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Towards integrated pest management of cocoa black pod disease in Sierra Leone: host genetic diversity, agroforestry systems, and biological control

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Cover: Healthy (left and centre left images) and diseased (centre right) cacao and agroforestry shade trees (right image) in Sierra Leone. Photographs: Mohamed Mambu Luseni.

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Towards Integrated Pest Management of Cocoa Black Pod Disease in Sierra Leone: Host Genetic Diversity, Agroforestry Systems, and Biological Control

Abstract

Cacao (*Theobroma cacao* L.) is a key agricultural commodity in West Africa, which produces approximately 70 % of global cocoa. In Sierra Leone, cocoa provides vital income for smallholder farmers, but productivity is constrained by with diseases such as cocoa black pod disease (CBPD) caused by Phytophthora species. Accurate genetic identification is essential for effective resistance breeding programs, conservation efforts, and distribution of true-to-type planting materials to farmers. This thesis takes an integrated approach to management of CBPD combining an investigation into the genetic diversity of *T. cacao*, a survey of the agroforestry approaches used by farmers and testing of biological control solutions for sustainable CBPD control in Sierra Leone. Genetic characterization of cacao germplasm reveals substantial variability within populations, with most variation occurring within rather than between accessions. CBPD prevalence across Sierra Leone's cacao-growing regions is strongly influenced by agroforestry system design. Dense shade and high canopy cover increase disease incidence through elevated humidity and reduced airflow, while greater tree-species diversity modestly reduces disease occurrence. Field applications of the biological control agent *Trichoderma atroviride* reduce CBDP incidence and increase yield, offering a promising sustainable alternative to the use of synthetic pesticides. The holistic approach taken in this thesis addresses key production constraints while promoting resilience and environmental sustainability in smallholder cacao systems.

Keywords: Cocoa Black Pod Disease, *Phytophthora megakarya, Phytophthora palmivora,* Cacao Agroforestry Systems, *Trichoderma atroviridae, Theobroma cacao,* smallholder farming, biocontrol, West Africa, IPM.

Mot integrerad skadedjursbekämpning av kakao-black pod-röta (CBPD) i Sierra Leone: Genetisk mångfald hos värdväxt, skogsjordbruk och biologisk bekämpning

Abstract

Kakao (*Theobroma cacao* L.) är en viktig jordbruksprodukt i Västafrika, som står för cirka 70 % av den globala kakaoproduktionen. I Sierra Leone är kakao en viktig inkomstkälla för småbrukare, men produktiviteten begränsas av sjukdomar som black pod-röta (CBPD) orsakad av Phytophthora-arter. Detaljerad genetisk identifiering är avgörande för effektiva förädlingsprogram för resistens, bevarandeinsatser och distribution till lantbrukare av kakaoplantor med önskvärda egenskaper. Denna avhandling använder en integrerad strategi för hållbar hantering av CBPD i Sierra Leone, genom att kombinera studier av den genetiska mångfalden hos odlad *T. cacao*, en kartläggning av skogsjordbruksmetoder som används av lantbrukare och test av biologisk bekämpning. Genetisk karakterisering av kakaokultivarer avslöjade betydande genetisk variation, där mest variation fanns inom snarare än mellan kultivarer. CBPD-förekomsten i Sierra Leones kakaoodlingsregioner påverkades starkt av designen på skogsjordbruket. Tät skugga och högt trädkrontäcke ökade sjukdomsförekomsten, troligtvis genom förhöjd luftfuktighet och minskat luftflöde. Större trädartsdiversitet minskade sjukdomsförekomsten något. Fältanvändning av ett biologiskt bekämpningsmedel med *Trichoderma atrovirid* minskade CBDP-förekomsten och ökade avkastningen, vilket erbjuder ett lovande hållbart alternativ till användningen av syntetiska bekämpningsmedel. Denna avhandling, med en holistisk ansats, adresserar viktiga produktionsbegränsningar samtidigt som den pekar på några sätt att främja motståndskraft och miljömässig hållbarhet i ett småskaligt skogsjordbruk av kakao.

Nyckelord: Kakao-black pod-röta, *Phytophthora megakarya*, *Phytophthora palmivora*, kakaoskogsjordbrukssystem, *Trichoderma atroviridae*, *Theobroma cacao*, småbrukare, biokontroll, Västafrika, IPM.

Preface

This thesis was completed as part of my PhD studies in Plant Protection Biology. The motivation for this research started from the need to develop sustainable management strategies for cocoa black pod disease in Sierra Leone. The work presented here reflects several years of field and laboratory research, focusing on genetic diversity, agroforestry systems, and biological control approaches.

Dedication

This thesis is dedicated first and foremost to Almighty Allah, whose boundless mercy, wisdom, and blessings have kept me going. Without His grace, this achievement would not have been possible.

I sincerely dedicate this work to my father, Pa Mambu Luseni, and late mother, Madam Mamie Kowa Mambu Luseni, whose unwavering support, sacrifices, and prayers have been the cornerstone of my success. Their faith in me has always given me strength and motivation.

To my beloved wife, Princess Konima Ndanema, and my family, thank you for your patience, tolerance, encouragement, and support throughout the many difficulties of this PhD journey. The support you provided helped me stay motivated and focused on achieving this goal.

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Bhattacharjee, R.; Luseni, M.M.; Ametefe, K.; Agre, P.A.; Kumar, P.L.; Grenville-Briggs, L.J. (2024). Genetic Diversity and Population Structure of Cacao (*Theobroma cacao* L.) Germplasm from Sierra Leone and Togo Based on KASP–SNP Genotyping. Agronomy. 14, 2458. doi:10.3390/agronomy14112458.

II. Luseni M. M, Lankinen Å, Kumar P. A, Bhattacharjee R, and Grenville-Briggs L. J. (2026). The Impact of Traditional Agroforestry Systems on Cocoa Black Pod Disease in Sierra Leone (manuscript).

III. Luseni M. M, Dotson. B, Kumar P. A, Bhattacharjee R, and Grenville-Briggs L. J. (2026). Biocontrol of Cocoa Black Pod Disease Using *Trichoderma atroviride* (manuscript).

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The contribution of Mohamed Mambu Luseni to the papers included in this thesis was as follows:

I. Participated in designing the study, data collection and writing and revision of the manuscript.

II. Participated in designing the study, data collection, data analysis and writing and revision of the manuscript.

III. Designed the study, collected the data, participated in data analysis, and writing and revision of the manuscript.

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Abbreviations

AuToDiDAC	Automatic Tool for Disease Detection and Assessment in Cacao
CAFS	Cacao Agroforestry Systems
CATIE	Centro Agronómico Tropical de Investigación y Enseñanza
CBPD	Cocoa Black Pod Disease
CSSV	Cacao Swollen Shoot Virus
CSSVD	Cacao Swollen Shoot Virus Disease
CSSVCU	Cacao Swollen Shoot Virus Disease Control Unit
DAPC	Discriminant Analysis of Principal Components
DBH	Diameter of Breast Height
EPA-SL	Environmental Protection Agency-Sierra Leone
FAO	Food and Agricultural Organization
GPS	Global Position System
ICCO	International Cocoa Organization
IDM	Integrated Disease Management
IITA	Institute of Tropical Agriculture, Nigeria
IPM	Integrated Pest Management
MAFS	Ministry of Agriculture and Food Security
MT	Metric Ton
PIC	Polymorphic Information Content
SLARI	Sierra Leone Agricultural Research Institute
WCF	World Cocoa Foundation

AI declaration

This thesis represents my original scholarly work, and I take full responsibility for the content, accuracy, and integrity of all material presented herein. All research design, data collection, analysis, interpretation of results, conclusions and recommendations are entirely my own.

Generative artificial intelligence (AI), specifically ChatGPT, was used in a limited capacity to support language editing and paraphrasing. Its use was restricted to improving the clarity, readability, and grammatical correctness of the text. No AI tools were used to generate research data, perform analyses, or develop scientific arguments or conclusions.

All outputs generated with the assistance of AI were carefully reviewed, validated, and edited by the author to ensure accuracy and appropriateness within the academic context. Responsibility for any errors or omissions remains solely with the author.

1. Introduction

1.1 Cacao

Cacao (*Theobroma cacao* L.) is a diploid tropical fruit tree (2n = 2x = 20; Bhattacharjee et al., 2024) native to the rainforests of tropical South America (Wood et al., 2021). Initially classified within the Sterculiaceae family, molecular phylogenetic studies now place cacao in the Malvaceae family, specifically in the subfamily Byttnerioideae (Colli-Silva et al., 2024). Cacao has a relatively small genome, estimated at 380 Mbp, with genotypes ranging between 411 and 494 Mb. The species possesses approximately 700 genes unique to its genome, which may underlie lineage-specific adaptations.

Genetic analyses reveal extensive diversity and admixture among cacao accessions, indicating significant inter- and intrapopulation gene flow. Morphologically diverse populations interbreed freely, contributing to the high genetic variability observed both within and between germplasm collections (Todd et al., 2025). While cacao exhibits partial self-compatibility, facilitating occasional self-fertilization, it is predominantly outcrossing due to floral morphology and pollinator behaviour, which promote recombination and sustain elevated heterozygosity (Motamayor et al., 2008; Santos et al., 2025).

Within the genus Theobroma, which comprises 22 species, cacao is the only widely cultivated species (Santos et al., 2023). Other species include *T. angustifolia*, *T. antioquia*, *T. bicolor*, *T. calodesmis*, *T. grandiflorum*, *T. leiocarpa, T. mammosum, T. microcarpa, T. obovato, T. pentagona, T. simiarum*, and *T. speciosum*. Cacao populations in South America evolved independently, forming distinct genetic lineages that have historically been classified as *T. cacao* ssp. *cacao* and *T. cacao* ssp. *sphaerocarpum*. These subspecies correspond broadly to the Criollo and Forastero groups, reflecting early divergence driven by geographic isolation and domestication within the Amazon basin (Zhang et al., 2020; Cornejo et al., 2023).

1.1.1 The Origins of Cacao

Archaeological evidence suggests that the Olmec and other early Mesoamerican civilisations fermented the sweet pulp of cacao fruits (cocoa) to produce an alcoholic beverage long before the development of chocolate drinks prepared from ground cacao seeds (Villarreal & Ruby, 2024). Residue analyses from pottery vessels recovered in the Ulúa Valley of Honduras indicate that cocoa pulp fermentation may have been practised at least 3,000 years ago, predating the later Mesoamerican tradition of grinding roasted cocoa beans to prepare chocolate beverages (Powis et al., 2019; Henderson et al., 2020). Cacao originates from the tropical rainforests of South America, particularly the Amazon Basin, where the Amazon, Guiana Shield, and Orinoco regions harbour a wide diversity of wild cacao populations that represent the primary centre of genetic diversity for the species (Thomas et al., 2022).

1.1.2 Cacao Varieties

Traditionally, cacao has been classified into three major groups based on morphological characteristics, genetic background, and geographic origin: Criollo, Forastero, and Trinitario (Bekele et al., 2020) (Figure 1). Criollo represents the domesticated Central American type known for superior flavour but low productivity (Wahyuni et al., 2021). Forastero comprises the more vigorous Amazonian populations widely cultivated for bulk cocoa production, and Trinitario is considered a hybrid group derived from crosses between Criollo and Forastero types (Zug 2020). A third group, Trinitario, originated from natural and artificial crosses between the Criollo and Forastero cacao types (Bekele, 2019). This group combines desirable characteristics from both parental lineages, including the fine flavour attributes associated with Criollo cacao and the vigour, disease tolerance, and higher productivity typical of Forastero types (ICCO, 2021). Criollo varieties typically produce pods containing large beans with white to pale pink cotyledons. These beans are characterised by low bitterness and a delicate aroma, which makes them highly valued for producing fine-flavour chocolates (Afoakwa, 2020; ICCO, 2022a). Criollo cacao is generally characterised by a high degree of self-compatibility and a relatively narrow genetic base, resulting in a largely homozygous genetic background compared with other cacao groups (Ceccarelli et al., 2022).

Molecular marker analyses, including simple sequence repeats (SSRs) and single-nucleotide polymorphisms (SNPs), have confirmed reduced genetic diversity and high genetic uniformity within Criollo germplasm (Bustamante et al., 2022). Criollo cacao has been cultivated for centuries in Central and parts of South America and is widely regarded as the earliest domesticated form of the cacao tree. Archaeological and genomic evidence indicate that early Mesoamerican civilisations selected and cultivated Criollo-type cacao, thereby establishing it as one of the first domesticated cacao lineages (Wallers, 2020; Powis et al., 2019). Despite its superior flavour quality, Criollo is not widely cultivated by farmers because it is highly susceptible to several major cacao diseases, including black pod disease, witches' broom, and frosty pod rot, which significantly limit its productivity under field conditions (Wickramasuriya and Dunwell, 2018).

Figure 1. Cacao varieties grown in Sierra Leone. (A) Trinitario, (B) Forastario, (C) Amazon and (D) Criollo. Photographs copyright Mohamed Mambu Luseni.

Early Forastero cacao cultivars are believed to have originated from the lower Amazon Basin, where wild populations of *Theobroma cacao* contributed to the genetic base of this group. Forastero types are characterised by high vigour, disease tolerance, and broad adaptability, which have made them the most widely cultivated cacao varieties worldwide (Ofori et al., 2024) and have been planted mostly in Brazil and Venezuela. Forastero cacao accounts for approximately 80% of global cocoa production and is genetically subdivided into two main groups: Lower Amazonian (Amelonado) and Upper Amazonian. It is widely cultivated for its high yield potential and greater tolerance to major diseases, including black pod disease and witches' broom, making it the backbone of commercial cocoa production worldwide (Bekele & Bekele, 2019).

Trinitario cacao is primarily a hybrid, heterogeneous, and evolutionarily dynamic, originating from the hybridization of Criollo x genotypes of Lower Amazonian Forastero but further shaped by multiple introgression events and continuous human selection. Contemporary genomic and population structure studies demonstrate that Trinitario is not a fixed lineage but an admixed group comprising diverse Forastero lineages combined with Criollo ancestry, resulting in high intra group variability (Gopaulchan et al., 2019). Recent research has emphasized that the traditional three group classification (Criollo, Forastero, Trinitario) is overly simplistic, as cacao diversity is now understood to consist of multiple genetic clusters with complex interbreeding histories, within which Trinitario represents a hybrid continuum rather than a discrete genetic entity (Nousias et al., 2024). The combination of fine flavour qualities inherited from Criollo and the vigour, productivity, and disease tolerance derived from Forastero that underpin Trinitario's global significance in fine-flavour cacao production are all explained by this complex genetic base (Dillon et al., 2024).

1.1.3 The global importance of cocoa

Globally, cacao is cultivated by approximately five to six million smallholder farmers (García et al., 2024), supporting the livelihoods of an estimated forty to fifty million people, particularly in West Africa, Latin America, and Southeast Asia (Prazeres et al., 2021). Cocoa production remains a critical source of income and employment for rural communities in major producing countries (ICCO, 2022b; World Cocoa Foundation, 2021; Afoakwa, 2020). Cacao is cultivated on an estimated 11.65 million hectares (Otekunrin, 2025) across 57 countries in the intertropical zone, reflecting its importance as a tropical cash crop (Agele et al., 2023). In 2023, world cocoa bean production was approximately 5.6 million metric tonnes of dried beans, underscoring the crop’s global significance in agricultural production and rural livelihoods (Otekunrin, 2025). Globally, the leading cocoa-producing countries are Côte d’Ivoire (1,980,000 tons), Ghana (950,000 tons), Cameroon (240,000 tons), and Nigeria (220,000 tons), collectively accounting for the majority of global production. Other significant producers include Indonesia, Brazil, Sierra Leone, Togo, Liberia, and several smaller tropical countries, reflecting the crop’s widespread cultivation in the intertropical zone (ICCO, 2023; FAO, 2023; World Cocoa Foundation, 2022).

In global trade, cocoa beans, the primary raw material for chocolate, are a major economic commodity, with the chocolate industry's global retail market value exceeding US$100 billion in 2021(Remi, 2021). The cocoa trade remains a vital source of revenue for developing countries, particularly in West Africa, Latin America, and Southeast Asia, supporting millions of smallholder farmers and their associated industries (ICCO, 2022; FAO, 2022; Afoakwa, 2020). Although cocoa consumption is relatively low in

Sierra Leone, it remains one of the country's main agricultural export crops (Fofanah, 2020). Production levels have varied over time, with official statistics indicating national cocoa yields of approximately 11,966 MT in 2013, 11,057 MT in 2014, and 7,487 MT in 2015, underpinning its role as an important cash crop and source of foreign exchange for rural farming households (Fofanah, 2020). It is mostly cultivated on small land areas (1-2 hectares), medium-scale farms (3-5 hectares), and large-scale farms (>5 hectares). Currently, cocoa has a growing presence in Sierra Leone's international trade statistics, largely due to recent efforts to enhance quality and increase production levels.

Cocoa is widely utilised across the globe, not only as a primary ingredient in chocolate products such as cakes, creams, beverages, and toppings but also in the cosmetic and personal care industry, where cocoa butter is incorporated into soaps, lotions, and other skincare products due to its moisturizing and emollient properties (Sawicka et al., 2024). The health benefits of consuming cocoa products have also been well acknowledged. Cocoa is rich in minerals, including iron, magnesium, calcium, phosphorus, copper, and manganese. It is also a good source of potassium and provides the body with carbohydrates, protein, and dietary fibre (Rojo-Poveda et al., 2020). In addition, its cholesterol content is negligible (Mbida et al., 2024). Cocoa beans contain a butter, a lipid fraction composed predominantly of monounsaturated fatty acids, such as oleic acid, along with saturated fats, including stearic and palmitic acids (Alotaibi et al., 2024). This unique fatty acid composition contributes to cocoa butter's stability, melting properties, and its functional applications in both the food and cosmetic industries (Loke, 2024).

1.2 Cacao agroforestry and cultivation systems

In Sierra Leone, as in other humid regions of West and Central Africa, farmers cultivate cacao using traditional cacao agroforestry, successional agroforestry, organic agroforestry, organic monoculture, or conventional monoculture. Cocoa production systems across the tropics exist along a continuum ranging from highly diversified, ecologically complex agroforestry systems to simplified, input-intensive monocultures.

1.2.1 Traditional cacao agroforestry

Traditional cacao agroforestry denotes one of the oldest and most widespread forms of cacao cultivation, especially in Sierra Leone and throughout West and Central Africa. This system is usually created by farmers purposefully keeping native trees to offer shade and preserve ecological services while selectively cutting secondary forests or lengthy fallow areas (Figure 2). High levels of biodiversity, soil fertility, and microclimate regulation are supported by the resulting multi-strata canopy structure, which is similar to natural forest ecosystems (Sonwa et al., 2019).

However, these systems are generally characterised by low external input use and limited management, which often leads to suboptimal yields due to excessive shade, ageing tree populations, and inadequate phytosanitary practices (Sonwa et al., 2019; Schroth et al., 2016; Konaté et al., 2024). This is indicative of a larger regional trend in the eastern region of Sierra Leone, where cacao is grown extensively. Instead of being farmed as a monoculture, cacao is incorporated into landscapes centred around forests. About 60% of the country's land area is covered with bush fallow vegetation and forest regeneration (EPA-SL, 2021). The majority of closed-canopy forests can be found in the east, where cocoa production is centred.

1.2.2 Successional cacao agroforestry

Successional cacao agroforestry represents a more recent and deliberately designed system that mimics natural ecological succession while optimising productivity. Successional systems are actively managed by introducing crops one after the other, starting with annual food crops, moving on to cacao, and finally adding a variety of perennial species like fruit and wood trees, instead of depending on inherited forest structures (Figure 2). This dynamic mechanism maximises nutrient cycling, continuous ground cover, and biomass recycling in a highly resilient and productive system.

Compared to monoculture systems, successional agroforestry systems can achieve high overall productivity per unit area while maintaining lower environmental impacts and greater resilience to climatic variability, according to recent studies. Before the cacao canopy closes, newly cleared fields in Cameroon are first interplanted with quick-growing food crops like corn (*Zea mays*) and white seed melon (*Cucumeropsis manii*), which increase fertility, improve soil cover, and generate short-term income (Akinbamijo et al., 2020). These systems create favourable microclimatic conditions for the establishment of cacao, optimise land use, and diversify income (Tscharntke et al., 2021). The overall system output and ecological sustainability are greatly increased, even though cocoa yields alone might not always match those of intensive monocultures (Somarriba et al., 2021; Tscharntke et al., 2011).

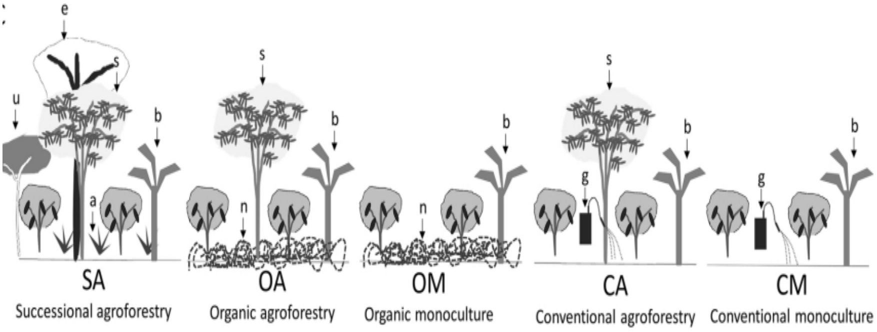

Figure 2: **Schematic representation of different cacao production systems and their key characteristics** with respect to agroforestry structure, vegetation composition, and management practices. The symbols represent: **s** – shade trees (fruit, leguminous, and timber species); **n** (leguminous perennial cover crops); **g** (biochemical inputs; Neem-based products); **b** (banana or plantain trees); **u** (*Cola nitida)*; and **e** (spontaneously growing trees and shrubs) that are selectively managed.

1.2.3 Organic cacao agroforestry

Organic cacao agroforestry systems build on the structural complexity of agroforestry while adhering to organic farming principles that exclude synthetic fertilisers and pesticides. Organic cacao agroforestry systems are based on the structural complexity of agroforestry and follow the rules of organic farming, which means they don't use synthetic pesticides or fertilisers (Figure 2). These systems rely on ecological processes like recycling organic matter, fixing nitrogen biologically, and controlling pests naturally to keep their productivity high. Compost, mulch, and other organic amendments help keep soil fertile. Cultural practices, resistant varieties, and biological control agents are all important for managing pests and diseases. Organic agroforestry systems are linked to better soil health, more biodiversity, and less pollution of the environment. However, yields are often average because of a lack of nutrients and less intense inputs. The environmental benefits per unit of output may also change depending on how well management is done and the local conditions (Wanger et al., 2015; Armengot et al., 2016).

1.2.4 Organic cacao monoculture

Organic cacao monoculture differs fundamentally from conventional agroforestry and conventional monoculture systems in that cacao is cultivated as a single crop without synthetic inputs but also without the ecological complexity provided by shade trees and species diversity (Figure 2). While this system meets organic certification standards, it lacks many of the ecological benefits associated with agroforestry, including microclimate buffering, biodiversity conservation, and natural pest suppression. Organic monocultures may be more vulnerable to pests, diseases, and climatic stress, and often exhibit lower yields compared to conventional systems. Nutrient management in these systems relies entirely on organic inputs, which may not always meet crop demand, thereby constraining productivity (Babin et al., 2020).

1.2.5 Conventional cacao monoculture

In this system, cacao is cultivated under full sun with little or no shade. Productivity is maintained through the application of synthetic fertilisers, fungicides, and pesticides (Figure 2). This method can result in significantly higher yields in the short term and is widely practised in major cocoa-producing countries such as Côte d’Ivoire. However, the ecological costs are substantial, including soil degradation, biodiversity loss, increased greenhouse gas emissions, and heightened vulnerability to pests and diseases. The reliance on chemical inputs also raises concerns regarding environmental contamination, human health risks, and long-term sustainability. Furthermore, the simplification of the production system reduces resilience to climate variability and may lead to declining productivity over time (Armengot et al., 2023; Mattalia et al., 2022; Suárez et al., 2022).

1.2.6 Conventional cacao agroforestry

In conventional Agroforestry systems, cacao is cultivated under a managed shade canopy composed of selected tree species. Productivity is enhanced through the use of synthetic fertilisers, fungicides, and, in some cases, herbicides (Figure 2). This approach is increasingly promoted in major cocoa-producing regions, including Côte d'Ivoire and Ghana, as a means of reconciling yield improvement with environmental sustainability.

Unlike traditional cacao agroforestry systems, which are often characterised by high and unmanaged shade levels, conventional agroforestry systems are deliberately designed and managed to optimise light availability, typically maintaining moderate shade levels (e.g., 30–50%). Shade trees are selectively retained or planted at controlled densities to minimise competition with cacao, often including economically valuable species such as timber or fruit trees. This results in a more uniform canopy structure compared to traditional systems, with improved light penetration and reduced humidity, thereby lowering disease pressure from pathogens such as *Phytophthora* spp. (Babin et al., 2020).

The integration of external inputs is a defining feature of conventional cacao agroforestry. Fertiliser application is used to address nutrient limitations and sustain higher yields. Fungicides, particularly copper-based and systemic products, are applied to manage diseases such as black pod. This combination of ecological and chemical management allows farmers to achieve higher productivity than in low-input systems at the same time, maintaining some of the ecosystem services associated with tree-based systems (Armengot et al., 2023).

Conventional agroforestry systems generally outperform traditional agroforestry systems due to improved management, better planting materials, and the use of inputs. The yields of cocoa are typically lower than those achieved in full-sun monoculture systems, reflecting a trade-off between light availability and ecological benefits. Decisively, the presence of shade trees contributes to greater system resilience by buffering temperature extremes, reducing evapotranspiration, and improving soil structure through litter inputs and root interactions (Schroth et al., 2016).

Ecologically, conventional cacao agroforestry provides intermediate benefits. While biodiversity levels are lower than in traditional or successional agroforestry systems due to reduced tree diversity and density. They are significantly higher than in monoculture systems. These systems also contribute to carbon sequestration and help mitigate deforestation pressures by maintaining tree cover within agricultural landscapes (Jagoret et al., 2014).

However, the reliance on synthetic inputs raises concerns regarding environmental sustainability. Excessive or poorly managed fertiliser use can lead to nutrient leaching and soil degradation. Repeated fungicide applications may contribute to environmental contamination and the development of pathogen resistance. The economic cost of inputs may limit adoption among smallholder farmers, particularly in low-income contexts such as Sierra Leone, where access to inputs and extension services remains constrained.

1.2.7 Cocoa production systems in Sierra Leone

Sierra Leone is located in West Africa, between latitudes 7° North and Longitudes 10° and 13° west and is bordered by the Atlantic Ocean in the South (Figure 3). It has a tropical climate and diverse topography conducive to the production of cacao. The eastern districts of Kailahun District, Kenema District, Kono District, and Bo District account for the largest share of the country's cacao-growing area (Gboku et al., 2017) (Figure 3). National production is estimated at approximately 14,000 tonnes annually, representing a small but economically important proportion of African cocoa output (Massaquoi et al., 2022).

Cacao farming is largely undertaken by smallholder farmers managing relatively small landholdings (Wainaina et al., 2021), typically between 1.0 and 1.6 acres (Effendy et al., 2019). These systems are supported by favourable climatic conditions characterised by constant rainfall, warm temperatures, and humid environments, along with fertile, well-drained soils rich in organic matter (Mihai et al., 2023; Thomas et al., 2024).

Cacao is often established either by intercropping seedlings with food crops such as rice during early stages or by thinning long-fallowed vegetation to retain shade trees (Angeles et al., 2023). However, management practices are generally characterised by low external input use, including minimal fertiliser and pesticide application, limited pruning, and irregular weeding and phytosanitation (Moinina et al., 2023). While cost-effective, these practices contribute to low productivity and increased vulnerability to pests and diseases.

Cocoa production in Sierra Leone and the wider West African region has historically been dominated by the Amelonado cacao variety, a traditional Forastero type introduced during the colonial period. Although adapted to local conditions, it has a narrow genetic base, making it susceptible to diseases such as black pod (Bhattacharjee et al., 2024). The impact of *Phytophthora* species, particularly *P. megakarya*, is severe, causing yield losses of 20–30% or more under favourable conditions (Adomako et al., 2021).

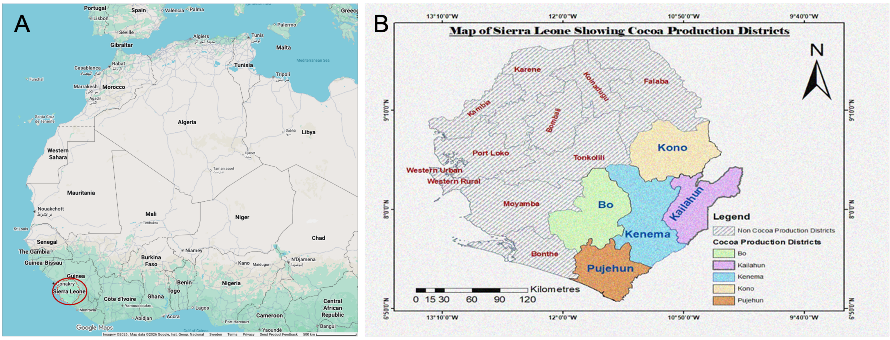

Figure 3: Distribution of cocoa production areas in Sierra Leone.

(A) Sierra Leone (marked with a red circle) is located in Western Africa and bordered by the Atlantic Ocean to the west, Guinea to the north and east, and Liberia to the south. Image reproduced with permission from GoogleMaps copyright 2026. (B) Within Sierra Leone, Kenema, Kailahun, and Kono are identified as the major cocoa-producing districts, along with the minor production areas Bo and Pujehun. The remaining districts are generally considered non-cocoa-producing regions, although small numbers of cacao trees can occasionally be found in Moyamba, Tonkolili, and Koinadugu. Map generated using AcrMap 10.8.2, Esri ArcGIS Desktop.

To address these challenges, breeding programmes have introduced hybrid varieties derived from crosses between Amelonado and Upper Amazon Forastero, aiming to improve yield and disease tolerance. However, resistance remains quantitatively controlled and influenced by environmental conditions (Ofori et al., 2023; Muñoz et al., 2021).

Despite these advancements, cocoa production continues to face significant constraints, including poor farm management, inadequate pruning, and insufficient disease control. Many farms experience severe black pod outbreaks, sometimes leading to abandonment and the creation of inoculum reservoirs (Merga 2022; Frimpong et al., 2021). Rehabilitation efforts through replanting and improved practices are ongoing but remain limited by labour constraints, low input use, and weak technical support (Somarriba et al., 2021; Esan et al., 2025).

In Côte d'Ivoire, the world's leading cocoa producer, cacao is predominantly cultivated in full-sun monocultures. While this approach increases short-term yields, it often results in biodiversity loss, higher pest and disease pressure, and increased reliance on agrochemicals (Armengot et al., 2023; Mattalia et al., 2022; Suárez et al., 2022). Conversion of forest land into monoculture plantations has also been associated with environmental degradation and reduced long-term productivity (Suprriadi et al., 2022).

Cacao cultivation typically occurs on small family-owned plantations (1–4 ha), where trees are planted in rows approximately 2.5 meters apart. Farmers may also cultivate understory crops such as cassava to diversify income and improve food security (Pulleman et al., 2022).

Over time, plantations require rehabilitation to restore productivity. This involves removing old trees and replanting new ones, guided by criteria such as tree age (>40 years), low yield (<500 kg ha^{-1} year^{-1}), and reduced plant density (<800 plants ha^{-1}) (Somarriba et al., 2021). Additional factors include pest and disease incidence, tree height, and shade levels (Asitoakor et al., 2024).

Rehabilitation strategies include the introduction of improved cultivars, fertiliser application, pruning, grafting, and enhanced crop protection measures (Sefa, 2025). However, these interventions require coordinated planning and technical support.

The sector highlights a critical need for integrated pest management (IPM) approaches that combine improved germplasm, better agronomic practices, and agroforestry management to sustainably enhance cocoa productivity and resilience in Sierra Leone.

1.3 Major Cacao pests and methods to control them

1.3.1 Mirid Bugs

The expansion of cacao crops in tropical Africa and Asia coincided with the appearance of significant insect pests that had adapted to the crop from their native host plants. Compared with Africa and Asia, where certain major pests are widely distributed and cause considerable crop damage, Latin America, where the crop is native, experiences a different pest landscape (Babin, 2018). The most pervasive and dangerous insect pests of cacao are mirid bugs (Djoukwe et al., 2018; Babin, 2018; Tormes and Mostoles, 2021).

However, only a small number of the 40 species in the family Miridae that harm cacao have a significant economic influence on global cocoa production (Babin, 2018). Mirids (*Distantiella theobroma* (Dist.) and *Sahlbergella singularis* Hagl. as well as stink bugs (*Bathycoelia thalassina* (Herrich-Schaeffer)), are well known for their role in causing cocoa losses in West Africa (Guessan-Bi et al., 2022). All the mirids that are harmful to cacao belong to the Odoniellini and Monaloniini tribes and the Bryocorinae subfamily (Tapi and Sonia, 2020). Mirids from the tribe Odoniellini (*S. singularis* Hagl. and *D. theobroma* Distant) are often robust insects with a brownish colour.

In contrast, those from the tribe Monaloniini are gracile and have vivid colours (Babin, 2018). On young pods, this damage may result in growth distortion, which occasionally causes yellowing and fruit abortion. Though the vegetative sections of trees have sustained the greatest damage, with numerous lesions and branch tips dying (Babin, 2018). On mature cocoa pods and shoots, mirid feeding lesions appear as black plugs of decomposing tissue (Ambele et al., 2023). To liquefy plant tissues, both nymphal and adult stages eat and inject saliva (Sonawane et al., 2025). For fungi pathogens, their feeding punctures serve as entry sites (Arné and Lee, 2020). Dieback disease is triggered by this secondary invasion of mirid feeding punctures, whereas invasion by stink bugs causes pods and beans to ripen prematurely and become deformed (Adu-Acheampong *et al.,* 2015; Sonawane et al., 2025).

Conventional pesticides are the primary approach for controlling mirids; however, treatments often used to manage mirids have also proven effective in indirectly controlling stink bugs (Adu-Acheampong *et al.,* 2015; Kumari et al., 2023). Field observations often provided significant evidence that mirids damaged some Trinitario selections (e.g., SC1) less than the common West African Amelonado (Adu-Acheampong et al., 2015). It was further verified that SC6 was among the selections resistant to the fungal-induced dieback disease in cocoa (Adu-Acheampong, 2015).

1.3.2 Cacao stem borer

The moth *Eulophonotus myrmeleon* Felder belongs to the Cossidae family of *Lepidoptera. E. myrmeleon,* a cacao stem borer that targets members of the Sterculiaceae family*,* has previously been reported as a threat in the Democratic Republic of the Congo, Ghana, Nigeria, Sierra Leone, Togo, and the Island of São Tomé (Kingsley-Umana et al., 2022). Their feeding activities weaken the stem, damage the vascular tissues, and stop the flow of sap, which causes the leaves to yellow, brown, and fall off, as well as the twigs to dry out; occasionally, this can even cause the tree to die Landry, 2023). If a branch is impacted, you can notice that some of the leaves on that branch are drying out while the others are still green. The tree may die as the infestation spreads to all its tissues. From a distance, the afflicted cacao farms will appear to have been destroyed by fire, even during the height of the rainy season (Kingsley-Umana *et al.,* 2022).

Because most of the insect's life cycle is spent hidden in the tree's stem or branches, managing *E. myrmeleon* with pesticides is difficult because chemicals used to control other pests, including capsids, are ineffective against this pest (Ambele et al., 2023).

1.3.3 Mealybugs as vectors of cacao viruses

The Pseudococcidae family of mealybugs, including *Planococcoides njalensis* (Laing), *Planococcus citri* (Risso), *Ferrisia virgata* (Cock.), and *Phenacoccus hargreavesi* (Laing), serve as vectors of the disease in West Africa (Bigger, 1981; Diallo et al., 2023). Although mealybugs are common on cacao, they are generally not considered significant pests of the crop. Mealybug feeds on fragile apical shoots, causing slowed growth and transforming those shoots into thin, brush-like growths (Sudiarta et al., 2025). Ultimately, flower cushions are aborted when colonised, wither, and dry up when repeatedly attacked (Najberek et al., 2023). Uneven fractures and pitting are the results of feeding on the rind of pods (Rakhesh et al., 2023). Common control techniques include applying a 0.5% neem oil emulsion to pest locations twice every two weeks, or applying Imidacloprid at 0.3 mL/L of water or Dimethoate 30 EC at 1.6 mL/L of water as needed (Kailas, 2023).

1.4 Major cacao diseases and methods to control them

Cacao is affected by a number of diseases that affect different parts of the plant, including the stem, leaves, roots, flowers, young fruits, and pods. The type of pathogen and environmental factors, particularly temperature and humidity, have an impact on the occurrence and severity of these diseases. Cocoa Black Pod Disease (CBPD) caused by species of the *Phytophthora*

Genus is the most important disease affecting cocoa. Cacao Swollen Shoot Virus Disease (CSSVD) is another serious disease. *Phytophthora* species also cause stem canker, which damages the stem and leads to the death of the tree. Other diseases include root rot, vascular-streak dieback, and witches' broom.

1.4.1 Cacao swollen shoot virus disease (CSSVD)

One of the most significant diseases affecting cocoa production in West Africa, particularly in Ghana, Côte d'Ivoire, Nigeria, Togo, and Sierra Leone (1963), is cacao swollen shoot virus disease (CSSVD; Agyeman-Boaten, 2018; Abrokwah et al., 2023). According to Monnier (2024), the disease has been documented outside Africa in Trinidad and Tobago, Sri Lanka, and Indonesia. CSSV is a para-retrovirus that infects plants and belongs to the viral family Caulimoviridae and genus Badnavirus (Agusto et al., 2024; Vieira et al., 2022). It has non-enveloped bacilliform particles that contain a circular double-stranded DNA genome (Ameyaw et al., 2023). Length measurements of the CSSV viral particle range from 121 to 130 nm, with a width of 28 nm (Monnier, 2024). Depending on the strain, the genome size ranges from 7.4 to 8.0 kb (CACAO, 2024). Sediment components present in CSSV's pure preparations determine its physicochemical properties (Ameyaw et al., 2023). It is vectored by mealybugs.

The feeding habits of mealybugs and the availability of the virus in host tissues to vectors are among the factors that determine how effectively they can spread the disease (CACAO, 2024). For instance, *Planococcoides njalensis* is considered one of the most efficient vectors of cacao viruses because its stylet penetrates the phloem tissues more rapidly than those of other mealybug species, such as *Planococcus citri* and *Ferrisia virgata*, enhancing virus acquisition and transmission efficiency (Dzahini-Obiatey et al., 2010). The type of virus (mild or virulent), the host stage of infection, and the plant tissues on which mealybugs feed are additional factors that influence transmission efficiency (Ahmed et al., 2023). The developmental stage of mealybugs is important for virus transmission efficiency. The early instar stages (young nymphs) are highly mobile and actively disperse across plant surfaces, enabling them to move between infected and healthy cacao tissues. Because of this greater mobility and feeding activity, young nymphs are generally more effective vectors of plant viruses than adult females, which are relatively sedentary and tend to remain localised on a single feeding site (Bertin et al., 2016). Consequently, the age structure of mealybug populations significantly influences the epidemiology and spatial spread of CSSV in cacao plantations (Ameyaw, 2020). The adult male mealybug is much smaller and different from the female; although it has two pairs of functional wings for flight, it is unable to feed since it lacks mouth parts, and as a result, does not transfer CSSV (Ahmed et al., 2023).

Large-scale eradication of infected cacao trees has been a principal strategy for controlling CSSVD in Ghana (Ameyaw, 2019). Records from the Cacao Swollen Shoot Virus Disease Control Unit (CSSVDCU) indicate that between 2001 and 2012, approximately 74,445,945 cacao trees, including visibly infected trees and apparently healthy trees located within a surrounding buffer zone, were removed to limit further spread of the virus. Of this total, 38,756,296 trees (about 52.06%) were destroyed in the Western Region, highlighting the region as the most severely affected cacao-growing area in the country during that period. These eradication measures were implemented to eliminate sources of inoculum and reduce the transmission of the virus by mealybug vectors, which facilitate the spread of the disease among neighbouring cacao trees (Ameyaw et al., 2014). The coordinated application of several strategies to maximise disease control in an ecologically and economically sustainable manner is referred to as integrated management of CSSVD (Namdeo Aiwale et al., 2025). One cannot overstate the importance of combining all recommended disease control practices into a single package to facilitate straightforward adoption and acceptance by farmers, especially given the challenges they face in adopting separate techniques for CSSVD control, as described above.

1.4.2 Cocoa black pod disease

Members of the Kingdom Stremenopila from the class Oomycetes and within them, members of the genus *Phytophthora* likely cause more production losses globally than any other disease of cacao. Oomycetes are fungal-like organisms with distictive reproduction and genetics and several members of this class, particularly from the genus *Phytophthora* are destructive pathogens of a wide range of plants including agricultural and horticultural crops and trees. Cocoa black pod disease, (CBPD) caused by several different *Phytophthora* species, is considered the most destructive disease of cacao worldwide (Adomako et al., 2021). The disease causes substantial yield losses, with global reductions in cocoa production commonly estimated at 20–30% annually, while under favourable environmental conditions, an individual farm may lose 30–90%, making it one of the most significant constraints to cocoa production worldwide (Adeniran et al., 2024). Of the causal agents, two species are mainly responsible for the disease in West Africa (Adomako et al., 2021). *Phytophthora palmivora* has several recorded hosts and is of universal importance in cacao-producing countries, causing global yield losses of 20-30% with tree deaths of up to 10% annually (Guest, 2007; Perrine-Walker, 2020; Misman et al., 2022; Morales-Cruz et al., 2020). However, individual farms in wetter cacao-growing areas may suffer total loss (Merga, 2022). *Phytophthora megakarya* (Ali et al., 2017; Morales-Cruz et al., 2019), which is generally considered more aggressive and therefore the most important and devastating, causes annual losses between 60% and 100% (Agele et al., 2023) when no control measures are taken (Frimpong et al., 2021). *P. megakarya* is endemic to neighbouring producing countries, Equatorial Guinea, Gabon, Cameroon, Togo, Nigeria, and Ghana, and it is still in an invasive phase in neighbouring Côte d'Ivoire (Frimpong et al., 2021). However, whilst CBPD is prevalent within Sierra Leone, the species responsible for the disease in this country is not known.

In addition to the more widely reported species affecting cacao in Africa, other *Phytophthora* species cause pod rot in the Americas. In particular, *Phytophthora capsici* and *Phytophthora citrophthora* are commonly associated with cocoa pod rot in Central America and South America. These pathogens can cause substantial yield losses when environmental conditions are favourable, particularly under high humidity, frequent rainfall, and warm temperatures that promote pathogen sporulation, dispersal, and pod infection (Obiakara et al., 2021; Timmer et al., 2000). Under such favourable climatic conditions, outbreaks of pod rot can rapidly spread within plantations, resulting in significant reductions in marketable cocoa production (Bowers et al., 2020). Several other species have also been reported as causal agents of pod rot in different cacao-growing regions. For example, *Phytophthora megasperma* and *Phytophthora katsurae* have been identified as occasional pathogens infecting cocoa pods. Although these species are generally considered less widespread or less economically significant than the principal pathogens, their occurrence highlights the diversity of *Phytophthora* species capable of infecting cacao (Adeniyi, 2019).

Fungal pathogens have also been reported to colonise cocoa pods and contribute to discolouration during disease development. Species of *Fusarium* and *Lasiodiplodia theobromae* are frequently associated with the browning and blackening of infected cocoa pods. These fungi are often considered opportunistic or secondary pathogens that invade pod tissues weakened by primary infections, mechanical injuries, or environmental stress (França et al., 2025; Huda-Shakirah et al., 2022). Their colonisation accelerates tissue necrosis and decomposition, resulting in the characteristic brown-to-black discolouration observed on diseased pods (Huda-Shakirah et al., 2022). Consequently, the presence of these fungi can intensify pod deterioration and may complicate the diagnosis of primary diseases affecting cocoa pods in the field (Nyadanu et al., 2020).

For small-scale cacao farmers in Sierra Leone, low yields due to diseases are a major challenge to cocoa production (Bhattacharjee et al., 2023; Bonuedi et al., 2020). Black pod disease, caused by various Phytophthora species and other pathogens, is a major constraint on cocoa production and quality (Marelli et al., 2019; Minimol et al., 2024).

A range of strategies has been proposed for the management of cacao diseases, each presenting specific advantages and limitations. These strategies include the application of chemical fungicides, the use of biological control agents, the implementation of phytosanitary practices such as removal of infected pods and field sanitation, and the development of disease-resistant cultivars through conventional breeding or modern biotechnological approaches (Agele et al., 2023). While individual control measures may provide partial protection, their effectiveness can be limited by factors such as cost, environmental concerns, pathogen adaptation, or inconsistent field performance (Miguélez-Sierra et al., 2024). Consequently, contemporary disease management in cacao increasingly emphasises integrated pest management (IPM) (sometimes also referred to specifically as integrated disease management (IDM)) approaches that combine multiple complementary strategies. By integrating chemical, biological, cultural, and host-resistance methods, disease control programs can maximise the strengths of each technique while minimising their limitations, thereby improving the sustainability and effectiveness of disease management in cacao production systems (Tahi et al., 2019). Implementation of any control intervention depends on early and accurate disease diagnosis, regardless of the management strategy (Marelli et al., 2019). The severity of cocoa pod diseases can be evaluated in the field using a standardised severity index (Hernández-Núñez et al., 2024). In many studies, cacao farmers and agricultural technicians assess disease intensity using a 0-7 severity rating scale that categorises the extent of visible symptoms on infected pods (Tan et al., 2017). This scale was adapted from a disease severity index originally developed to assess anthracnose infection in mango and subsequently modified for use in cacao pathology studies (Tan et al., 2017). The scoring system enables systematic estimation of disease progression by assigning numerical values corresponding to increasing levels of tissue infection and lesion coverage on the pod surface (Hernández-Núñez et al., 2024). Such severity scales are widely used in plant pathology because they facilitate consistent disease monitoring, comparison among treatments, and statistical analysis in field experiments evaluating disease management strategies (Bock et al., 2022). However, due to bias and human error in visually grading diseases, such judgments are frequently inaccurate (Tan et al., 2017). Recent advances in digital agriculture have led to the development of automated tools for detecting and assessing plant diseases (Sandotra et al., 2023). One such system, AuToDiDAC (Automatic Tool for Disease Detection and Assessment in Cacao), was designed to automatically inspect cocoa pods for symptoms of black pod rot and accurately estimate disease severity. The system employs image analysis and machine learning to identify visual disease symptoms and quantify the extent of infection on cocoa pods (Tan et al., 2017). By providing objective, standardised assessments, AuToDiDAC helps reduce the subjectivity and variability inherent in conventional visual disease scoring conducted by farmers and field technicians (Tan et al., 2017). Consequently, automated diagnostic tools such as AuToDiDAC can enhance the efficiency, consistency, and accuracy of disease monitoring in cacao plantations, thereby supporting more informed disease management decisions (Harvyanti et al., 2023).

1.4.3 Stem canker disease

In parts of West Africa, such as Ghana, after CSSVD and black pod disease, *Phytophthora* stem canker is the third most significant disease (Ofori et al., 2022; Frimpong et al., 2021) that affects the cacao plant. Inoculum for black pod disease comes from stem canker, and vice versa (Jagadeesh et al., 2022). The two species that produce black pod disease *P. palmivora* and *P. megakarya*, also cause stem canker (Adomako et al., 2021). These pathogens infect the bark, flower cushions, and chupons. The infection site on the stem develops water-soaked margins that range in colour from dark brown to black. The primary roots may be affected by cankers near the trunk's base. The bark conceals canker sores, which frequently ooze reddish gum or infect flower cushions, killing the flowers, especially the newly developed flowers. If left untreated, the disease can kill young cacao trees, weaken mature trees, and destroy flower cushions, thereby reducing potential output (Debnath et al., 2023; Merga, 2022). Whole trees die as a result of the canker sores girdling the stem. In Ghana’s major cacao-growing regions, substantial tree mortality has been reported in recent years, largely associated with an increased incidence of various canker and stem diseases, which weaken tree structure and reduce productivity. These diseases are aggravated by environmental stress and poor farm management and have contributed to declining tree health and reduced yields in several cocoa-producing areas (Ploetz, 2019; Guest & Keane, 2020; Abdulai et al., 2022). Girdling cankers represent a serious constraint to cocoa production, as they damage the vascular tissues of the trunk and branches, ultimately leading to rapid tree decline and mortality. In severely affected plantations, it has been estimated that up to 10% of cacao trees may die annually as a result of these cankers, highlighting their significant impact on long-term plantation productivity and sustainability (Ploetz, 2019; Guest & Keane, 2020; Bailey & Meinhardt, 2020). The severity of cacao diseases caused by *Phytophthora* species varies depending on several interacting factors, including the pathogen species present, the genetic background of the cacao cultivar, and local climatic conditions such as rainfall, humidity, and temperature. In West Africa, changes in planting material, particularly the gradual replacement of traditional Amelonado varieties with hybrid genotypes, have been associated with increased incidence of stem cankers and related diseases in some production systems, possibly due to differences in host susceptibility and management practices (Ploetz, 2019; Guest & Keane, 2020; Opoku et al., 2021). All sections of the cacao plant are vulnerable to attack by *Phytophthora*. It is futile to focus solely on preventing pod infections while ignoring the tree's other sources of oomycete infection.

Although stem canker doesn't directly affect pods, it does harm the overall health of the cacao trees, which has an impact on the production and longevity of production trees. It is important to try to stop the oomycete from spreading via the pod stalk of infected pods to the stem. Cultural control procedures, including routine weeding and the removal of mummified fruits left over from the previous season, are ineffective. Canker diseases in cacao can be effectively reduced through the annual application of potassium phosphonate, an inorganic salt widely used for the management of *Phytophthora*-related diseases. Potassium phosphonate acts both directly, by inhibiting pathogen development, and indirectly, by stimulating host defence responses, thereby reducing disease severity and improving tree survival in infected plantations (Ploetz, 2019; Opoku et al., 2021). Canker management in cacao may also involve mechanical removal of infected bark followed by the application of copper-based fungicides to the exposed tissues. Scraping the affected surface to expose the canker removes infected tissue and facilitates better penetration of the fungicide, thereby suppressing *Phytophthora* pathogens and limiting further disease development (Guest and Keane, 2020; Opoku et al., 2021).

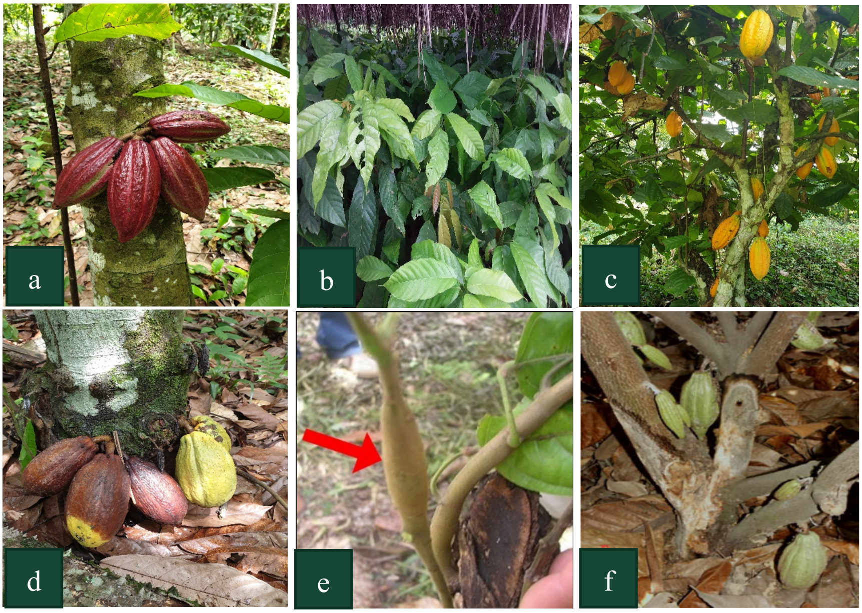

Figure 4: Major diseases of Cacao in Sierra Leone. Top left (a) healthy pods; reference point), top middle (b) healthy seedlings free from, top right (c) stem covered with ferns, bottom left (d) cocoa pod infected with black pod disease, bottom middle (e) Swollen shoot caused by CSSV, and bottom right (f) stem canker caused by *Phytophthora*. Photographs copyright Mohamed Mambu Luseni.

1.5 Integrated Pest Management in Cacao

Integrated Pest Management (IPM) represents a holistic and sustainable approach to managing pests and diseases in cacao production systems (Agulanna et al., 2025). IPM involves the strategic combination of multiple compatible control methods to maintain pest populations below economically damaging thresholds while minimising risks to human health, beneficial organisms, and the environment (Angon et al., 2023). In cacao systems, where multiple biotic constraints such as insect pests, viral diseases, and *Phytophthora*-induced black pod disease interact with environmental conditions, IPM provides a framework for achieving long-term, resilient disease management (López-García, 2024).

1.5.1 Host Plant Resistance

The implementation of IPM in cacao is based on several key components. Host plant resistance forms a fundamental pillar of IPM. The use of genetically diverse and disease-resistant cacao varieties can significantly reduce susceptibility to major pathogens such as *Phytophthora megakarya* and *Phytophthora palmivora* (Baruah et al., 2022). Breeding programmes that exploit genetic diversity, including local and introduced germplasm, contribute to the development of tolerant or resistant cultivars (Imán et al., 2025). This aligns with the need to enhance adaptive capacity in cacao systems, particularly under changing climatic conditions.

1.5.2 Sanitation and cultural control

Cultural and agroforestry practices play a critical role in suppressing pest and disease incidence. Practices such as regular removal of infected pods (phytosanitation), pruning to improve aeration, optimal shade management, and appropriate plant spacing reduce disease inoculum and limit the spread of pathogens (Fruit, 2023; Marathe, 2022). In addition, soil fertility management and organic matter inputs contribute to overall plant health and resilience against infections (Awazi et al., 2025).

1.5.3 Biological control

Biological control is an increasingly important component of IPM in cacao systems. The use of antagonistic microorganisms, particularly species of *Trichoderma*, has shown promising results in suppressing Phytophthora infections through mechanisms such as mycoparasitism, competition, and induction of host plant resistance (Chóez-Guaranda et al., 2023; Pakora et al., 2018). Other beneficial organisms, including endophytes and rhizosphere-associated microbes, also contribute to enhancing plant defence mechanisms and reducing disease severity (Bharadwaj et al., 2025). The integration of biological control agents offers an environmentally friendly alternative to chemical inputs and supports sustainable cocoa production (Valenzuela-Cobos et al., 2023).

1.5.4 Chemical control

Chemical control, while still widely used, is considered a complementary component within IPM rather than a primary strategy. The judicious use of fungicides, particularly copper-based compounds and systemic fungicides, can provide effective protection against black pod disease when applied at critical periods. However, overreliance on chemical control can lead to issues such as pathogen resistance, environmental contamination, and increased production costs. Therefore, chemical interventions should be applied based on disease monitoring and economic thresholds, and in combination with other IPM components.

1.5.5 Monitoring and early disease detection

Monitoring and early disease detection are essential for effective IPM implementation. Regular field scouting, the use of disease severity scales, and emerging digital tools such as image-based diagnostic systems enhance the accuracy and timeliness of disease assessment (Singh et al., 2025). Early detection enables targeted interventions, reducing unnecessary input use and improving control efficiency.

1.5.6 Farmer knowledge and participatory approaches

Farmer knowledge and participatory approaches are also critical to the success of IPM. Adoption of integrated strategies depends on farmers' understanding of pest and disease dynamics, as well as access to training and extension services. Participatory approaches that incorporate local knowledge and socio-economic realities can enhance the adoption and sustainability of IPM practices in smallholder cacao systems (Antwi-Agyei et al., 2025).

In the context of Sierra Leone, where cacao production is largely dominated by smallholder farmers operating within agroforestry systems, the integration of host genetic diversity, improved agroforestry management, and biological control strategies offers a promising pathway for sustainable management of black pod disease. This thesis, therefore, focuses on these key components as central elements of an integrated pest management framework for cacao.

2. Aims and objectives

The overall aim of this thesis is to gain a better understanding of cacao production and cocoa black pod disease management in Sierra Leone. To make progress towards the development of a comprehensive integrated pest management program for cocoa black pod disease in Sierra Leone, this study has three main research aims:

I. To survey the genetic diversity within *Theobroma cacao* L. populations in Sierra Leone.
II. To assess the impact of traditional agroforestry systems on cocoa yield and cocoa black pod disease in Sierra Leone.
III. To investigate the effectiveness of *Trichoderma atroviride* in controlling cocoa black pod disease in Sierra Leone.

The specific aims of the individual papers included:

Paper I:

1. Characterise and document the cacao germplasm in Sierra Leone to allow future conservation and utilisation by specifically:
    a. Using KASP-SNP genotyping, understand the genetic diversity and population structure of cacao germplasm currently used by farmers in Sierra Leone
    b. Compare that to the genetic diversity and population structure of cacao germplasm used by farmers in Togo.
    c. Use this data to identify potential mislabelling of germplasm that should be corrected for proper utilisation.

Paper II:

1. Evaluate how widespread the incidence of cocoa black pod disease is in Sierra Leone.
2. Investigate if there are differences in cacao density and the density, diversity and shade properties of companion trees within cacao agroforestry systems in Sierra Leone and test if the occurrence of cocoa black pod disease is linked to any of these factors.

3. Assess if there are differences in cocoa yield among the cacao farms and districts and, at the same time, investigate how yield is affected by cocoa black pod disease.

Paper III:

1. Investigate if a commercial preparation of *Trichoderma atroviride* can improve cocoa yield and effectively control cocoa black pod disease in smallholder farms in Sierra Leone by answering four interlinked questions:
    a. Is there an overall effect of *T. atroviride* on pod yield?
    b. Is there an overall effect of *T. atroviride* on cocoa black pod disease?
    c. Is the effect of *T. atroviride* both direct (applied to the trunk) or systemic (seen in the canopy after trunk treatment), and are these different effects?
    d. Is the effect of *T. atroviride* different depending on the cultivar being treated or the site?

3. Methods

3.1 Study areas

All studies included in this thesis were conducted in the Eastern region of Sierra Leone, where cacao is primarily grown in Kenema (07° 52’ 36” N, 011° 11’ 15” W), Kailahun (08° 17’ 00” N, 010° 34’ 00” W), and Kono (08° 38’ 00” N, 010° 59’ 00” W) with minor occurrences in Bo (07° 57’ 53” N, 011° 44’ 18” W) and Pujehun (07° 21’ 02” N, 011° 43’ 05” W) Districts Figure 5).

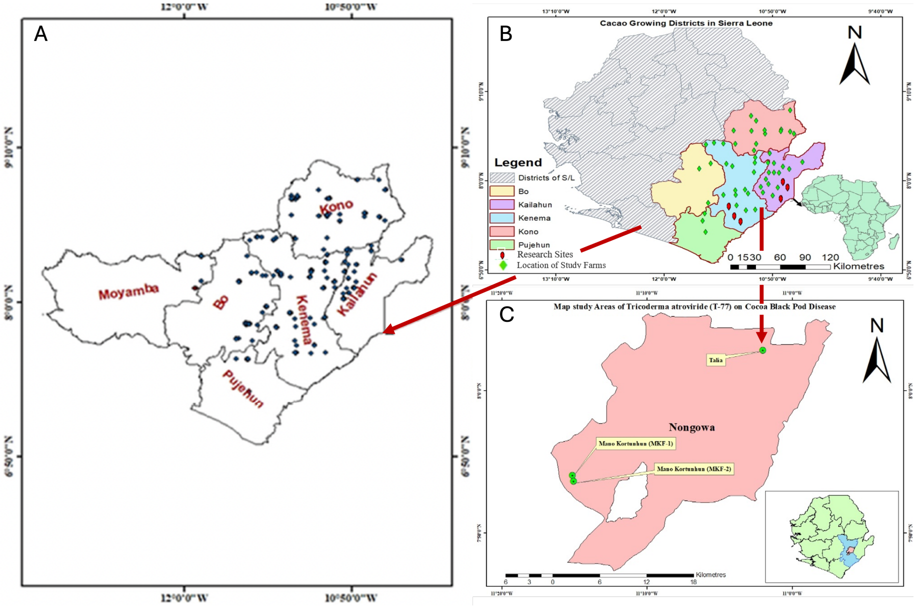

Figure 5: Location of the study areas used in this thesis. (A) Leaf samples for KASP-SNP genotyping were collected from a wide range of farms from both the major (Kenema, Kailahun and Kono) and minor (Bo and Pujehun) cacao growing districts of Sierra Leone, from cacao farms and from trees in the Njala University lower nursery gardens in Moyamba. (B) Assessments of agroforestry systems and cocoa black pod disease were carried out in field stations across the five cacao-producing districts in Sierra Leone (S/L) red diamonds denote research gardens and green diamonds show farm sites. (C) Three farms (shown as green diamonds) within the chiefdom of Nongowa in the heart of the major cocoa growing district, Kenema, were used to assess the efficacy of *Trichoderma atroviride* as a biocontrol agent against cocoa black pod disease. Maps generated using ArcMap 10.8.2, Esri ArcGIS Desktop.

3.2 Field assessments

The data for papers **I** and **II** are based on the assessment of genotypes and cacao agroforestry systems practiced by farmers. This was conducted to better understand and develop cacao farmers' integrated pest management in Sierra Leone. The genotypic characterisation of cacao germplasm from Sierra Leone and Togo, and the potential for resistance breeding, provide a clear foundation for the data presented in paper **(I)**. A total of 235 leaf samples were collected in Sierra Leone from diverse sources, including farmers' fields, research sites, the lower nursery garden of Njala University, and plantations of agricultural companies such as Tropical Farms. Sample collections were strictly based on the leaf type, pod structure, and pod colour. To prevent excessive duplication, samples with the same identification were obtained at most twice. I recorded the GPS coordinates for the district, chiefdom, community, and farm. All samples were preserved and labelled.

In paper (**II**), I selected 50 sample sites of farmers' fields and six research sites that varied in farm structure, species and density, species phenotypic characteristics, and management strategies. At each plantation, I constructed a 2,500 m^2 quadrant measuring 50 m x 50 m. All agroforestry tree species in the selected quadrant were identified, and measurements of their phenotypic traits (height, canopy, canopy area, and DBH) were made. Other features, such as elevation, tree diversity, and evenness, were measured. To determine the incidence of black pod disease, five cacao trees were also chosen at random. I recorded the GPS values for each quadrant, its agroforestry and cacao trees.

Biological control of CBPD (paper **III**) has been requested from cacao growers in the region. For this goal, three sites of 300 cacao trees were selected, with 100 cacao trees at each site. Two treatments (Treated and untreated, serving as the control) were used. *T. atroviride* used as a treatment was sprayed on the trunk at a height of 2.5 m to see if there is both a direct and a systemic yield effect in the canopy. Water was sprayed on the trunks of untreated (controlled) cacao trees. Data collection was divided into 2 zones: below 2.5 m (trunk area with direct treatment) and above 2.5 m (canopy area not directly treated) of each cacao tree for both treated and control (untreated) trees. Healthy and diseased pods were separated by site, treatment, and canopy and trunk.

3.3 Cacao genetic diversity assessment

3.3.1 Markers used for genetic diversity analysis

Molecular marker systems have become indispensable tools in cacao genotyping. It enables the precise characterisation of genetic diversity, population structure, and trait-associated loci. DNA-based markers are particularly valuable because they are not influenced by environmental conditions or plant developmental stage, making them reliable for breeding, conservation, and disease resistance studies. Over the past three decades, several classes of molecular markers have been developed and applied in cacao research, each with specific advantages, limitations, and applications depending on the objective of the study.

3.3.1.1 Restriction Fragment Length Polymorphisms (RFLPs)

Among the earliest marker systems used in cacao are RFLPs. RFLPs are based on variations in DNA fragment lengths generated by restriction enzyme digestion. These markers are co-dominant and highly reliable, allowing differentiation between homozygous and heterozygous genotypes. In cacao, RFLPs were instrumental in early genetic mapping and diversity studies, particularly in distinguishing major genetic groups such as Criollo, Forastero, and Trinitario. However, their use has declined due to technical complexity, high DNA requirements, and low throughput compared to more modern techniques (Motamayor et al., 2002).

Random Amplified Polymorphic DNA (RAPD) markers were subsequently adopted due to their simplicity and low cost. RAPDs amplify random segments of DNA using short primers and do not require prior sequence information. They have been used in cacao for preliminary diversity assessments and germplasm characterisation. However, RAPDs are dominant markers and suffer from poor reproducibility, which limits their reliability in advanced genetic studies and breeding programmes (Faleiro et al., 2002).

3.3.1.2 Amplified Fragment Length Polymorphisms (AFLPs)

AFLPs represent an improvement over RAPDs, combining restriction digestion with selective PCR amplification. AFLPs generate many polymorphic markers with high reproducibility and genome coverage. In cacao, AFLPs have been used for genetic diversity analysis, linkage mapping, and population structure studies. Despite their high resolution, AFLPs are technically demanding and are also dominant markers, which limits their ability to distinguish heterozygous genotypes (Vos et al., 1995). Simple Sequence Repeats (SSRs), also known as microsatellites, are among the most widely used markers in cacao genotyping. SSRs consist of short, tandemly repeated DNA sequences that are highly polymorphic due to variation in repeat number. These markers are co-dominant, highly reproducible, and locus-specific, making them ideal for genetic diversity studies, parentage analysis, and germplasm identification. In cacao, SSR markers have been extensively used to characterise genetic resources, assess population structure, and support breeding programmes. Their high level of polymorphism makes them particularly useful for distinguishing closely related genotypes (Lanaud et al., 1999; Motilal et al., 2013).

3.3.1.3 Inter-Simple Sequence Repeat (ISSR)

ISSR markers have also been used in cacao for genetic diversity analysis. These markers amplify regions between microsatellite loci and do not require prior sequence information. ISSRs are more reproducible than RAPDs and can generate moderate levels of polymorphism. However, like RAPDs and AFLPs, they are dominant markers and are therefore less informative for detailed genetic analysis.

3.3.1.4 Single Nucleotide Polymorphisms (SNPs)

SNPs represent the most advanced and widely used marker system in modern cacao genomics. SNPs are single base-pair variations in the DNA sequence and occur abundantly throughout the genome. They are highly amenable to high-throughput genotyping platforms, allowing the simultaneous analysis of thousands of loci. In cacao, SNP markers are used for genome-wide association studies (GWAS), quantitative trait loci (QTL) mapping, genomic selection, and marker-assisted breeding. Their high density and scalability make them particularly suitable for identifying genes associated with important traits such as disease resistance, yield, and quality (Livingstone et al., 2015; Cornejo et al., 2018).

3.3.2 The choice of markers for genetic diversity studies

The choice of marker system in cacao genotyping depends largely on the research objective. For example, SSRs are preferred for germplasm characterisation and diversity studies due to their high polymorphism and co-dominant inheritance. SNPs are the markers of choice for high-resolution mapping, genomic selection, and large-scale breeding programmes because of their abundance and compatibility with automated platforms. AFLPs and ISSRs may be used for preliminary assessments where resources are limited, while RFLPs are now largely of historical importance.

In the context of cacao breeding and disease resistance studies, particularly for traits such as resistance to *Phytophthora* spp., SNPs and SSRs are the most commonly used. These markers facilitate the identification of QTLs associated with resistance and enable marker-assisted selection, thereby accelerating the development of improved varieties. The integration of molecular markers into cacao breeding programmes enhances selection efficiency, reduces breeding cycle duration, and supports the development of resilient and high-performing genotypes.

DNA marker technologies have transformed cacao genotyping by providing precise, reliable, and high-throughput tools for genetic analysis. The continued advancement of genomic technologies is expected to further improve our understanding of cacao genetics and accelerate the development of improved cultivars suited to the challenges of disease pressure and climate change. In this study, KASP-SNP genotyping was chosen as a reliable and economical method for describing population structure and genetic diversity in cacao germplasm. Reliable genotyping platforms are necessary for the effective detection of SNPs, which are abundant and persistent markers across the genome. The Kompetitive Allele-Specific PCR (KASP) technique uses fluorescence-based discrimination and allele-specific amplification to enable precise, high-throughput, and repeatable SNP detection. KASP tests were used because they are technically simple, with a reasonable price, and ideal for screening a large number of samples as compared to other genotyping techniques. Additionally, a panel of 20 KASP-SNP markers had already been developed by Intertech AB and tested on cacao materials from Nigeria. To guarantee sufficient genome coverage, a panel of these 20 informative SNP markers dispersed throughout the *Theobroma cacao* genome was used in this investigation. This method enabled precise identification of genetic variation, detection of potential mislabelling among accessions, and a reliable estimate of population structure in germplasm collections from Sierra Leone and Togo.

3.3.3 Leaf sample collection

A total of 235 cacao leaf samples were collected from farmers' fields (150 samples), agricultural companies (49 samples), research gardens (23 samples), and Njala University plots (13 samples). These leaf samples were placed in 50 ml macro tubes, poured with silica gel for preservation, and labelled. The District, Chiefdom, community, sample label code, and GPS readings were appropriately recorded for each sample.

3.3.4 DNA extraction and genotyping

DNA quality and quantity were first assessed using 0.8% agarose gel electrophoresis to ensure suitability for downstream analysis. A panel of 20 high-quality and highly polymorphic SNP markers was selected for genotyping cacao leaf samples. These SNPs were derived from an initial set of 1536 markers previously identified from expressed sequence tag (EST) datasets representing diverse cacao tissues and transcriptomic profiles. From this initial pool, 100 SNPs were shortlisted based on call rate, genome-wide distribution across the ten chromosomes, heterozygosity, and prior validation in cacao genetic studies. These markers had previously been converted into Kompetitive Allele-Specific PCR (KASP™) assays at LGC Genomics for use in cacao diversity studies in West Africa.

For the present analysis, a subset of 20 SNPs was selected from the 100-marker panel based on polymorphism level and performance in low-density genotyping applications. Marker selection was further supported by simulation analyses to ensure optimal discrimination power. Genotyping was performed using the KASP™ high-throughput PCR SNPline workflow in 384-well plates with a 1 µL reaction volume. Each reaction comprised approximately 10 ng of template DNA, marker-specific assay mix containing allele-specific primers, and KASP-TF™ master mix, which includes fluorescence resonance energy transfer (FRET)-based reporters (FAM and HEX), Taq polymerase, nucleotides, $MgCl_2$, and buffer components. Following PCR amplification, fluorescence signals were detected and analysed using KRAKEN™ software (LGC Biosearch Technologies), and genotype calls were assigned based on cluster plot separation into homozygous or heterozygous classes for each biallelic SNP locus.

This SNP–KASP genotyping approach provides a cost-effective, reliable, and scalable method for cacao genetic analysis, particularly suitable for applications such as diversity assessment, population structure analysis, and marker-assisted selection in breeding programmes.

3.4 Collection of agroforestry system data

At each of the 50 farmers' fields and six research sites, a square quadrant measuring 50 x 50 m and covering an area of 2,500 m^2 was demarcated. Data on species, density, canopy diameter, height, and diameter of breast height at 1.3 m were measured for phytogeography. To identify each species, taxonomists from Njala University and the Sierra Leone Agricultural Research Institute (SLARI), who are actively involved in forest-related work, were engaged. Trees of suitable heights and DBH of undersized agroforestry trees were measured using a measuring tape with a 50 m length. Trees at higher elevations were measured using the triangulation method with clinometer observations.

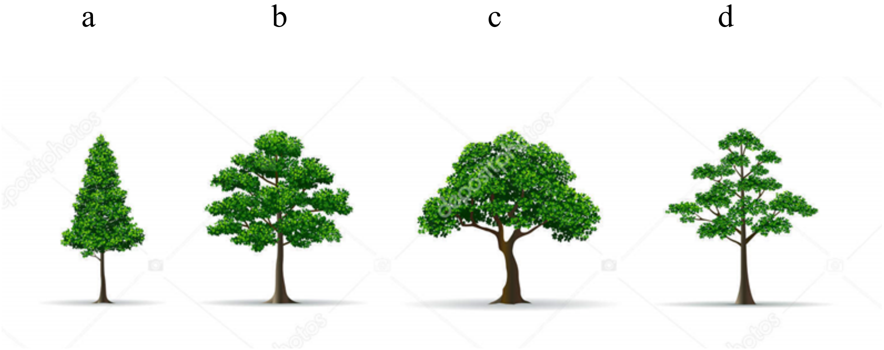

Figure 6. Four major agroforestry tree crown architectures commonly associated with cacao plantations. (a) pyramidal, **(b)** vase-shaped, **(c)** broad-spreading, and **(d)** weeping forms.

3.5 Assessment of the occurrence of cocoa black pod

For the purpose of collecting data on disease pods, five cacao trees were randomly chosen in each quadrant. These five trees were subsequently harvested when the pods had reached physiological maturity. Those with black pod disease were identified and separated from those without it. I recorded the GPS reading of the four corners of each quadrant.

3.6 Treatment with *Trichoderma atroviride*

The biocontrol agent *Trichoderma atroviride* was selected for this study based on its well-documented efficacy and experience from other countries against plant pathogens, particularly *Phytophthora* species responsible for cocoa black pod disease. Along with the scientific evidence of its proven antagonistic mechanisms of mycoparasitism, competition, and induction of plant defence, its selection was also guided by practical considerations relevant to smallholder cultivation systems. The product was readily available to SLARI-KFTCRC for field testing at farmers' fields in Sierra Leone as a commercial preparation (Microsaf® which is a preparation of *T. atroviride* strain 77) which is sold in Sierra Leone by Tradin Organic. This means that the findings have direct relevance and application by enabling both examination under actual farmer field circumstances and potential uptake by farmers themselves at the end of the study.

At each farm, a 50 x 50 m quadrat was established, containing approximately 278 cacao trees planted at a spacing of 3 x 3 m. Within each quadrant, 100 cacao trees were randomly selected, with 50 cacao trees treated with *T. atroviride* and 50 trees serving as untreated controls.

Several cacao cultivars were present at the sites, including Forastero, Criollo, Trinitario, Amazon, and Amelonado, with several known to be susceptible to CBPD. To allow cultivar comparisons across farms, plots included Amazon, Amelonado, and Trinitario trees. Cultivar distribution was similar among the three sites (MKF1, MKF2, and Talia).

Before treatment, agroforestry shade trees were pruned to 19-26 m to improve light penetration and airflow, and farms were weeded regularly. Infected pods were removed two weeks before the first treatment. A commercial formulation of *T. atroviride* 77B (Microsaf® T; 2×10^9 CFU g^{-1}) was applied at 10 g in 10 L water using a knapsack sprayer at 9-day intervals from August to December, totalling 16 applications. Water was applied on the cacao trees selected as control.

4. Results and Discussion

4.1 Knowledge gaps and current constraints for farmers

In West Africa, the research focus has previously been on countries which contribute the highest percentages of cocoa to the global market, including Ghana and Côte d'Ivoire, and smaller producers such as Sierra Leone have therefore often been overlooked.

Cocoa production serves as an important source of livelihood for smallholder farmers across many tropical regions, especially in West Africa (Agele et al., 2023). In Sierra Leone, cacao is an essential cash crop that supports rural incomes and contributes to national export revenues (Bonuedi et al., 2020). However, regardless of its economic significance, cocoa yields in smallholder systems continue to be relatively low (Vaast & Somarriba, 2014). There are several problems and limitations for farmers in Sierra Leone. The first is that they often have limited access to improved genetic planting materials or are unsure of the cultivars or genetic background of the trees in their farms due to mislabelling or missing records on inheritance of older farms (Olasupo & Aikpokpodion, 2019). Secondly, farmers may not be using the correct agronomic management practices, which can be based on a lack of knowledge of the best agroforestry practices to follow, inadequate record keeping and limited training opportunities (Kouassi et al., 2023). Finally, farmers often do not have access to cost effective disease control options with a high level of efficacy. Synthetic pesticides are expensive and generally not available in Sierra Leone, and biological control options are not widely used due to a lack of research into their efficacy and limited training opportunities for farmers (Adomako et al., 2021). Among these constraints, the lack of reliable planting materials, along with the prevalence of pests and diseases, represent the most critical barriers, often leading to considerable yield losses and poor bean quality (Aneani et al., 2017).

The materials at farmers' fields are highly susceptible to *Phytophthora* species that cause black pod disease and stem canker (Polanco et al., 2023). Due to infection with black pod disease, the expected yield from plantations has been significantly reduced (Polanco et al., 2023), creating a dilemma for the Sierra Leonean cocoa industry. This demonstrates that most planting materials used by farmers in their plantations don't possess desirable characteristics such as high yield and resistance to black pod disease (Aikpokpodion & Adeogun, 2011; Sauvadet et al., 2021). Variations in genetic material and incorrect labelling can complicate the interpretation of experimental data. Variations in genetic materials can also affect farmer yield because it can be challenging for farmers to forecast how a particular variety will behave under their unique growing circumstances. Genetic material mislabelling may lead to inaccurate inferences from the data by persuading farmers to plant genotypes inappropriate for their growing environment (Bohr et al., 2024; Jaleta et al., 2020). As a result, yields are reduced and pest and disease incidence increase (Vaast & Somarriba, 2014). Therefore, more research is needed to precisely determine the genotypes farmers are using, the occurrence of cocoa black pod disease and to test and develop strategies that enable farmers to better control this disease with minimal inputs.

The lack of improved planting materials and the circulation of mislabelled seedlings represent major constraints to cocoa productivity in smallholder farming systems (investigated in **PAPER I**). In many cacao growing regions, including Sierra Leone, farmers often obtain seeds or seedlings from informal sources such as neighbouring farms, local markets, or by collecting pods themselves (Bhattacharjee et al., 2023; Osei-Adu et al., 2016). Although this practice facilitates access to planting materials, it frequently results in the propagation of genetically unverified and heterogeneous planting stocks (Daouda et al., 2021). Consequently, farmers may unknowingly cultivate cacao trees with low yield potential, poor adaptation to local environmental conditions, and high susceptibility to pests and diseases (Daouda et al., 2021).

A further challenge within these informal systems is the widespread distribution of mislabelled planting materials (**PAPER I**). Seedlings that are claimed to be improved or disease-tolerant varieties may not correspond to their true genetic identity due to poor nursery management, lack of certification systems, or mixing of planting materials during propagation and distribution (Bordeaux et al., 2023; Olasupo & Aikpokpodion, 2019). This can lead to reduced productivity and increased susceptibility to diseases such as black pod disease (Gutiérrez et al., 2021; Septiani et al., 2024). The results in **PAPER I** show that cacao planting materials used by farmers in Sierra Leone and Togo are mislabelled, exhibiting poor production performance, and are susceptible to black pod disease.

Several plant diseases caused by fungal and oomycete pathogens represent additional major constraints to cocoa production worldwide (Ramírez-Camejo et al., 2025). The humid tropical conditions under which cacao is typically cultivated provide favourable environments for pathogen survival, multiplication, and dissemination (Oro et al., 2020). Pathogens infect various parts of the cacao tree, including pods, leaves, and stems, resulting in reduced productivity and poor bean quality (Lim et al., 2023). Yield losses associated with plant diseases in cacao can be substantial, particularly in regions where environmental conditions favour rapid pathogen development (Bailey et al., 2022) (**PAPERS II, III**). Also, limited financial capacity among smallholder cacao farmers restricts their ability to purchase synthetic fungicides for the control of CBPD and other cacao diseases; consequently, disease management largely relies on non-chemical practices such as cultural control, rather than regular use of synthetic inputs (Agele et al., 2023). In many traditional cacao farming systems, disease severity is further intensified by structural and management related factors (Mvondo et al., 2023). Ageing plantations with declining tree vigour are generally more susceptible to pest and pathogen attack (Hougni et al., 2021). Additionally, inadequate farm sanitation, such as the failure to remove infected pods and plant debris, facilitates the accumulation and spread of pathogen inoculum within farms (Oro et al., 2019; Purwantara, 2008). Limited adoption of improved crop management practices, including pruning, shade regulation, reduction of shade trees, and the use of improved planting materials, further exacerbates disease incidence and severity (Tscharntke et al., 2023). These interacting constraints highlight the need for integrated pest and disease management strategies that combine improved host-resistant vegetative materials, sound agronomic practices, and environmentally sustainable control measures to enhance cacao productivity and resilience in smallholder farming systems (Grant et al., 2025).

4.2 Cocoa yield across farms and districts

In **PAPER II** I assessed cocoa productivity, estimated as the weight of healthy pods harvested from sampled trees. Yield varied between 3.39 and 6.31 kg among the 250 cacao trees assessed across the 50 surveyed farms, with an overall mean yield of 4.68 ± 0.61 kg (SD). Research station plots were excluded from this analysis to focus on yield performance under farmer-managed field conditions. I also assessed yield with and without treatment with the biocontrol agent *Trichoderma atroviride* in 3 farms in Kenema (**PAPER III**). The absence of significant yield variation among districts suggests that cocoa productivity may be influenced more strongly by local farm management practices and ecological conditions than by broader geographic location **(PAPER II).** Similar patterns have been reported in cacao agroecosystems in other parts of West Africa, where yield variability often occurs at the farm level due to differences in soil fertility, shade management, pest and disease pressure, and agronomic practices rather than regional differences alone (Asante et al., 2021; Asare et al., 2016). Studies conducted in cacao agroforestry systems have shown that environmental and management factors interact to influence productivity, resulting in heterogeneous yields even within the same ecological zone (Doe et al., 2023).

The observed yield range in this study is also consistent with the generally moderate productivity levels reported for smallholder cacao systems in West Africa. For example, recent studies indicate that average cocoa yields in many smallholder farms remain below their potential due to limitations such as nutrient depletion, suboptimal shade management, and disease incidence (Figueiredo et al., 2024; Hougni et al., 2021; Amponsah-Doku et al., 2022). Furthermore, empirical surveys of cocoa production systems in Côte d'Ivoire reported average yields of approximately 376 ± 36 kg ha^{-1} $year^{-1}$, highlighting the persistence of a widespread yield gap in many traditional cacao systems (Kouassi et al., 2023).

The relatively similar yield levels across districts may also reflect comparable agroforestry structures and management practices across farms**,** particularly with respect to shade tree composition, pruning regimes, and pest and disease management. Evidence from cacao agroforestry research shows that certain shade tree species and farm management practices can enhance cocoa productivity by improving soil fertility and microclimatic conditions (Dumont et al., 2014; Kouassi et al., 2023). At the same time, inappropriate shade density or poor nutrient management can constrain productivity, contributing to variability at the farm scale rather than across broader geographic regions (Figueiredo et al., 2024).

Overall, the findings suggest that improving farm-level management practices may be more effective for enhancing cocoa productivity than interventions targeting regional differences alone (Figure 7**).** Strategies such as optimized shade-tree management, improved soil fertility management, and effective disease control could play a critical role in closing existing yield gaps and improving the sustainability of cocoa production systems in West Africa.

4.3 Towards integrated disease management in cacao

4.3.1 Genetic diversity in Sierra Leone cacao germplasm

Cacao planting materials with host-resistant traits play a crucial role in improving yield stability and disease management among smallholder farmers, particularly in regions where CBPD meaningfully constrains production (Nyadanu et al., 2019). Using resistant varieties reduces crop losses and limits dependence on chemical control measures, thereby promoting more sustainable cocoa production systems (Agele et al., 2023). Well-characterised materials provide reliable and uniform genetic resources for scientific research, breeding programs, and the evaluation of disease resistance under both controlled and field conditions (Bekele and Phillips-Mora, 2019).

Though the distribution and use of mislabelled cacao planting materials give serious consequences for both farmers and research (**PAPER I**). Farmers who unknowingly cultivate misidentified varieties/clones may not obtain the expected levels of resistance or productivity, resulting in poor field performance and economic losses. Similarly, for researchers, inaccurate labelling can compromise experimental reliability, distort genetic analyses, and undermine the reproducibility and interpretation of scientific findings (Bhattacharjee et al., 2024).

In this context, proper varietal/clonal labelling is increasingly recognised as a critical component of integrated management of CBPD. Accurate identification enables clear differentiation between resistant and susceptible genotypes, which is essential because cacao exhibits significant variation in response to *P. palmivora*, with resistance governed by specific genetic factors and quantitative trait loci (Muñoz et al., 2021; Winters et al., 2024). Consequently, correct labelling supports the strategic selection of tolerant planting materials that inherently reduce disease incidence.

Accurate varietal identification further enhances the effectiveness of integrated disease management practices. Since disease development depends on host–pathogen interactions that vary among genotypes, management strategies of agroforestry trees (**PAPER II**) can be tailored accordingly (Capador-Barreto et al., 2025). Susceptible varieties typically require more intensive interventions, including fungicide application and strict sanitation, whereas resistant varieties can be managed with fewer biocontrol inputs (**PAPER III**). This targeted approach improves control efficiency while reducing production costs and environmental impacts.

Moreover, proper labelling supports effective field sanitation and epidemiological management. The ability to identify susceptible trees enables the selective removal of infection sources, thereby reducing inoculum pressure on cacao farms. Observed differences in disease incidence among cacao clones further highlight the importance of correct genotype identification for managing disease spread and evaluating resistance under field conditions (Puig et al., 2022).

From a breeding perspective, varietal labelling ensures traceability of germplasm and prevents misidentification, both of which are essential for selecting resistant parent lines and developing improved hybrids (**PAPER I**). Recent molecular studies emphasize the importance of distinguishing resistant and susceptible genotypes at genetic and transcriptomic levels, reinforcing the need for accurate labelling in resistance breeding programs (Baruah et al., 2024).

The cacao accessions collected from Sierra Leone and Togo included several clones with identical accession names, even when samples were obtained from different trees within the same farm or across different farms. Many genotypes bearing similar names were collected from trees located on separate farms, suggesting that these trees may either represent the same clonal lineage or that some planting materials may have been mislabelled.

Among the 235 cacao genotypes collected in Sierra Leone, 144 accessions had unique names, with 66 accessions originally sourced from Ghana, 77 from the University of Reading, and the origin of one genotype was unknown. The cacao collection from Togo comprised 77 accessions, each with a unique name, originating from several countries, including Cameroon (4 accessions), Côte d'Ivoire (11 accessions), Ghana (17 accessions), Nigeria (28 accessions), and Togo (9 accessions).

A small number of clones represented hybrid or shared origins between countries, including Cameroon and Ghana, Côte d'Ivoire and Cameroon, Ghana and Côte d'Ivoire, and Ghana and Nigeria. In addition, five genotypes lacked accession names and are presumed to correspond to other clones. Furthermore, eleven accession names were common to both Sierra Leone and Togo, sourced from the University of Reading and Nigeria, respectively.

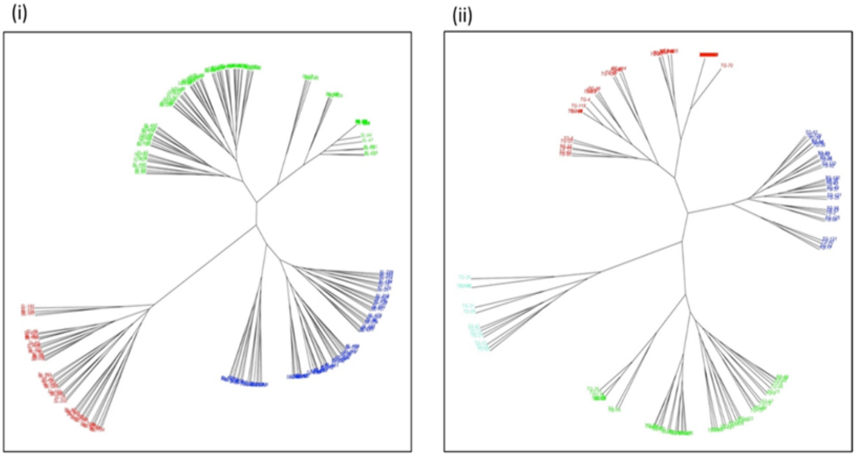

Figure 7. Phylogenetic tree of 376 cacao accessions from Sierra Leone and Togo. The blue and green colors represent accessions from Sierra Leone and Togo, respectively. Phylogenetic tree depicting genetic relationships among cacao accessions from (i) Sierra Leone and (ii) Togo. Colors of each tree represent genetic groups within each country.

For the genetic diversity Parameters, an analysis using 20 KASP–SNP markers revealed that both the Sierra Leone and Togo cacao populations exhibited moderate diversity, with slightly higher variation observed in the Sierra Leone accessions, and that combining the two populations increased overall diversity, as indicated by higher PIC, observed heterozygosity, and minor allele frequencies, suggesting that the pooled population provides a broader genetic base for breeding and conservation efforts (Table 1).

Table 1. Descriptive statistics based on 20 KASP–SNP markers across cacao accessions.

Cacao Population	N	He	Ho	MAF	PIC
Sierra Leone	235	0.30	0.24	0.21	0.22
Togo	141	0.29	0.22	0.19	0.21
Combined	376	0.30	0.26	0.23	0.24

N: Number of accessions.

The population structure analysis of the combined cacao population revealed that the 376 accessions from Sierra Leone and Togo could be divided into four major subpopulations (K = 4), with 73.4% of accessions confidently assigned and the remainder showing admixture (Figure 7). Sierra Leonean germplasm exhibited less admixture and was structured into three subpopulations (Figure 8), compared to that of Togo. Both the STRUCTURE and DAPC analyses consistently identified four clusters in the combined population, with Cluster 3 comprising the largest number of accessions, predominantly from Sierra Leone, followed by Clusters 2, 1, and 4. These results indicate substantial genetic differentiation among subpopulations and notable admixture, highlighting the complementary genetic diversity between Sierra Leonean and Togolese cacao germplasm.

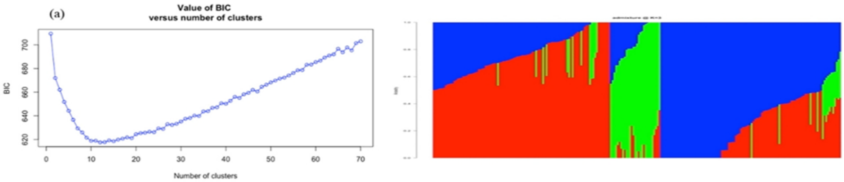

Figure 8. Cacao population structure from Sierra Leone. Plot of mean likelihood of delta K against the number of K groups; subpopulations at K = 3. The colours represent three subpopulations of 235 cacao accessions: red-subpopulation 1, green-subpopulation 2, blue-subpopulation 3.

4.3.2 Agroforestry systems and CBPD in Sierra Leone

Agroforestry management plays an important role in shaping the ecological conditions within cacao farms and can influence both pest and disease dynamics. Cacao is traditionally cultivated under shaded systems, where a diversity of associated tree species contributes to microclimate regulation, improved soil fertility, and biodiversity conservation (Vásquez et al., 2022). Well-managed agroforestry systems can enhance ecosystem resilience by promoting beneficial organisms, improving soil health, and stabilizing farm productivity (Diyaolu & Folarin, 2024). However, poorly managed shade systems may create favourable environmental conditions for certain pests and pathogens, particularly when excessive canopy cover increases humidity and reduces air circulation within the cacao canopy (Wartenberg et al., 2018).

The observed diversity of agroforestry trees in the surveyed cacao systems highlights the structural and ecological complexity of cacao-based agroforestry in Sierra Leone. A total of 916 agroforestry trees across 112 species and 41 families across 56 quadrats indicates relatively high species richness within cacao farms **(PAPER II)**. Such diversity is typical of traditional cacao agroforestry systems in West Africa, where farmers maintain a mixture of shade, fruit, and timber species to support ecological functions and diversify farm income (Dumont et al., 2014; Braga et al., 2019).

The higher number of species recorded on farms (89) compared with research stations (43) suggests that farmer-managed systems often maintain greater plant diversity than more controlled experimental plantations **(PAPER II)**. Smallholder cacao farms typically integrate multiple useful tree species that provide food, medicine, timber, and shade (Dawoe et al., 2016). This management practice results in complex, heterogeneous vegetation structures that contribute to biodiversity conservation, microclimate regulation, and enhanced ecosystem services within cacao agroforestry systems (Vaast & Somarriba, 2021). In contrast, research stations frequently manage shade trees more selectively to facilitate experimental uniformity and crop management.

The average of seven species per quadrant and a Shannon diversity index of 1.57 (Table 2) indicate moderate species diversity within the cacao agroforestry systems **(PAPER II)**. Similar diversity levels have been reported in cacao agroforestry systems across West and Central Africa, where shade-tree richness typically ranges from 5 to 15 species per hectare-equivalent sampling area. This reflects the multifunctional role of shade trees in providing ecosystem services, improving microclimatic conditions, and supporting biodiversity conservation in smallholder cacao landscapes (Vaast & Somarriba, 2021; Abdulai et al., 2022). Moderate diversity is often beneficial because it supports ecological services, including nutrient cycling, microclimate regulation, and biodiversity conservation (Tilman et al., 2014).

The dominance of a few species *Cola nitida*, *Elaeis guineensis*, *Musa sapientum*, *Terminalia ivorensis*, and *Citrus sinensis*, which together accounted for a large portion of the recorded trees **(PAPER II)**, reflects typical farmer preferences in West African cacao landscapes. These species are commonly retained because they provide economic benefits (e.g., kola nuts, oil palm products, fruits, or timber) in addition to shade for cacao. The high occurrence of *C. nitida* in particular suggests its cultural and commercial importance in the region.

Species belonging to the Sterculiaceae family, the same botanical family as cacao, were also identified; however, their low abundance, except for *C. nitida*, indicates limited representation in the agroforestry structure. This pattern may reflect farmers' selective retention of species based on economic value or compatibility with cacao production.

Table 2. **Analysis of selected agroforestry and environmental variables**. Mean (± sd) of selected agroforestry and environmental variables at farms located across five cacao-producing districts in Sierra Leone. Tree data was collected from a 2,500 m^2 quadrant per farm. Different letters indicate a significant ($P < 0, 05$) difference. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, N = number, DBH = diameter at breast height

Variable	Bo	Kailahun	Kenema	Kono	Pujehun	F/Chisq$_{dis}$
N farms	3	14	16	14	3	
Cacao density (N trees per 2,500 m^2)[2]	148 (7.02) b	261 (36.3) a	263 (25.6) a	282 (22.1) a	164 (12.6) b	23.7 ***
Elevation (m)[1,2]	237 (107) b c	242 (76.6) b	167 (52.6) c	389 (81.1) a	72.0 (17.7) d	30.9 ***
N trees (per 2,500 m^2)[1,2]	21.0 (2.65)	16.9 (8.70)	13.6 (4.57)	14.5 (4.20)	19.7 (4.16)	1.48
Shannon diversity (per 2,500 m^2)[2]	1.57 (0.57)	1.70 (0.61)	1.64 (0.53)	1.50 (0.41)	1.04 (0.67)	1.07
Shade tree index (per 2,500 m^2)[1,2]	1.80 (0.44) ab	1.91 (0.36) a	1.81 (0.17) a	1.66 (0.19) ab	1.40 (0.12) b	3.60 *
Tree DBH (m) (per 2,500 m^2)[1,2]	0.90 (0.15) b	1.29 (0.61) ab	1.09 (0.28)b	1.69 (0.50) a	1.22 (0,23) ab	4.76 **
Farm age category (year)[3]	22.0 (20.8) ab	20.0 (13.0) ab	27.3 (11.1) a	16.0 (8.3) b	28.3 (18.5) ab	9.93 *
Farm size category (ha)[3]	2.0 (0)	3.0 (1.5)	4.0 (2.3)	3.0 (2.2)	4.7 (3.1)	6.44

[1] Log-transformed

[2] F-test

[3] Chisq-test

Therefore, appropriate management of shade tree density, species composition, and canopy structure is essential to balance ecological benefits with black pod disease suppression **(PAPER II)**.

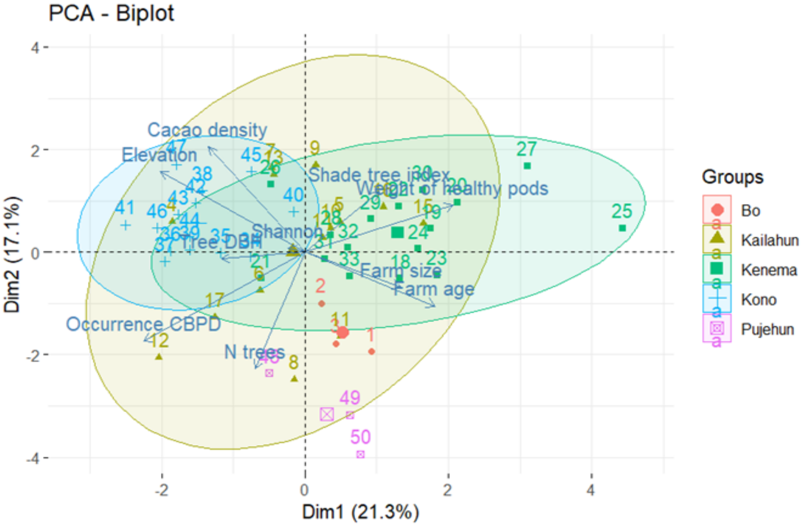

Figure 9. Biplot of occurrence of cocoa CBPD, cacao yield and selected agroforestry and environmental variables at farms located in five cacao-producing districts in Sierra Leone. Tree data was collected from a 2,500 m^2 quadrant per farm. Ellipses indicate grouping of farms within a district. No ellipses are shown for Bo and Pujehun because of the low sample sizes.

The findings from **Paper II** demonstrate that cocoa production districts in Sierra Leone exhibit considerable variation in agroforestry structure and management practices, including differences in shade tree density, species composition, and canopy architecture (Figure 9). Such heterogeneity is a defining characteristic of cacao agroforestry systems and has important implications for disease epidemiology, productivity, and ecosystem functioning. Recent studies increasingly emphasise that agroforestry structures strongly shape microclimatic conditions within cacao farms, thereby influencing both pest and pathogen dynamics (Gidoin et al., 2014; Suárez et al., 2022).

Shade trees play a critical role in regulating the microclimate of cacao plantations by modifying temperature, light availability, and humidity under the canopy (Vásquez et al., 2022). Research on West African cacao systems has shown that different shade tree species produce distinct microclimatic conditions that can significantly influence cacao growth and disease development (Kohl et al., 2024; Olwig et al., 2023). For example, studies conducted in Ghana have demonstrated that shade tree morphology and spatial arrangement alter below-canopy microclimates and influence cacao growth performance and resilience to environmental stressors (Kohl et al., 2024). Similarly, cacao agroforestry systems in Côte d'Ivoire have been shown to improve environmental stability and support sustainable production through the integration of diverse shade tree species (Kouassi et al., 2024).

Differences in shade management and canopy structure may partly explain the observed variation in disease patterns among districts in Sierra Leone (Figures 9, 10). Agroforestry systems with dense or poorly managed shade may create microclimatic conditions favourable for *P. palmivora* and *P. megakarya* development and their spore dispersal (Gidoin, 2013; Oro et al., 2020). Conversely, moderately shaded systems with well-regulated canopy structures can improve airflow and reduce the persistence of free moisture on pods, thereby limiting pathogen infection (Gidoin, 2013; Jiménez-Pérez et al., 2019). Empirical evidence from Ghana indicates that the composition and density of shade trees can significantly influence both mirid infestations and black pod disease incidence, with certain tree species associated with lower levels of disease and pest damage (Asitoakor et al., 2024).

Beyond disease regulation, tree diversity within cacao agroforestry systems also contributes to broader ecological processes that influence farm resilience (Sari et al., 2023). Diverse agroforestry systems can enhance soil fertility, support beneficial organisms, and improve ecosystem services, including pollination and nutrient cycling (Allen et al., 2024; Kristanto et al., 2024). Studies have demonstrated that maintaining tree diversity in cacao farms can improve soil health, enhance long-term productivity, and support biodiversity conservation (Allen et al., 2024; Mbile et al., 2025). Furthermore, cacao-based agroforestry systems have been identified as important climate adaptation strategies because shade trees buffer temperature extremes and reduce climatic stress on cacao (Salamanca et al., 2023; Ariza et al., 2023)

However, the benefits of agroforestry systems depend strongly on management practices. Excessive shade or poorly regulated canopy structures may reduce light penetration and increase relative humidity, thereby favouring Oomycetes (Jiménez-Pérez et al., 2019; Oro et al., 2020). Conversely, strategic pruning and canopy regulation have been shown to improve cacao productivity and flowering by optimizing light availability and canopy ventilation (Esche et al., 2023). These findings emphasize that the effectiveness of agroforestry systems in reducing disease risk depends not only on tree diversity but also on appropriate management of shade tree density and structure.

The heterogeneity observed among cacao production districts in Sierra Leone, therefore, likely reflects differences in farmer management practices, local ecological conditions, and historical land-use patterns. Farms with balanced shade tree diversity and well-regulated canopy structures may provide favourable microclimatic conditions that support cocoa productivity while limiting disease development. In contrast, farms with excessive shade or poorly managed canopy architecture may experience increased disease pressure due to prolonged humidity and reduced airflow around cocoa pods.

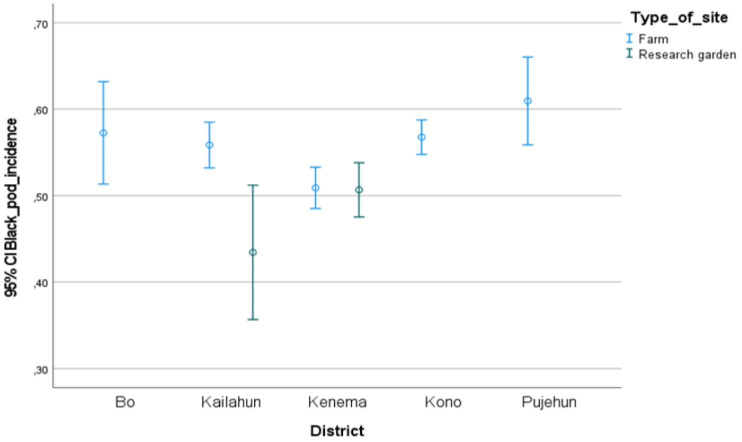

Figure 10: Mean occurrence of CBPD in Sierra Leone. Data recorded across 50 farms and six sites located at two research stations in five districts. Disease incidence was assessed as the proportion of infected pods collected from five cacao trees per quadrat at each farm or site and represents ± 95% confidence interval.

CBPD is caused by several species of the genus *Phytophthora* (including *P. palmivora*, *P. megakarya*, and others), which infect cocoa pods at multiple developmental stages, leading to rapid pod rot, significant yield reductions, and compromised bean quality. Under favourable conditions, the pathogen spreads rapidly within plantations through rain splash, infected plant debris, and soil inoculum (Ndoungué et al., 2017; Nembot et al., 2018), resulting in yield losses commonly ranging from 30–90% in West and Central African systems and, in unmanaged conditions, losses can exceed 60–70% of potential yield without effective management strategies (Kongor et al., 2024).

The epidemiology of black pod disease is tightly linked to environmental and management factors. High rainfall and prolonged wet periods increase zoospore dispersal and infection rates, whereas microclimate within shaded canopies influences disease dynamics in both monocultures and agroforestry systems (Mvondo et al., 2023; Tapia et al., 2025). Recent field studies have shown that environmental variables such as rainfall patterns, topographic position, and within-tree microclimates significantly influence BPD incidence and severity, highlighting the importance of climatic and site-specific assessments in disease modelling and early warning systems (Mvondo et al., 2023; Tapia et al., 2025).

Research advances in cacao host resistance and molecular interactions have identified key transcriptomic responses and small RNA regulators that differentiate resistant and susceptible genotypes during Phytophthora infection, paving the way for enhanced genetic strategies in breeding programs that integrate phenotypic resistance with agronomic traits (Septiani et al., 2024). Also, genetic diversity studies underscore the value of integrating diverse germplasm and conserved defence pathways (e.g., phenylpropanoid biosynthesis) to enhance resilience to black pod pathogens across genetic backgrounds (Catie, 2024).

Management strategies emphasise integrated approaches that combine cultural practices (e.g., sanitation, phytosanitary pod removal, and canopy management), targeted fungicide applications, and biological agents. Recent field evaluations demonstrate that integrating synthetic fungicides, such as copper-based formulations, with rigorous cultural practices significantly reduces disease severity, whereas biofungicides based on *Trichoderma* spp. show promising efficacy in reducing the development of black pod disease under both laboratory and field conditions, offering environmentally friendly alternatives to conventional chemical control (Jibat and Alo, 2023).

In agroforestry and cropping systems, evidence suggests that well-managed cacao agroforestry systems do not inherently increase pest and disease incidence relative to full-sun monocultures; rather, management intensity and microclimatic control are key determinants of disease pressure. This has important implications for sustainable cocoa production in systems that promote diversification and ecosystem services while avoiding disease outbreaks (Armengot et al., 2020).

Collectively, this data along with the literature underscores that black pod disease remains a major constraint to cacao productivity globally, with complex interactions between pathogen biology, host genetics, environmental conditions, and management practices. The findings of **PAPER II**, which show that all cacao trees under investigation exhibited visible BPD symptoms, (Figure 10) align with global reports of the disease's pervasive nature, reinforcing the need for integrated pathogen monitoring and tailored management interventions across Sierra Leone's cacao landscapes.

4.3.3 Biocontrol of CBPD using *Trichoderma atroviride*

Controlling BPD remains a major challenge for smallholder farmers. Traditional management approaches rely largely on cultural practices such as regular harvesting and removal of infected pods, field sanitation, pruning to improve canopy aeration, and the application of chemical fungicides. Yet, these strategies are often constrained by limited farmer resources, high fungicide costs, and inadequate access to extension services. In addition, the frequent use of chemical fungicides raises environmental and health concerns and may contribute to pathogen resistance.

Consequently, there is increasing interest in sustainable disease management approaches that can be integrated into smallholder cacao production systems. Biological control has emerged as a promising alternative, particularly through the use of beneficial microorganisms. Species belonging to the genus Trichoderma have been widely studied for their ability to suppress plant pathogens through multiple mechanisms, including mycoparasitism, competition for nutrients and space, and the production of antifungal metabolites. Furthermore, Trichoderma species can stimulate plant growth and enhance plant defence responses, making them valuable components of integrated disease management strategies. For example, *T. atroviride* was found in **PAPER III** to stimulate healthy and total production of pods in cacao tree canopies compared with trunks.

The use of Trichoderma-based biological control offers a promising and sustainable strategy for managing CBPD, while reducing reliance on synthetic fungicides and supporting environmentally resilient cultivation systems. These multifunctional fungi also promote plant growth and enhance soil health, making them particularly suited to integrated disease management in agroforestry and low-input smallholder systems where chemical control is often impractical or ecologically harmful (Chen et al., 2025).

Recent field and laboratory studies demonstrate the efficacy of Trichoderma spp. against Phytophthora species. Novel bioformulations based on Trichoderma spores and bioactive metabolites have shown promising results in reducing CBPD. Several studies demonstrate that these formulations significantly suppress disease incidence on cocoa pods through mechanisms (Olorunfemi et al., 2023). Research conducted in West Africa, particularly in Côte d'Ivoire, indicates that biological control approaches, such as the use of antagonistic microorganisms, including Trichoderma and other beneficial microbes. These can significantly reduce cacao disease severity while avoiding the environmental and health risks associated with repeated applications of synthetic fungicides (Nguena et al., 2022). Beyond direct pathogen suppression, Trichoderma spp. enhance plant health by stimulating systemic acquired resistance, priming host defence signalling pathways (e.g., salicylic acid and jasmonate/ethylene), and improving root growth and nutrient uptake (Kredics et al., 2024; Singh et al., 2018). These effects not only mitigate disease but also support plant resilience against abiotic stresses, which is crucial for cocoa productivity in variable tropical climates (Gutiérrez-Moreno et al., 2025). Integration of Trichoderma into cacao agroecosystems aligns with the broader principles of sustainable agriculture that prioritize biological control agents for disease suppression, soil health improvement, and reduced chemical inputs (Guzmán-Guzmán et al., 2025). The utility of Trichoderma in agroforestry-based cacao systems is particularly relevant. These systems often feature complex canopy structures and diverse microclimates that influence disease dynamics. Biological control agents, such as Trichoderma, are compatible with shaded, biodiverse settings, in which enhancing beneficial microbial communities is more sustainable than recurrent fungicide use (Gutiérrez-Moreno et al., 2025). When combined with improved cultural practices such as phytosanitation, selective shade management, and soil fertility optimization Trichoderma contributes to an integrated pest management framework that enhances both resilience and yield stability in smallholder cacao landscapes (Neeraja et al., 2025).

Collectively, the evidence underscores that Trichoderma-based biological control is not only effective against black pod disease but also complements agroecological approaches that sustain productivity, biodiversity, and environmental quality. Continued development of optimised bioformulations, selection of strains suited to local environments, and integration with other agroforestry practices will be essential to realising the full potential of Trichoderma in sustainable cocoa production (Chen et al., 2025).

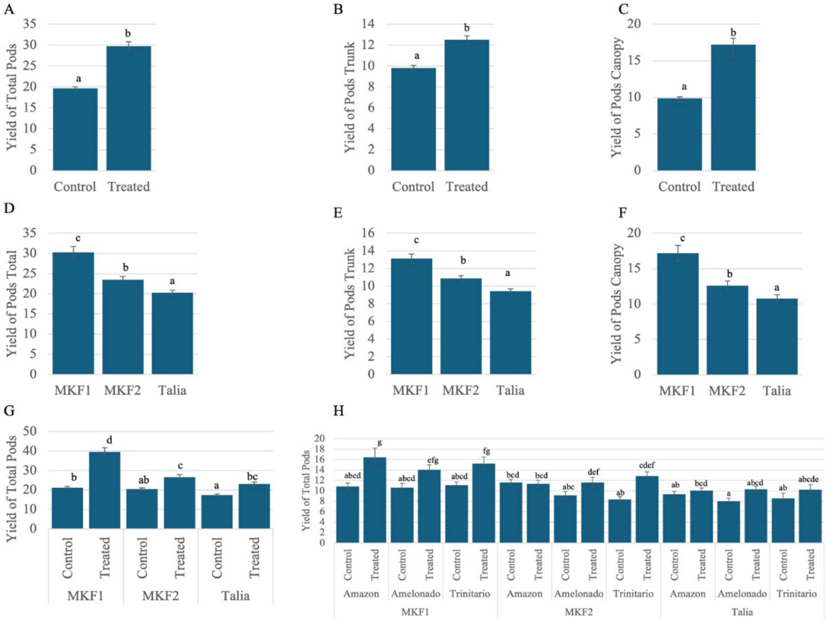

Figure 11. Significant differences in Cacao Pod Yield after treatment with *T. atroviride*. The overall effects of *T. atroviride* treatment on pod yield in total (A) in the treated trunk region (B), or in the canopy region (C). Total yield of pods across sites (D), trunk yield of pods across sites (E), and canopy yield across sites and differences in total yield with treatment across sites (G). (H) The interaction of treatment, site and cultivar. Letters indicate statistical differences in Log2 transformed data (p = 0.05) using Tukey's test. Only significant differences are visualised.

The biocontrol experiment used 300 cacao trees, 150 of which were treated with *T. atroviride* applied to their trunks, with the canopy left untreated. This treatment was administered across three distinct locations, each comprising 100 cacao trees. At each site, 100 trees were chosen at random (50 treated and 50 untreated). *T. atroviride* was applied at the trunk's base to a height of 2.5 m. The canopy sections of the trees were purposefully left untreated. The controlled trees were treated with water. The results of this study revealed insights into the number of pods in the healthy, diseased, and total pod populations of the canopies and trunks of selected trees **(PAPER III,** Figure 11**)**. Descriptive statistics revealed clear differences in pod distribution between the canopy and trunk of cocoa trees. Across all sampled observations, *T. atroviride* treatment had a significant positive effect on all yield parameters, including total pod yield, trunk yield, and canopy yield (Figure 11, A-C). Yield also differed significantly between sites (Figure 11, D-F), and the interaction between site and treatment was significant, with treatment identified as the largest contributor to variation in the dataset (Figure 11, G). While the three-way interaction among site, cultivar, and treatment significantly affected total pod yield, the contribution of cultivar to this effect was minimal (Figure 11, H).

The application of *T. atroviride* significantly reduced CBPD incidence across treated trees, confirming the effectiveness of this biocontrol agent against *Phytophthora* spp. under field conditions. This finding is consistent with recent studies demonstrating that Trichoderma species can suppress *Phytophthora* pathogens through multiple mechanisms, including mycoparasitism, antibiosis, and competition for nutrients and space (Poveda, 2021). The observed reduction in disease incidence in the directly treated trunk region suggests that *T. atroviride* effectively colonized infection sites and inhibited pathogen development locally.

Importantly, the extension of disease reduction to the canopy, which did not receive direct application, indicates a systemic or indirect effect of the treatment. This supports growing evidence that Trichoderma spp. can induce systemic resistance in host plants by activating plant defense pathways, including jasmonic acid and salicylic acid mediated responses (Vinale et al., 2020). The more pronounced disease reduction observed in the canopy compared to the trunk further suggests that induced resistance mechanisms may play a stronger role than direct antagonism in disease suppression at distal plant parts. Similar systemic effects have been reported in cacao and other crops, where Trichoderma applications enhance plant immunity beyond the site of application (Bamisile et al., 2021).

The small but significant variation in disease incidence among sites highlights the influence of environmental and management factors on CBPD dynamics. Differences in microclimatic conditions such as humidity, temperature, and canopy structure are known to affect Phytophthora infection and disease development (Adu-Ampomah et al., 2019). The absence of significant on-site effects in the treated trunk region suggests that direct application of *T. atroviride* may buffer environmental variability by providing consistent local protection. In contrast, the observed site-dependent variation in canopy infection and total disease incidence indicates that local environmental conditions and tree physiology may influence systemic effects.

Furthermore, the significant interaction between site and treatment for total and canopy disease incidence suggests that the efficacy of *T. atroviride* is context-dependent. This aligns with previous findings that the performance of biological control agents can vary across environments due to differences in microbial communities, climate, and host plant conditions (Köhl et al., 2019). Nevertheless, the relatively small magnitude of this interaction indicates that *T. atroviride* remains broadly effective across different field conditions.

Generally, these results demonstrate that *T. atroviride* provides both direct and systemic protection against CBPD, with consistent performance across sites and enhanced disease suppression in untreated plant parts. This highlights its potential as a sustainable and environmentally friendly alternative to chemical fungicides for managing CBPD in smallholder cacao systems

5. Conclusions

This study provides a comprehensive and integrated understanding of cocoa production systems in Sierra Leone by linking genetic diversity, agroforestry structure, and biological control strategies for the management of CBPD, while incorporating comparative germplasm insights from Togo. The use of 20 KASP–SNP markers proved effective for characterizing genetic relationships among cacao accessions and confirmed the robustness of Kompetitive Allele-Specific PCR markers derived from next-generation sequencing platforms for rapid and reliable germplasm identification. However, the molecular analysis revealed substantial mislabeling among introduced cacao materials, likely arising from historical errors during germplasm exchange, incorrect labeling, and loss of field identification tags. Such inconsistencies pose significant risks to breeding programs, germplasm conservation, and the accurate selection of parental lines. These findings underscore the critical need for rigorous germplasm verification, the adoption of standardized nomenclature aligned with the International Cacao Germplasm Database, and adherence to naming conventions from recognized repositories such as the International Cocoa Quarantine Centre, Reading, to ensure consistency in germplasm management across West Africa.

The study further confirms that CBPD remains a major constraint to cocoa productivity in Sierra Leone, particularly under humid tropical conditions favorable for pathogen development. Agroforestry structure emerged as a key determinant of disease dynamics. Farms with higher diversity of companion tree species consistently exhibited lower disease incidence, suggesting that diversified systems can create less conducive microclimatic conditions for the development of Phytophthora species. In contrast, farms dominated by large, mature shade trees with high DBH were associated with increased disease incidence, likely due to dense canopies that maintain high humidity and reduced airflow. These findings highlight the importance of optimizing shade management and promoting species diversity to balance ecological benefits with disease suppression in cacao agroforestry systems.

In addition to agroforestry practices, this research demonstrates the strong potential of biological control using *T. atroviride* as part of an integrated CBPD management strategy. Commercial formulations of *T. atroviride* significantly reduced disease incidence and improved pod health and yield under field conditions. Notably, the consistency of results across three independent farms, despite being conducted within a single growing season, supports the reliability and practical applicability of this approach. The combined use of *T. atroviride* and improved field sanitation further enhanced disease control, emphasizing the value of integrated management practices. Evidence of improved pod health in untreated canopy tissues suggests the activation of systemic or induced resistance mechanisms, consistent with the known ability of Trichoderma species.

Importantly, the application of *T. atroviride* did not influence the proportional representation of cacao cultivars (Amazon, Amelonado, and Trinitario) across study sites, indicating that the treatment effect was independent of cultivar distribution. This confirms that treatment efficacy should be evaluated based on plant health and productivity indicators such as the number of healthy pods, diseased pods, and total yield rather than changes in cultivar frequency. While inherent genetic differences among cultivars may influence susceptibility to CBPD, the broad-spectrum activity of Trichoderma suggests its suitability across diverse genetic backgrounds. Consequently, pod performance metrics remain the most reliable indicators for assessing the success of biological control interventions in cacao systems.

6. Future perspectives

The findings of this study highlight the importance of integrating accurate genetic resource management, well-designed agroforestry systems, and effective biological control strategies to enhance cocoa productivity and resilience against CBPD. Further investigation to strengthen germplasm identification will improve breeding efficiency, while promoting diversified agroforestry and scalable biocontrol solutions will support sustainable disease management for smallholder farmers.

6.1 Characterisation of cacao germplasm

Future research should involve high-density genotyping approaches, such as genome-wide SNP arrays or whole-genome resequencing, to provide a more comprehensive understanding of genetic diversity and population structure within cacao germplasm collections. While the use of 20 KASP–SNP markers in this study successfully detected genetic relationships and mislabeling among accessions, higher-resolution molecular tools would allow more accurate identification of duplicates, introgression patterns, and potential resistance traits.

6.2 Multi-location phenotyping of cacao cultivars

Further studies should conduct multi-environment field trials to evaluate cacao cultivars under diverse agroecological conditions. Phenotyping across multiple locations would help determine genotype × environment interactions, identify cultivars with improved resistance to CBPD, and assess yield stability across production regions. Long-term trials would also enable a more rigorous evaluation of how different cacao genotypes perform under varying climatic and management conditions.

6.3 Integration of disease management and agroforestry

Additional research is needed to investigate the optimal composition and spatial arrangement of shade trees in cacao agroforestry systems. While this study suggests that higher tree-species diversity may reduce CBPD incidence, more detailed studies should examine how canopy structure, shade density, and species composition influence microclimatic conditions such as humidity, temperature, and airflow within cacao plantations. Understanding these interactions will inform the design of agroforestry systems that balance biodiversity conservation, climate resilience, and disease suppression

6.4 Long-term evaluation of biocontrol strategies

Although the application of *T. atroviride* combined with sanitation practices showed promising results in reducing diseased pods and improving pod health, further studies should evaluate the long-term effectiveness and consistency of biological control under farmer-managed conditions**.** Future research should explore optimal application methods, treatment frequency, compatibility with other integrated pest and disease management strategies, and the persistence of Trichoderma populations in field environments.

6.5 Interactions between host genetics and biocontrol

Another important research direction is to investigate potential interactions between cacao genotypes and biological control agents**.** Some cacao cultivars may respond differently to microbial inoculants due to variations in plant defence mechanisms or in interactions with the root microbiome. Studying these relationships could help identify cultivar–biocontrol combinations that maximize disease resistance and productivity.

6.6 Socioeconomics and adoption among farmers

Finally, future research should include socioeconomic assessments of farmer adoption of improved agroforestry management practices and biological control technologies**.** Understanding farmer perceptions, economic feasibility, and practical constraints will be critical for translating research findings into scalable disease management strategies for smallholder cocoa producers.

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Popular science summary

Improving cocoa production in Sierra Leone from better planting materials to sustainable disease control

Cocoa is the crop behind chocolate, cosmetic and other pharmaceutical products but for millions of smallholder farmers, it is also important source of income for many farmers in Sierra Leone, West Africa. Yet production is still low because of pests and diseases, especially cocoa black pod disease. Species of microbes from the Phytophthora genus cause this disease, which costs farmers an enormous amount of money every year. At the same time, many farmers rely on planting materials of their farms of uncertain origin and manage their farms under diverse agroforestry systems without clear guidance on how these systems influence disease. This research investigates these challenges by integrating three fundamental areas: cocoa genotyping, agroforestry practices, and biological control.

One important finding of this research is that many cocoa varieties grown by farmers are wrongly identified and mislabelled. Trees with the same name were found to be genetically different, while in some cases genetically identical trees were given different names. This suggests extensive mislabelling and uncertainty of all planting materials. As a result, farmers may innocently cultivate cocoa trees that are low-yielding or extremely susceptible to disease. In identifying the true genetic identity of cocoa trees, this research provides a basis for selecting and distributing improved planting materials. This means that for farmer's access to verify and well-characterized cocoa varieties can give them reliable yield and improved resistance to black pod disease.

The research also shows that cocoa black pod disease is widespread through Sierra Leone. On average, more than half of the cocoa pods on farms were infected with black pod disease, confirming the scale of the problem. However, the study highlights that farm management practices play a key role in influencing black pod disease levels. In particular, farms with a high number of trees with larger tree sizes tended to have higher levels of black pod disease. This is likely because dense shade and deprived airflow create humid conditions favouring the spread of the pathogen. Simultaneously, the study found that farms with a larger diversity of tree species experienced lower disease incidence. This suggests that biodiversity within cocoa farms help lessen disease pressure, possibly by having a positive effect on the ecosystem.

In addition to disease, farm arrangement also affects yield. The study found that high cocoa tree density reduces productivity, while lower density increases yield. This means that overcrowded farms limit tree performance due to competition for light, nutrients, and water. Together, these findings indicate that effective agroforestry management is not simply about increasing or reducing shade, but about achieving the right balance. Farmers need to maintain moderate shade, avoid overcrowding, and promote a diversity of tree species to create a healthier and more productive system.

A key contribution of this research is the demonstration of biological control as a practical solution for farmers. The study evaluated the use of the beneficial fungus *Trichoderma atroviride* to control black pod disease under field conditions. The results showed that treating cocoa trees with *Trichoderma atroviride* significantly reduced disease pods and increased pod yield. Importantly, the effect was not limited to the area where the treatment was applied. The fungus similarly indicating a systemic effect by providing protection in other parts of the tree. Making it more promising as well as a practical tool for farmers.

The use of biological control offers numerous advantages specifically for farmers. It reduces need on chemical fungicides, which tend to lower production costs in the long term, and is environmentally friendly. For small-scale farmers, this method is valuable because it can be integrated into the present farming practices without expensive inputs.

This research provides a comprehensive strategy for improving cocoa production for smallholder farmers in Sierra Leone. It shows that in order to increase productivity and reducing disease, it require a combination of activities: using the right planting materials, managing farm structure effectively, and using sustainable disease control methods. These findings are directly applicable to farmers, extension services, and policymakers working to support the cocoa sector.

In applying these results, farmers can improve yields, cut down losses caused by black pod disease, and increase their income. At a broader level, this research contributes to more sustainable cocoa production systems supporting both livelihoods and environmental health of the farmers.

Populärvetenskaplig sammanfattning

Kakao är grödan bakom choklad, kosmetika och många läkemedelsprodukter. För miljontals småbrukare i Sierra Leone i Västafrika är kakao också en viktig inkomstkälla. Trots detta är produktionen av kakao låg, vilket beror på förekomsten av skadedjur och sjukdomar, särskilt kakao-black pod-röta. Denna sjukdom orsakas av arter av släktet *Phytophthora*, och kostar lantbrukarna enorma summor pengar varje år. De olika sorter av kakao som lantbrukare har att tillgå för sina odlingar är av osäkert ursprung. De har inte heller någon tydlig guidning i hur kakao bäst bör odlas inom skogsjordbruk så att sjukdomen hålls borta. Denna forskning undersöker dessa utmaningar genom att integrera tre grundläggande områden: kakaogenotypning, odlingsmetoder inom skogsjordbruk och biologisk bekämpning.

Ett viktigt resultat av denna forskning är att många av de kakaosorter som odlas av lantbrukare är felaktigt identifierade och felmärkta. Träd med samma namn visade sig vara genetiskt olika, medan genetiskt identiska träd i vissa fall fick olika sortnamn. Detta tyder på omfattande felmärkning och osäkerhet kring allt planteringsmaterial. Som ett resultat kan lantbrukare av misstag odla kakaoträd som ger låg avkastning eller är extremt mottagliga för sjukdomar. Genom att identifiera den sanna underliggande genetiken hos kakaoträd ger denna forskning en grund för att välja och distribuera förbättrade planteringsmaterial. Detta innebär att lantbrukare kan få tillgång till verifierade och välkarakteriserade kakaosorter som kan ge dem tillförlitlig avkastning och förbättrad resistens mot black pod-röta.

Forskningen visar också att kakao-black pod-rötan är utbredd i Sierra Leone. I genomsnitt var mer än hälften av alla undersökta kakaobaljor infekterade i en studie utförd hos olika småbrukare, vilket bekräftar problemets omfattning. Studien belyser dock att skötselmetoder av odlingen spelar en nyckelroll för förekomst av sjukdomen. I synnerhet gårdar med ett stort antal träd och större träd tenderade att uppvisa mer sjukdom. Detta beror troligen på att tät skugga och bristande luftflöde skapar fuktiga förhållanden som gynnar spridningen av patogenen. Samtidigt fann studien att gårdar med en större mångfald av trädarter hade lägre sjukdomsgrad. Detta tyder på att biologisk mångfald inom kakaoodlingar bidrar till att minska sjukdomstrycket, möjligen genom en positiv påverkan på ekosystemet. Odlingsmetoder påverkade också avkastningen. Studien fann att hög täthet av kakaoträd minskar avkastningen jämfört med lägre densitet. Detta innebär att täta planteringar begränsar trädens produktivitet, tex på grund av konkurrens om ljus, näringsämnen och vatten. Sammantaget tyder dessa resultat på att effektivt skogsjordbruk inte bara handlar om att öka eller minska skugga, utan om att uppnå rätt balans. Jordbrukare behöver sörja för odlingar med optimal skugga utan att plantera för tätt och främja en mångfald av trädarter för att skapa ett hälsosammare och mer produktivt system.

Ett viktigt bidrag från denna forskning är demonstrationen av biologisk bekämpning som en praktisk lösning för lantbrukare. En studie utvärderade användningen av nyttosvampen *Trichoderma atroviride* för att bekämpa black pod-röta under fältförhållanden. Resultaten visade att behandling av kakaoträd med nyttosvampen signifikant minskade sjukdomsförekomsten och ökade avkastningen. En viktig upptäckt var att effekten inte var begränsad till det område där behandlingen applicerades. Detta betyder att svampen kan ge skydd i andra delar av trädet, vilket gör den mer lovande som ett praktiskt verktyg för lantbrukare.

Användningen av biologisk bekämpning erbjuder många fördelar, särskilt för lantbrukare. Det minskar behovet av kemiska svampmedel, vilket tenderar att sänka produktionskostnaderna på lång sikt, och är miljövänligt. För småbrukare är denna metod värdefull eftersom den kan integreras i nuvarande odlingsmetoder utan dyra insatser.

Denna forskning erbjuder en övergripande strategi för att förbättra kakaoproduktionen för småbrukare i Sierra Leone. För att öka produktiviteten och minska sjukdomar kan en kombination av åtgärder tillämpas: att använda lämpliga kakaosorter, ett välanpassat skogsjordbruksystem och att använda hållbara metoder för sjukdomsbekämpning. Dessa resultat är direkt tillämpliga för småbrukare, rådgivare och beslutsfattare som arbetar inom kakaosektorn.

Genom att tillämpa dessa resultat skulle småbrukare kunna förbättra avkastningen, minska förluster orsakade av black pod-röta och därmed öka sina inkomster. Utifrån ett bredare perspektiv bidrar denna forskning till mer hållbara kakaoproduktionssystem som gynnar både lantbrukarna ekonomiskt och miljömässigt.

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Genetic Diversity and Population Structure of Cacao (*Theobroma cacao* L.) Germplasm from Sierra Leone and Togo Based on KASP–SNP Genotyping

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Abstract: Cacao (*Theobroma cacao* L.) is a tropical tree species belonging to the Malvaceae, which originated in the lowland rainforests of the Amazon. It is a major agricultural commodity, which contributes towards the Gross Domestic Product of West African countries, where it accounts for about 70% of the world's production. Understanding the genetic diversity of genetic resources in a country, especially for an introduced crop such as cacao, is crucial to their management and effective utilization. However, very little is known about the genetic structure of the cacao germplasm from Sierra Leone and Togo based on molecular information. We assembled cacao germplasm accessions (235 from Sierra Leone and 141 from Togo) from different seed gardens and farmers' fields across the cacao-producing states/regions of these countries for genetic diversity and population structure studies based on single nucleotide polymorphism (SNP) markers using 20 highly informative and reproducible KASP–SNPs markers. Genetic diversity among these accessions was assessed with three complementary clustering methods, including model-based population structure, discriminant analysis of principal components (DAPC), and phylogenetic trees. STRUCTURE and DAPC exhibited some consistency in the allocation of accessions into subpopulations or groups, although some discrepancies in their groupings were noted. Hierarchical clustering analysis grouped all the individuals into two major groups, as well as several sub-clusters. We also conducted a network analysis to elucidate genetic relationships among cacao accessions from Sierra Leone and Togo. Analysis of molecular variance (AMOVA) revealed high genetic diversity (86%) within accessions. A high rate of mislabeling/duplicate genotype names was revealed in both countries, which may be attributed to errors from the sources of introduction, labeling errors, and lost labels. This preliminary study demonstrates the use of KASP–SNPs for fingerprinting that can help identify duplicate/mislabeled accessions and provide strong evidence for improving accuracy and efficiency in cacao germplasm management as well as the distribution of correct materials to farmers.

Keywords: cacao; STRUCTURE; DAPC; hierarchical clustering; genetic diversity

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1. Introduction

Cacao, *Theobroma cacao* L., is a tropical tree native to the humid tropics of the central and northern parts of South America [1]. It is a diploid (2n = 2x = 20) vegetatively propagated species domesticated approximately 3000 years ago [2,3]. It is the major ingredient used in the multi-billion-dollar chocolate and confectionary industry, as well as for other intermediary products such as cacao butter, cacao powder, cacao cake, and cacao liquor. The coastal countries in West Africa, known as the West Africa cacao belt [4], from Sierra Leone, Guinea, and Liberia to southern Cameroon, apart from the Benin Republic, are responsible for the production of about 70% of the world's cacao (http://faostat.fao.org) (accessed on 23 September 2023). The world's chocolate and confectionary industry is heavily dependent on cacao beans from West African countries, both due to the high production and high quality of beans (bulk cacao, may not be specialty cacao) compared to those produced in other cacao-producing regions such as Asia or Central and Southern America [5]. In 2020, cacao was the primary agricultural export commodity for Cote d'Ivoire, Ghana, Nigeria, Cameroon, and Sierra Leone, and the second most important for Guinea, Togo, and Liberia, thus contributing significantly towards the Gross Domestic Products of these countries (www.statista.com) (accessed on 23 September 2023).

It is estimated that Brazilian cacao (of the Amelonado type, which is also known as the Lower Amazon Forastero type) was first introduced into West Africa in the 19th and early 20th centuries by the Portuguese [6], and since then, it has been cultivated by smallholder farmers in this region. Cote d'Ivoire and Ghana remain the highest producers of cacao in the world, accounting for over 60% of global world production of around 4.9 million tons in the 2021/2022 cacao season [7,8]. Among cacao-producing countries in the world, Togo and Sierra Leone rank 15th and 17th with a production of 22,522 and 14,670 metric tons, respectively [9]. In 2017, the World Bank Trade Statistics recorded export earnings of about USD 14,461 million from cacao beans in Sierra Leone [10], even with low productivity. Over the years, cacao production has increased in both Sierra Leone and Togo, which corresponds to an increase in the area under cultivation. However, future yields are expected to be adversely affected by changing climatic conditions. Similar to other West African countries, cacao cultivation in Sierra Leone and Togo faces the challenges of old trees, aged farmers, black pod disease, mirids, poor access to improved planting materials, and other challenges (such as cacao swollen shoot virus) related to its cultivation and management [11,12].

The cacao germplasm introduction in Sierra Leone and Togo followed the same trend as introductions in other West African countries, which is from a common source—Fernando Po [13]. However, in Sierra Leone, there may also have been introductions from other sources (the West Indies), raising questions as to the contributions of various sources [14]. There is, therefore, an interesting possibility that present-day cacao in West Africa (apart from recent introductions through the University of Reading) and germplasm exchanges between West African countries such as introductions from Ghana to both Sierra Leone and Togo at experimental stations of national institutes is of dual origin. In Togo, cacao germplasm is conserved in a gene bank consisting of clones introduced from countries in the sub-region (Ghana, Côte d'Ivoire, Cameroon, and Nigeria) and international collections (University of Reading, United Kingdom). In both of these countries, historical phenotypic data or any other type of data were unavailable to understand the nature of these genotypes (personal communications). It is, therefore, crucial to understand and assess genetic relationships and genetic diversity among cacao germplasms within and between these two countries to effectively conserve and efficiently use them for further breeding and crop improvement. Several efforts have been put forward to understand and assess the genetic diversity currently available in major cacao-growing countries in the world, including several West African countries, using both molecular markers and morphological traits [15–19]. However, there are no studies to date that have targeted germplasms collected from different cacao-growing regions in Sierra Leone and Togo to assess genetic diversity and understand their population structure.

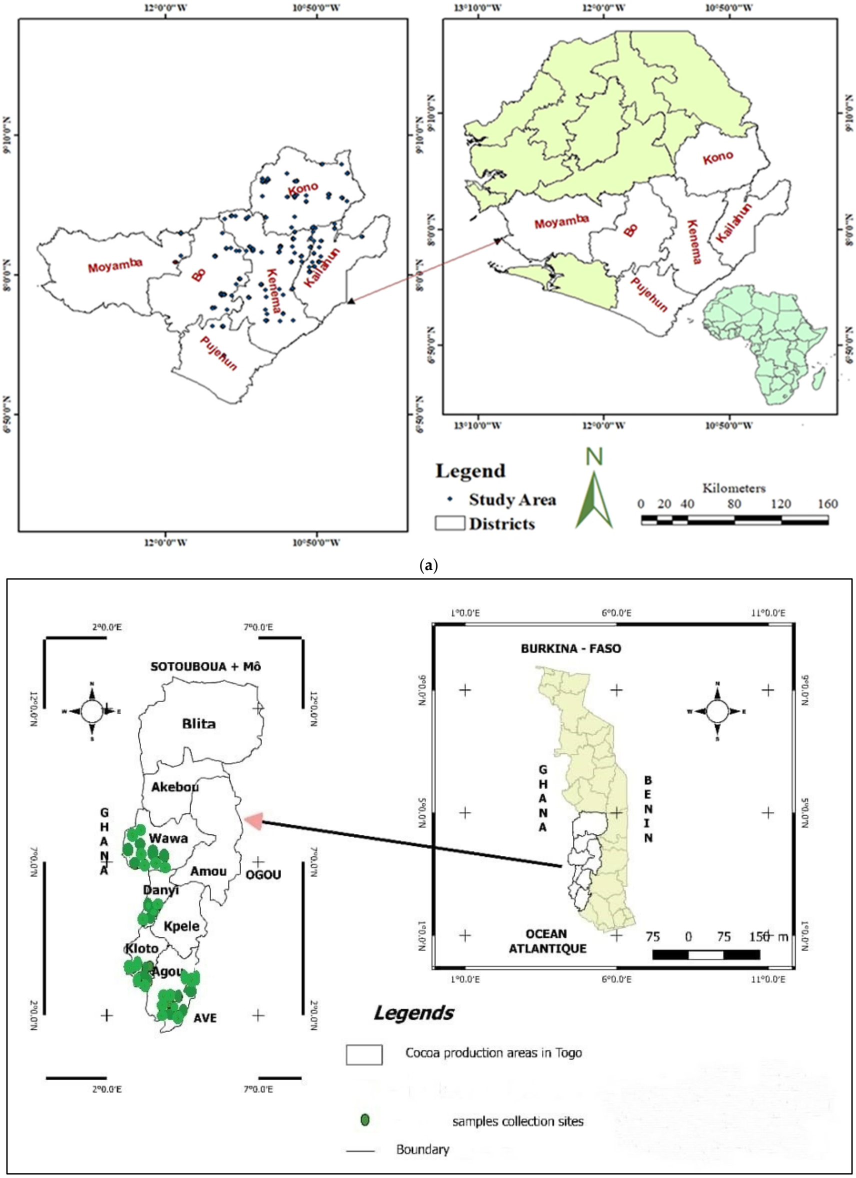
There is a general agreement that cultivated cacao in West Africa has a narrow genetic base and faces issues of mislabeling [15–18]. A 2019 review that compared both modern and historical introductions did not detect significant genetic diversity or improvements in yield or pest and disease resistance during the last 20 years [20]. Any improvements in yield resulted from better management practices, although some of the recent varieties or hybrids developed in major West African countries produced significantly higher yields with better pest and disease resistance under intensive cultivation. Similar improvements in yield as well as other traits are needed in other countries so that there will be enough supply for the world's increasing demand for dry cacao beans. Therefore, it is critical that cacao germplasms available in other West African cacao-producing countries be characterized, documented, identified, conserved, and utilized. This will allow searches for promising unique genetic materials that can be used to develop strong, local breeding programs in areas that currently depend on Cote d'Ivoire, Ghana, or Nigeria for improved varieties or planting materials.

Assessments of genetic diversity and identification of mislabeled accessions in germplasm collections have been conducted by using single nucleotide polymorphism (SNP) markers in several crops, a fast, high-throughput, and affordable tool for whole-genome genetic diversity analysis. SNPs have been successfully used to characterize crops such as maize [21] and soybean [22]. Similarly, SNP markers have been used for fingerprinting cacao germplasm collections in several studies [23–27]. In West Africa, Takrama et al. [28] used 54 SNPs to fingerprint 160 cacao trees from the germplasm collection at the Cocoa Research Institute of Ghana (CRIG) for accurate identification of individual genotypes. However, high-throughput sequence-based KASP (kompetitive allele-specific PCR)-SNPs have been used in only two studies for cacao germplasm from West Africa [17,18]. KASP–SNP is an effective method compared to traditional SNP genotyping using electrophoresis systems because of a low genotyping error rate, cost-effectiveness, and flexibility to automation [29]. In this present study, a subset of 20 KASP–SNPs from a set of 100 KASP–SNPS used in genotyping cacao genotypes from Nigeria and Ghana was used for genotypic characterization of cacao from Sierra Leone and Togo. This is the first study to use a subset of 20 selected KASP–SNPs to understand the extent of genetic diversity and population structure within and among cacao germplasms.

2. Materials and Methods

2.1. Cacao Sampling

In Sierra Leone and Togo, we sampled 235 and 141 cacao accessions, respectively, from different cacao growing regions (Table S1a,b). In Sierra Leone, leaf samples were collected from cacao farms operating across the three major cacao-producing districts: Kenema (N: 8°02.805′, W: 11°02.032′), Kailahun (N: 8°21.570′, W: 10°23.477′), and Kono (N: 8°36.196′, W: 10°56.458′). In addition, leaf samples were collected from minor cacao-producing districts such as Bo (N: 8°10.653′, W: 11°41.720′) and Pujehun (N: 7°08.684′, W: 11°22.652′). Leaf samples were also collected from trees in the Njala University research garden in the Moyamba district (N: 8°06.493′, W: 12°04.950′) (Figure 1a). Similarly, in Togo, leaf samples were collected from 15 cacao plantations located in the areas of Agou (N: 6°49′18″, E: 0°52′04″), Kloto (N: 7°04′, E: 0°44′), Danyi (N: 6°49′40.2″, E: 0°43′07″), and Litimé (N: 8°06′; E: 1°00′) (Figure 1b). Individual trees were tagged and geo-referenced from which fresh young cacao leaf samples were collected and dried using silica gel (note: the individual tagged trees are maintained in the seed gardens or farmers' farms; no trees were destroyed during this study). The dried leaf samples were then shipped to the Bioscience Center of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, where six leaf discs of approximately 5 mm in diameter from dried leaf samples of each genotype were punched into labeled 8-strip 1.1 mL propylene tubes with strip caps, with up to 12 strips placed in 96-well boxes (Thistle Scientific, Warwickshire, UK). A total of four labeled 96-well boxes were then shipped to a genotyping service provider (Intertek, Kista, Sweden) for automated DNA extraction and genotyping with 20 SNP markers using the KASP assay. Two blank controls were included in each box during genotyping.



(**a**)

(**b**)

Figure 1. (**a**). Map of Sierra Leone showing the districts and locations where leaf samples were collected (note: map generated using ArcMap 10.8.2 (Esri ArcGIS Desktop) and GPS information collected during sampling). (**b**). Map of Togo showing the districts and locations where leaf samples were collected (note: map generated using QGIS 3.32.3 and the GPS information collected during sampling).

2.2. DNA Extraction, Preparation and Genotyping

DNA quality and quantity were checked on a 0.8% agarose gel. We used 20 high-quality SNPs to genotype the cacao leaf samples. These 20 SNPs were chosen from a set of 1536 SNPs identified from previous studies that used expressed sequence tags (ESTs) of a wide range of cacao tissue and organs displaying transcriptomic differences [30,31]. A subset of 100 SNPs was then selected from the initial set based on call rate, representativeness across the ten chromosomes and heterozygosity, and their use by cacao researchers in past studies [25,32,33]. The selected 100 SNPs were then converted using KASP™ assays at LGC Genomics (http://www.biosearchtech.com/support/education/kasp-genotyping-reagents) (accessed on 20 August 2022) for earlier studies on cacao genetic diversity in West Africa [17,18]. For this present study, 20 highly polymorphic, high-quality KASP–SNPs (Table S2) were carefully selected from the original 100, while genotyping was carried out at Intertek, Sweden. We performed simulations on 100 KASP–SNPs to select these 20, which are routinely used in cacao breeding for low-density genotyping at Intertek. The KASP assay protocol followed the KASP manual [34] in which genotyping was carried out with high-throughput PCR SNPline workflow by using 1 µL reaction volume in 384-well PCR plates. The KASP genotyping reaction mix consisted of three components, including sample DNA (10 ng), marker assay mix comprising target-specific primers, and KASP-TF™ master mix containing two universal fluorescence resonant energy transfer cassettes (FAM and HEX), passive reference dye (ROX™), Taq polymerase, free nucleotides, and $MgCl_2$ in an optimized buffer solution. The SNP assay mix is specific to each marker and consists of kompetitive allele-specific forward and reverse primer. After PCR, the plates were fluorescently read, and allele calls were made by using KRAKEN™ software (LGC Biosearch Technologies, Hoddesdon, UK) and scored on a Cartesian plot, (cluster plot), assigning each DNA sample to a class: homozygous for either allele 1 or 2 or heterozygous in the case of biallelic SNPs.

2.3. Data Analyses

For data analyses, all cacao genotypes across both target countries were combined with an assumption that genotypes with the same name may be genetically different and genotypes with different names may be genetically similar. Genetic diversity analyses based on minor allele frequency (MAF), polymorphism information content (PIC), expected heterozygosity (He), and observed heterozygosity (Ho) parameters were conducted with vcftools and plink 1.9 [35]. A genetic distance matrix-based identity by state (IBS) generated with plink 1.9 [35] served as the basis for hierarchical clustering. An unrooted phylogenetic tree was constructed to visualize how closely accessions were related within each country by using the ape (analyses of phylogenetics and evolution) library package [36] and phangorn, an R package [37]. The dissimilarity matrix was then used to construct network relationships among cacao accessions from both Sierra Leone and Togo with QGRAPH [38] implemented in R. In addition, as a complementary analysis, Discriminant Analysis of Principal Component (DAPC) was carried out using 'genind object' and the find.clusters function in the adegenet package [39]. In order to properly assign the accessions to groups, the Bayesian information criterion (BIC) was used to assign accessions to groups and determine the optimal number of clusters to be retained. A binary file was generated from the filtered VCF file and was then subjected to cross-validation for population structure analysis [35]. A cut-off value of 50% ancestry suggested through the Admixture analysis was used to estimate membership probabilities of all accessions for the groups identified [36]. The model-based clustering approach implemented in ADMIXTURE assumes linkage equilibrium among loci and Hardy–Weinberg equilibrium within ancestral populations [40]. However, such assumptions may not apply in clonally propagated tree species like cacao due to the presence of clonal duplicates in germplasm collections. To validate the clustering pattern obtained from ADMIXTURE and hierarchical clustering algorithms, DAPC, an assumption-free multivariate clustering method, was used. Genetic differentiation among and within groups (individual country level and combined) was estimated via an analysis of molecular variance (AMOVA), and its significance was tested with a non-parametric approach with 999 permutations by using GenAlex v. 6.503 [41]. Coefficients of genetic differentiation among populations (Sierra Leone, Togo, and combined) were calculated based on pairwise F_{ST} (fixation index) to estimate genetic distances and relationships among populations used in this study.

3. Results

3.1. Cacao Accessions from Sierra Leone and Togo

The cacao accessions collected from both Sierra Leone and Togo represented several clones with the same name (Table S3) even when they were collected from different trees either at the same farm or from different farms. The GPS coordinates as well as the village/farm names and district names for each genotype are provided in Tables S1a and S1b. Most of the cacao genotypes from Sierra Leone with similar names were collected from trees located at different farms, indicating that these trees either belong to the same clone or there are issues of mislabeling. The situation is the same in Togo, although genotypes with the same names were present at the same farm as well as at different farms.

The 235 cacao genotypes from Sierra Leone represented 144 accessions with unique names. These 144 accessions were sourced from Ghana (66 accessions) or the University of Reading (77 accessions), while the source of one genotype was unknown (Table S3). Similarly, the genotypes from Togo represented 77 accessions with unique names. These seventy-seven accessions were sourced from Cameroon (four accessions), Cote d'Ivoire (eleven accessions), Ghana (seventeen accessions), Nigeria (twenty-eight accessions), and Togo (nine accessions), with a few clones representing both Cameroon and Ghana (ICS 6 × NA33; ICS16), Cote d'Ivoire and Cameroon (IFC1 × SNK13), Ghana and Cote d'Ivoire (NA2), or Ghana and Nigeria (SCA 12 × NA32; C23; C26 × SCA 6), and five genotypes whose accession names were lost (Table S3). For the accessions with lost names, it is assumed that these may represent PA7/A19 clones (Table S3). There are eleven accessions/accession names common between both Sierra Leone and Togo, of which PA7, ICS60, IMC47, Na34, and Pa35 were sourced from the University of Reading and Nigeria in Sierra Leone and Togo, respectively. C20 and C70 listed Ghana as the source country for both Sierra Leone and Togo; C26 and C42 listed Ghana and Nigeria as the source country for Sierra Leone and Togo, respectively; C23 listed Ghana for Sierra Leone and Nigeria/Ghana for Togo as the source country; Pound 7 listed the Gene Bank of SLARI and Ghana as the source for Sierra Leone and Togo, respectively; and PA7 listed both the Gene Bank of SLARI and Nigeria as the source (Table S3).

3.2. Genetic Diversity Parameters

The 20 KASP–SNP markers used herein were distributed across the ten chromosomes of cacao, with two SNPs on each chromosome (Table S2). The average PIC (polymorphic information content), He (expected heterozygosity), Ho (observed heterozygosity), and MAF (minor allele frequency) values for 235 cacao accessions collected in Sierra Leone were 0.22, 0.30, 0.24, and 0.21, respectively, while for 141 accessions collected in Togo, these values were 0.21, 0.29, 0.22, and 0.19, respectively. For the combined population of 376 cacao accessions, the average PIC, He, Ho, and MAF values were 0.24, 0.30, 0.26, and 0.23, respectively (Table 1). Table S4 and Figure S1 present summary statistics and the distribution of He, Ho, MAF, and PIC values for the combined population, respectively. Of the 20 SNPs used in this study, many had a minor allele frequency (MAF) above 0.2 and showed high PIC values with a peak distribution above 0.2. Similarly, low observed and expected heterozygosity was recorded. The observed heterozygosity for the combined population was higher than individual country-level observed heterozygosity, which was also represented by higher PIC values (Table 1).

Table 1. Descriptive statistics based on 20 KASP–SNP markers across cacao accessions.

Cacao Population	N	He	Ho	MAF	PIC
Sierra Leone	235	0.30	0.24	0.21	0.22
Togo	141	0.29	0.22	0.19	0.21
Combined	376	0.30	0.26	0.23	0.24

N: number of accessions.

3.3. Population Structure and Genetic Relationships

The model-based population structure analysis of the combined cacao population (235 accessions from Sierra Leone and 141 from Togo) showed that the delta K values from the mean log-likelihood probabilities stagnated at K = 4 (Figure 2a), although the optimal number of clusters obtained initially was K = 2 (Figure S2). The 376 cacao accessions were divided into four subpopulations at K = 4 (Figure 1b). Based on an 80% membership probability threshold, 270 accessions (73.37%) were successfully assigned to the four sub-populations. In comparison, 106 accessions with a probability of <80% were designated as an admixed population (Table S3). Sub-population 1 consisted of 123 accessions (Sierra Leone: 94 accessions; and Togo: 29 accessions). Sub-populations 2, 3, and 4 constituted 3.72%, 22.07%, and 13.30% of the accessions, respectively, with ten accessions from Sierra Leone and four accessions from Togo in sub-population 2, thirty-six accessions from Sierra Leone and forty-seven accessions from Togo in sub-population 3, and twenty-six accessions from Sierra Leone and twenty-four accessions from Togo in sub-population 4 (Table S3, Figure 1b). The admixed group consisted of 69 accessions from Sierra Leone and 37 accessions from Togo. Few additional smaller peaks observed (Figure 2b) implied the presence of subgroups within the four major subpopulations. Therefore, individual STRUCTURE analysis was performed for accessions representing Sierra Leone and Togo. Sub-clustering of cacao germplasms from Sierra Leone and Togo showed that delta K values stagnated at K = 3 and K = 4, respectively (Figure 2a,b). A higher degree of admixture was observed in the cacao germplasm from Togo than in the cacao germplasm from Sierra Leone. The 235 cacao accessions from Sierra Leone were divided into three subpopulations with 102 accessions in subpopulation 1, 29 in subpopulation 2, and 93 in subpopulation 3; whereas, only 11 accessions (4.7%) were in an admixed group (Table S3; Figure 3a). In contrast, one hundred and forty-one cacao accessions from Togo were grouped into four subpopulations, with four accessions in subpopulation 1, thirty-six in subpopulation 2, fifty in subpopulation 3, and twenty-five in subpopulation 4. The admixed group consisted of 26 accessions, representing 18.4% admixture among the accessions (Table S3; Figure 3b).

Using the Bayesian information criterion (BIC) implemented in DAPC, a maximum of K = 4 was obtained, which corresponded to the four groups obtained for the combined population (Figure 4) and for the germplasm collection in Togo (Table S3). Designation of cluster membership showed that cluster 3 included the largest number of accessions (158), followed by cluster 2 with 116 accessions and cluster 1 with 86 accessions, with cluster 4 having the fewest accessions (16). Of the 158 accessions in cluster 3, 105 accessions (66.5%) were from Sierra Leone and 53 (33.5%) from Togo (Table S3). Cluster 2 included 51 accessions from Sierra Leone and 65 accessions from Togo, whereas cluster 1 had 68 accessions from Sierra Leone and 18 accessions from Togo. The smallest cluster, cluster 4, represented eleven accessions from Sierra Leone and five from Togo.

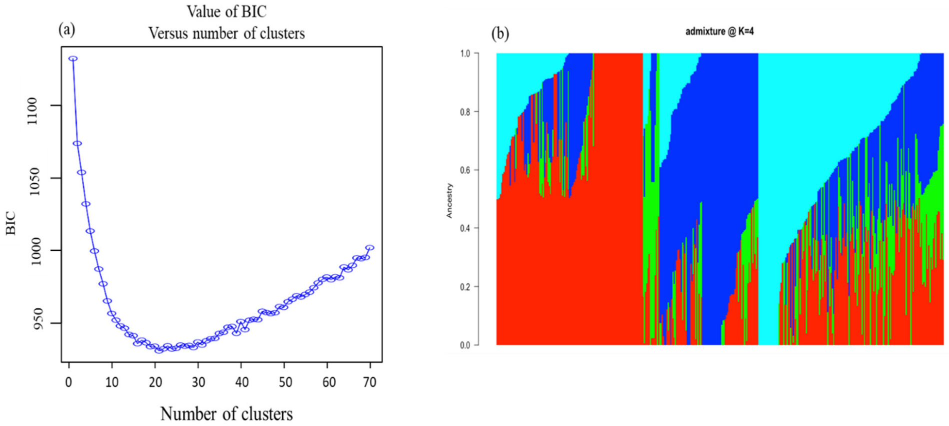


Figure 2. Graphical representation of the population structure of 376 cacao accessions. (**a**) Plot of mean likelihood of delta K against the number of K groups. (**b**) Subpopulations at K = 4. The colors represent four subpopulations of 376 accessions: red: subpopulation 1, blue: subpopulation 2, light blue: subpopulation 3, and green: subpopulation 4.

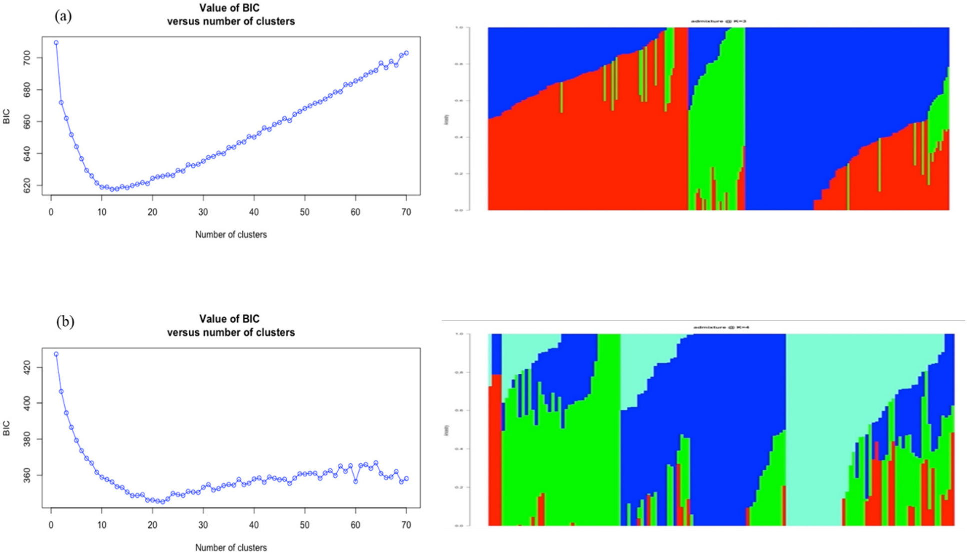


Figure 3. Population structure (**a**) Sierra Leone: plot of mean likelihood of delta K against the number of K groups; subpopulations at K = 3. The colors represent three subpopulations of 235 cacao accessions: red—subpopulation 1, green—subpopulation 2, blue—subpopulation 3. (**b**) Togo: plot of mean likelihood of delta K against the number of K groups; subpopulations at K = 4. The colors represent four subpopulations of 141 cacao accessions: red—subpopulation 1, green—subpopulation 2, blue—subpopulation 3, turquoise—subpopulation 4.

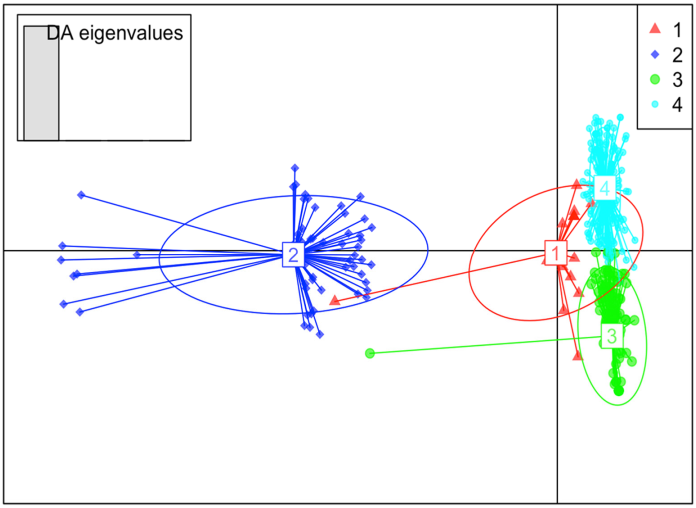
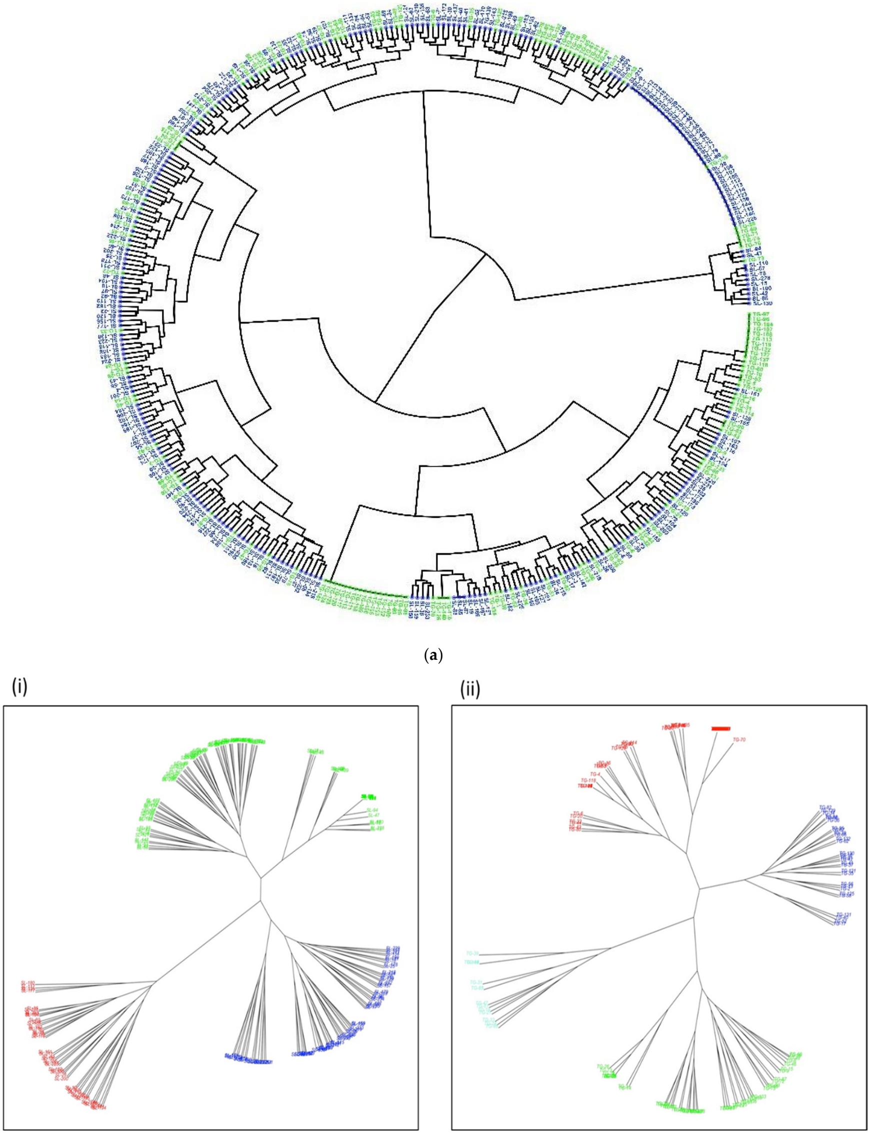


Figure 4. Discriminant analysis of principal components (DAPC) using 20 KASP–SNP markers. The axes represent the first two linear discriminants (LD). Each color represents a cluster, while each dot represents an individual. Numbers represent different subpopulations identified by DAPC analysis.

Contrary to the results of STRUCTURE and DAPC, the hierarchical clustering assigned all 376 cacao accessions to two major clusters with several sub-clusters representing a higher degree of admixture among accessions from both countries (Figure 5a). The hierarchical clustering performed for cacao accessions from Sierra Leone and Togo independently is displayed in Figure 5b. The cacao accessions from Sierra Leone split into three main clusters (Figure 5b(i)), while those from Togo divided into four main clusters (Figure 5b(ii)) (Table S3), consistent with results obtained from our STRUCTURE and DAPC analyses. For Sierra Leone, cluster 1 was the largest, with 119 cacao accessions, while 72 accessions and 44 accessions were grouped into clusters 2 and 3, respectively. For Togo, cluster 3 was the largest, with 52 cacao accessions followed by cluster 1 (46 accessions), cluster 2 (29 accessions), and cluster 4 (14 accessions) (Table S3). A network analysis between cacao accessions from Sierra Leone and Togo (Figure 6) showed strong genetic relationships, indicating that these accessions share a similar genetic background across the two countries. The central core of the QGRAPH (Figure 6) represents a set of accessions collected from Sierra Leone and Togo that are genetically similar to each other. While some peripheral genotypes depict more genetic divergence, this was more evident for accessions from Sierra Leone than for those from Togo.

A comparison of all three methods (STRUCTURE, DAPC, and hierarchical clustering) did not reveal any identical clustering patterns among these 376 accessions, except for two accessions that were both from Sierra Leone with the same accession name (C77) (Figure S2; Table S2). However, the DAPC and hierarchical clustering showed similar patterns of clustering among 135 out of 376 cacao accessions, in which one hundred and twenty-seven accessions were from Sierra Leone and eight accessions were from Togo. Meanwhile, the DAPC and STRUCTURE analyses showed similar patterns of grouping among 61 out of 376 cacao accessions (30 from Sierra Leone and 31 from Togo) (Figure S3).



(**a**)

(**b**)

Figure 5. (**a**) Phylogenetic tree for 376 cacao accessions from Sierra Leone and Togo. The blue and green colors represent accessions from Sierra Leone and Togo, respectively. (**b**) Phylogenetic tree depicting genetic relationships among cacao accessions from (i) Sierra Leone and (ii) Togo. Colors of each tree represent genetic groups within each country.

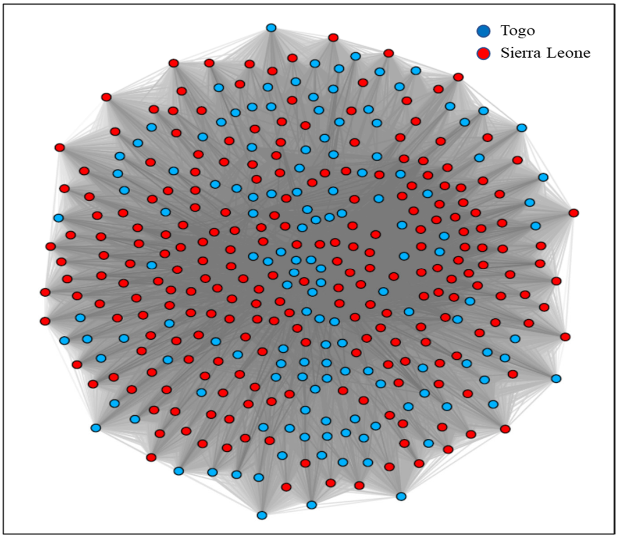


Figure 6. Genetic networks obtained by using QGRAPH. This diagram visualizes networks among cacao accessions from Sierra Leone and Togo, with the node size depicting genetic relationships among different accessions based on observed heterozygosity and allelic richness.

3.4. Analyses of Molecular Variance and Genetic Differentiation

The AMOVA analysis revealed a variability of 86% within accessions and 14% among populations (Combined data) (Table 2). The overall F_{ST} value was 0.601. A significant level of population divergence based on pairwise F_{ST} ($p < 0.0001$) was also observed among different populations, while strong genetic relationships with much less divergence were observed within populations (Table 3). The average F_{ST}-based population differentiation was highest for the combined population (0.096) and lowest for cacao accessions from Sierra Leone (0.049). The pairwise F_{ST} values ranged from 0.010 (combined population vs. Togo) to 0.045 (Sierra Leone vs. Togo).

Table 2. Analysis of molecular variance (AMOVA) among and within three different genetic populations (Sierra Leone, Togo, and combined).

Source	d.f.	SS	MS	Est. Var.	% Var.	*p* Value
Among populations	3	189.16	63.05	0.362	14	0.001
Among accessions	372	690.62	1.86	0.000	0	0.001
Within accessions	376	809.50	2.15	2.153	86	0.001
Total	751	1689.28		2.515	100	
Fixation index (F_{ST})	0.601					0.001

d.f.: Degree of freedom, SS: Sum of squares, MS: Mean sum of squares, Est. Var.: Estimated variance, % Var.: Percent Variance.

Table 3. Pairwise fixation index (F_{ST}) values within and among cacao accessions collected from Sierra Leone and Togo.

	Sierra Leone	Togo	Combined Population
Sierra Leone	−0.0024		
Togo	0.0451	−0.0040	
Combined Population	0.0201	0.0101	−0.0015
Average F_{ST}	0.049	0.073	0.096

4. Discussion

The results revealed that genotyping cacao germplasms collected from Sierra Leone and Togo by using 20 KASP–SNPs was efficient in assessing the genetic diversity and population structure. These KASP–SNPs represented a subset of 100 KASP–SNPs carefully selected based on their distribution across twenty cacao chromosomes (two SNPs per chromosomes), polymorphic nature, reproducibility, and higher efficiency from earlier studies used in fingerprinting cacao germplasms from Ghana [17], Nigeria [18], and other West African countries (unpublished). The biallelic nature of these selected SNPs has a lower error rate in allele calling, with higher accuracy and efficiency [17].

The germplasms studied represent collections maintained in different seed gardens as well as farmers' fields in both countries. Currently, cacao cultivation is unstructured in Sierra Leone, with cacao trees labeled as 'Forestero' being the main variety cultivated there [11]. However, other varieties known as 'Amazon cacao', 'Ghanian cacao', and 'Ivorian cacao' are also grown, with a significant exchange of planting materials among the farmers. The situation is similar in Togo. A thorough assessment of the available genetic diversity and population structure is necessary for the enhancement and effective use of well-characterized, diverse germplasms in the cacao improvement programs of both countries.

In this present study, diversity indices revealed the presence of substantial genetic diversity in cacao germplasm from both Sierra Leone and Togo indicated by average He (0.30 and 0.29, respectively) and Ho (0.24 and 0.22, respectively), whereas the PIC value was 0.24. Similar He values were reported for cacao collections from Nigeria [18] and Ghana [17], although those studies recorded higher PIC values and observed heterozygosity than was observed in our study. This difference could be explained by the number or selection of KASP–SNPs used or may reflect real differences among the populations. Nonetheless, average He and Ho values for the combined (Sierra Leone and Togo) population in this current study were comparable to those obtained for each country individually, indicating that the cacao germplasms present in both countries may share the same genetic background. This study did reveal that eleven accession names are common between both countries and that there is a presence of mislabeled cacao accessions in seed gardens and farmers' fields, as has already been reported in most West African countries [15–17,28]. It is also clear that a common set of cacao genotypes is shared by major cacao-producing West African countries, i.e., Cote d'Ivoire, Ghana, Nigeria, and Cameroon, likely extending to other smaller countries in the region, suggesting that mislabeled and duplicated cacao germplasms may be shared more widely than previously reported.

Our assessment of genetic relatedness, based on three different analyses (model-based population structure, IBS-based clustering, and DAPC), revealed that the combined population of accessions from Sierra Leone and Togo and the accessions specifically from Togo were composed of four main subpopulations, whereas the one from Sierra Leone was composed of three main subpopulations. The fact that this clustering was supported by a Bayesian approach, a genetic distance-based method, and a DAPC-based analysis, provides strong support for the observed population structure and genetic relationships among accessions.

In studies where reference genotypes are not used and historical pedigree data are unavailable, ancestry information can still provide a framework for determining admixtures and the contributions of genotypes to open pollination or any other sort of natural hybridization [42]. The fact that no common clustering was observed across the three clustering methods used in our study could be because DAPC revealed more clusters than ADMIXTURE, but the latter method assigned genotypes based on ancestries. The DAPC approach relies on discriminant functions that seek to maximize divergence between clusters while minimizing within-cluster diversity [39]. Similar inconsistency has been observed in other studies, such as cassava [42]. In clonally propagated crops such as cacao, genotypes represent complex inter-generational hybridization or open pollination, resulting in complex genetic relationships and clinal patterns of genetic differentiation [39]. Still, there is a general agreement in cluster assignment across all three methods, specifically for DAPC and ADMIXTURE approaches, wherein >75% genotypes were assigned to specific clusters.

It is worth noticing that in our combined analysis, most cacao accessions (73.37%) were assigned to one of the four subpopulations with probabilities >0.8, and only 106 of 376 cacao accessions (28.19%) were classified as admixtures. In a recent study of cacao germplasm from Nigeria, the rate of admixtures or off-types ranged from 10% to 73% among the clones in seed gardens [18]. Such admixtures in cacao germplasm from both Sierra Leone (4.7%) and Togo (18.4%) probably reflect genotypes with different allelic patterns and labeling errors present in the introduced germplasms from other West African countries. This is also supported by our findings that mislabeling was less frequent in accessions from Sierra Leone (33.33%) than in those from Togo (45.45%). Furthermore, the presence of a higher level of admixed accessions in cacao genotypes from Togo may also reflect recent breeding advances involving open pollination and bi-parental crossing among accessions coupled with strong selection pressure, as observed in other clonally propagated crops [43], which is not the case in Sierra Leone. Labeling errors pose a higher risk of misidentification of clones, which proliferates when beans/pods from such clones are shared or used to establish new seed gardens, when neighboring farmers share materials with each other, or when farmers import materials from neighboring countries without understanding their genetic potential, which is a common practice in both Sierra Leone and Togo. Olasupo et al. [18] explained how the presence of 58% mislabeled accessions in an old seed garden translated into 100% mislabeling in a newly established seed garden in Nigeria. The presence of mislabeling in germplasms from the University of Reading in Sierra Leone and at the seed gardens of the National Institute in Togo indicates a loss of labels or human errors in recording them, occurrences commonly observed in clonally propagated crops with long life cycles such as tree crops [17,18]. There are serious implications when mislabeled or misidentified germplasm is introduced, as it often leads to poor predictions of their performance or their value in improvement programs. This may be one explanation for relatively low yields among cacao accessions introduced from neighboring countries, in addition to the prevalence of pests and diseases.

Network analysis was used to unravel the genetic relationships among the cacao accessions from Sierra Leone and Togo. In the absence of pedigree records or comparisons to international reference clones, the dissection of genetic relationships among cacao accessions from these two countries through network analysis was a worthwhile approach. Network analysis has been successfully used in other clonally propagated food crops such as cassava [42] and white yam [44]. In tree crops such as cacao, which is an outbreeding species with a long-life cycle, open pollination is common, with the source of pollen typically unknown. The extent of genetic diversity observed within cacao accessions in our study can, in part, be attributed to this factor as well as to the presence of a good number of unique accessions in both countries. Hence, these unique cacao accessions could be considered potential parents for local cacao improvement once preliminary agronomic evaluations and trait profiling have been conducted in multiple sites. There is also a need for field conservation of unique cacao accessions in both countries, with long-term support for best management practices and irrigation facilities to mitigate losses associated with biotic and abiotic stresses. It may also be necessary to mainstream DNA fingerprinting of introduced germplasm from neighboring countries and international collections for the regular auditing of cacao accessions for their true-to-typeness and also to check for pollen contamination during hybridization through open or manual pollination.

The low level of genetic diversity of the cacao germplasm historically introduced into West Africa is well known [13,17,18], a situation further compromised by mislabeling and unintentional duplication in both seed gardens and farmers' fields. Mislabeling (the use of the same name for different genotypes or the use of different names for identical genotypes) is common in many clonally propagated crops [42,44,45]. The number of SNPs used herein was relatively low but based on their high polymorphic, informative nature;

our results provide a first look at these limitations within the cacao germplasm of Sierra Leone and Togo.

We suspect that additional studies based on the use of a larger set of SNPs may provide a deeper look into the sub-populations observed in our study, but patterns of genetic diversity and mislabeling may change little. When possible, the original donors should be contacted to determine the correct application of accession names through genotyping original source material.

5. Conclusions

In this study, we made use of 20 KASP–SNP markers to assess the genetic diversity of cacao germplasms from Sierra Leone and Togo. The genetic relationships elucidated among the accessions in each country as well as the identification of mislabeling have provided key information to support future cacao improvement by identifying diverse and correctly named parents. This study also confirmed the reliability and accuracy of KASP–SNPs generated from next-generation sequencing-based genotyping coupled with complementary statistical analyses to generate knowledge on genetic diversity and population structure. In this study, we identified a high degree of mislabeling in most of the introduced materials, which has been attributed to errors from the sources of introduction, labeling errors, and lost labels.

Further detailed research is needed for multi-location phenotyping as well as genotyping with high-density DNA markers or the whole-genome re-sequencing of cacao germplasm in Sierra Leone and Togo to validate and refine our initial findings. The presence of duplicates/mislabeling has serious consequences for improvement programs, and caution should be taken to ensure future accuracy in labeling and consistent identification of clones/accessions before establishing seed gardens or distributing planting materials to farmers. It is recommended that accession naming in both countries follow the International Cocoa Germplasm Database convention [46] and maintain the International Cocoa Quarantine Center, Reading (ICQC, R) names when acquiring germplasms from the University of Reading or other major collections.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14112458/s1, Table S1. (a) List of cocoa genotypes from Sierra Leone with clone name, GPS information, district name, chiefdom name and village/farm name. Table S1 (b) List of cocoa genotypes from Togo with clone name, GPS information, district name, chiefdom name, and village/farm name; Table S2. Details of 20 KASP–SNPs with their IDs and their position in each linkage group; Table S3. Cacao accessions from Sierra Leone, their grouping based on population structure, DAPC and Hierarchical Clustering (HC), and the source of introductions of cacao germplasm in the two countries; Table S4. Descriptive statistics of He, Ho, MAF, and PIC values for the combined population of 376 cacao accessions. Figure S1. Distribution of He, Ho, MAF, and PIC values for the combined population (376 cacao accessions); Figure S2. Graphical representation of the population structure of 376 cacao accessions. Plot of mean likelihood of delta K against the number of K groups. Figure S3. Comparison of three complementary approaches: STRUCTURE, DAPC, and Hierarchical Clustering for grouping 376 cacao accessions.

Author Contributions: Conceptualization, R.B., P.L.K. and L.J.G.-B.; Methodology, R.B., P.A.A., M.M.L. and K.A.; Data analysis, P.A.A., M.M.L. and K.A.; Writing original draft preparation, R.B.; Writing—review and editing, M.M.L., K.A., P.L.K., P.A.A. and L.J.G.-B.; Supervision, R.B., P.L.K. and L.J.G.-B. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: All data supporting the findings in this study are available within this article and supplementary files. The raw SNP data can be made available upon request to the corresponding author.

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Cacao (*Theobroma cacao*) is a key agricultural commodity in Sierra Leone, but productivity is constrained by cocoa black pod disease caused by *Phytophthora* species. This thesis takes an integrated approach to management of cocoa black pod disease by combining an investigation into the genetic diversity of *T. cacao*, to aid in accurate genetic understanding, a survey of the agroforestry approaches used by farmers and testing of biological control solutions for sustainable disease control in Sierra Leone.

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