasas 1964). Although similar floral characteristics may be found in other genera in the tribe, *Theobroma's* and *Herrania*'s fleshy and indehiscent fruit is unique for them. All other *Byttnerieeae*, on the other hand, have dry and completely or slightly dehiscent fruit. Because of this combination of characteristics, a strong affinity between *Theobroma* and *Herrania* has been established. The concept of a sister-group connection between them has recently been validated by sequences of the chloroplast gene ndhF. The growth forms of *Herrania* and *Theobroma* are easily distinguished by the leaves. *Herrania* species have palmately compound leaves, whilst *Theobroma* species have simple leaves. (Whitlock & Baum 1999).

Table 8: Comparison of morphological characteristics between *Theobroma* and *Herrania* genera (adapted from Cuatrecasas 1964)

	Theobroma	***Herrania***
Stem	sympodial	monopodial
Branching	3-5 verticillate branching, dysmorphic and copious branches	unbranched with apical growth
Leaves	dysmorphic, simple leave blades, petal lamina rounded, not more than twice as long as the hood	uniform, petal lamina much longer than the hood, linear
Fruit	Smooth or rugose, angular, staminal filament symmetrical	strongly costate, staminal filament asymmetrical parted in two branches

4.8. Uses and potential of cacao crop wild relatives

The economic importance of the cocoa and chocolate industries does not need to be emphasized. The pulp of most wild cacao species is consumed by the indigenous, who use it to make refreshing beverages, or they consume it raw as is. Although the seeds of most species may be used to make chocolate, only one of them has become commercially important and cultivated in this regard: *Theobroma cacao*. The cocoa butter removed by pressure during the chocolate-making process is an important by-product of cacao seeds. Cocoa butter is used in both cosmetics and pharmacy business, and also because of its cardiotonic and diuretic characteristics, cacao extracts and theobromine are essential in medicine (Cuatrecasas 1964).

4.8.1. *Theobroma speciosum* Willd ex. Spreng.

A study focusing on the antioxidant properties of flowers from *Theobroma speciosum* (Mar et al. 2021). Although there are many known uses of the cacao seeds and pulp there are many other so-called uncommon or unusual plant parts such as fruit peels, plant buds, rhizomes, or even unripe fruits which may offer potential benefits. A recent study by Mar et al. (2021) showed these results: Sugars (glucose and fructose), organic acids (citric and malic acid), and phenolic substances were discovered (protocatechuic acid, quercetin, quercetin pentoside, and quercetin-3-O-glucoside). K, Mg, P, and Ca were the most common minerals found in flowers. The aqueous extract included phenolic compound-rich fractions linked to its antioxidant potential. The edible flowers of *Theobroma* species might be used as an antioxidant-rich alternative food source (Mar et al. 2021).

4.8.2. *Theobroma grandiflorum* K. Schum.

In Brazil, *Theobroma grandiflorum* is known as *cupuasú* because of the flavourful mucilage, surrounding the seeds (Malhotra 2017). Juices, ice creams, jams, sweets, pastries, and liquors all benefit from the intense tropical taste of *cupuasú* seed-surrounding pulp. The seed pulp accounts for up to 45 % of the fruit's fresh weight. *Cupuasú* seed fat is not the same as cocoa butter, but it may be used in a variety of cosmetic and culinary items, such as ice cream and pastries (Alves et al. 2007).

4.8.3. *Theobroma bicolor* Pittier

It's known as *patashte* in Mexico and is used in traditional drinks. *Balamte* (jaguar tree), *pataxte, white cocoa,* and *malacayo cocoa* are some of the names used in Guatemala, where its seeds are utilized in drinks, while its shells are used for handicraft items. It is called *macambo* or *maraca* in Colombia and Peru, and its pulp is used to produce juices and marmalades. Its toasted seeds are eaten as snacks or prepared to make chocolate named *bacalate*. *White cocoa* is its popular name in Ecuador, where the pulp is eaten raw by the Kichwa people, and the roasted seeds are used to prepare several dishes (Ponce-Sánchez et al. 2021). Seeds of *T. bicolor* have a high nutritional value, similar to that of nuts sold in foreign markets. Proteins, lipids, carbohydrates, fibres, and copper, magnesium, manganese, potassium, and zinc, are high in seed components. In the last five years, global nut consumption has increased, and *T. bicolor* seeds from Peru have penetrated worldwide markets, notably those in the United States. Similarly, seeds from kichwa lowland villages in Ecuador have lately begun to be sold in Quito (Ponce-Sánchez et al. 2021)

4.8.4. *Theobroma subincanum* Mart.

The seeds of *Theobroma subincanum* are larger morphologically, with woody and tough teguments thicker than those of *T. cacao*. This trait might be a problem during the industrial squeezing process, preventing them from being used to their full potential. The study done by Bruni (2002) revealed a significant amount of cycloartenol (about 30 %) in the endosperm. This data emphasizes the distinction between *T. subincanum* and other *Theobroma* species (Bruni et al. 2002). *T. subincanum* seeds can thus be recommended for a variety of applications. The high topocepherol concentration of *T. subincanum* seeds or their components, which are similar to that of commercial wheat germ oil, implies that they might be used as a low-cost renewable source of vitamin E isomers for industrial processing in cosmetics, medicines, and nutraceuticals. Furthermore, even if the teguments of *T. subincanum* are woodier and more robust than those of *T. cacao*, the quality of *T. subincanum* products resulting from industrial processing of the whole seed would not be compromised (Bruni et al. 2002).

5. Conclusions

There is very little literature that has been published on the potential of wild relatives of *Theobroma cacao*, but that does not mean that there is no potential. A list of potentially wanted characteristics of wild relatives of cacao was obtained in the context of breeding. Many hybrids of *T. cacao* cultivars have been successfully cultivated, and some showed resistance to destructive diseases and pests. From used literature, it is apparent that wild relatives of cacao are not being used in breeding programmes because the focus is not only on the resistant traits but on the quality of yield, which wild relatives do not reach in comparison to Criollo, Forastero, or Trinitatio cultivars. In publications have been mentioned which genes have the ability to resist various diseases, so gene sequencing of material from cacao wild relatives could help to understand their potential more in-depth. There is also one factor that compromises the research, which occurs, for example, in Peru (also a hotspot of cacao wild relatives) where there is prohibited to export plant material for research purposes. Hybrids between *T. grandiflorum* × *T. obovatum* were resistant to witches' broom disease and produced a high number of pods. Wild relatives of cacao have the potential to become a solution to some constraints cocoa production and cultivation faces. More advanced biotechnologies and gene sequencing could be beneficial in finding genes which are specifically resistant to different threats. The valid point is that the development of new varieties or cultivars of cacao hybrids is a time-consuming process that can take up to 20 years in the case of one variety. A list of all accepted species from *Theobroma* and *Herrania* species, considered wild relatives of *T. cacao*, has been made and is unique for this thesis. The list could be used as a guide for choosing the right species for breeding programmes, since it provides information about distribution, morphological characteristics and, breeding potential for each species.

6. References

Acebo-Guerrero Y, Hernandez-Rodriguez A, Heydrich-Perez M, El Jaziri M, Hernandez-Lauzardo AN. 2012. Management of black pod rot in cacao (*Theobroma cacao* L.): a review. Fruits **67**:41–48. Int Soc Horticultural Science-Ishs, Leuven.

Adomako B. 2007. Causes and extent of yield losses in cocoa progenies. Tropical Science **47**:22–25.

Almeida A-AF de, Valle RR. 2007. Ecophysiology of the cacao tree. Brazilian Journal of Plant Physiology **19**:425–448. Brazilian Journal of Plant Physiology.

Alves R, Sebbenn A, Artero A, Clement C, Figueira A. 2007. High levels of genetic divergence and inbreeding in populations of cupuassu (*Theobroma grandiflorum*). Tree Genet Gen 3: 289-298. Tree Genet Genomes **3**:289–298.

Andres C, Gattinger A, Dzahini-Obiatey HK, Blaser WJ, Offei SK, Six J. 2017. Combatting Cocoa Swollen Shoot Virus Disease: What do we know? Crop Protection **98**:76–84.

Appiah AA, Opoku IY, Akrofi AY. 2004. Natural occurrence and distribution of stem cankers caused by *Phytophthora megakarya* and *Phytophthora palmivora* on cocoa. European Journal of Plant Pathology **110**:983–990.

Barreto TR, Silva ACM da, Soares ACF, Souza JT de. 2008. Population densities and genetic diversity of actinomycetes associated to the rhizosphere of *Theobroma cacao*. Brazilian Journal of Microbiology **39**:464–470. Sociedade Brasileira de Microbiologia.

Bartley BGD. 2005. Genetic diversity of cacao and its utilization. Genetic Diversity of Cacao and Its Utilization:1–341.

Beckett ST, editor. 2009. Industrial chocolate manufacture and use4th ed. Wiley-Blackwell, Chichester, U.K. ; Ames, Iowa.

Beer J. 1987. Advantages, disadvantages and desirable characteristics of shade trees for coffee, cacao and tea. Agroforestry Systems **5**:3–13.

Bhattacharjee R. 2018. Taxonomy and classification of cacao. Pages 3–18.

Boroughs H, Hunter R. 1961. The Effect of Temperature on the Germination of Cacao Seeds.

Bowers JH, Bailey BA, Hebbar PK, Sanogo S, Lumsden RD. 2001. The Impact of Plant Diseases on World Chocolate Production. Plant Health Progress **2**:12. Scientific Societies.

Bruni R, Medici A, Guerrini A, Scalia S, Poli F, Romagnoli C, Muzzoli M, Sacchetti G. 2002. Tocopherol, fatty acids and sterol distributions in wild Ecuadorian *Theobroma subincanum* (*Sterculiaceae*) seeds. Food Chemistry **77**:337–341.

Ceccarelli V, Fremout T, Zavaleta D, Lastra S, Imán Correa S, Arévalo-Gardini E, Rodriguez CA, Cruz Hilacondo W, Thomas E. 2021. Climate change impact on cultivated and wild cacao in Peru and the search of climate change-tolerant genotypes. Diversity and Distributions **27**:1462–1476.

Cuatrecasas J. 1964. Cacao and Its Allies: A Taxonomic Revision of the Genus *Theobroma*. Smithsonian Institution.

Curry G, Koczberski G, Omuru E, Nailina RS. 2007. Farming Or Foraging? Household Labour and Livelihood Strategies Amongst Smallholder Cocoa Growers in Papua New Guinea.

Davison EM. 1998. *Phytophthora Diseases* Worldwide. Plant Pathology **47**:224–225.

De Souza J, Monteiro F, Ferreira M, GRAMACHO K, Luz E. 2018. Cocoa diseases: witches' broom. Pages 239–269.

de Souza PA, Moreira LF, Sarmento DHA, da Costa FB. 2018. Cacao—*Theobroma cacao*. Pages 69–76 in Rodrigues S, de Oliveira Silva E, de Brito ES, editors. Exotic Fruits. Academic Press. Available from https://www.sciencedirect.com/science/article/pii/B9780128031384000101 (accessed April 1, 2022).

Dzahini-Obiatey H, Domfeh O, Amoah F. 2010. Over seventy years of a viral disease of cocoa in Ghana: From researchers' perspective. Afr. J. Agric. Res. **5**.

Evans HC, Holmes KA, Reid AP. 2003. Phylogeny of the frosty pod rot pathogen of cocoa. Plant Pathology **52**:476–485.

Fulton R. 1989. The cacao disease trilogy: black pod, monilia pod rot, and witches'-broomDOI: 10.1094/PD-73-0601.

Guest D. 2008. Black Pod: Diverse Pathogens with a Global Impact on Cocoa Yield. Phytopathology **97**:1650–3.

Guest D, Keane P. 2018. Cacao diseases: vascular-streak dieback. Pages 287–301.

Hanada R, Pomella A, Soberanis W, Loguercio L, Pereira J. 2009. Biocontrol potential of *Trichoderma martiale* against the black-pod disease (*Phytophthora palmivora*) of cacao. Biological Control **50**.

Harlan JR, de Wet JMJ. 1971. Toward a Rational Classification of Cultivated Plants. TAXON **20**:509–517.

Hebbar PK. 2007. Cacao Diseases: A Global Perspective from an Industry Point of View. Phytopathology® **97**:1658–1663. Scientific Societies.

Heywood V, Casas A, Ford-Lloyd B, Kell S, Maxted N. 2007. Conservation and sustainable use of crop wild relatives. Agriculture, Ecosystems & Environment **121**:245–255.

Hunter JR. 1959. Germination in *Theobroma cacao*.

Igawa TK, de Toledo PM, Anjos LJS. 2022. Climate change could reduce and spatially reconfigure cocoa cultivation in the Brazilian Amazon by 2050. PLoS ONE **17**.

International Plant Names Index. (n.d.). Available from https://www.ipni.org/ (accessed April 10, 2022).

Jean-Philippe M, Carolina SB, Stefan ER, Chigozie VN, Juan CMA, Rodrigo C. 2017, October 4. Hybrid plant products from *theobroma* species and methods of making the same. GB2549006A. Available from https://patents.google.com/patent/GB2549006A/en (accessed April 9, 2022).

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

Lee Chin Tui. 1996. Insecticides resistance in cocoa pod borer *Conopomorpha cramerella* Snellen and the strategy to prevent its development.

Lot H, Djiekpor E, Jacquemond M 1991. 1991. Characterization of the genome of cacao swollen shoot virus. Journal of General Virology **72**:1735–1739. Microbiology Society,.

Malhotra S. 2017. Genetic resources of cocoa (*Theobroma cacao* L.) and its utilization-An Appraisal. Indian Journal of Genetics and Plant Breeding **77**:199–213.

Mar JM, da Silva LS, Moreira WP, Biondo MM, Pontes FLD, Campos FR, Kinupp VF, Campelo PH, Sanches EA, Bezerra J de A. 2021. Edible flowers from *Theobroma speciosum*: Aqueous extract rich in antioxidant compounds. Food Chemistry **356**:129723.

Marelli J-P, Guest DI, Bailey BA, Evans HC, Brown JK, Junaid M, Barreto RW, Lisboa DO, Puig AS. 2019. Chocolate Under Threat from Old and New Cacao Diseases. Phytopathology® **109**:1331–1343. Scientific Societies.

Micheli F, Guiltinan M, Gramacho KP, Wilkinson MJ, Figueira AV de O, Cascardo JC de M, Maximova S, Lanaud C. 2010. Chapter 3 - Functional Genomics of Cacao. Pages 119–177 in Kader J-C, Delseny M, editors. Advances in Botanical Research. Academic Press. Available from https://www.sciencedirect.com/science/article/pii/B978012380868400003X (accessed April 1, 2022).

Motamayor JC, Lachenaud P, Mota J, Loor R, Brown J, Schnell R. 2008. Geographic and Genetic Population Differentiation of the Amazonian Chocolate Tree (*Theobroma cacao* L). PloS one **3**:e3311.

Motamayor JC, Risterucci A, Heath M, Lanaud C. 2003. Cacao domestication II: Progenitor germplasm of the Trinitario cacao cultivar. Heredity **91**:322–30.

Muller E. 2016. Cacao Swollen Shoot Virus (CSSV): History, Biology, and Genome. Pages 337–358 in Bailey BA, Meinhardt LW, editors. Cacao Diseases: A History of Old Enemies and New Encounters. Springer International Publishing, Cham. Available from https://doi.org/10.1007/978-3-319-24789-2_10 (accessed March 13, 2022).

N'Guessan KF, N'Goran JAK, Eskes AB. 2008. Resistance of cacao (*Theobroma cacao* L.) to *Sahlbergella singularis* (*Hemiptera*: *Miridae*): investigation of antixenosis, antibiosis and tolerance. International Journal of Tropical Insect Science **28**:201–210.

Ortiz Federica 2016. *Theobroma cacao* L:32.

Phillips-Mora W, Leal A, Mata Quirós A, Motamayor JC. 2012. Catálogo.

Plants of the World Online | Kew Science. (2022). Available from https://powo.science.kew.org/ (accessed April 10, 2022).

Ponce-Sánchez J, Zurita-Benavides MG, Peñuela MC. 2021. Reproductive ecology of white cacao (*Theobroma bicolor* Humb. & Bonpl.) in Ecuador, western Amazonia: floral visitors and the impact of fungus and mistletoe on fruit production. Brazilian Journal of Botany **44**:479–489.

Purdy L, Schmidt R. 1996. STATUS OF CACAO WITCHES' BROOM: Biology, Epidemiology, and Management. Annual Review of Phytopathology **34**:573–594.

Rajab YA, Hölscher D, Leuschner C, Barus H, Tjoa A, Hertel D. 2018. Effects of shade tree cover and diversity on root system structure and dynamics in cacao agroforests: The role of root competition and space partitioning. Plant and Soil **422**:349–369.

Ramtahal G. 2012, July 24. INVESTIGATIONS OF HEAVY METALS IN COCOA IN TRINIDAD AND TOBAGO.

Santos RC, Pires JL, Correa RX. 2012. Morphological characterization of leaf, flower, fruit and seed traits among Brazilian *Theobroma* L. species. Genetic Resources and Crop Evolution **59**:327–345.

Silva CRS, Venturieri GA, Figueira A. 2004. Description of Amazonian *Theobroma* L. collections, species identification, and characterization of interspecific hybrids. Acta Botanica Brasilica **18**:333–341.

Sotelo A, Alvarez RG. 1991. Chemical composition of wild *Theobroma* species and their comparison to the cacao bean. Journal of Agricultural and Food Chemistry **39**:1940–1943.

Stuppy, W. 2014. An Introduction to the Morphology of Fruits. Pages 31-32. Royal Botanic Gardens, Kew.

Stocker TF, Qin D, Plattner G-K, Tignor MMB, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM. 2013. Climate change 2013 the physical science basis: Working Group I contribution to the fifth assessment report of the intergovernmental panel on climate change. Page 1535.

Teh C-L, Pang JT-Y, Ho C-T. 2006. Variation of the response of clonal cocoa to attack by cocoa pod borer *Conopomorpha cramerella* (*Lepidoptera*: *Gracillariidae*) in Sabah. Crop Protection **25**:712–717.

The IUCN Red List of Threatened Species. (2022). Available from https://www.iucnredlist.org/en (accessed April 12, 2022).

The WFO Plant List | World Flora Online. (2022). Available from https://wfoplantlist.org/plant-list (accessed April 10, 2022).

Thresh JM, Owusu GK. 1986. The control of cocoa swollen shoot disease in Ghana: an evaluation of eradication procedures. Crop Protection **5**:41–52.

Tropicos - Home. (2022). Available from http://legacy.tropicos.org/Home.aspx (accessed April 10, 2022).

Wessel. 1983. The Cocoa Pod Borer Moth (*Acrocercops cramerella* Sn.) Review of research in Indonesia 1900–1918.

Whitlock BA, Baum DA. 1999. Phylogenetic Relationships of *Theobroma* and *Herrania* (*Sterculiaceae*) Based on Sequences of the Nuclear Gene Vicilin. Systematic Botany **24**:128–138. American Society of Plant Taxonomists.

Williams G. 1953. Field Observations on the Cacao Mirids, *Sahlbergella singularis* Hagl. and *Distantiella theobroma* (Dist.), in the Gold Coast. Bulletin of Entomological Research **44**:101–119. Cambridge University Press.

Willson KC. 1999. Coffee, cocoa and tea. CABI Pub., New York.

Wood GAR, Lass RA. 2008. Cocoa. John Wiley & Sons.

References for figures

Figure 1: Leaves of *Theobroma cacao*

A) Chang, M. 2021. *Theobroma cacao* leaves. Available from https://www.inaturalist.org/photos/170504771 (accessed April 2022).

B) Sanchez, J. 2021. Leaves of a matured tree. Available from https://www.inaturalist.org/photos/170658071 (accessed April 2022).

C) Villota, F. 2019. Leaf in detail. Available from https://www.inaturalist.org/photos/39234727 (accessed April 2022).

Figure 2: Flowers of *Theobroma cacao*

A) Amelia, C. 2021. Cauliflory. Available from https://www.inaturalist.org/photos/116178624?size=medium (accessed April 2022).

B) Villa Restrepo, E. 2019. Flowers. Available from https://www.inaturalist.org/photos/37191425?size=medium (accessed April 2022).

C) Chathuranga, N. 2021. Flower in detail. Available from https://www.inaturalist.org/photos/144309645 (accessed April 2022).

D) Lee, C. 2008. Fruit developing from the flower. Available from https://www.inaturalist.org/photos/77748268 (accessed April 2022).

Figure 3: Pods and seeds of *Theobroma cacao*

A) Mody, K. 2019. Matured cacao pod. Available from https://www.inaturalist.org/photos/165435733 (accessed April 2022).

B) Castañeda, D. 2020. Dried cacao seeds. Available from https://www.inaturalist.org/photos/103100620 (accessed April 2022).

C) López, A. 2017. Seeds inside of the husk covered in pulp. Available from https://www.inaturalist.org/photos/165435733 (accessed April 2022).

D) Mody, K. 2019. Matured cacao pods. Available from https://www.inaturalist.org/photos/178046693 (accessed April 2022).

Figure 4: Fruit of selected *Theobroma* species

A) Chapalbay, R. 2019. *T. grandiflorum*. Available from https://www.inaturalist.org/photos/40658195?size=large (accessed April 2022).

B) Caniso, M. 2019. *T. microcarpum*. Available from https://www.inaturalist.org/photos/40543319 (accessed April 2022).

C) Higgins, S. 2013. *T. bicolor*. Available from https://www.inaturalist.org/photos/63634803 (accessed April 2022).

D) Ferreira, M. 2019. *T. speciosum*. Available from https://www.inaturalist.org/photos/106000013 (accessed April 2022).

E) Queiroz, M. 2021. *T. subincanum*. Available from https://www.inaturalist.org/photos/112890981 (accessed April 2022).

F) Lopez, H. 2021. *T. simiarum*. Available from https://www.inaturalist.org/photos/138048172 (accessed April 2022).