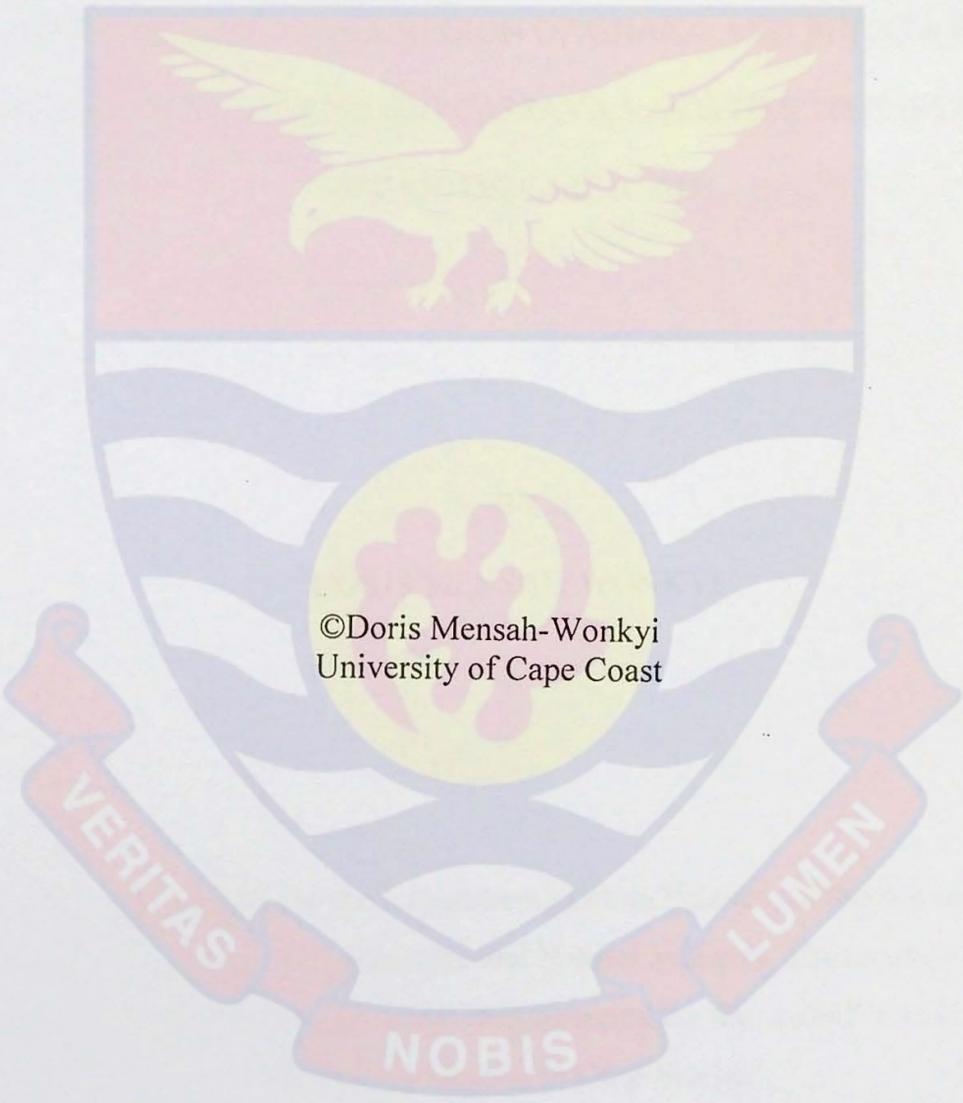

UNIVERSITY OF CAPE COAST

GENETIC DIVERSITY OF CASSAVA MOSAIC GEMINIVIRUSES (CMGS) IN THE VOLTA REGION OF GHANA AND RESISTANCE SCREENING OF IMPROVED CASSAVA VARIETIES TO CASSAVA MOSAIC DISEASE (CMD)

BY

DORIS MENSAH-WONKYI

A thesis Submitted to the Department of Crop Science, of the School of Agriculture, College of Agriculture and Natural Sciences, University of Cape Coast in Partial Fulfilment of the Requirements for the Award of Doctor of Philosophy Degree, in Crop Science.

MAY 2022

DECLARATION

Candidate's Declaration

I declare that this thesis is the outcome of my own unique study, and no part of it has been submitted for another degree at this University or anywhere.

Candidate's Signature: ..... Date: 19/12/22

Name: Doris Mensah-Wonkyi

Supervisors' Declaration

We hereby certify that the preparation and presentation of this thesis were supervised in conformity with the University of Cape Coast's thesis supervision criteria.

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ABSTRACT

Cassava Mosaic Disease is one of the major virus diseases limiting cassava production in Ghana and Africa. The disease accounts for low yields of cassava with over forty (40) improved cassava varieties (ICVs) bred and released to improve cassava production in Ghana. Household surveys in six cassava-growing districts in the Volta Region indicates that all 180 respondents had experienced CMD on their farms but did not know the cause and majority (74.4%) do not control the disease. A field survey in the six districts showed CMD was prevalent and severe with high incidence of coinfections with ACMV and EACMV after serological analysis. PCR detected ACMV (78.9%), EACMV (1.1%) and co-infection (20%) in all the districts but not in all the farms surveyed. Next generation sequencing of field samples using Illumina MiSeq sequencing platform led to the assembly of twelve full length CMGs genomes (6 ACMV DNA-A components, 3 ACMV DNA-B components and 3 DNA-A EACMV components) which aligned closely to already published isolates identified in West Africa. TAS ELISA screening of 21 ICVs evaluated in 2018/2019 detected ACMV in all varieties but not 'Hemaa' and 'AGRA' from the coastal savannah. EACMV was detected in varieties 'Ampong, TEK, Sika, Lamesese, Abasafita, Amansan, and Esam' from the Coastal savannah and varieties 'Ampong, Lamesese, Botan, Sika, AGRA, IFAD, and Capevars' from the Forest zone. Graft challenge of ICVs in the screen house using CMD-infected rootstocks showed that all the ICVs are resistant to EACMV except Amansan, Abrabopa and AGRA. Generally, all the ICVs are showed mild to moderate infection to CMD following screening using TAS-ELISA, PCR and graft challenge with infected rootstocks in two years.

KEY WORDS

Agro-ecological zone

Begomovirus

Cassava mosaic virus

Geminiviridae

Genotype

Improved cassava varieties

Indexing

Severity

ACKNOWLEDGEMENTS

What God cannot do does not exist. I want to thank God for his faithfulness, grace and protection throughout this study.

I express my gratitude to my supervisors, Professor Elvis Asare-Bediako, Profrssor G. C van Der Puije, Dr. Wilfred Elegba and Dr Andrew Sarkodie-Appiah for their expert assistance and continuous efforts to help me complete this study.

My sincere appreciation to Mr. Francis Gankui of the Ministry of Food and Agriculture, Ho, Mr. Thomas Baagmae of Logba, Mr. Tetteh of Asuansi Farm Institute and Mr Arthur of Wenchi Farm Institute and UCC; Teaching and Research Farm for their help during the survey work and evaluation experiment.

I also wish to express my deepest thanks to Dr. Kwabinah Asare of Cocoa Research Institute of Ghana and Mr Lenin Apuri of the Soil Science Department of the University of Cape for supporting me with the data analysis.

My profound gratitude to Mr. Shadrach Kwoffie, Mr. Benjamin Otu Owusu of Biotechnology laboratory, University of Ghana and Mr Robert Appiah of Biotechnology and Nuclear Agriculture Research Institute for helping me out with my laboratory activities.

I am very grateful to Professor Kwame Offei, Director of the Biotechnology Centre-University of Ghana for providing space and logistics during the laboratory analysis

I am indebted to my mother Madam Abigail Annan for her support, understanding and pushing me through my education to this level. Also, special thanks go to my husband, Mr. Akwasi Afriyie for his patience, encouragement and prayers during the study. I am also grateful to my guardian (Mr and Mrs

Amissah) and my siblings (Wonkyi's and Amissah's) for their support and prayers.

My gratitude goes to the University of Cape Coast, Biotechnology and Nuclear Agriculture Research Institute, Wenchi and Auansi farm Institutes for providing the improved planting materials for the study. Again to staff and management, and students (2018/2019) of Ohawu Agricultural College and Mr Thomas Baagmea for providing land and labour during the evaluation

Special thanks to a good friend Dr. Ogyiri Adu and Amma Kissiwa Amoateng for their encouragement throughout the programme

I want to recognize the staff of the Biotechnology and Nuclear Agriculture Research Institute especially the Biotechnology Centre for their support, advice and encouragement.

Lastly a big appreciation to the Samuel and Emelia Brew-Butler, SGS/GRASAG-UCC Research Grant for sponsoring part of my study.

DEDICATION

To my dear late Grand Parents and Dad (Mr Joseph Oboe Annan, Madam Elizabeth Victoria Sampson and Dr. Thomas Mensa-Wonkyi), my mother, Madam Abigail Annan and my husband Mr Akwasi Afriyie

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LIST OF ABBREVIATIONS

ACMV	Africa cassava mosaic virus
BNARI	Biotechnology and Nuclear Agriculture Research Institute
CMD	Cassava Mosaic Disease
CMG	Cassava mosaic geminiviruese
CSIR	The Council for Scientific and Industrial Research
EACMV	East Africa cassava mosaic virus
FBO	Farmer based organizations
ICTV	International Committee on Taxonomy of Viruses
NARP	National Agricultural Research Project
NRTCIP	National Root Tuber Crop Improvement Project
MAP	Month after planting
PM	Planting materials
RELC	Research-Extension-Farmer Linkage Committee
RTIMP	Root Tuber Improvement Marketing Programme
WAAPP	West Africa Agricultural Productivity Programme

CHAPTER ONE

INTRODUCTION

Cassava (*Manihot esculenta Crantz*) is a major staple food for over 800 million people around the world (Howeler, Thomas, Holst Sanju_an, Sanju_an, Quir_os, Isebrands & Gonz_alez, 2013) and over 500 million people in Sub Saharan Africa, (Atwijukire, Hawumba, Baguma, Wembabazi, Esuma, Kawuki, & Nuwamanya, 2019). The roots are a major source of starch and processed into diverse food products (Sanchez, Salcedo, Ceballos, Dufour, Mafla, Morante, Jaramillo, 2009).

According to Oparinde, Abdoulaye, Manyong, Birol, Asare-Marfo, Kulakow, & Ilona, (2016), cassava serves as the second highest provider of calories in diets after maize in Africa. Due to its ability to survive drought and grow well on poor or marginal soils, it is considered a food security crop (Kleih, Phillips, Wordey, & Komlaga, 2013).

Worldwide production of the crop stands at 291 million tons with Africa contributing over 50% estimated around 177 million tons (FAO, 2019). Out of this total global production, Ghana accounts for 18 million tons (FAOSTAT, 2016). Cassava contributes 22% out of the total 47% agricultural gross domestic product to the economy of Ghana (Angelucci, 2013). The crop is grown by most farming families in Ghana and provides more than 50% of daily calories to consumers (Manyong, Dixon, Makinde, Bokanga, & Whyte 2000). Presently, cassava is largely grown in every part of the country occupying 887,000 hectares with an achievable yield of 45 t ha^{-1} (Bayitse *et al* 2017).

Even though the crop is known to tolerate and produce under low soil fertility and easy to multiply planting materials without any financial assistance

to the farmer, its production is hampered by several constraints, mainly biotic constraints (Hillocks, 2002).

Problem Statement

Diseases and pests are major constraints to cassava production in Africa (Bayitse *et al*., 2017). Common insect pests of cassava include Cassava mealy bugs, (*Pheanacoccus manihoti*), green spider mite (*Mononychillus tanajoa*) (Herren, & Neuenschwander 1991), and whiteflies (*Bemisia tabaci*) (Perrings, 2001).

The most devastating disease that affects production of cassava in Africa and Ghana is the Cassava Mosaic Disease (CMD), caused by *Cassava Mosaic Germiniviruses* (family *Geminiviridae*; genus *Begomovirus* (Leg & Fauquet, 2004). Yield losses due to CMD range between 20% to 90% (Moses, Asafo-Agyei, Adubofuor, Adusei, 2008) and in severe cases 100% yield loss is recorded (Thresh & Cooter, 2005; Moses, 2009). Economically, CMD causes annual yield loss estimated at US$ 1.9 – 2.7 billion (Legg, Owor, Sseruwagi, & Ndunguru, 2006). CMD is transmitted through the whitefly vector and use of infected planting materials (Thresh, Otim-Nepa & Nichols, 1994). Disease incidence and severity has been observed to be higher in crops grown from infected cuttings (Thresh *et al.,* 1994).

The most effective means of controlling CMD in Africa has been through the use of resistant varieties (Thresh, Otim-Nepa, Legg, & Fargette, 1997). Thus, since the first report of the disease in Ghana by Dade (1930), more than twenty improved and resistant cassava varieties have been released to control CMD (National Crop Variety Catalogue 2019). However, reports from Research Extension Farmer Linkage Committee (RELC) (Volta, Central and

Eastern Regions) indicate that CMD is still endemic in farmers' cassava farms (RELC report, 2016-2018). Again, personal observation at the Ohawu Agricultural College in the Volta Region and Asuansi multiplication Centre in the Central Region where some of these improved/resistant varieties are maintained and multiplied for local farmers confirms the RELC report.

To identify reasons for the persistence of CMD in Ghana, especially in the Volta Region after the release of several improved and resistant varieties over 20 years, answers to the following questions would be helpful:

- Are farmers aware of the existence/incidence and effects of CMD on their farms?
- Are farmers aware of the mode of transmission and management of CMD?
- Are they aware of the availability of CMD-resistant or tolerant varieties?
- Do farmers have access to improved varieties?
- Are farmers growing CMD-resistant or tolerant varieties?
- Are the CMD-resistant or tolerant varieties still resistant to the Cassava Mosaic Geminiviruses s in the field?
- Are the improved varieties susceptible to strains of *Cassava Mosaic Geminiviruses* (CMGs) present in the Volta Region?
- Are there new strains/species of CMGs in the Volta region?

Justification

Over the years a lot of effort has been made by the government and other international agency-led programmes to increase cassava production in Ghana. One of the regions that received such support is the Volta Region (Akumatey, 2017; Torvikey, 2017). These programmes include the Root and Tuber

Improvement & Marketing Programme (RTIMP), West Africa Agricultural Productivity Programme (WAAPP), the Caltech Venture Programme with the most current being the One District, One Factory drive by the government of Ghana (Akumatey, 2017; Torvikey, 2017). All these programmes and interventions have increased the production of cassava in the region. For example, through the Caltech Programme, about 3000 hectares of land was acquired for planting and processing cassava at Hodzo in the Ho municipality (Akumatey, 2017). This programme saved the country an estimated 200 million USD used for importing cassava flour and ethanol (Akumatey, 2017).

According to the National Board for Small Scale Industries, the Volta Region has become the hub for cassava production with a number of private investments such as Reagvin Ventures Limited acquiring about 4000 hectares of land for production and processing of cassava (Akumatey 2017). Therefore, it is important to investigate farmers' awareness of CMD, management practices, knowledge and use of improved/resistant cassava varieties and the status of resistance of the improved/resistant cassava varieties in the Volta Region.

Furthermore, this study will determine the prevalence and distribution of CMD in farmer fields, characterization of genetic diversity of CMGs and the development of effective and sustainable management strategies against the disease. Information generated on the status of CMD in the Volta Region will be useful in directing breeding goals, policy and development of strategies to control or manage CMD in the Volta Region and other cassava growing regions of Ghana.

General Objective

To assess the prevalence, distribution and genetic diversity of *Cassava Mosaic Geminiviruses* in the Volta Region of Ghana.

Specific objectives:

- To determine farmer awareness of CMD, management practices, knowledge and use of released improved/resistant cassava varieties
- To determine the prevalence and spatial distribution of CMD and associated *Cassava Mosaic Geminiviruses* in the Volta Region.
- To determine the genetic diversity of Africa Cassava Mosaic Virus and East Africa Cassava Mosaic Virus species in the Volta Region.
- To assess the resistance/tolerance level of selected improved cassava varieties to CMD in the coastal and forest agro-ecological zones and screen house conditions.

CHAPTER TWO

LITERATURE REVIEW

Cassava: Origin, distribution, production and uses

Cassava was introduced to West Africa in the year 1588 by the Portuguese to feed the Africa's slave trade (Okogbenin, Egesi, & Fregene, 2006). Cultivation of cassava started early in the Fernando Po around the 16th century, however the spread to other West African countries delayed until the 18th to 20th century (Oslen & Schaal 1999; Hershey, 2012; Njoku, 2012). In the latter half of the18th century, cassava had become the most widely grown and used crop in the coastal plains. The Portuguese planted the crop near their trading port, forts and castle in Ghana because it was a major source of food consumed by both the Portuguese and African slaves (Oslen & Schaal 1999).

The crop is drought-tolerant and able to grow appreciably well in low soil fertility, thus, it is the most cultivated root crop by small holder farmers in the tropics (FAO 2013; Liu, Liu, Peng, He, 2014; Saranraj, Behera, & Ray, 2019).

The production of cassava stands at 291 million tonnes, since 2017, with Africa contributing 177,948 million tons (FAO, 2019). The highest producer globally is Nigeria contributing 59,485,947 MT representing 20.4%, followed by Congo DR (31,596,046 MT), Thailand (30,973,292 MT), Indonesia (19,046,000 MT), Brazil (18,876,470 MT) and Ghana (18,470,762 MT) respectively which all accounts for 6.32% of the world's output (FAO, 2019).

In Africa, Nigeria ranks first followed by DR Congo, Ghana, Angola and Mozambique. In terms of land under cultivation, Africa uses about 56.5% for cultivation of cassava with Nigeria having the largest land under cultivation

followed by DR Congo, Uganda, Mozambique, Angola and Ghana respectively (FAO 2019).

In Ghana, cassava cultivation spread from the southern part to the middle and the northern part of the country by 1930. However, its spread to other regions such as the Brong Ahafo, and the Northern Regions was delayed until after the 1983 drought (Korang-Amoako, Cudjoe, & Adjakloe, 1987). Currently, cassava is grown in all the regions of Ghana on small scale basis (MoFA, 1997; Torkpo, Gafni, Danquah, & Offei, 2018).

Cassava cultivation

Presently, cassava is cultivated in the tropical and sub-tropical regions of Africa, Asia, and Latin America, between latitude 30°N and 30°S of the equator and 26 to 2300 m above sea level (Alves, 2002). The crop is grown in areas such as North-eastern Brazil, Colombia's northern coast, Peru's coastal region, West Africa and Asia which are characterized by severe drought, poor and intermittent precipitations, high potential evapotranspiration, poor soil fertility and high risk of pest and diseases (Okogbenin, Setter, Ferguson, Mutegi, Ceballos, Olasanmi, Fregene, 2013).

In Sub-Saharan Africa, land area under cultivation grew from 6.16 million ha in 1968 to 20.2 million ha in 2017, rising at an average annual rate of 2.59% (FAO, 2019). In Ghana, cassava production is estimated at more than 12 million metric tons per year (MoFA, 2009). Ghana's cassava food production grew from 1047 per 1000 tons in 1968 to 6745 per 1000 tons in 2017 with an average annual rate of 4.36% (FAO, 2019).

Cassava cultivation requires warm and humid climate with average temperatures of 25 - 27 ° C, annual rainfall of 500 mm to 5000 mm and below

an altitude of 150m Norman, 2017). According to MoFA-SRID, (2014), Ghana has an annual rainfall between 800 and 2,200 mm making it suitable for cassava cultivation. Cassava does well in oxisols and ultisols which are highly acidic and marginal with pH of 4.0-8.0 performing well without any agrochemical input. (O'Hair, 2005; El- Sharkawy, 2004). Although cassava does relatively well in soils with low fertility low supply of nitrogen can decrease light interception and reduce growth of the cassava canopy while poor supply of potassium reduces water usage (El-Sharkawy, 2007). However, nutrient application has been shown to increase yields in cassava (Adekayode & Adeola, 2009; Sogbedji, Agboyi, Detchinli, Atchoglo, & Mazinagou, 2015).

Status of cassava research and development of improved varieties in Ghana

Cassava is a very important food security crop in Ghana although production is limited by pests and diseases. As a result, the majority of interventions are targeted at resistance breeding carried out by the Council for Scientific and Industrial Research (CSIR), Biotechnology and Nuclear Agriculture Research Institute (BNARI), the public Universities in collaboration with the Ministry of Food and Agriculture (Ofori, AI-Hassan, Afuakwa, & Noamesi 1997).

In the 1930s, when cassava production in Ghana was threatened by the cassava mosaic virus disease, the government imported different varieties from other West African countries, East Africa, the Caribbean and Asia (Ofori *et al.*, 1997). Genetic crosses between these imported varieties and local varieties were made which resulted in the introduction of four varieties, Queen, Gari, Williams and Ankra in 1935 (Ofori *et al.*, 1997). These varieties were highly resistant to CMD and produced high yields.

By the 1950s, the varieties Queen, Gari and Williams showed susceptibility to CMD with the exception of Ankra which was still resistant and, thus, extensively grown throughout the country. The break down in resistance of the aforementioned three varieties resulted in the setting up of a second breeding programme which lasted till the mid-1960s. By the end of the programme, four different genotypes (K357, K162, K680 and K491) which were moderately resistant to CMD and high yielding (19 t/ha) were introduced for adoption. These genotypes displayed resistance against the CMD until the 1970s (Ofori *et al.*, 1997).

A third breeding programme seeking to curb the cassava mealy bugs and the cassava green spider mite pests was initiated in collaboration with FAO and IITA (Ofori *et al.*, 1997). Two biological agents, a parasitoid wasp (*Epidinocarsis lopezi*) and predatory insects (*Diomus sp.* and *two Hyperdspis spp*) were introduced to curb the cassava mealy bugs and the cassava green spider mite. Apart from the introduction of these biological agents, three other genotypes (TMS 50395, TMS 4 1425, and TMS 30572) were introduced to manage the pests as well CMD (Ofori *et al.*, 1997).

In 1988 and 1991, the National Root & Tuber Crop Improvement Project (NRTCIP) and National Agricultural Research Project (NARP) was set up to work in collaboration with other research institutions like Crop Research Institute, Soil Research Institute, Savannah Agriculture Research Institute, Plant Genetic Resources Institute among others to improve cassava varieties. In 1996, three new varieties which identified as high yielding and CMD resistant were released. These varieties namely were "Afisiafi", "Gblemo duade" and "Abasafitaa" (Ofori *et al.*, 1997).

Thereafter, other tolerant and/or resistant varieties have been released to control or manage CMD. These varieties include "Afisiafi, Abasafitaa, Tekbankye, Doku duade, Agbelifia, Essam bankye, Bankyehemaa, Capevars bankye, Bankye botan, Eskamaye, Filindiakong, Nyerikobga, Nkabom, IFAD, Ampong, Broni Bankye, Sika bankye, Otuhia, CRI-Amansan Bankye, CRI-AGRA Bankye, CRI-Dudzi, CRI-Abrabopa, CRI-Duade Kpakpa and CRI-Lamesese" (Appiah, 2015). In 2015 the Crop Research Institute released other varieties which are resistant to CMD and high yielding known as "CRI-Amansan Bankye, CRI-Duadzi, CRI-Abrabopa, CRI-Duade Kpakpa and CRI-Lamesese". Similarly, the University of Cape Coast released some varieties to control the disease which include "Broni Bankye, Bankye Botan and Cape vars Bankye" which have stood the test of time in relation to CMD (Asare *et al.*, 2014; Personal Communication with Mr Emmanuel Ogyir-Adu, Assistant farm manager for the School of Agriculture and Research farm, University of Cape Coast 12/08/2019).

Another focus for cassava improvement has been to increase micronutrient content, especially vitamin A (Ceballos, Iglesias, Pérez, & Dixon, 2004). Breeding for yellow flesh cassava varieties with higher levels of vitamin A has led to the release of four varieties by the University of Cape Coast in partnership with BNARI- GAEC (Amernope, Asare-Bediako, Tetteh, Asare, Taah, & van der Puije, 2019).

Pests and diseases of Cassava

Insect pests and diseases are a major cause of low yields in cassava production accounting for 50% of losses (Uzokwe, Mlay, Masunga, Kanju, Odeh, Onyeka, 2016; Fermont, Van Asten, Tittonell, Van Wijk, & Giller, 2009).

Insect pests include cassava green mite, cassava whitefly, mealy bugs and, grasshopper. A number of these insect pests are the major cause of plant disease transmission especially viruses and bacteria (Night, Asiimwe, Gashaka, Nkezabahizi, Legg, Okao-Okuja, Obonyo, Nyirahorana … Mutumwinka, 2011; Yu-sheng, Hu, Fang-hao1, & Gui-fen, 2019). Being polyphagous, these insect pests can feed on about nine different plant families with cassava being the most preferred (Uzokwe, Mlay, Masunga, Kanju, Odeh, Onyeka, 2016).

Cassava green mite is also a major constraint to cassava production in Africa reducing yields by 10-80% (Chernoh, 2014). Uganda was the gateway for entry of the pest into Africa in 1970s, and it has become common especially in the major cassava producing areas (Chernoh, 2014). Control is by the use of clean materials for planting at the onset of the raining season (Chernoh, 2014).

The cassava mealy bugs were first seen in Africa in 1970 and cause yield losses as high as 80% (FAO, 2019). The bugs are found in almost all the African countries including Ghana. The sap-sucking pests cause destruction to plants by shortening the internodes, distort upper shoot, curling and yellowing (FAO, 2019). Although existing and new cassava varieties show little resistance to the mealy bugs (Parsa & Winotai, 2012), use of parasitic wasp (*Anagyrus lopezi)* as biological control agent has been effective in the management of the pest (Parsa & Winotai, 2012).

The whitefly (*Bemisia tabaci*) is an important insect pest of cassava which feeds on several plant families transmitting about five plant viruses including cassava mosaic virus and cassava brown streak virus during feeding (Wenbo, Wosulab, Hasegawac, Casingad, Shirimab, Fiaboee…Feia, 2019).

The variegated grasshopper is another important pest of cassava which feeds on leaves and stem of the cassava plant thus reducing the rate of sprouting or dieback of stems (Mansaray, Samura, Sundufu, & Massaquai, 2012). They are sometimes classified as polyphagous because they feed on different plant families like the grass and forb families (Mansaray *et al.,* 2012). Farmers can use appropriate chemicals such as "knock off" to manage the variegated grasshopper (Mansaray *et al.,* 2012.

Another cause of low yields in cassava is bacterial disease, the most common and devastating being the cassava bacterial blight (CBB) (Sedano, Moreno, Mathew, Léon, Fabio, Cano, Ballvora, Camilo Carrascal, 2017). A study by Wydra & Verdier (2002) in Ghana and other African countries revealed that, the disease is found in all agro-ecological zones in Africa. It is caused by the bacterium *Xanthomonas axonopodis* pv. *manihoti* (Xam), which causes yield losses in fresh roots and planting materials resulting in low accumulation of starch in the tubers (Fanou, Valerien, Wydra, & Erfurt, 2018). The bacterium is able to cause and sustain infection by surviving in planting materials and epiphyte crops. The progress of the disease is influenced by climatic factors as well as high population of grasshopper (Fanou *et al.,* 2018).

Cassava root rot is the most common fungal disease of cassava caused by the pathogen *Fusarium spp.* The disease affects the root by reducing the rate of nutrient synthesis and storage (Ngobisa, Djidjou, Ntsefong, Mbenoun, Zok, & Fontem (2015). It is prevalent in soils with high amount of moisture which increases the rate of rotting (Ngobisa *et al.,* 2015). A study by Ngobisa *et al.,* (2015) revealed that the disease was ranked second in terms of constraints to cassava production. Different fungi associated with the occurrence of the

disease include *Botryodiplodia theobromae Colletotrichum sp. Fusarium sp. Pestalotia sp. Sphaerostilbe repens Trichoderma viride* and *Geotrichum sp.* (Ngobisa *et al.,* 2015). An infected plant shows symptoms of swollen roots with internal brown colour indicating rotting (Ngobisa *et al.,* 2015).

Virus diseases of cassava

The most common virus diseases of cassava in Africa are the Cassava Mosaic Disease (CMD) and Cassava Brown Streak Disease (CBSD) (Legg & Fauquet 2004; Patil *et al*. 2014).

Cassava Mosaic Disease is widespread in Africa and caused by Cassava Mosaic Geminiviruses belonging to the family *Geminiviridae;* genus *Begomovirus* (Bock & Woods, 1983; Patil & Fauquet, 2009; Swanson & Harrison, 1994; Thresh, Otim-Nape, & Fargette, 1998). Symptoms of CMD include mosaic patterns on the leaves which occur early during leaf development. Other symptoms include distortion of leaves, curling, small sized leaves and stunting (CABI, 2020).

The second important virus disease of cassava is cassava brown streak disease is caused by cassava bown streak viruses which belong to the *Potyviridae* family; genus *Ipomovirus* (Mbanzibwa, Tian, Tugume, Mukasa, Tairo, Kyamanywa…Valkonen, 2009; Winter, Koerbler, Stein, Pietruszka, Paape, Butgereitt, 2010).

Symptoms of the virus include root lesions consisting of dry necrotic rotten patches, brown stem lesions, and leaf yellowing (Hillocks, Raya, & Thresh, 1996). Unlike CMD, CBSD is widespread in the eastern and central parts of Africa causing yield losses as high as 70% (Patil & Fauquet, 2009; Hillocks, Raya, Mtunda, & Kiozia, 2001).

Cassava Mosaic Disease (CMD)

Storey (1936), first proposed the viral etiology of CMD in Tanzania through grafting of cassava where the causative organism was confirmed. The structure, composition and genomic nature were demonstrated by Stanley and Gay (1983). In 1993, only three distinct CMG species associated with CMD were identified serologically, namely *African cassava mosaic virus* (ACMV) (Bock & Woods, 1983) *East African mosaic virus* (EACMV) (Swanson & Harrison, 1994), and *Indian cassava mosaic virus* (ICMV) (Hong, Robinson, & Harrison, 1993). Currently, seven more cassava mosaic geminivirus (CMG) species have been identified with CMD in Africa (De Bruyn, Harimalala, Zinga, Mabvakure, Hoareau, Ravignné…Lefeuvre, 2016). These are, *East African cassava mosaic Cameroon virus* (EACMCV) (Fondong, Pita, Rey, de Kochko, Beachy, & Fauquet, 2000a), *East African cassava mosaic Malawi virus* (EACMMV) (Zhou, Robinson, & Harrison, 1998), *East African cassava mosaic Zanzibar Virus* (EACMZV) (Maruthi, Susan, Colvin, Briddon, Simon, 2005), *South African cassava mosaic virus* (SACMV) (Berrie, Palmer, Rybicki, Rey, 1998), *East African cassava mosaic Kenya virus* (EACMKV) (Bull, Briddon, Sserubombwe, Ngugi, Markham, & Stanley 2006), *African cassava mosaic Burkina Faso virus* (ACMGSFV) (Tiendrebeogo, Lefeuvre, Hoareau, Harimalala, De Bruyn, Villemot…Lett, 2012) and *Cassava mosaic Madagascar virus* (CMMGV) (Harimalala *et al*., 2015).

CMD was first discovered in Tanzania in 1894 (Hillocks &. Thresh 2000). The virus was thought to be of African origin with the name krauselkrankheit and spread throughout the continent (Fauquet & Fargette 1990). The infection was not severe in most countries until it caused devastating

losses to cassava in East Africa in the 1920s (Fauquet & Fargette 1990). The disease spread to other countries including Nigeria, Sierra Leone and Ghana (Fauquet & Fargette 1990). By 1987, the spread of CMD was confirmed in most cassava producing areas (Fauquet & Fargette 1990).

Before the year 2000, the ACMV and EACMV species were thought to be distinct occupying specific geographical areas on the continent. The ACMV for instance was believed to occur in Southern, Central and Western Africa while EACMV was limited to the coasts of East Africa, Madagascar, Malawi and Zimbabwe specifically the eastern parts of Africa (Swanson & Harrison, 1994). Currently, ACMV and EACMV species are now widespread commonly occurring in the same geographical regions. For example, the EACMV species can be found in the Western and Central parts of Africa (Fondong, Pita, Rey, de Kochko, Beachy, & Fauquet 2000a; Pita, Fondong, Sangare, Otim-Nape, Ogwal, & Fauquet, 2001a).

In Ghana, CMD was reported in 1926 (Doku, 1966) and is one of the important diseases of cassava with high incidence and severity in most cassava growing areas (Cudjoe *et al.,* 2005; Lamptey, Okoli, & Frimpong-Manso 1998; Torkpo & Offei 2007, Torkpo, Gafni, Danquah, & Offei 2018). In Ghana, the Africa Cassava Mosaic Virus and East Africa Cassava Mosaic Virus are the two species of CMGs that have been identified (Lamptey *et al.*, 2012; Offei *et al.*, 1999).

Spread and transmission of CMD

CMD has spread across Africa by transmission through the whitefly (*Bemisia tabacci* (Gennadius) vector (Maruthi, Colvin, Seal, Gibson, & Cooper,

(2002b) and mechanically through use of infected planting materials (Alabi, Kumar, & Naidu, 2011).

Besides *Manihot esculenta,* CMD can be transmitted between other plant members of the *Euphorbiaceae* family (*Jatropha curcas*, *Manihot glaziovii* and *Ricinus communis)* (Lister, 1959), *Fabaceae* family (*Centrosema pubescens*, *Glycine max*, *Leucaena leucocephala*, *Pueraria phaseoloides* and *Senna occidentallis*) (CABI, 2020) as well as *Nicotiana benthamiana*, *N. clevelandii* and *Datura stramonium* (Lister 1959; CABI, 2020).

The use of infected planting material is one of the effective means of spread of CMD. In Africa and Ghana, most farmers re-use material from their own cassava farms or collect from other farmers' fields. Others obtain planting materials from outside their locality (and name the material after that locality), from extension officers or from non-governmental organizations (NGOs) (Thresh *et al.,* 1998). This practice has led to an increase in virus inoculum even in regions where there is little or no vector population (Thresh *et al.,* 1998; Chikoti, Melis, & Shanahan, 2016).

The lack of farmer awareness on the devastating effect of CMD on cassava yields (Chikoti *et al.*, 2016) and unavailability of disease-free materials to use as a check (Thresh *et al.,* 1998; Szyniszewska, Chikoti, Tembo, Mulenga, Gilligan, van den Bosch, & McQuaid, 2021) have accounted for the spread of CMD across Africa and Ghana.

For secondary transmission of CMD, the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) (Maruthi, Jeremiah, Mohammed, & Legg, 2017) has been the main vector although other whitefly species, such as *B. afer* can also transmit CMD (Palaniswami, Nair, Pillai, & Thankappan 1996; Fondong, Pita,

Rey, de Kochko, Beachy, & Fauquet, 2000a). There have been arguments against the transmission of CMGs by whiteflies and the percentage of CMD infection attributed to the vector (Dubern 1994). However, vector transmission studies have shown that 3% of CMD infection out of an infection level of 27% in cassava fields in Tanzania could be attributed to *B. tabacci* (Legg & Raya, 1998). Furthermore, Maruthi, Colvin, Seal, & Thresh (2002a, 2002b) recorded 60% to 79% of CMD infection as a result of *B. tabacci* transmission.

As a polyphagous vector, *B. tabacci* can infect over 500 species including herbaceous and annuals from about 74 families (Brown, Frohlich, & Rosell 1995; Cossa, 2011). The hosts of *B. tabacci* include cassava, pepper, avocado, banana, cabbage, soybean, tomato among others (Palaniswami, & Henneberry, 2011). Indirectly, whiteflies are known to transmit more than 150 plant viruses with majority belonging to the genus begomovirus (family *geminiviridae*) (Khan, Ghani, Ghaffar, & Tamkeen, 2011; Navas-Castillo, Fiallo-Olive, & Sanchez-Campos., 2011; Horowitz, Antignus, & Gerling, 2015; Smith, Seijo, Vallad, Peres, & Druffel, 2015). Thus, *B. tabacci* exist as a species complex causing extensive damage to important crops directly or indirectly.

Biologically, certain characteristics of *B. tabacci* such as, high reproductive rate, multivoltism (multiple generations per year), ability to migrate long distances and broad host range (Gerling, Alomar, & Arno, 2001) have made control and management a challenge worldwide. According to Legg., French, Rogan, Okao-Okuja, Brown, (2002) and Legg, Sseruwagi, Boniface, Okao-Okuja, Shirima, & Bigirimana (2013), high incidence of the two major virus diseases of cassava (Cassava Mosaic Disease and Cassava

brown streak virus) in sub-Saharan Africa can be attributed to increase in whitefly population.

Economic importance of CMD in Africa

Whitefly-transmitted geminivirus diseases can cause substantial, and sometimes complete, yield losses of important food and industrial crops (Morales & Anderson, 2001). According to Thresh and Cooter (2005) the effect of CMD on yield is dependent on the variety. Cours (1951) reported a direct relationship between CMD severity and vegetative growth and tuber root yield. Fargette, Fauquet and Thouvenel (1988) observed that the effect of CMD is dependent on the source of planting materials and/ or the timing of whitefly infection. Plants grown from CMD-infected cuttings display severe symptoms than plants of the same variety infected by whiteflies (Thresh *et al.*, 1994). Healthy or clean materials however experience little to no damage (Fargette *et al.*, 1988).

CMG-induced symptoms range from moderate to extreme based on the strain of viruses, isolates, cassava cultivars and environmental factors. Cassava plants, which display mild symptoms, usually grow with leaves displaying moderate, light-green mosaic symptoms (Fauquet & Fargette, 1990; Pita *et al.*, 2001a). Plants with serious symptoms exhibit excessive leaf shrinking along with distortion at the bottom of the leaflets. It is therefore important to consider the incidence, severity, prevalence, productivity and sensitivity of cassava varieties in order to estimate losses due to CMD (Fargette *et al.*, 1988, Otim-Nape, Shaw, & Thresh 1994; Legg, & Fauquet 2004).

CMD reduces cassava yields in Africa by 15 to 24 percent, equivalent to 12 to 13 million tons per year (Thresh *et al.*, 1997). The extreme CMD

epidemic in Uganda for instance resulted in a decline in the area planted to cassava from 26,000 ha in 1989 to 3,000 ha in 1990 (Otim-Nape *et al.*, 2000).

Genome organization of Cassava Mosaic Geminiviruses

The genome of CMGs is bipartite, made up of DNA-A and DNA-B each 2.7 - 2.8kb in size (Hanley-Bowdoin, Settlage Orozco, & Robertson, 1999; Stanley *et al.*, 2005). Among the two, the DNA-A was sequenced first and encodes six genes (AV2, AVI, AC1, AC2, AC3 and AC4). The AV1 which codes for the coat protein is important in vector transmission and also plays a role in genome encapsulation.

The DNA-B has less conserved regions making it more diverse than the DNA-A which has more conserved region (Rybicki, 1994; Harrison & Robinson, 1999). The DNA-B set of genes (BV1 nuclear shuttle protein and BC1, movement protein) are responsible for intra and intercellular movement (Etessami, Callis, Ellwood, Stanley, 1988; Ntui, Kong, Khan, Igawa, Janvi, Nakamura, 2015).

Both genomes have an intergenic region which is highly variable but contains the invariable nonanucleotide sequence TAATATT/AC which is required for initiation of replication by the Rep protein (Hanley-Bowdoin, Settlage, Orozco, Nagar, & Robertson 1999). Replication in geminiviruses depends on their host nuclear DNA replication machinery since they do not encode their own DNA polymerases (Gutierrez, 2000). CMGs replicate their circular genomes in two phases; the ssDNA genome is first converted into an intermediate dsDNA product by cellular enzymes (Saunders, Lucy & Stanley, 1992) before the viral Rep protein together with other cellular factors produce dsDNA and ssDNA viral forms through rolling circle mechanism (Stenger,

Revington, Stevenson, & Bisaro 1991; Heyraud, Matzeit, Kamman, Schaefer, Schell, & Gronenborn 1993a; Stanley, 1995). To achieve systemic infection, viruses must transport their genomes from an infected cell via the plasmodesmata to other parts of the plant, overcoming borders between non-vascular and vascular tissues (Carrington, Kasschau, Mahajan, & Schaad, 1996).

The role of recombination and pseudo recombination in viral pathogenicity

There is great diversity among *geminivirus* genomes and for that matter begomoviruses due to recombination or pseudo-recombination (Preiss & Jeske 2003, Saeed & Samad, 2017). Recombination is the sharing of genetic material between two nucleotide sequences (Posada & Crandall, 2001), an essential mechanism that affects biological evolution of species, especially viruses. Cassava Mosaic Geminiviruses (CMGs) provide evidence of recombination in their DNA-A and DNA-B genome components (Padidam, Beachy, & Fauquet 1999; Pita, Fondong, Sangare, Kokora, & Fauquet 2001b).

For CMGs, the ACMV species demonstrates a high degree of homology regardless of where it is located compared to EACMV-like viruses, for which there is frequent recombination and considerable variation (Pita *et al.*, 2001a). Recombination between the DNA-A of ACMV and EACMV resulted in the formation of the hyper-virulent recombinant EACMV-Uganda, which caused severe CMD epidemic in Uganda (Deng, Otim-Nape, Sangaré, Ogwal, Beach, & Fauquet, 1997; Zhou, Liu, Calvert, Munoz, Otim-Nape, Robinson, & Harrison 1997). Similarly, evidence for recombination was detected in the EACMV-Cameroon virus (EACMCV) (Fondong *et al.*, 2000a).

Pseudo-recombination is the sharing of all genomic materials between similar isolates and separate species (Stanley & Townsend, 1986; Hou & Gilbertson, 1996). Evidence of this was reported by Pita *et al.*, (2001a) in Uganda where pseudo-recombination in EACMV resulted in the EACMV-UG2 DNA-A and EACMV-UG3. Briddon, Liu, Pinner and Markham (1998) also found an artificial pseudo-recombination between ACMV-Nigeria and ACMV-Kenya which resulted in the ACMV-Nigeria (DNA-A) and ACMV-Kenya (DNA-B) being non-infectious. However, ACMV-Kenya (DNA-A) and ACMV-Nigeria (DNA-B) exhibited severe mosaic symptoms on cassava with their progeny being transmissible by *B. tabaci*. Mixed infections of CMGs (ACMV and EACMV) have become common in farmer fields increasing the probability for recombination and evolution of new virulent species.

Furthermore, the presence of satellite molecules have contributed to diversification and emergence of new strains that are able to overcome host resistance (Garcia-Arenal & McDonald, 2003). This genetic recombination usually occurs in the intergenic region (Tsai, Hu, Shung, Lee, Wang, Kenyon, 2011).

Detection and characterization of Cassava Mosaic Geminiviruses

CMGs are detected and characterized using two methods namely; serological and nucleic acid based methods. The nucleic acid method is based on characterization of nucleotide sequences in genomic DNA molecules of CMGs while the serological method is based on the reaction of CMGs with a panel of monoclonal antibodies (Hong *et al.*, 1993; Stanley & Gay, 1983; Zhou *et al.*, 1997; Swanson & Harrison, 1994).

Serological methods

Serological studies give proof of the diversity of geminiviruses that infect cassava by the use of triple (TAS) and double (DAS) antibody sandwiches (Sequeira & Harrison, 1982; Swanson & Harrison, 1994). These tests can be done by using two types of antisera, either polyclonal or monoclonal. The polyclonal antisera contain antibodies that binds specifically to targets of interest, hence it can detect several viruses. Polyclonal antisera can be used to distinguish several pathogenic strains although sensitivity could be problematic (Hull, 2009). On the other hand, monoclonal antisera contain antibody that detects one specific virus, meaning its antibody is for only one epitope (Hull, 2009). This method has been used extensively and effectively to distinguish between ACMV, EACMV and other plant viruses (Zhou *et al.*, 1997).

Nucleic acid-based methods

This is by use of either RNA or DNA to diagnose viruses. Nucleic acid-based methods have been extensively used for detection of CMG's diagnosis (Zhou *et al.*, 1997).

While nucleic acid hybridization tests are feasible, the increased specificity and sensitivity of polymerase chain reaction (PCR) make it the recommended assay in several cases. Coupled with the occurrence of recombination in CMGs, PCR is mostly recommended (Zhou *et al.*, 1997). About three methods have been used in PCR (Zhou *et al.*, 1997).

One strategy is based on the use of primers based on nucleotide sequences that do not exist in other CMG's meaning only the target virus is detected (Deng *et al.*, 1994).

In another method, degenerate primers based on sequences that exist in many CMG's are used in PCR and the viruses are characterized by the pattern of fragments obtained by endonuclease treatment restriction

Heteroduplex mobility assays (HMA's) is another useful method used in differentiating CMGs (Berry & Rey, 2001). This strategy is sensitive, fast and can detect virus mixtures in cassava grown in the field. However, this technique is not commonly used for the detection of CMG's because it is restricted by use of a single primer pair that might not identify recombination of large DNA fragments in many genomic regions (Berry & Rey, 2001). Hence it may require the use of such a wide variety of reference samples to classify unidentified isolates more easily and in detail.

Management of CMD

Generally, there are three approaches to reducing losses due to CMD which include removal of infected crops; delaying infection (by planting late or early) to later stages of crop growth and reducing the extent of damage sustained after infection has occurred (Thresh & Cooter, 2005). According to Thresh (2003), CMD can be managed by implementing phytosanitary measures, use of disease-resistant varieties, cultural practices, vector control and mild-strain defense (Thresh, 2003).

Phytosanitary Measures

This involves improving the health status of planting material as well as removal of other sources of inoculum from further spread through whitefly vector activity.

This involves crop hygiene; the removal of all diseased cassava plants or other host plants to reduce the potential transfer of pests and pathogens to

newly planted fields , the use of CMD-free stem cuttings as a vegetative planting material and the exclusion (roguing) of diseased plants from within the field (Thresh & Cooter, 2005). Also, it is important to clear crop residues and other host plants of Cassava Mosaic Geminiviruses (CMGs) such tree cassava (*Manihot glaziovii*), *Jatropha spp.* and others from newly planted fields (Sserubombwe, Briddon, Baguma, Ssemakula, Bull, Bua, Alicai, Omongo, Otim-Nape, & Stanley, 2008).

Another form of phytosanitation on farms involves the removal of the apical portions of cassava plants known as de-topping (Pacumbaba, 1987). This method encourages the growth of fresh leaves which may not show virus symptoms due to the systemic nature of the infection (Pacumbaba, 1987). However, this practice helps in the build-up of whitefly population probably due to the presence of fresh leaves (Ariyo, Dixon, Atiri, 2003).

Planting CMD-free materials

Using healthy materials for planting is an effective method to control virus diseases. Healthy cuttings develop more readily and grow faster than infected ones which can translate to yield (Thresh & Cooter, 2005). The use of disease- free materials should be followed by sanitation to prevent or delay the onset of CMD (Thresh & Cooter, 2005)

However, there are a number of challenges that must be addressed for this management practice to be effective. First is the easy accessibility to sufficient stock of CMD-free planting materials which are affordable to farmers (Thresh & Cooter, 2005). Another challenge associated with the use of CMD-free material is latency of CMD symptoms in resistant varieties especially during the selection of materials (Thresh & Cooter 2005). It is therefore

important to select cuttings from an area of the field where there is low incidence of CMD or stock grown in relative isolation (Thresh & Cooter 2005). This method has proven to produce low incidence of CMD in cassava plants (Otim-Nape, Bua, Thresh, Baguma, Ogwal, Ssemakula, Acola…Martin, 2000). Another approach to solving this challenge is by the selection of cuttings from symptomless plants and the upper portion of the stem because recovery from CMD starts from the upper part of the plant (Fondong, Thresh, & Fauquet, 2000b).

Rouging

Rouging is one of the approved practices for managing CMD. Rouging involves getting rid of diseased plants in a crop stand which can serve as a source of inoculum for spread of disease. This was evident in the studies carried out by Colvin, Otim-Nape, Holt, Omongo, Seal, Stevenson, Gibson…Thresh (1999); Fondong, Thresh, and Zok, (2002), where incidence and severity of whitefly and CMD were higher in diseased plants than healthy plants. Weekly rouging of CMD-infected plants in the field for two to three months after planting is encouraged (Guthrie, 1990) in both cassava multiplication centres and farmers' fields. Furthermore, rouging is more effective when adopted by many farmers in a well-planned programme. However, in fields where CMD-susceptible varieties are planted, rouging becomes difficult due to the high incidence of CMD (Thresh & Cooter 2005).

Use of resistant varieties

Management of virus diseases by use of resistant varieties has shown to reduce incidence and severity of begomovirus diseases in cassava (Colvin,

Nagaraju, Moreno-Leguizamon, Govindappa, Reddy, Padmaja…Muniyappa 2012).

Resistance breeding studies of cassava started in the 1920s through intraspecific crosses between cassava varieties and other species, notable between *M. esculenta* × *M glaziovii* (Thresh & Cooter 2005). Breeding programmes initiated by IITA generated a collection of genotypes termed Tropical Manihot Series (TMS) which was deployed to farmers in Nigeria and parts of Africa (Nweke, Spencer & Lynam, 2002). Some of these varieties (TMS 303337, TMS 30395, TMS 30572 and TMS 30003) showed some resistance to CMD but resulted in yield losses when they were infected (Thresh & Cooter 2005). To improve the level of resistance, IITA embarked on another breeding programme using landraces from Nigeria and other African countries. These resistant varieties were called Tropical Manihot esculenta (TME) and exhibited stability to CMD infection under compromised environmental conditions (Thresh & Cooter 2005). The crossing of the TMS and TME lines led to the introduction of the resistance genes CMD1 and CMD2 into the local varieties. Besides these varieties and hybrids generated through inter and intraspecific crosses, CMD-resistant materials were collected from farmers' fields (Thresh & Cooter 2005).

Famers' knowledge on viral disease

Management of plant viruses is a major problem to farmers due to the similarity in symptoms caused by viruses with soil deficiency-like symptoms (Schreinemachers, Balasubramaniam, Boopathi. Viet, Ha. Kenyon…Wu 2015). This makes it challenging for farmers to identify and manage virus diseases in the field. For example, a farmer who may have experienced symptoms of a

particular virus may not have knowledge when such a virus presents a different etiology and epidemiology (Van den Bosch, Jeger., & Gilligan, 2007; Jones 2014; Islam, 2017).

A number of surveys have been conducted to identify and assess farmers' knowledge and perceptions on plant viruses and their management. Findings from these surveys show that majority of farmers could not correctly diagnose and/ or manage the disease (Adams, Lefkowitz, King, Harrach, Harrison, Knowles…2017; Islam, 2017) resulting in misapplication of pesticides causing financial loss to the farmer since such infected crops are left unmanaged. Even those who had received some training on the same disease could not diagnose and manage the virus. These findings support with a similar study by Asare-Bediako, Wonkyi-Mensah, van der Puije, Amenorpe and Osei (2017) on Tomato Yellow Leaf curl Virus, where 92.6 % out of 150 farmers were able to identify the symptoms of the disease using pictures but could not diagnose the correct causative virus.

Knowledge and access to improved crop varieties

CMD is transmitted by whiteflies and use of infected planting materials. The transmission of CMD by whiteflies is known to be low and directly proportional to the number of whiteflies (Houngue *et al.*, 2019). Paramount in the management of CMD is the use of clean and resistant varieties. This is very essential since there is a close correlation between infected materials and incidence of CMD (Houngue *et al.*, 2019). A study by Gibson (2006) revealed that many farmers have knowledge on the names of local varieties compared to the resistant varieties. These names varied from locality to locality based on factors such as degree of disease resistance, yielding ability of the variety, its

origin, the name of the individual who introduced the variety to the region for the first time, morphological traits of the variety or growth habit, flowering habit, maturity period among others.

It was widely observed that the farmers still cultivate their own varieties of cassava which was mainly due to scarcity of resistant materials for planting (Gibson, 2006). It is known that farmers who are in Farmer Base Organizations (FBOs) and involved in multiplying these resistant varieties distribute them to their members. They however do this at a fee to generate income for their Farmer Base Organization (Gibson, 2006). Those who could not afford, sourced their planting materials from their own farms or from family and friends (Gibson, 2006). However, most farmers prefer to plant local available varieties other than adopting resistant and improved varieties provided by stakeholders due to low adoption of improved varieties (Gibson, 2006).

Factors influencing adoption of Improved Crop Varieties (ICVs)

Studies on factors that affect adoption of improved crop varieties (ICV) have centred on demographics or household factors, farm characteristics and institutional factors (Ouma & De Groote, 2011; Nmadu, Sallawu, & Omojeso, 2015; Denkyirah, Okoffo, Adu, Aziz, & Ofori, 2016).

A number of demographic characteristics influence the adoption of ICVs. These include age of farmer, number of years in farming, family size, level of education among others (Thomson, Gelson, & Elias, 2014).

Age is a major factor that indirectly affects farmers' adoption level since older farmers have more experience. Kaliba, Verkuijl and Mwangi (2000) and FAO (2013) reported that farmers' adoption level decreases with age. This means that, older farmers are more risk averse, conserved and therefore prefer

known or local varieties. This however contradicts with Islam *et al.* (2012) who reported that experienced farmers are likely to adopt new varieties. Danso-Abbeam, Antwi Bosiako, Ehiakpor, & Nantui (2017), in a study with maize farmers observed that experience in farming was a major contributing factor to adoption of improved varieties. He revealed that farmers with less experience will adopt only improved crop varieties while those with more years of experience will continue to plant their local varieties and still adopt improved varieties.

Family size is a very important factor that determines farmers adoption. Most agricultural technologies come with a change in how farmers practice agriculture and may require more farm hands (frequent weeding, spacing, pesticide and fertilizer application etc). According to Danso-Abbeam *et al.* (2017), the probability of maize farmers adopting Improved Maize Varieties (IMVs) was 1.13, which means that anytime one individual is added to a household, the adoption level of the household in adopting ICV increase by 1.13 times. In a study by Sodjinou, Glin, Nicolay, Tovignan and Hinvi (2015), it was seen that households with high number of people agreed to adopt the cultivation of organic cotton compared with households with lower number of people. Afolami, Obayelu, & Vaughan (2015) gathered that households that have technological gadget like television, mobile phones, radios, among others increase farmer's accessibility to information and therefore positively influenced adoption level.

In terms of education, there is a positive and significant relationship between adoption and education. Danso-Abbeam *et al.* (2017), Diiro, Ker, & Sam (2015) and Gebresilassie & Bekele, (2015) affirmed that farmers who are

educated can easily process, understand and look for information on improved varieties which can increase their level of adoption. Again, access to seminars, workshops, farmers day schools among others will help farmers be aware of new varieties and their benefits, thus, increasing their confidence to adopt improved crop varieties.

With respect to farm conditions, long distance from farmers house to field has been seen to be a major limiting factor influencing adoption. This is because, farmers may have to walk long hours carrying improved planting materials to their fields or spending money transporting improved planting materials. This according to Danso-Abbeam *et al.* (2017) discourages farmers from adopting improved crop varieties as shown by negative relationship between distance and level of adoption. Again, distance from farmer's community to where farmer can access improved varieties and other inputs also affects adoption level (Fisher & Carr, 2015). Returns (income and yield) from previous varieties also influence adoption level. Farmers who made less profits from growing local varieties compared to improved varieties are more likely to adopt new and improved varieties (Danso-Abbeam *et al.*, 2017).

Extension contact is very important in shaping the decision-making process of farmers. Farmers get first line information on improved varieties, their benefit and how to get access to improved varieties from extension services. Extension interaction helps to inform and provide answers to questions regarding agricultural innovation (Danso-Abbeam *et al.*, 2017). The extension contact should be coupled with on-farm trials, demonstration farms and other hands-on activities to build farmers technical abilities and skills. These lead to an increase in adoption of technologies by farmers (Danso-Abbeam *et al.*,

2017). Another institutional factor is the membership to farmer-based organization (FBO). Stakeholders as well as extension officers are encouraged to help farmers' form and mange farmer-based organizations (Salifu Francesconi, Kolavalli, 2010; AgSSIP, 2007). A study by Mmbando and Baiyegunhi (2016) found a positive relationship between membership to FBO and level of adoption of improved maize varieties in Tanzania. This relationship was confirmed by Danso-Abbeam *et al.*, (2017) in Ghana. However, Ahmed and Anang (2019) realized that the influence of FBO on the level of adoption depends on knowledge and effectiveness of the leadership of the group. If the leaders have enough knowledge and information on the said technology and are able to influence their members then, this positive relationship will be achieved.

In conclusion, many farmers do not have knowledge on the causative species and management of virus diseases, a number of them are able to identify symptoms of plant virus disease in the field. Therefore, this study recommends that breeding for resistance to virus diseases should be in collaboration with farmers and farmer groups or cooperatives. Furthermore, education of farmers on the benefits of adopting new and improved varieties through extension agents should be an integral part of breeding programmes.

CHAPTER THREE

ASSESSING FARMERS' KNOWLEDGE OF CASSAVA MOSAIC DISEASE (CMD), MANAGEMENT PRACTICES, SOURCES AND USE OF IMPROVED CASSAVA VARIETIES IN THE VOLTA REGION OF GHANA

Introduction

Cassava is one of the most economical and widely grown root crops in tropical and sub-tropical Africa (Ambang, Amougou, Ndongo, Nantia, Nyobe, Ongono, 2007). Owing to its ability to withstand harsh environmental conditions, it remains the most adopted staple food in the world providing calories to about 800 million people (El-Sharkawy *et al.*, 2006; FAO, 2013). West Africa contributes 58% of Africa's total production (FAOSTAT, 2016).

Ghana is Africa's third-largest producer of cassava and has a global share of 6.3% (MoFA, 2009; Shipman, 2017; Nsiah-Frimpong *et al.*, 2021). According to MoFA, (2009), the per capita consumption of cassava for Ghana is 152.9 kg per year (Shipman, 2017; MoFA, 2009). Cassava contributes 46% to Ghanas' agricultural GDP (Nag, 2017). It has been identified as 'food for the poor because of its in-ground storage capacity and flexibility that allows flexible harvesting to ensure all-year-round food supply (FAO, 2013; Sanginga & Mbabu, 2015;Torkpo *et al.*, 2017). Domestic production grew from 15,989,940 to 19,137,94 metric tons in 2017 attributable to the flagship program (Planting for food and Jobs) of the Government of Ghana (MoFA, 2017; Nsiah Frimpong *et al.*, 2021). Despite its potential, the productivity of cassava is still below the optimum level in African smallholder farming systems (Elegba, Appiah, Azu,

Afful, Agbemavor, Agyei-Amponsah…Danso, 2013; Nsiah Frimpong *et al.*, 2021).

In Ghana, Cassava mosaic virus disease is one of the main reasons for the low productivity of cassava (Moses, Oppong, & Lamptey, 2015; Nsiah Frimpong *et al.*, 2021). This is done to the increased cross-border trade, the movement of people from one country to another and the exchange of planting materials among farmers and countries resulting from regional integration (Echodu, Edema, Wokorach, Zawedde, Otim, Luambano…Asiimwe 2019).

With the rising pest-and disease-related losses, the acceptance of improved crop varieties and proper management practices in Ghana have not been inspiring, presumably, because farmers have little to or no access to quality and improved crop varieties and little awareness of pests and diseases as well as relevant management practices. This accession has been confirmed in studies by Akyeampong (2009), Cudjoe, Gyamenah and Braima (2005) and Manu-Aduening, Lamboll, Mensah & Gibson (2007).

Farmers' knowledge is described as the body of knowledge established by farmers through interaction between indigenous and scientific knowledge, and for which they continually adjust their knowledge based on changes in environmental, socio-cultural and political conditions (van Mele, 2000). This knowledge can either increase or decrease plant disease incidence. For example, losses in Faba beans due to chocolate spot disease reached 100% as a result of farmers ascribing their causes to increased soil humidity (Kiros Meles & Abang, 2008). This means that human survival is partly dependent on farmers' awareness of crop diseases (Bentley & Thiele, 1999).

The epidemiology of a disease in an area is partly influenced by perception and knowledge of farmers (Sherwood 1997). It is an area where farmers, extensionists and researchers can reap tremendous benefits from closer cooperation through exchange of knowledge (Sherwood, 1997; Meles-Kiros & Abang, 2008). Perceptions and knowledge of farmers will inform researchers about farmers' attitude in the management of plant disease (Sherwood, 1997).

As shown by Bentley and Thiele (1999), scientists often have to contact farmers to find out about their knowledge of certain diseases to promote further partnership between farmers and scientists in the effective management of diseases. This partnership also helps in the integration of traditional and scientific information systems that support farmers and scientists in developing effective disease management practices (van Mele, 2000). Morse and Buhler (1997) reiterated the point that the synthesis of scientific and traditional knowledge is important to achieving sustainable agriculture and for that matter pest management.

Beyond synthesis of the two types of knowledge in the implementation of new management strategies or technologies, another critical issue is adoption (Bentley & Thiele, 1999; van Mele, 2000). Adoption of a new technology by farmers is usually driven by a combination of many factors (Bentley & Thiele, 1999; van Mele, 2000). Adoption plays a crucial role in the socio-economic aspect of plant disease management (Bentley & Thiele, 1999; van Mele, 2000). The critical issues are the attitudes, perceptions and awareness of farmers about the latest technologies (Bentley & Thiele, 1999; van Mele, 2000). There have been variations in choices of attribute depending on the farmer's economic position,

geographical area and his / her agricultural objective (Bentley & Thiele, 1999; van Mele, 2000).

A number of management strategies including release of resistant and improved varieties have been put in place to limit spread of CMD but these improved cassava varieties are mostly poorly adopted, farmers have not shifted and continue to use existing local planting materials vulnerable to pests and diseases (Salum, 2016). According Salum, (2016), only about 2% out of 500,000 farmers in Zanzibar adopted improved varieties for the management of CMD. Low adoption of technologies is one reason why new technologies have weak impacts (Michelles, 2005).

In managing plant diseases, it is vital to assess the awareness, perceptions and management practices of farmers, which are useful for developing research goals, finding disparities between the knowledge of farmers and scientists in order to plan strategies and other communication avenues to bridge information gap between the mentioned stakeholders (van Mele, 2000). Knowing the knowledge gaps among farmers is an essential step in the process of developing a pest management programme (van Mele, 2000).

In this study, we assessed farmers' knowledge of CMD and management practices in six key cassava-growing districts in the Volta Region of Ghana. In addition, we assessed farmers' knowledge on the sources and use of improved varieties to manage or control CMD in Ghana. Although farmers had some knowledge on CMD, they did not implement management practices on their fields to control CMD.

Materials and methods

Study areas

The research was carried out in six key cassava growing districts in the Volta Region of Ghana. The districts are Akatsi South, Addaklu District (coastal savannah), Hohoe Municipality, Afadjato South Municipality (forest zone), Krachi East and Krachi Nchumuru districts (guinea savannah). These districts were selected because they have the characteristics of majority of most of the major agro ecological zones in Ghana (coastal savannah, forest zone and the guinea savannah).

Description of study districts

Akatsi South District

Akatsi South District was created from the old Akatsi District and has a population of 93,477 people (MoFA, 2020). It lies between latitude 6° S - 7° N and 0° W - 1° E, in the south-eastern part of the Volta Region. The district is surrounded by the Keta, Ketu East, South Tongu and Adaklu-Anyigbe Districts. It has a rural economy with agriculture accounting for about 75.5%, commerce services and industry accounting for 18.35%, 3.5 % and 2.7% respectively (MoFA, 2020). In the district, crop production is primarily at the subsistence level with cassava, maize, sweet potatoes, cowpea, pepper, tomato, garden eggs, okra, groundnut and tobacco being the common crops grown. There are a number of projects that have been introduced by the government and NGOs to boost the production of cassava. Such programmes include Export Marketing and Quality Awareness Project (EMQAP), Root and Tuber Improvement and Marketing Programme (RTIMP) and West Africa Agricultural Productivity Programme (WAAPP) (MoFA, 2020).

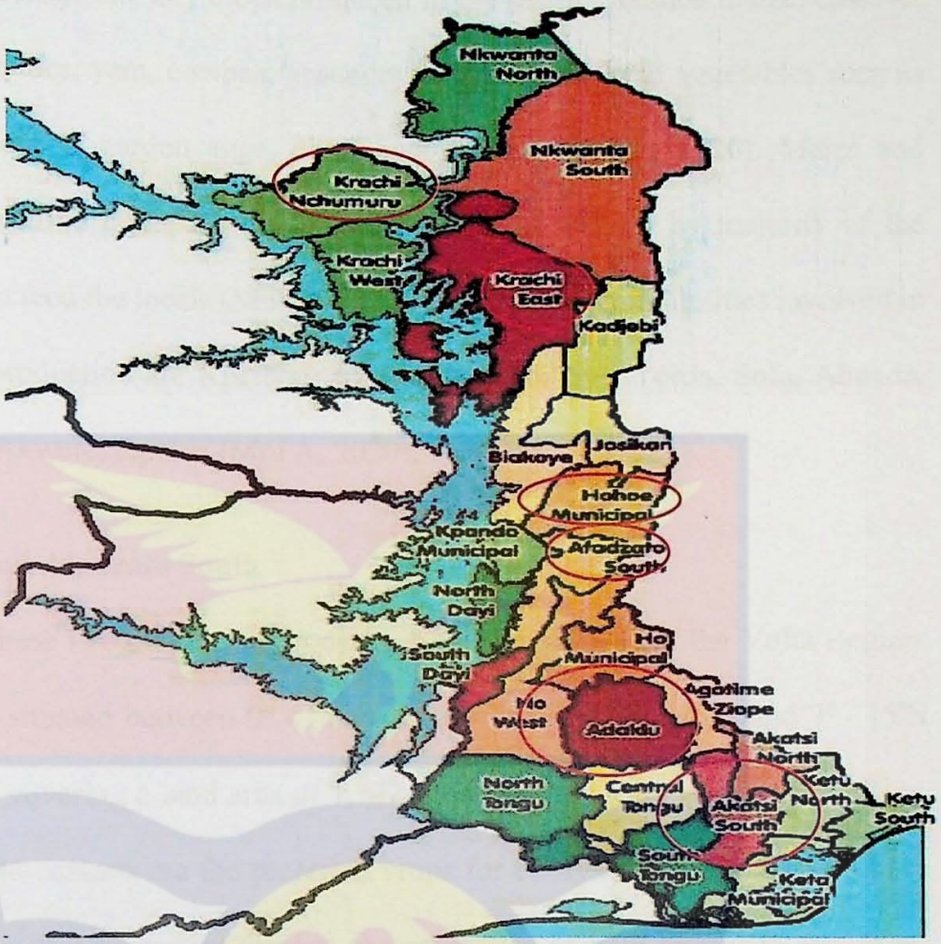


Figure 3.1: Map of the Volta Region of Ghana indicating the study areas

Adaklu District

Adaklu is a new created district with its mother district being Adaklu-Anyigbe. It lies between latitude 6° - 161 and latitude 6° - 371 North of the Equator and longitude 0° - 241 and 0° - 501 East of the Greenwich meridian. It has both coastal and transitional ecology (MoFA, 2020). Throughout the year, temperatures are high with mean monthly temperatures varying from 22°C to 32°C. Average temperatures during the dry season are so high that the planting of food crops are kept at a minimum (MoFA, 2020). The district's mean annual rainfall is about 850 mm-1000 mm with the peak in June and the lowest in December and increasing from the coastal to the transitional part. Agricultural production in the district is dominated by production of crops and livestock

(MoFA, 2020). The key crops produced in the district include maize, cassava, sweet potatoes, yam, cowpea, groundnuts, exotic and local vegetables such as tomato pepper, garden eggs, okro, and melons (MoFA, 2020). Maize and cassava are the main commodities grown in the district by majority of the farmers to feed the locals (MoFA, 2020). Some of the communities involved in cassava production are Kpeleho, Aziedukope, Hlihave, Torda, Sofa, Ahunda, Ablonu, Akwete, Kpetoe (MoFA, 2020).

Hohoe and Afadzato South

These two districts are located in the central part of the Volta Region. They are situated between 0° - 15'E and 0° - 45'E and 6° - 45'N and 7° - 15'N latitudes, covering a land area of 1,172 square kilometers (117, 200 ha). Rice, maize, and cassava are the preferred crops for cultivation. Both districts have four major soil types that display characteristics of both savannah and forest zones, which facilitate the cultivation of crops such as cocoa, coffee, oil palm among others. It has a bimodal rainfall cycle with annual rainfall ranging from 1100 mm to 1500 mm. Heavy rainfall begins from April to July, while the minor season starts from September to November (MoFA, 2020). Some communities involved in the production of cassava include Santrokofi, Have, Loba, Gbi Woebe, Goviefe kowu, Fodome, Fodome Ve, Alavanyo, Nyagbo, Tafi, and Akpafu (MoFA, 2020). Cassava production for the two districts is around 75,000 metric tons. There are a number of programmes to support cassava production this includes the Root and Tuber Improvement and Marketing Programme (RTIMP) and West Africa Agricultural Productivity Programme (WAAPP) (MoFA, 2020). Cassava is used for starch, gari, tapioca and biscuits (MoFA, 2020).

Krachi-Nchumuro

The municipal was carved out of the old Krachi West. It lies between Latitude 70 °-4 ° N and 80 °-25 ° N and longitude 25 ° W and 20 °E of the Volta Region of Ghana (Ghana districts, 2009). It is one of the most important marketing hubs for agricultural products in the Volta Region as well as in Ghana and a major producer of crops such as yam, cassava, maize, rice, sorghum, soybean and vegetables (MoFA, 2020). It occupies a land area of 2969 square kilometers. The mean maximum temperature is 30 ^{0}C which is recorded in March, while the mean minimum temperature is 25.5 ^{0}C recorded during the rainy season in August (MoFA, 2020. The annual rainfall indicates incoherencies ranging from 1,735,20 mm to 970,50 mm (MoFA, 2020). The district produces approximately 174,000 tons of cassava per annum with the potential to increase to 800,000 metric tons within one year (MoFA, 2020). Cassava in the district is usually processed into starch, gari, tapioca, animal feed and for domestic purposes (MoFA, 2020). Programmes like the Root and Tuber Improvement and Marketing Programme (RTIMP) and West Africa Agricultural Productivity Programme (WAAPP) have been introduced to improve cassava production (MoFA, 2020).

Sample size and sampling techniques

A total of 180 cassava farmers were selected from the six districts for the study using different sampling procedures such as multistage random sampling, purposive sampling and convenience sampling to select the respondents. These sampling methods were selected taking into consideration factors such as time, cost and degree of accuracy. The multistage random sampling was used to select the districts in Volta Region, communities within

the districts and households within the communities. Six districts were randomly selected from several cassava-producing districts in the Volta Region during the first level. In the second level, 18 cassava-producing communities (3 from each of the six districts) were purposively selected. Ten cassava-growing households from each community were chosen by convenience (Danso-Abbeam *et al.*, 2017).

Data collection

Pretesting of the questionnaires and the interview schedules were done in Ohawu township in the Ketu North District since it has characteristics similar to the study areas. Due to the language barrier, an interpreter was sought to assist in the data collection. Data on farmers' awareness and knowledge on CMD, management practices, use of improved varieties and sources of planting materials were obtained by the use of questionnaires and interview schedules. Cards bearing pictures of plants showing typical CMD symptoms were shown to farmers to aid disease identification. Before showing these cards, farmers were asked questions to gauge their knowledge of CMD. Demographic data were collected for all the respondents.

Table 3.1: Agro-ecological zones, districts and communities for the study

Agro-ecological zones	Districts	Communities
Coastal savannah	Akatsi South	Gefia
		Abedrafor,
		Atidzive
	Adaklu	Kpeleho
		Aziedukope
		Hlihave
Forest	Hohoe	Santrokofi
		Fodome
		Gbi-Woebe
	Afadzato South	Have Ando
		Goviefe kowu
		Logba
Guinea savannah	Krachi east	Kparikpari
		Anyanbor
		Yariga
	Krachi Nchumuru	Chinderi
		Lumbusu
		Boreae

Data analysis

Data were analysed with statistical packages for social sciences (SPSS), version 16 and Microsoft Office Excel, version 2010. Socio-economic characteristics were defined using descriptive statistics such as percentage, frequency, distribution tables, chi-square, among others.

Results

Demographic characteristics of cassava farmers

Table 3.2 summarizes the socioeconomic characteristics of cassava farmers in the study communities. From the results, 128 respondents (71.1 %) were male and 52 respondents (28.9 %) were female.

A majority (60%) of farmers interviewed were old (between the ages 50-69) with only 17 respondents (9.4%) between the ages of 30 - 39. Farmers between the ages of 40 - 49 constituted 30.6% of respondents. Of the total number of farmers interviewed, 47.2% had non-formal education with 52.8% having some level of formal education. Out of the 47.2% that had non-formal education, 96.5% representing 82 respondents had literacy training with 3.5% representing three farmers having numeracy training. Interestingly, a majority (58.3%) of respondents with formal education were primary school graduates while only 4.2% of the respondent had tertiary education. About 26.3% of farmers had Junior High School Certificate or Middle School Certificate and 11.5% had Senior High School Certificate.

Table 3.2: Demographic characteristics of cassava farmers

Variable	Frequency	Percentage
a) Sex of the respondent		
Male	128	71.1
Female	52	28.9
Total	180	100
b) Age of respondent (years)		
30- 39	17	7.4
40-49	55	30.6
50-59	80	44.4
60-69	28	15.6
Total	180	100
c) Education Level		
Non formal education	85	47.2
Formal	95	52.8
Total	180	100

Table 3.2 continued

d) Non formal education		
Artisanal	82	96.5
Numeracy	0	0
Literacy	3	3.5
Total	85	100
e) Formal education		
Primary	55	58.3
JHS/MLCS	25	26.3
SSS	11	11.5
Tertiary	4	4.2
Total	95	100

Farm characteristics and access to extension services

The number of years farmers have been engaged in cassava production (Table 3.3, see Appendix Bi), ranged from 1- 5 years (16.7%), 6 -10 years (36.1%), 11-15 years (21.7%) and 16-20 years (15%). Only 19 (10.5%) respondents have been engaged in cassava production for more than 20 years. In the area of land use (Table 3.3, see Appendix Bi), 32.8% of farmers interviewed had land sizes ranging from 1-2.9 acres. About 109 respondents representing 60.5% had land sizes between 3.0-4.9 acres and 6.8% having land sizes that were above 5 acres.

In this study, we found out that none of the respondents planted only improved varieties (Table 3.3, see Appendix Bi). Majority of the farmers (77.2%) planted local varieties, while 22.8% of farmers planted both local and improved varieties. Some of the local varieties planted were limited to specific areas while others were planted in more than one district or across the entire six districts.

A crosstab between the districts and the local varieties planted revealed that 38 farmers (21%) planted the variety "Ho", followed by "Saboba" (19.4%) planted at the two Northern districts of the Volta Region and "Grace" (15%),

which is grown in the middle and the coastal savannah districts but not the two northern districts.

Furthermore, 14 (7.8%) of the respondents grew "Dogbo and Gabon" in the two coastal savannah and forest zones respectively. Eleven respondents (6.1%) planted "Awarante" only in the two northern districts. Only 7 (3.9%), 6 (3.3%), 3 (1.7%) and 3 (1.7%) of the respondent planted "Hushiga" "Mafi" Abibiagi and Gbedevi respectively in only Adaklu whereas 5 (2.8%) planted Dekpo in only Akatsi south. About 17 (9.4%) respondents from the two Coastal Savannah districts grew "Hushivi".

In Ghana, cassava is cultivated as a monocrop or intercropped with maize or legumes such as cowpea (Ennin, Asafu-Agyei, Dapaah, & Ekyem, 2001; Ennin, Clegg, & Francis, 2002). In this study, the majority of farmers (56.7%) intercrop cassava compared to 43.3% of farmers that plant cassava as a monocrop (Table 3.3, see Appendix Bi). Out of the 102 farmers, 63.7% intercropped cassava with maize. 13.7% with either cowpea or groundnut and 8.8% with vegetables.

There are different purposes for which farmers' plant cassava (Table 3.3 Appendix B). From the results, most of the respondents (145) cultivate cassava for the production of different products (Gari, dough, fufu etc.). Only 32 farmers cultivate cassava solely for use as gari.

A cross-tabulation indicates the majority of these farmers are from the Akatsi South and the Adaklu Districts. Very few (3) farmers in Akatsi South indicated dough was the only reason they cultivate cassava.

The majority of farmers (51.7%) do not have access to extension services with about 45.6% sometimes having access to extension services

(Figure 3.2a). Similarly, the majority of farmers (92.8%) do not belong to a farmer-based organisation (FBO). Only 7.2% of farmers are members of an FBO (Figure. 3.2b)

Table 3.3: Crosstab between districts and the LV planted

		Districts						
		Akatsi South	Adaklu	Hohoe	Afadjato South	Krachi East	Nchumuru	Total
Local varieties	Abibiagi	0	3	0	0	0	0	3
	Awarante	0	0	0	0	3	8	11
	Dekpor	5	0	0	0	0	0	5
	Dogbo	3	4	9	7	0	0	14
	Gabon	0	0	0	5	0	0	14
	Gbedevi	0	3	12	0	0	0	3
	Grace	4	2	9	9	0	0	27
	Ho	4	2	9	9	7	7	38
	Hushiga	0	7	0	0	0	0	7
	Hushivi	14	3	0	0	0	0	17
	Mafi	0	6	0	0	0	0	6
	Saboba	0	0	0	0	20	15	35
	Total	30	30	30	30	30	30	180

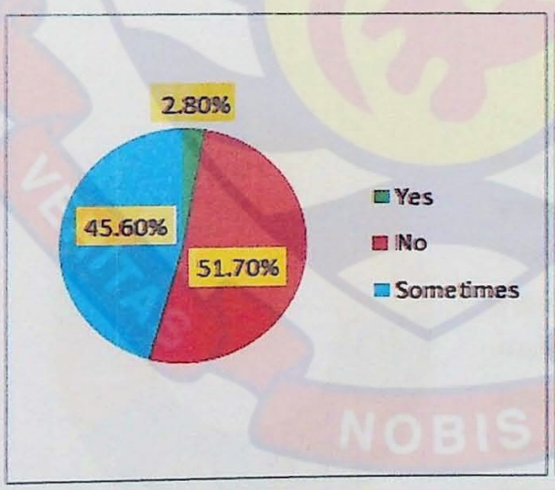


Figure 3.2a Access to extension service

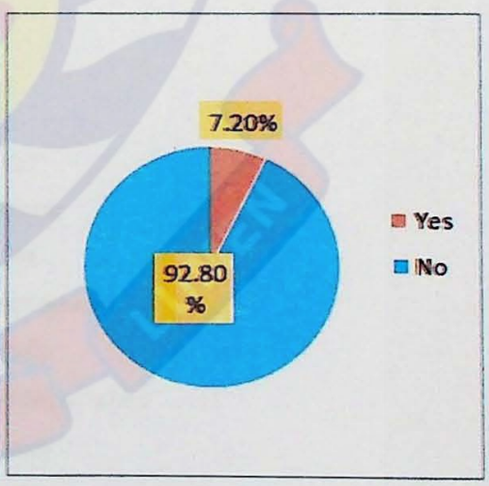


Figure 3.2b Member of FBO

Farmers knowledge of CMD

The awareness and knowledge of farmers with regards to the existence, cause, symptom identification and growth stages at which Cassava Mosaic Disease occurs are presented in Table 3.5.

All 180 farmers surveyed had knowledge or had experienced CMD on their farms. Farmers were very aware of the existence of the disease and were able to identify and mention the symptoms as presented in Fig 3.2 (see Appendix Bii). Majority of farmers (177) associated mosaic patterns on the leaves of cassava plants to CMD followed by yellowing of leaves (166), curling (151) and stunted growth (99). Although rotting of storage roots is not a known symptom of CMD; about 66 respondents mentioned it as a symptom they experienced in addition to CMD. About 44 farmers attributed the presence of whiteflies as a symptom of CMD with only a few farmers attributing necrosis (25) and shedding of leaves (9) to CMD (Figure 3.3).

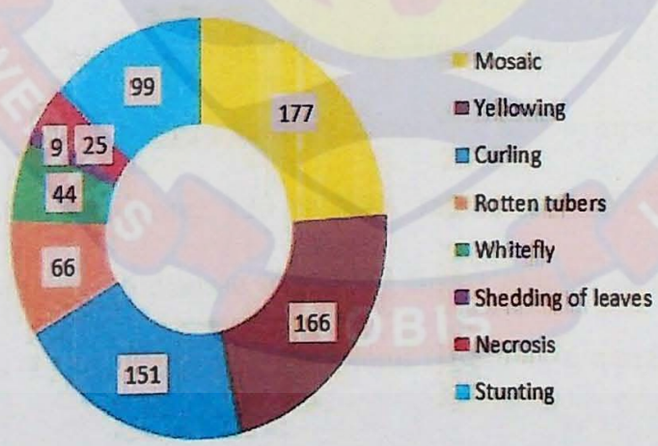


Figure 3.3. Symptoms mentioned by respondents as CMD ****Multiple response

However, only 51 (28.3%) respondents indicated awareness aware of the causes of CMD with 129 (71.7%) not aware (Table 3.5). Of the number of farmers who indicated awareness aware of the causes of CMD, 47% (24) attributed the cause to climatic conditions (drought, high temperatures among others). About 14 (27.5%) and 11 (21.6%) linked CMD to challenges in soil fertility and whiteflies respectively. Only two (3.9%) of the respondents attributed CMD to the spraying of weedicide.

On the mode of CMD transmission, most of the respondents (64.4%) did not know the mode of transmission. Approximately 19.4% and 11.1% stated that transmission is by wind and infected soil. Interestingly, only 5.1% of respondents listed planting material/whiteflies as the mode of transmission (Table 3.5).

The most popular local names for CMD in the districts surveyed were "Zongolachichi"(Eczema because of the whitefly), "Ayifle", "Kwata" (leprosy) and the least popular "Kokobi".

From the responses, 48.3% of farmers observe CMD symptoms during the major season with 41.7% observing symptoms in both minor and major seasons. Only 10% of farmers observe CMD symptoms in only the minor season. Furthermore, 130 farmers representing 72.2% first observed the symptoms of the disease within the first 3 months of growth compared to 50 farmers (27.8%) observing symptoms within 4 - 6 months after planting.

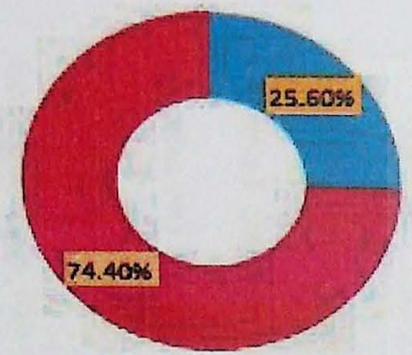
Table 3.4: Farmers knowledge on CMD

Variable	Frequency (f)	Percentage (%)
Do you know the cause of CMD?		
Yes	51	28.3
No	129	71.7
Total	180	100
What is the cause		
Whiteflies	11	21.6
Soil-borne	14	27.5
Spraying of weedicide	2	3.9
Climate	24	47
Total	51	100
Mode of transmission		
No idea	116	64.4
Wind	35	19.4
Whiteflies	9	5.1
Planting material	0	0
Infected soil	20	11.1
Total	180	100
Do you know the name?		
Yes	119	66.1
No	61	33.9
Total	180	100
Name of CMD in respondents communities		
Ayifle	21	17.6
Dayo	4	3.4
Kwafo	8	6.7
Kwata	20	16.8
Mosaic	7	5.9
Nsoson	9	7.6
Zongolachichi	31	26
Kokobi	2	1.7
Kwatari	3	2.5
Edzekpo	14	11.8
Total	119	100
What season do you see CMD		
Minor	18	10
Major	87	48.3
Both	75	41.7
At what growth stage/Month do you encounter the disease?		
<1-3MAP	130	72.2
>3-6MAP	50	27.8
7-9MAP	0	0
Others	0	0
Total	180	100

Management of CMD by farmers

From the study, we observed that a greater number (134) of farmers representing 74.4% did not control CMD while only 46 (25.6%) did apply some control measures (Figure. 3.4). The reasons given by farmers for not applying measures to control CMD are that, they did not have any idea of control measures and the ability of infected plants to recover, a phenomenon known as recovery (Fargette, Jeger, Fauquet, Fishpool, 1994) (Figure. 3.5). A crosstab between education level and management of CMD revealed that most farmers with non-formal or formal education do not apply control measures on their farms to manage CMD (Table 3.7 see Appendix Biv). Table 3.8 (see Appendix Bv) however revealed that the 36 farmers who did control CMD were made up of, 15 farmers with primary qualification, 9 respondents with JHS/MLSC, 8 farmers with SHS and 4 respondents with tertiary education.

Management of CMD included the application of pesticides, rouging and de-topping (Figure 3.6). A crosstab between management practices and their effectiveness indicates that the majority of farmers believe rouging is an effective approach to manage CMD while the opposite is true for the use of pesticide and de-toping (Table 3.9 see Appendix Bvi).



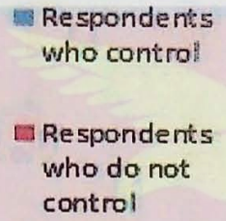


Figure 3.4. Number of respondents who control CMD

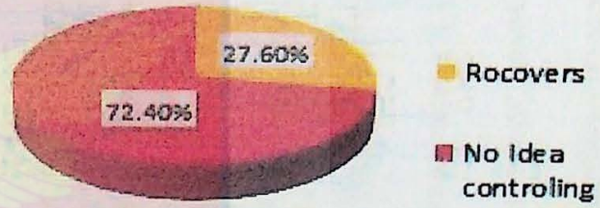


Figure 3.5. Reasons farmers do not control

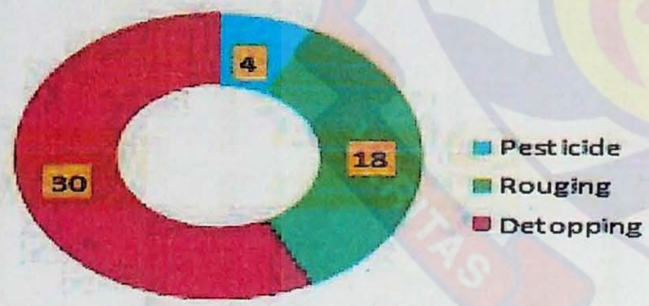


Figure 3.6: Management of CMD

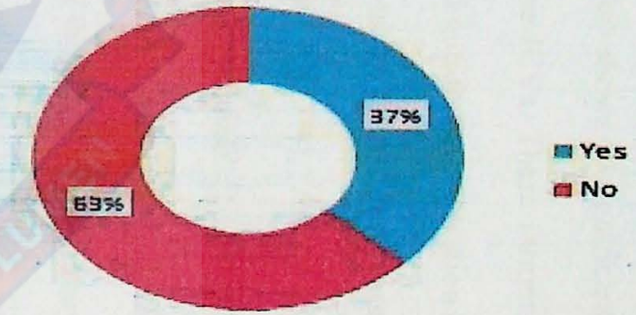


Figure 3.7: Success of Management

Farmers' awareness of Improved Varieties (ICVs)

Most farmers (69.4%) were aware of the availability of improved varieties (Table 3.10). Chi-square analysis shows a significant ($p < 0.05$) number of farmers who were aware of the ICVs. These farmers had formal education compared to 52 farmers who had non-formal education (Table 3.11). However, 30.6% of respondents were not aware of improved cassava varieties (ICV). The most mentioned ICVs were "Sika" (36.7%), "Ampong" (34.4%) and "Ankrah" (27.2%). Others mentioned include "Afisiafi" (10.6%), "Bosom esia" (10%), "Abasafita" (3.3%) AGRA (0.6%) and "Abrabopa" (0.6%). However, about 4.4% of farmers did not know any of the ICVs although they were aware of its availability from friends. Of the 125 respondents who were aware of ICVs, 34.4% had not planted any of the ICVs before giving reasons such as woody storage roots, unpalatability, have little information about ICVs, difficulty to access planting materials and low demand for these varieties. About 65.6% of farmers have planted ICVs before and the highest cultivated ICVs were "Ampong" (17.8%), "Sika" (13.3%), "Ankrah" (11.7%), "Afisiafi" (8.9%), "Bosomensia" (5%) and "Abasafita" (2.2%). Also 95.1% of those who had planted these varieties did experience CMD symptoms with only 4.9% not experiencing CMD symptoms. Currently not all the respondents still plant ICVs on their farms (Table 3.10; Figure 3.8). Chi-square analysis of the yield of improved cassava varieties and whether farmers still plant these improved varieties was significant ($p < 0.05$) (Table 3.12).

From Figure 3.9 it can be seen that majority of farmers who plant ICVs face challenges like to access planting materials (PM), cost of transporting

planting materials to farms, the incidence of CMD, woody/colour issues, less demand and access to extension services.

Table 3.5: Respondents awareness of improved varieties

Aware of improved varieties	Frequency (f)	Percent (%)
Yes	125	69.4
No	55	30.6
Total	180	100
Mention them if yes (*** Multiple responses)		
Abasafita	6	3.3
Afisiafi	19	10.6
Ampong	62	34.4
Bosomensia	18	10
Ankrah	49	27.2
Sika	66	36.7
AGRA	1	0.6
Abrabopa	1	0.6
None	8	4.4
Have you planted any before		
Yes	82	65.6
No	43	34.4
Total	125	100
Why haven't you planted before *Multiple responses		
Woody	7	3.9
Less info about them	37	20.6
Difficult accessing	39	21.7
Not demanded	15	8.3
Which have you planted ***Multiple responses		
Abasafita	4	2.2
Afisiafi	16	8.9
Ampong	32	17.8
Bosomensia	9	5
Ankrah	21	11.7
Sika	24	13.3
Any CMD symptoms when planted?		
Yes	78	95.1
No	4	4.9
Total	82	100
Did you record high yield		
Yes	14	17.1
No	15	18.3
A bit higher than IV	31	37.8
Not sure	22	26.8
Total	82	100
Do you still plant IV		
Yes	45	54.9
No	37	45.1
Total	82	100

Table 3. 6: Chi-square analysis of farmer awareness of improved varieties and the highest education level attained.

	Highest education					
	Non-formal	Formal	Total	Chi-square value	Df	Asym. sig
Aware of improved varieties						
Yes	52	73	125	4.219[a]	1	0.04
No	32	23	55			
Total	84	96	180			

Df = degrees of freedom; Asym. Sig= p value

Table 3.7: Chi-square analysis of yield of improved cassava varieties and whether farmers still plant improved varieties

	Do you still plant it					
	Yes	No	Total	Chi-squre	Df	Asympt. Sig
Did you record a high yield				74.237[a]	3	0.000
Yes	14	0	14			
No	0	15	15			
A bit higher than ICVs	30	1	31			
Not sure	1	21	22			
Total	45	37	82			

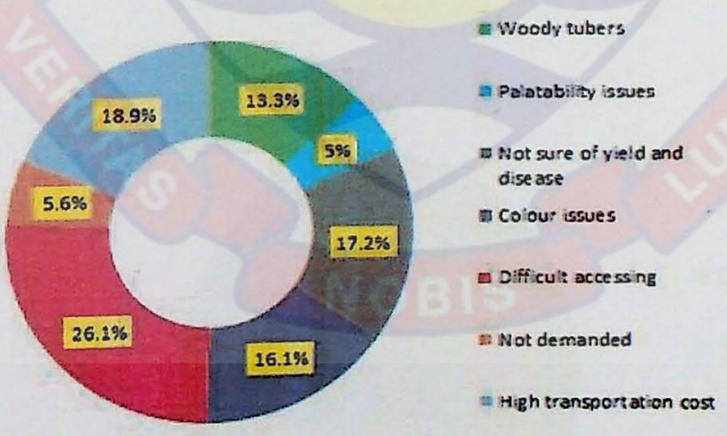


Figure 3.8. Reasons cassava farmers have stopped planting ICVs

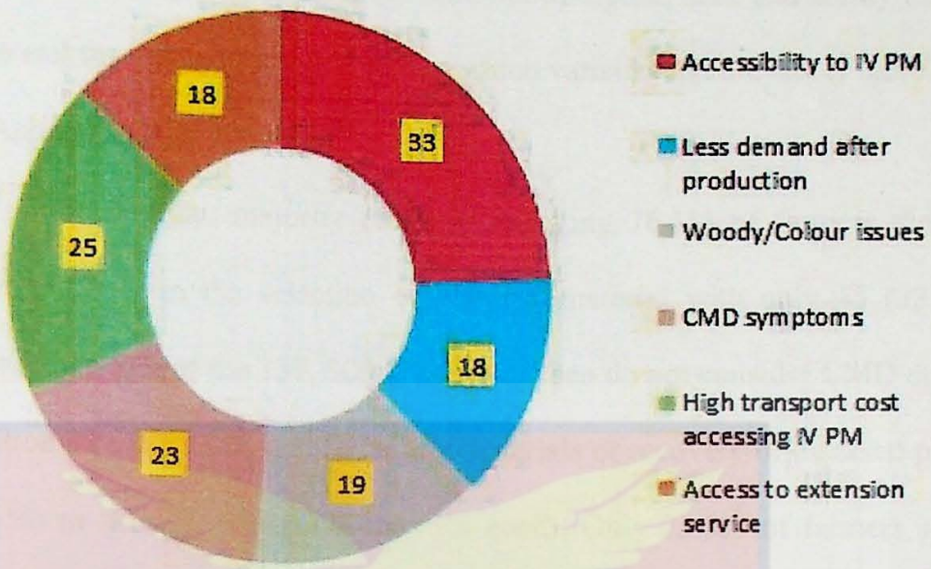


Figure 3.9: Constraints of farmers who still plant ICVs

Source and Selection of Planting Materials

The present study revealed that farmers source their planting materials from three sources, which include their farms, family and friends and the Ministry of Food and Agriculture (Figure 3.10). Farmers in the study areas consider many factors such as early yielding, appearance and healthy-looking

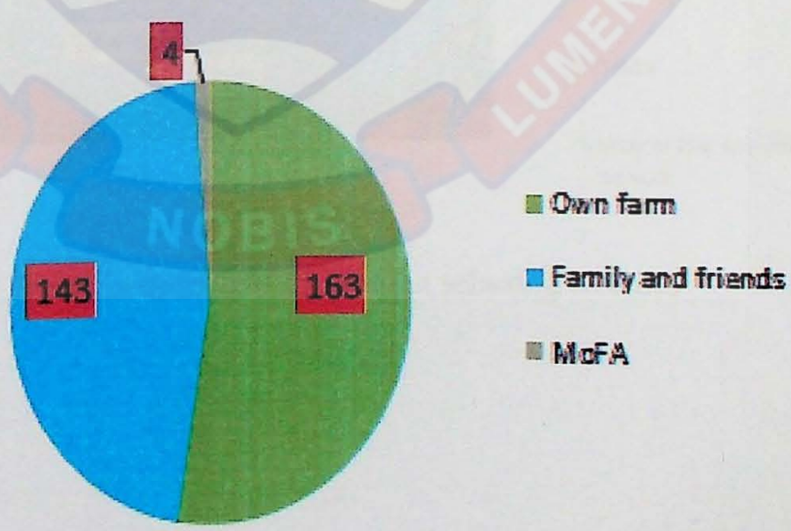


Figure 3.10: Farmers sources of planting materials (Multiple responses)

PM, high yielding, availability, demand, multipurpose, taste and ability to stay in the soil for long periods in deciding which varieties to cultivate (Figure 3.11 see Appendix Bi).

Interestingly, majority (137) representing 76.1% of farmers did not consider CMD in the selection of planting material with only 43 (23.9%) considering. Out of the 137, 100 (72.9%) of then do not consider CMD during selection due to a shortage of planting materials or recovery of diseased plants (21.1%) or drought and soilborne (3% each). Only 23.8% of farmers select symptom-free cuttings in order to obtain high yields (Table 3.13 See appendix B vii).

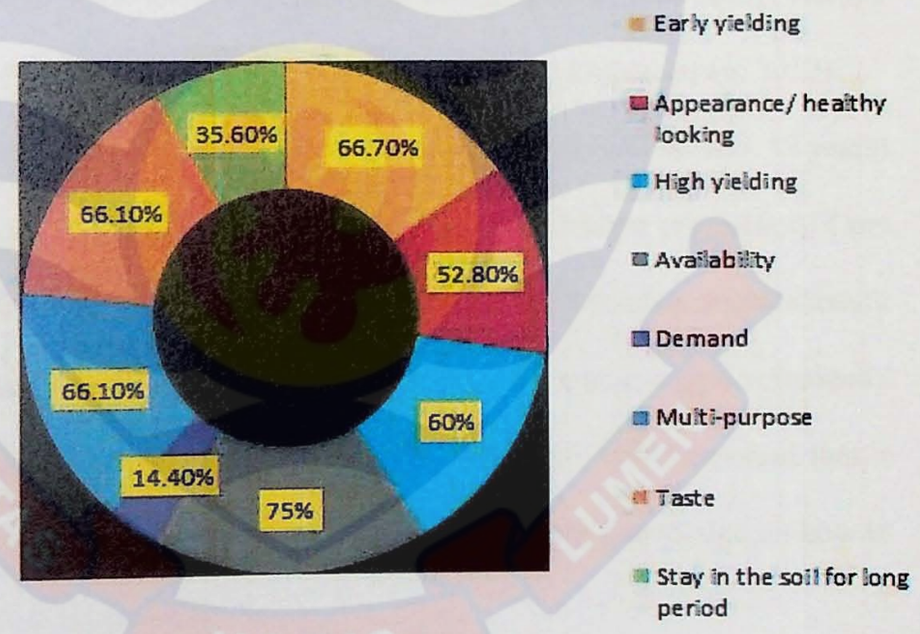


Figure 3.11: Factors respondents consider in selecting varieties

Discussion

Socio-economic and farm characteristics of respondents

Male farmers as revealed in this study dominate cassava production in Ghana. This probably is due to the high demand for labour and energy required in cassava production particularly by small-scale farmers due to low implementation of mechanisation. This observation confirms an earlier report by Nweke, Dunstan, Spencer, & Lynam (2001) which shows that females provide less than half of the entire labour input in cassava production. In contrast, reports by Oyegbami, Oboh, & Omueti (2010) and Thompson (2013) show that a greater percentage of women are involved in cassava production. This observation could be due to higher numbers of women involved in the downstream activities of the cassava value chain such as processing, storage and sale of products (Nweke *et al.,* 2001; IFAD. 2012; Onyemauwa, 2012).

The majority of cassava farmers are middle-aged (around 40 – 59 years) which suggests that fewer young people engage in cassava production. Low yields and the energy-demanding nature of cassava production might account for the low number of young people engaged in cassava production in the study areas (Ogundari & Ojo, 2006). Nonetheless, Akorade (2004) reported that it takes around 7 -12 years for farmers to acquire adequate knowledge on how to improve cassava production.

Formal education is important in the adoption of improved crop varieties or technology. The inability of farmers to read and write can hinder the adoption of improved cassava varieties (Nwabueze & Odunsi, 2007). In this study, majority of farmers had formal education mostly at primary level with low numbers from the tertiary while quite a number of the respondents had no formal

education. This might have negatively affected cassava production in the study areas due to the low adoption of improved cassava varieties with higher yields and resistance to CMD. A low level of education among farmers can result in the persistent use of conventional farming practices that lower cassava productivity (Anyanwu, Kalio, Manilla, & Ojumba, 2012).

The majority of farmers interviewed planted old or traditional varieties with a few cultivating both old and improved varieties. A number of improved varieties that are planted in the districts surveyed include Afisiafi, Ankrah, Abasafita, Sika and Ampong (Table 3.3). The most planted ICV were Ankrah, Sika and Ampong. Ankrah is one of the first ICV released in Ghana to control CMD (Ofori, *et al.,* 1997), thus it is widely known and cultivated. The introduction of the West Africa Agricultural Productivity Programme (WAAPP) in Ghana in 2007 led to the promotion of the varieties Sika and Ampong in the Volta Region Region (MoFA, 2020). Several factors such as level of education and age influence the adoption of new and improved cassava varieties (Abbeam–Danso *et al*., 2017; Muhammad-Lawal, Salau, & Ajayi, 2012). Furthermore, lack or scarcity of planting materials of improved varieties can lead to low adoption (Gibson, 2006).

The majority of cassava farmers interviewed intercropped cassava with maize since it is a key cereal providing a staple diet for many households in sub-Saharan Africa (Undie, Uwah, & Attoe, 2012). In addition, farmers Iin the present study cultivated cassava for domestic use and other purposes (e.g. processing). Farmers in Ghana and across Africa prefer the cultivation of cassava varieties that can be processed into different products besides food (Acheampong, Owusu, & Gyiele 2013; Bentley, Olanrewaju, Madu,

Olaosebikan, Abdoulaye, Wossen…Tokula, 2017; Nakabonge, Samukoya, & Baguma, 2018).

Farmer Based Organizations (FBOs) are pivotal to sustaining agriculture productivity because they improve access to markets, facilitate extension services to farmers, distribution of farm inputs and empower their members to contribute to policies that affect their livelihood (Swanson & Rajalahti 2010, Salifu, Funk, Keefe, & Kolavalli, 2012).

In the present study, the majority of farmers do not receive any extension services and are not members FBOs, a situation that, results in a lack of cohesion among farmers/FBOs (Bampoe, 2015). The absence of or a low number of active FBOs in the surveyed districts could be due to lack of funding support or absence of agricultural productivity programmes. The implementation of the WAAPP programme in Ghana resulted in the formation of a number of FBOs (Bampoe, 2015). However, farmers in the study area attested that after the WAAPP programme, resources are not enough to keep and sustain the groups. Thus, funding to support the activities of FBOs and training of Agricultural Extension Agents (AEAs) is needed to improve agronomic practices implemented by cassava farmers in Ghana.

Farmers' Knowledge on Cassava Mosaic Virus Disease

The results show that all respondent farmers had heard of or encountered CMD in their fields and as a result were able to identify the symptoms of CMD (Figure 3.2, see Appendix Bii). This could be as a result of the ease with which CMD symptoms can be identified (Sherwood, 1997). The sensitization and training of farmers usually through improvement programmes on how to identify and manage virus diseases such as CMD in the field is important to

control the spread into new geographic regions or hosts (Bampoe, 2015, Gibson, 2006). However, due to similarities in symptoms caused by plant viruses and soil deficiency effects, it can be a challenge for farmers to identify virus symptoms in their fields. It is worth noting that only a few of the respondents mentioned mosaic as the name of the disease with most farmers assigning names ("Ayifle", "Kwata" (leprosy), "Edzekpo Dayo", "Kwafo, "Nsoson" (worms or margot), "Kokobi" and "Zongolachichi" (ezema) to CMD in their local language. Similarly in millet farmers referred to the Neck blast disease (fungal disease) as "batcha" which means mockery because diseased millet plants might not bear seeds (Kiros-Meles, & Abang, 2008).

CMD is caused by nine distinct virus species known as Cassava Mosaic Geminiviruses and transmitted through the use of infected cuttings or whiteflies (*Bemisia tabacci*) (Zhou, Robinson, & Harrison 1998, Fondong *et al.*, 2000a, Bull *et al.*, 2006, De Bryun *et al.*, 2016). Transmission through infected cuttings is a major cause of reduced yield and can lead to 100% yield loss (Jericho, Thompson, Gerntholtz, & Viljoen, 1999). The observation of CMD symptoms during the major, minor or both seasons can be attributed to the use of infected material (Chikoti, Mulenga, Tembo, & Sseruwagi, 2019) although the severity of the symptoms may vary because of high temperature, viral strain, disease pressure or host susceptibility (Chikoti, Ndunguru, Melis, Tairo, Shanahan, & Sseruwagi, 2013a; Chikoti, Tembo, Chisola, Ntawuruhunga, & Ndunguru, 2015; Thresh, Otim-Nape, & Fargette, 1998). In this study, most of the farmers did not know the cause of CMD and attributed it to the climate (drought) or soil borne (nutrient) deficiencies, which exhibit similar symptoms (van den Bosch, Jeger, & Gilligan, 2007; Jones, 2014). Education of farmers on how to identify

symptoms and the use of clean planting materials is crucial for effective management of the CMD in Ghana and Africa.

It is worthy to note that the stage of growth at which symptoms appear is very important because it can affect the yield of cassava (Thresh *et al.*, 1994). Studies have shown that expression of CMD symptoms in the first month after planting is (< 1MAP) likely due to planting of infected material and can lead to significant (above 80%) yield loss (Thresh *et al.*, 1994: Fauquet & Fargette 1990; Adriko, Sserubombwe, Adipala, Bua, Thresh, & Edema, 2012). In contrast, the transmission of CMD by whiteflies results in the expression of symptoms later compared to infection via planting material (Fauquet & Fauquet 1990; CABI, 2020). In the present study, we realized that most of the respondents (72.2%) observed CMD symptoms between 1 - 3 MAP with only a few (27.8%) observing symptoms between 4 - 6 MAP. This observation suggests that CMD infection in the study areas can be mainly attributed to the use of infected planting materials and not whiteflies.

Management of CMD by Farmers

The control and management of Cassava Mosaic Disease (CMD) just as any other plant virus disease requires knowledge of the causes, mode of transmission and symptoms (Chikoti *et al.*, 2019). Several approaches have been used to control CMD, which include planting improved cassava varieties (ICVs), de-topping, which encourages the growth of fresh leaves. However, the practices of de-topping according to Ariyo *et al.*, (2003) helps in the build-up of whitefly population. The most effective approach of controlling CMD has been roguing (CABI, 2020). According to Thresh & Cooter (2005) roguing is more effective when adopted by many farmers in a well-planned programme.

However, the practice becomes more difficult and ineffective where CMD-susceptible varieties are planted. It was surprising to find out that many of the respondents (74.4%) in the surveyed districts did not implement management practice to control CMD on their farms. The few numbers that practiced roguing still left some CMD-infected plants on the field to produce storage roots for harvest. The cultivation of infected material year after year leads to an increase in mixed infections with multiple Cassava Mosaic Geminiviruses (CMGs) on the field resulting in severe symptoms (Fondong *et al.*, 2000a, Patil & Fauquet, 2009,). The increase in mixed infections can result in the recombination and evolution of new geminivirus species (Pita *et al.*, 2001a, b, Zhou *et al.*, 1998, Lefeuvre *et al.*, 2007). It was interesting to know that farmers that had higher levels of education (Senior High school or above) implemented management practises to control CMD on their fields. This finding highlights the importance of education in the control of CMD in Ghana and Africa.

Awareness of Improved Cassava Varieties (ICVs)

Farmers' awareness and access to planting materials are essential drivers in the adoption of improved cultivars (Acheampong & Owusu, 2015; Abbeam–Danso, 2017). Besides awareness, other factors such as the involvement of farmers in the selection of traits for the development of new cultivars (participatory approach to breeding) can improve the adoption of ICVs (Alene, Khataza, Chibwana, Ntawuruhunga, & Moyo 2013; Acheampong & Owusu, 2015; Abbeam –Danso, 2017; Chikoti *et al.*, 2019). In this study, the majority of respondents were aware of the existence of ICVs and could mention a number of examples such as "Sika" "Ampong" "Ankrah "Afisiafi" "Bosome nsia" "Abasafita" AGRA and "Abrabopa. It was confirmed that the education level

of cassava farmers significantly influenced their awareness of ICVs (Table 4.1). Similar significant relationships have been observed between the education level and adoption of improved maize varieties among farmers (Gorfe, 2004; Million & Belay, 2004; Acheampong, & Owusu, 2015; Abbeam-Danso, 2017). In addition, the flow of information among friends and family members of farmers played a role in their attitudes and behavior in adopting ICVs.

It should be noted that after the WAAPP programme, multiplication centres (e.g. Ohawu Agricultural College) were established to multiply ICVs especially Ampong and Sika. However, the distant location of these centres makes it costly for farmers to access and transport planting materials, limiting the adoption of ICVs. Therefore, the nearer the multiplication source of ICVs to farmers, the more likely such varieties would be adopted (Salasya, Mwangi, Mwabu, & Diallo, 2007).

Although some ICVs have undesirable characteristics such as being woody and a change in color after milling, farmers still planted these ICVs. For example, farmers in Ajagbale in Nigeria continued to plant the cassava variety TME-419 due to its high yielding nature although it had some undesirable characteristics (Bentley *et al.*, 2017). This finding shows that, once farmers adopt ICVs, they would cultivate for a long period. It was interesting to note that, respondents who grew ICVs in the study areas still reported symptoms of CMD on their farms. The continuous use of planting materials saved from farmers' fields' season after season can lead to accumulation of CMGs, thereby increasing the CMD disease pressure as confirmed by Asgedom, Struik, Heuvelink, & Araia (2011) and Adriko, Sserubombwe, Adipala, Bua, Thresh, & Edema (2011).

Source and Selection of Planting Materials

In addressing CMD, it is very important to identify the source of planting materials grown by farmers because this is the main mode of spread (Jericho *et al.,* 1999). In Africa, most farmers use planting materials saved from their own farms or from family and friends, which has facilitated the widespread of CMD (McQuaid, Gilligan, & Bosch, van den, 2017; Houngue, Pita, Cacaï, Zandjanakou-Tachin, Abidjo, 2018). Use of planting materials from previous seasons leads to an increase in the severity of the disease because of the systemic nature of CMD (Ntawuruhunga, Legg, Okidi, Okao-Okuja, Tadu, Remington, 2007). In the present study, farmers explained that CMD symptoms reduce over time, thus they do not consider CMD during the selection of planting materials particularly when there is a shortage of planting materials (Table 3.13 see appendix B vii). In cassava, the phenomenon of recovery where CMD-infected plants recover from virus symptoms or produce virus-free stem cuttings multiplied from infected parents has likely contributed to the use of infected materials by farmers in Africa (Fargette *et al.*, 1994).

Preference is an important factor in the adoption of a particular variety since farmers are likely to adopt or use variety when such varieties meet their requirement as observed by Houngue *et al.*, (2018). Respondents in the present study mentioned many factors or characteristics they consider in selecting a particular variety to grow (Figure 3.11). These characteristics include the availability of planting materials, early yielding, high yield, multi-purpose use, taste, healthy appearance/healthy-looking, demand by buyers and its ability to be harvested at any time when there is no market. This finding confirms earlier reports by Acheampong *et al.*, (2013) that the varieties farmers in Ghana select

is dependent on the above-mentioned characteristics and traits. Most of the improved varieties lack farmer-preferred traits and focused on high yield under ideal, and well-managed conditions (Reeves & Cassaday, 2002). However, most of the tropical soils for cassava cultivation are marginal and do not produce the expected high yields. Plant breeders must involve farmers in the development of new and improved varieties from the start. Furthermore, siting of multiplication centres close to cassava farms will make them accessible for farmers, eventually improving the adoption of these ICVs.

Conclusion

Generally, respondents in the six major cassava-producing districts in the Volta Region have knowledge of CMD, can identify and describe symptoms and the season and month in which the disease occurs. However, very few farmers could give the correct cause(s) and mode of transmission of the disease. This lack of knowledge may have contributed to the widespread of CMD in the surveyed region.

It was alarming to discover that the majority of farmers did not implement control measures to manage CMD in their fields. This is disturbing because the lack of management of the disease could lead to the build-up of Cassava Mosaic Geminiviruses in cultivated cassava varieties, facilitating recombination between geminivirus species and leading to the evolution of novel virus species. Educating farmers in the use of control measures such as rouging and de-topping and avoidance of using PMs from family and friends in the management of CMD will help reduce the incidence of the disease in the Volta Region. Besides the education of farmers, the situation of multiplication centres close to farms and the involvement of farmers in the selection of desired

traits and development of new cultivars will result in higher adoption of these ICVs by farmers in Ghana and Africa.

CHAPTER FOUR

PREVALENCE OF CASSAVA MOSAIC GEMINIVIRUSES IN THE VOLTA REGION OF GHANA.

Introduction

Cassava Mosaic Disease (CMD), first reported in 1894 is a major constraint to cassava production in Africa (Storey & Nichols, 1938). CMD is caused by Cassava Mosaic Geminiviruses (genus: *Begomovirus*, family: *Geminiviridae*) (Legg & Fauquet, 2004). CMD is transmitted by the whitefly *Bemisia tabacci* (Gennadius) vector (Thresh *et al*., 1998; Maruthi *et al*., 2005).

Currently, nine CMG species have been identified and characterised in Africa (Stanley & Gay, 1983; De Bryun *et al*., 2016, Zhou *et al*., 1998; Berrie *et al*., 1998; Tiendrebeogo *et al*., 2012; Harimalala, *et al*. 2015). CMGs occur as a single infection or as co-infection with more than one CMG species (mixed infections) leading to more severe symptoms due to high accumulation of viral DNA (Pita *et al*., 2001a; Ogbe, Legg, Raya, Muimba-Kankalongo, Theu, Kaitisha, Phiri, & Chalwe, 1997; Ogbe, Thottappilly, Dixon, & Mignouna, 2003). A classic example of co-infection of cassava with African Cassava Mosaic Virus (ACMV) and a recombinant strain of East African Cassava Mosaic Virus (EACMV) led to a severe CMD pandemic in Uganda in the early 1990s (Zhou *et al*., 1998; Zhou *et al*., 1997; Fondong *et al*., 2000a; Pita *et al*., 2001a). The new variant EACMV-UG2 was prevalent in Eastern Africa occurring mostly in dual infections with EACMV and ACMV (Fondong *et al*., 2000a; Pita *et al*., 2001a; Ogbe *et al*., 1997, 2003). Consequently, the EACMV-UG2 was identified in South Africa (Jericho, 1999). Mixed infections of cassava with ACMV and EACMV species have become common in Africa (Fondong *et*

al., 2000a; Ndunguru, 2005; Harimalala, *et al*., 2015; Torkpo *et al*., 2017) and increases the probability for recombination and evolution of new CMG species.

A number of factors including an increase in whitefly population, new biotypes and the movement of cassava planting materials across different growing areas have accounted for the spread of CMD and CMGs in Africa (Padidam *et al*., 1999; Legg *et al*., 2015). Whitefly vectors transmit CMGs in a persistent manner irrespective of the coat protein, thus increasing prevalence and incidence (Storey & Nichols, 1938; Dubern, 1994; Brown, Frohlich, & Rosell, 1995; Morales & Jones 2004; Polston, De Barro, & Boykin, 2014; Maruthi, Colvin, Seal, & Thresh, 2002a; Morales & Jones, 2004). Furthermore, climatic factors such as temperature, rainfall and wind affect the abundance of whitefly vectors (Fauquet & Fargette, 1990). The exchange of infected stem cuttings by farmers is a major source of local and long distance spread of CMGs across Africa. This practise can result in the introduction of new CMG strains into previously uninhabited environments resulting in higher virulence or recombination of CMG species (Mabasa, 2007).

To understand the magnitude of CMD in Ghana, a survey of farmers' fields is important to identify and characterise CMG species diversity and prevalence of CMD in farmers' fields. Field surveys are key aspects of epidemiological studies of plant diseases because they provide baseline data for plant disease identification and characterisation (Campell & Benson, 1994).

In addition, due to variations in CMD expression in different locations, field surveys can provide useful information for development of effective management strategies (Ndunguru, 2005). To that end, protocols that allow for quick and accurate identification of CMGs and subsequent characterisation

facilitate virus studies and management. The Enzyme Linked Immunoassay Assay (ELISA) is one of the most widely used immunoassay methods for the detection of antigen-antibody interaction due to its high specificity, standard configuration and convenient reading (Dunne, 2004; Satija, Punjabi, Mishra, & Mukherji, 2016). The double antibody sandwich (DAS) ELISA (Sequeira & Harrison, 1982) and the triple antibody sandwich (TAS) ELISA (Swanson & Harrison, 1994) have been used to detect Cassava Mosaic Geminiviruses in the field. Both tests have been efficiently used to distinguish between ACMV and EACMV species.

The DAS and the TAS are performed using either monoclonal only or monoclonal and polyclonal antibodies respectively. These react with the plant viruses in distinguishing between two or more viruses (Zhou *et al.*, 1997). DAS has a simple protocol, time saving and there is no cross reactivity from secondary antibody, it however lacks signal amplification since only a primary antibody is used in the absence of a secondary antibody (CUSABIO, 2007-2020). TAS, however, has high flexibility, sensitivity, and specificity since different antibodies bind to the same antigen for detection (Seepiban, Charoenvilaisiri, Warin, Bhunchoth, Phironrit, Phuangrat…Gajanandana, 2017; CUSABIO, 2007-2020).

In Ghana, after the first report of EACMV by Offei, Owuna-Kwakye, & Thottappilly (1999), other studies have confirmed the high level of mixed infections of ACMV and EACMV in farmer fields (Elegba *et al.*, 2013; Torkpo *et al.*, 2017). Due to the increasing diversity of CMGs in sub-Saharan Africa and Ghana (Sseruwagi, Sserubombwe, Legg, Ndunguru, & Thresh, 2004; Torkpo *et al.*, 2017), there is the need to continually assess the prevalence and

incidence of CMGs in the field to aid effective disease management. Sseruwagi *et al.*, (2004) suggests that effective monitoring and management of CMGs should be done every two to four years.

Therefore, the principal objective of this study was to determine the prevalence, spatial distribution and diversity of Cassava Mosaic Geminiviruses in the Volta Region of Ghana years after Torpkp *et al.* (2017).

Specifically, the study sought to determine the:

1. Prevalence and spatial distribution of CMD in the Volta Region
2. Abundance of whitefly population in farmers' fields.
3. Diversity of Cassava Mosaic Geminivirus species in the Volta Region.

Materials and Methods

Field survey for prevalence and spatial distribution of CMGs

A field survey for prevalence and spatial distribution of CMGs was conducted in all six (6) districts in the Volta Region (Table 3.1) between June and August of the 2019 cropping season. The eighteen (18) communities surveyed in this study are indicated in Figure 4.0.

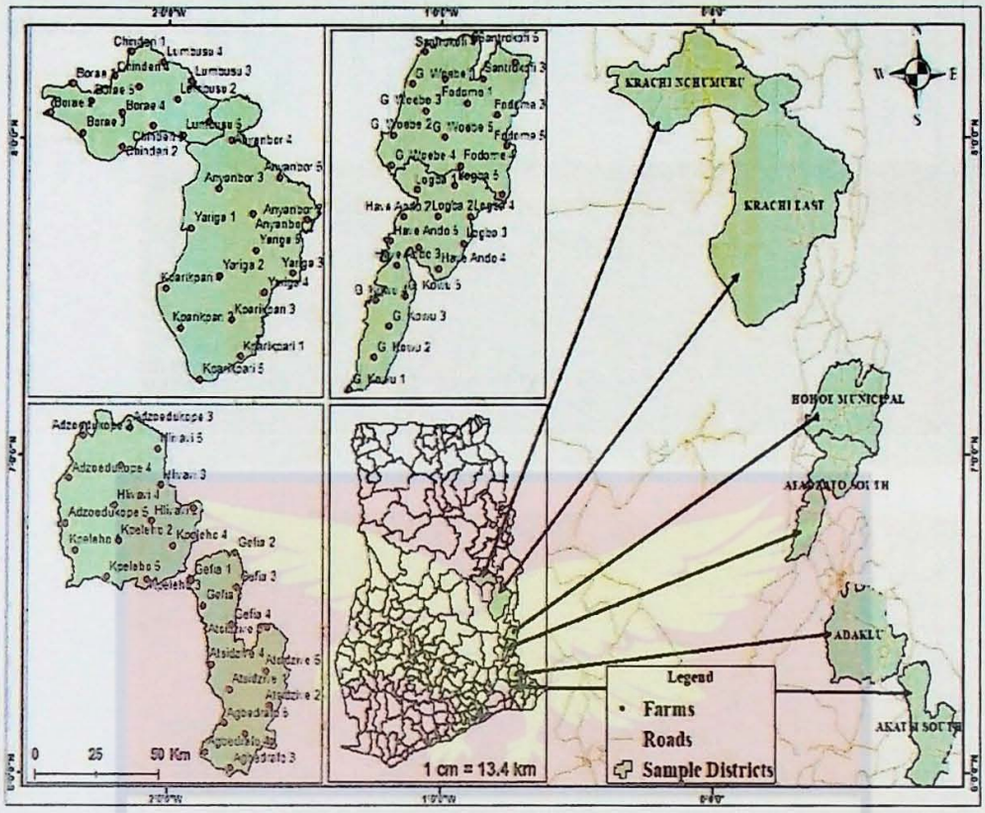


Figure 4.1: Map of Ghana showing the surveyed area

Sampling techniques and Sample size

The random sampling method was used to select cassava farms in the six communities. Five farms were selected from each 3 community per each six districts, and 30 plants (25 symptomatic and 5 asymptomatic) were sampled in each farm. Leaf material was collected from the younger leaves of 3 to 4-month-old plants (Figure 4.1).

In selecting the farms, an interval of 30 km and above was considered as suggested by Cochran (1977). The stratified random sample design (SRSD) was used in sampling the test plants. With this method, fields were divided into equal strata, and the test plants were selected from each of the sectors. Averagely, each farm surveyed measured one acre or more. In total, data were collected on 2700 test plants (30 * 90 farms: 2250 symptomatic and 450 asymptomatic). In each of the districts, 375 diseased plants and 75 healthy

plants were tested.

Figure 4.2: Typical asymptomatic plant (A) and symptomatic plant (B) in farmers fields

Detection of CMGs using Triple Antibody Sandwich (TAS) ELISA

Young leaf samples (symptomatic and asymptomatic) were collected from all 2700 plants assessed. The samples were transported to the laboratory in labelled Ziplock bags on ice. The leaf materials were transferred to a freezer at a temperature of -18 ° C until processed. For detection of CMGs using ELISA, microtitre plates were coated with the IgG (20 µL diluted in 20ml of coating buffer (1.59 g Na2CO3 + 2.93 g NaHCO3 + 0.20 g NaN3). 100 µL of the mixture was added to each well on the plate, covered, and incubated at 37 °C for 4 hours. After which the plate was washed three times with Phosphate-buffered saline (PBS) + 0.5 ml Tween 20 per liter and blocked with 100 µL of 2% skimmed milk. The plate was then covered and incubated for another 30 min at 37 °C, after which it was washed and tapped dry.

Sap from diseased and healthy leaves was extracted from 0.5 g leaf samples with 5ml of extraction buffer (8.0 g NaCl, 0.2 g KH2PO4, 1.15 g Na2HPO4, 0.2 g KCl at pH 7.4), Tween 20 and 2% Polyvinyl pyrrolidone in a ratio of 1: 5 (1g plant sample in 5 ml of buffer). 100 µL of the extracted samples were loaded in duplicate wells on the plate. Similarly, the positive and negative controls were loaded onto the same plate. The plates were covered with rubber film and kept overnight in a fridge at 4 °C. Plates were washed three times with washing buffer (PBS-T). The monoclonal antibody (MAb) was diluted in conjugate buffer (1: 1000) and 100 µL of the mixture loaded into each well and incubated at 37 °C for four hours. The plate was washed as previously described and RAM-AP diluted in 1:1000 in conjugate buffer was loaded (100 µL) into each plate and incubated for an hour. The plate was washed three times at three minutes interval and 100 µL aliquots of a freshly prepared substrate (1 mg/ml

para-nitrophenyl-phosphate in substrate buffer) was added to each well. It was then covered and incubated at 37 °C for one hour to aid colour development. The plate was visually assessed, and the absorbance read using a spectrophotometer (Multiskan Ascent VI.25-Version 1.3.1) at a wavelength of 405 nm.

Data collection

Prevalence and spatial distribution of CMD

Data was collected on the whitefly population, disease incidence, and symptom severity in 90 farms (15 farms per district) in the Volta Region. GPS coordinates for each farm was recorded for all 90 farms visited. Total whitefly population was assessed by counting the number of adult whiteflies on the five topmost, fully expanded leaves of the selected plants (Fargette, Fauquet, & Thouvenel 1988; Asare, Galyuon, Asare-Bediako, Sarfo, & Tetteh, 2014). The percentage disease incidence was obtained by counting the number of plants showing the disease in each stratum in each farm (Cochran, 1977; Sseruwagi *et al*., 2004). CMD severity was assessed using a scoring scale rated 1 to 5, (Table 4.1) where 1 = no disease symptoms and 5 = severe chlorosis, distortion of leaves and retarded growth (IITA, 1990).

Table 4.1: CMD rating scale with corresponding symptom expression

Rating	Symptoms
1	No symptoms observed
2	Mild chlorotic pattern on entire leaflets or mild distortion at base of leaflets appearing green and healthy
3	Strong mosaic pattern on entire leaf, and narrowing cum distortion of lower 1/3 of leaflets
4	Severe mosaic distortion of 2/3 of leaflets and general reduction of leaf size
5	Severe mosaic distortion of 4/5 or more of leaflets, twisted and misshapen leaves

(IITA, 1990)

TAS-ELISA detection of CMD

Based on the ELISA absorbance values, samples were either declared positive or negative for the virus. Samples with an absorbance value more than twice that of the negative control were considered positive for the virus (Charoenvilaisiri, Seepiban, Kumpoosiri, Rukpratanporn, Warin, Phuangrat… Gajanandana, 2021).

Data Analysis

Mean whitefly counts were transformed using the square root transformation approach. Data on percentage incidence was calculated according to Imran, Shakeel, Azhar, Farooq, Saleem, Saeed, Nazeer, Riaz, Naeem, & Javaid (2012). The values were transformed using angular transformation before statistical analysis. Data on the severity of CMD for individual farms were first calculated using a formulae by to Chomdej, Chatchawankanpanich, Kositratana, & Chunwongse (2007) before analysis:

$$\text{Disease incidence} = \frac{Number of infected\ plants}{Total\ number\ of plants} \times 100 \text{ (Imran } et\ al.\text{, 2012)}$$

Disease severity index=

$$\frac{\sum(Rating\ scale \times Number of plants)}{Total\ Number\ of\ Plants \times Heighest\ Rating} \times 100 \text{ (Chomdej } et\ al.\text{, 2007)}$$

The values generated were subjected to ANOVA using GenStat Statistical package version 11 (VSN International) and means separated by the use of LSD at 5 % significance level. Graphs were drawn using Microsoft excel on 95% confidence interval.

Results

Abundance of whitefly population in farmers' fields in the Volta Region

Mean whitefly population was significantly higher ($F_{5, 80} = 58.59$ $P < 0.05$) in Krachi Nchumuru (10.01 ± 0.22 b), Krachi East (9.80 ± 0.29 b) and Akatsi South (9.03 ± 0.16 b) compared to Hohoe (5.58 ± 0.33 a), Afadjato South (5.92 ± 0.36 a) and Adaklu (5.93 ± 0.25 a) (Table 4.2). The lowest whitefly population was recorded in the Hohoe district (5.58 ± 0.33 a) while the highest whitefly population was recorded in Krachi Nchumuru district (10.01 ± 0.22 b).

Table 4.2: Mean whitefly population at six districts

Districts	Mean Whitefly Population/Plant
Hohoe	5.58 ± 0.33 a
Afadjato South	5.92 ± 0.36 a
Adaklu	5.93 ± 0.25 a
Akatsi South	9.03 ± 0.16 b
Krachi East	9.80 ± 0.29 b
Krachi Nchumuru	10.01 ± 0.22 b
LSD (0.05)	0.77

Prevalence and spatial distribution of CMD in the Volta Region

CMD incidence across the six districts in the Volta Region varied significantly ($F_{5, 80} = 15.51$ $P < 0.05$). The highest incidence of CMD was recorded in Krachi Nchumuru (96.67 ± 0.23 %) followed by Krachi East (96.47 ± 0.31 %), Akatsi South (86.67 ± 1.59 %), and Adaklu (82.40 ± 1.95 %) (Table 4.3). Similar to the whitefly population, CMD incidence was lowest in the Hohoe district (77.2).

Table 4.3: Mean incidence of CMD across the six districts

Districts	Mean Incidence (%)
Hohoe	77.20 ± 1.64 a
Afadjato South	77.67 ± 1.68 a
Adaklu	82.40 ± 1.95 ab
Akatsi South	86.67 ± 1.59 b
Krachi East	96.47 ± 0.31 c
Krachi Nchumuru	96.67 ± 0.23 c
$LSD_{(0.05)}$	4.93

Mean Severity of Cassava Mosaic Disease

The six cassava-growing districts in the Volta Region had varying levels of CMD severity ($F5, 80 = 14.53$ $P< 0.05$) (Figure 4.2). CMD severity ranged from 2.93 in Adaklu district to 4.37 in Hohoe district. In Akatsi South, Krachi East, and Krachi Nchumuru districts, CMD severity scores of 3.42, 3.52, and 3.69 respectively were recorded. Cassava plants in Hohoe displayed severe symptoms of CMD.

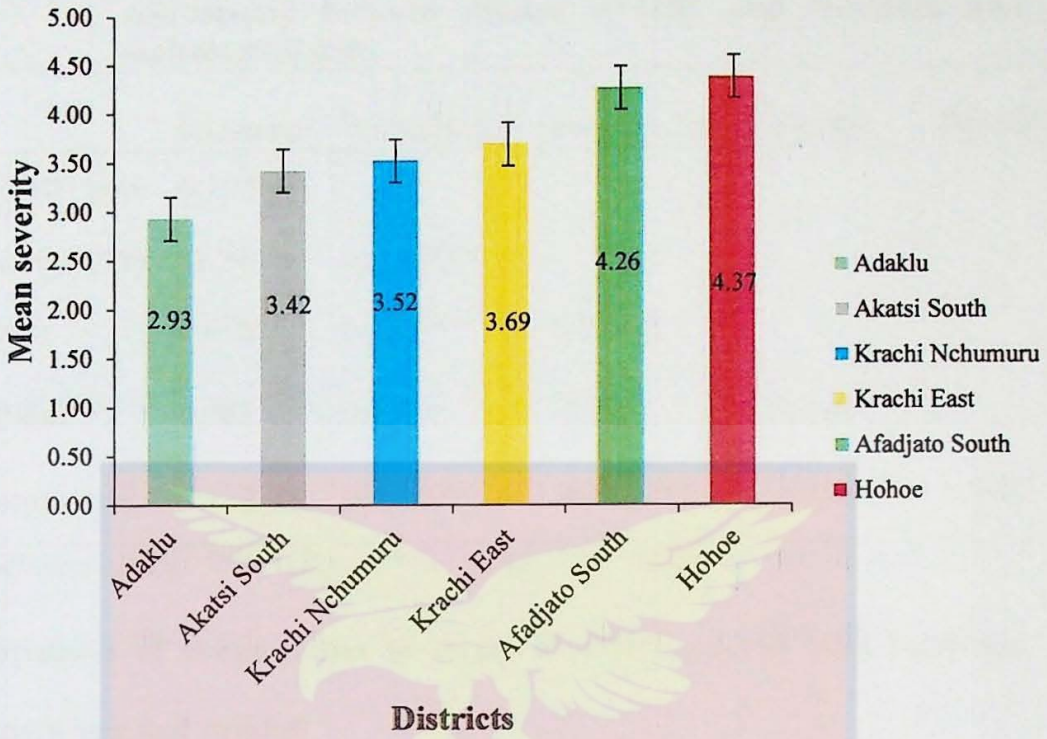


Figure 4.3: Mean severity of CMD across the six districts

Relationship between Variables

To ascertain the relationships between different variables that measure the Cassava Mosaic Disease, the Pearson coefficient of correlation was estimated between whitefly population, incidence, severity, and climatic data. The result (Table 4.4) indicated that there existed a strong positive significant correlation ($r=0.719$; $P<0.05$) between whitefly population and incidence, suggesting that the increase in disease incidence is possibly caused by the increase in whitefly population. Moreover, there existed a significant weak negative correlation ($r=-0.177$; $P<0.05$) between rainfall and disease severity and temperature. This suggests that increase in temperature or rainfall may possibly decrease the disease severity.

Table 4.4: Correlation coefficients for pairwise comparison of the relationship between disease severity and incidence and metrological data

	Incidence	Whitefly pop	Severity (AP)	Month	Rainfall
Whitefly pop	0.719***				
Severity (AP)	-0.291**	-0.302***			
Month	0.476***	0.362***	0.224**		
Rainfall	0.065	0.052	-0.177	0.186	
Temperature	0.132	0.314***	-0.519***	-0.601***	0.050

*Significant codes: '***'0.00; '**' < 0.001; '*' <0.05*

Relatedness of communities in terms of CMD severity and incidence, temperature and rainfall

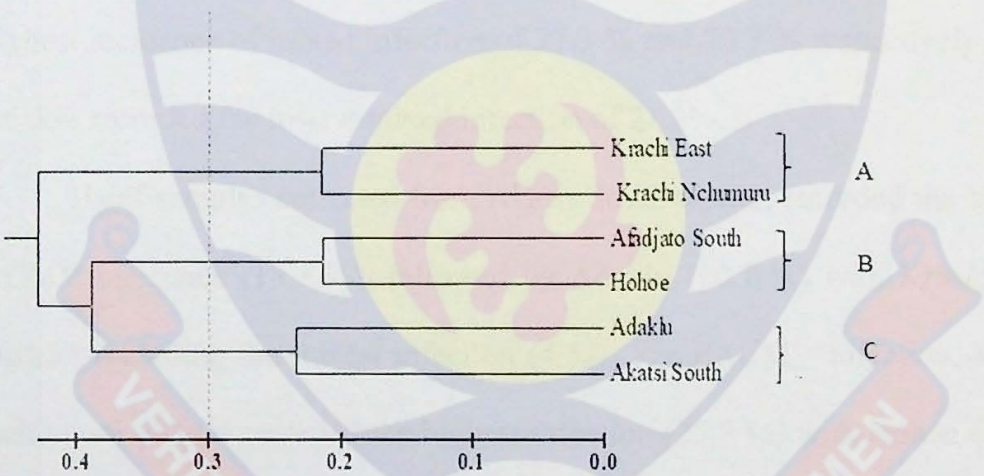


Figure 4.4: Dendrogram showing relatedness of communities in terms of CMD severity and incidence, temperature and rainfall constructed from PowerMarker using seventeen polymorphic markers with UPGMA tree method

The relatedness of the six communities were examined using five parameters, namely the incidence, severity, whitefly population, rainfall and temperature. There were three (3) main clusters observed at 0.3 dissimilarity coefficient. Cluster A shows a close similarity between Krachi East and Krachi Nchumura. Cluster B shows a close similarity between Afadjato South and

Hohoe as well as Cluster C showing the closeness of Adaklu and Akatsi South (Figure 4.3).

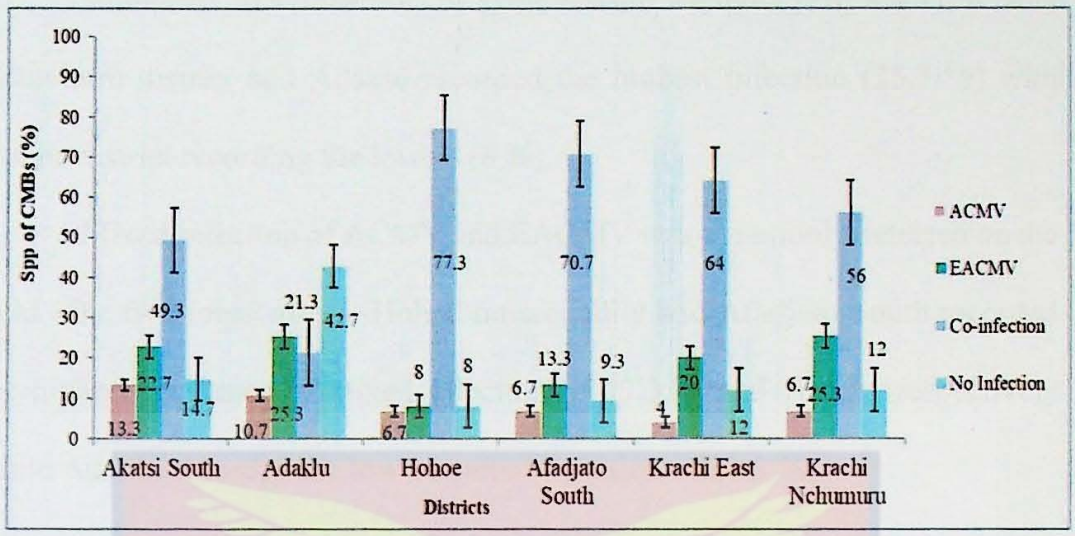
Cassava Mosaic Geminivirus species diversity in the Volta Region

Figure 4.4A shows the diversity of CMG species detected in symptomatic samples by TAS ELISA test. Both ACMV and EACMV species were detected either singly or as co-infections in leaf samples collected from farmers' fields in the six districts (Figure 4.4A). EACMV was detected in a higher number of samples screened than ACMV in all six districts except Hohoe. A higher percentage (more than half) of leaf samples were co-infected with ACMV and EACMV than either species alone in all six districts surveyed. The two forest zones; Hohoe municipality and Afadjato South recorded the highest incidence of mixed infection of 77.3 % and 70.7 % respectively while Adaklu recorded the lowest mixed infection of 21.3%.

Leaf samples collected from Akatsi South district recorded the highest ACMV incidence (13.3 %), followed by Adaklu (10.7 %) with Krachi East District recording the lowest infection (4 %). For EACMV infection, Krachi Nchumuru district recorded the highest infection (25.3 %) with Hohoe district recording the lowest (8%).

Of the 450 asymptomatic samples collected in farmers' fields in the Volta region, 165 samples were positive for CMGs. EACMV was detected in 80 samples, while ACMV was detected in 40 samples. A total of 45 samples were infected with both ACMV and EACMV species (Figure 4.4B)

A



B

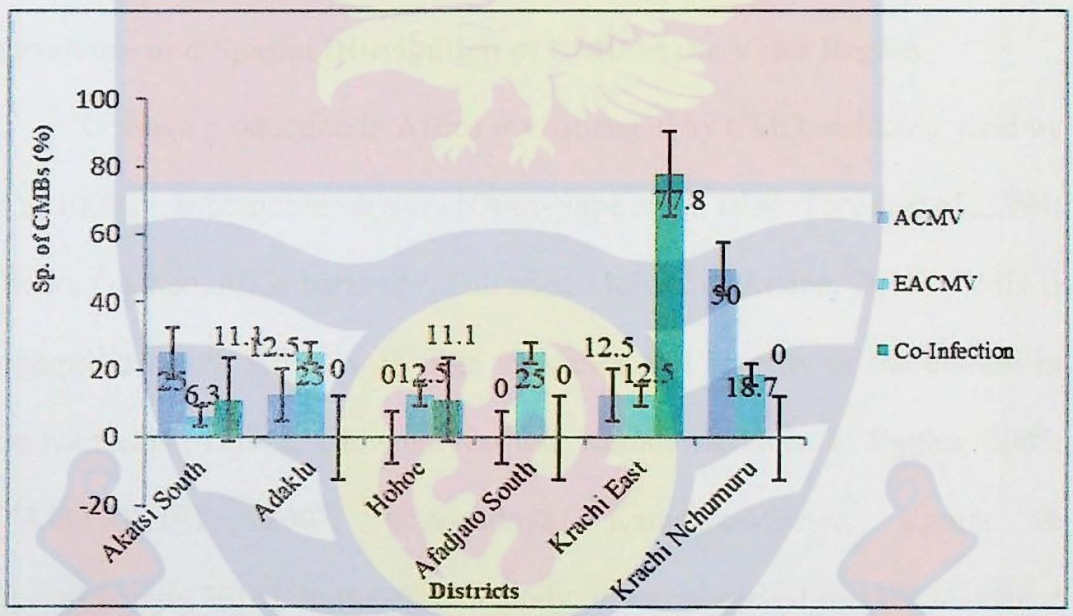


Figure 4.5: Cassava mosaic geminivirus species detected in (A) symptomatic and (B) asymptomatic leaf samples in the Volta

Generally, the percentage of ACMV in all the districts was low and varied significantly. Akatsi South districts recorded the highest ACMV incidence (13.3 %), followed by Adaklu (10.7 %), Hohoe, Afadjato South and Krach Nchumuru (6.7 %) with Krachi East District recording the lowest infection (4 %).

With EACMV infection in symptomatic samples (Fig 4.4A), Krachi Nchumuru district and Adaklu recorded the highest infection (25.3 %) with Hohoe district recording the lowest (8 %).

Mixed infection of ACMV and EACMV was commonly detected on the field. The two forest zones; Hohoe municipality and Afadjato South recorded the highest incidence of mixed infection of 77.3 % and 70.7 % respectively while Adaklu recorded the lowest mixed infection of 21.3 %.

Discussion

Prevalence and Spatial Distribution of CMD in the Volta Region

Cassava production in Africa is challenged by CMD, reducing yield by up to 100% in susceptible varieties (Otim-Nape *et al.*, 1994; Thresh *et al.*, 1994; Atieno, Ogendo, Midatharahally, Hillocks, Mulwa, & Arama, 2016). CMD is widespread across Africa with high incidence and severity of the disease in cassava fields (Yajima, Chiwona-Karltun, Kambewa, Huis, & Jiggins, 2005; IITA/SARRNET, 2007; Changadeya, Kamowa-Mbewe, Kumar & Ntawuruhunga, 2016). In the present study, it was observed that the incidence and severity of CMD were high in the Volta Region. Cassava plants in the field displayed typical CMD symptoms such as mild chlorotic pattern, severe mosaic and distortion of leaves and reduced leaves.

Whitefly population in farmers' fields varied across the selected districts. The abundance of whiteflies in cassava fields is an important factor that influences the spread of CMD across Africa (Otim-Nape, Bua., Thresh., Baguma., Ogwal., Semakula...Martin, 1997; Legg & Ogwal, 1998). Most of the farmers planted varieties with differences in their morphology and biochemistry, which may have played a role in the suitability of growth and

survival of the whiteflies. Again, variation in whitefly population can be attributed to environmental factors such as temperature and rainfall distribution across the various agro-ecological zones, which influences vector multiplication and spread. In Ghana, forest and transitional zones are characterised by low temperatures and high rainfall compared to the coastal and the savannah zones (Stanturf, Melvin, Warren, Charnley, Polasky, Goodrick…Nyako, 2011). For instance, high whitefly incidence has been observed in areas with high temperatures compared to the wet areas (Legg & Raya, 1998). High temperatures and medium precipitation enhance whiteflies reproduction, development, and longevity (Fauquet & Fargette, 1990; Fargette, Colon, Bouveau, & Fauquet, 1996; Sseruwagi *et al.*, 2004; Jeremiah, Ndyetabula, Mkamilo, Haji, Muhanna, Chuwa…Legg, 2015; Mbewe, Kumar, Changadeya, Ntawuruhunga, & Legg, 2015). On the contrary, Mabasa (2007) reported that lower temperature and high rainfall encourage high vegetative growth that provides shelter for whiteflies and therefore increases their population. Nonetheless, in the Volta Region, districts located in the coastal savannah and savannah regions (Akatsi South, Krachi East and Krachi Nchumuru respectively) recorded higher whitefly numbers compared to those in the forest zone. For example, in the forest zone (Hohoe and Afadjato South) which has characteristic low temperatures and high rainfall, low whitefly numbers were recorded. The observation at Adaklu may be explained by the fact that the district has some characteristics of both coastal, transition and forest zone and therefore the low numbers.

In this study, data on whitefly numbers were collected from cassava fields between 3–6 MAP. Whitefly population has been reported to increase

between 3–7 MAP likely due to high vegetative growth at this stage (Sseruwagi, Otim-Nape, Osiru, & Thresh, 2003). Thus, the age of cassava plants influences whitefly population in cassava fields. According to Plantwise 2014, areas with frequent cassava virus infections has an average whitefly population threshold of 3-5 per plant compared to 5-10 in areas with no cassava virus infections. However, according to Theu and Sseruwagi (2003), Changadeya *et al.* (2016), a high whitefly population does not correlate with high CMD incidence. Thus, the prevalence of CMD in the six districts surveyed in the Volta Region could be due to other factors besides whiteflies. From the field survey as reported in the Chapter 3, we noticed that majority of farmers' plant local varieties, which are susceptible to CMD. The growing of susceptible local varieties and the exchange of planting materials among farmers increase the risk of spreading CMD across districts in the Volta Region. The high incidence of CMD in the Volta Region as observed in this study corroborates earlier reports by Torkpo *et al.* (2017) and Oteng-Frimpong, Levyd, Torkpo, Danquah, Offei, Gafni (2012).

In the present study, districts with low whitefly population had low CMD incidence and vice versa, corroborating the findings of Chikoti, Shirima & Legg (2013b). Therefore, the use of CMD-free planting materials is an important strategy to reduce the incidence of CMD especially in cassava-growing regions with low whitefly populations.

Common CMD symptoms identified in farmers' fields include leaf chlorosis, mosaic patterns, leaf deformation, leaf distortion and stunting. CMD severity in the study area was high with a mean severity score of 3.7 compared to the 2.7 reported by Torkpo *et al.* (2018). According to Mabasa, (2007) and Legg, Okao-Okuja, Mayala, & Muhinyuza (2001), an area or region with

severity score of 3.0 and above is considered an epidemic region. Thus, CMD in the Volta Region, one of the major cassava producing areas in Ghana is at epidemic levels and this warrants effective management strategies to minimise the impact of the disease.

Several factors notably high incidence of mixed infections and the sensitivity of local cultivars to the different CMD species present in the field might have contributed to the high symptom severity scores. In mixed infections, the risk of synergistic interaction between the different geminivirus species or strains is high. In addition, the high prevalence of the EACMV species which induces high symptom severity (Fondong *et al*., (2000a), Pita *et al*., 2001a; Maruthi *et al*., 2002b) could explain the high CMD severity on the field. Co-infection is known to increase severity of CMD due to increased accumulation of the viral DNA of either of the strains or both as well as a synergy between the two viruses (Fondong *et al*., 2000a; Pita *et al*., 2001a). This explains why Hohoe and Afajato South districts, which had the highest co-infection, had the highest CMD symptom severity scores while Adaklu with lowest mixed infection showed only mild symptoms.

Cassava Mosaic Geminivirus diversity in the Volta Region

In Africa, the most common and widespread CMGs are the EACMV and ACMV, which are location specific (Fondong *et al*., 2000a; Pita *et al*., 2001a; Ogbe *et al*., 1997, 2003). Torkpo *et al*., (2017), confirmed the above observation in Ghana where majority of samples screened for CMGs were positive for ACMV and negative for single infections with EACMV. However, similar to our results in this present study, they observed a high level of co-infection with ACMV and EACMV. This finding is alarming and highlights the

increasing levels of co-infections and severe CMD symptoms observed in cassava fields in Ghana. Furthermore, the abundance of whitefly population across the study area coupled with infected materials could account for the increased dual infections similar to reports by Harimalala *et al.*, (2015) and Tembo, (2016).

Interestingly, some of the asymptomatic samples tested positive to single infections of ACMV, EACMV or co-infection with both CMGs. As observed with CMD infections, there is a latent period for plants to develop symptoms, which is associated with virus titers in the plants (Fargette, Thouvenel, & Fauquet, 1987; Fargette *et al.*,1994). This observation also shows the importance of confirming viral observation with serological or DNA based nucleic acid assay.

Conclusion

CMD is prevalent in the Volta Region of Ghana. The incidence of CMD in most farms surveyed was high reaching almost 100% with plants displaying severe symptoms. The widespread use of local susceptible varieties and high whitefly population in the study areas account for the high incidence and severity of CMD recorded in the Volta Region. The high incidence of co-infection of plants with ACMV and EACMV is a cause for concern because this increases the probability for synergistic interactions or recombination between species leading to evolution of new CMG species.

CHAPTER FIVE

GENETIC VARIABILITY OF ACMV AND EACMV IN THE VOLTA REGION

Introduction

Cassava is known to be vulnerable to plant virus diseases because of its vegetative propagation (Legg *et al.*, 2015). Cassava Mosaic Disease is one of the most destructive virus diseases of cassava in sub-Saharan Africa prevalent across Africa (Thresh *et al.*, 1997). The disease was first reported in the East Africa specifically Tanzania in the year 1894 (Waeburg, 1894, Legg, & Fauquet, 2004) and has now spread to most Africa countries and its off-shore islands (Patil *et al.*, 2009).

CMD is caused by nine cassava mosaic geminviruses which belong to the Genus *Begomovirus,* family *Geminiviridae* (Patil & Fauquet, 2009; De bryun *et al.*, 2016). These viruses are transmitted by whiteflies and infected planting material (Legg & Thresh, 2003). The continuous use of infected planting materials has been identified as the major cause of the emergence of different species with different biological characteristics and virulence (Legg & Fouquet, 2004). Apart from the reuse of infected planting materials, the system of farming is a major cause of the emergence of CMGs. In the Sub Saharan Africa, most cassava farmers plant cassava with other crops such as maize, vegetables, legumes among others as security against crop failure (FAO, 2013). These human-mediated activities are known to increase the risk of recombination among CMGs in the fields.

CMGs have a single stranded DNA genomes with a size range of 2500-2900 nucleotides (Stanley & Gay, 1983; Hull, 2009). It has a bipartite genome

consisting of a DNA-A genome made of 2700nt and a DNA-B genome of size 2700nt (Hull, 2009). The nucleotides sequence in the DNA-B genomes of CMGs is more diverse since it has less conserved elements compared to DNA-A (Rybicki, 1994; Harrison & Robinson, 1999).

Infections with single CMG induce fewer symptoms (Pita *et al.*, 2001a). However, mixed infection of two or more CMGs induces very severe symptoms that can reduce crop yield significantly due to synergism and cause an epidemic in extreme cases as reported in Uganda in the early 1990's (Fondong *et al.*, 2000a; Pita *et al.*, 2001a; Ogbe *et al.*, 1997, 2003).

In Ghana, CMGs have been identified as devastating and prevalent causing yield loss of 20% to 90% (Moses, 2009) and occurring in many cassava farms (Oteng-Frimpong *et al.*, 2012; Torkpo *et al.*, 2017). Over the last three decades, about 750 CMG genomes have been characterized and published in the public data base (Bock & Woods, 1983; Stanley & Gay, 1983). The characterisation of genome in CMGs in cassava fields will bring to light the available germiniviruses infecting cassava production in the region Again this is vital to reduce yield losses and ensure food and income security for small scale farmers in Ghana. Furthermore, this information is important in the design and implementation of CMD management strategies in Ghana.

The variability of ACMV and EACMV has been ascertained in some parts of Ghana since 2007 and 2012 (Oteng-Frimpong *et al.*, 2012 & Torkpo *et al.*, 2017). However, with recombination and/or pseudo-recombination being the force behind virus evolution, and the substitution rate of geminiviruses ranging between 10^{-4} and 10^{-3} per site per year (Shackelton, Parrish, Truyen, & Holmes, 2005; Duffy & Holmes, 2008; Lopez-Bueno, Tamames, Velazquez,

Moya, Quesada, Alcami 2009), it is important to identify and characterise genetic diversity of CMGs in cassava fields in Ghana after almost 10 years since the last reported study.

DNA-based diagnostic methods are efficient in characterising genetic diversity of CMGs (Padidam *et al.*, 1995; Pita *et al.*, 2001a) and these include polymerase chain reaction (PCR) amplification, restriction fragment length polymorphism (RFLP) analysis and DNA sequencing (Rojas *et al.*, 1993; Padidam *et al.*, 1995; Brown, Idris, Torres-Jerez, Banks, & Wyatt, 2001). Zhou *et al.*, (1997) opined that in PCR, shared or unique sequences can be used since different geminiviruses share similar nucleotide sequences in different parts of their DNA-A molecules compared to their DNA-B (Rojas *et al.*, 1993; Deng, McGrath, Robinson, & Harrison, 1994; Zhou *et al.*, 1997). The PCR-amplified fragments are further digested using a restriction enzyme like endonucleases through the Restriction Fragment Length Polymorphism (RFLP) process. The RFLP is normally used together with Rolling cycle amplification (RCA), which is one of the effective methods used for the identification and characterization of various monopartite and bipartite geminiviruses attacking plants (Inoue-Nagata, Albuquerque, Rocha, Nagata, 2004; Haible, Kober & Jeske, 2006; De Bruyn *et al.*, 2016). RCA is known to increase the concentration of the virus DNA (Rector, Tachezy, & Van Ranst, 2004) prior to sequencing to better understand the genetic structure and diversity of the population (Ndunguru, Legg, Aveling, Thompson, & Fauquet 2005).

In this study, genetic diversity of CMGs infecting cassava in six districts in the Volta Region was determined using molecular tools such as PCR and sequencing. Twelve full length genomes were assembled and characterised

using nucleotide sequence identity and phylogenetic analysis. Information from genetic diversity studies will be useful for designing effective CMD management strategies in Ghana.

Materials and methods

The molecular assay to determine the diversity of CMGs infecting cassava in the selected areas was carried out at the Biotechnology Laboratory of the College of Agriculture and Consumer Sciences, University of Ghana, Legon.

Sample collection

Six districts (Akatsi South District, Adaklu District, Hohoe Municipality, Afadjato South District, Krachi Nchumuru District and Krachi East District were selected from the major cassava producing areas in the Volta Region. In each district, three communities were selected for sample collection. Five farms were randomly selected in each communities taking into consideration proximity to each other (a minimum interval of 5 km to 50 km) (Cochran, 1977; Sseruwagi *et al*., 2004; Ndunguru *et al*., 2005; Mbasa, 2007). In each farm, young leaves materials were collected from all 25 symptomatic plants and pooled into one sample. A total of 90 (15 farms*6 districts) symptomatic fresh leaf samples from 90 (3 communities, 5 farms per each community for 6 districts) cassava farms, aged 1 - 3 months were selected from June to August 2019. Sampling was done by plucking fresh symptomatic leaves into a zip-lock polythene bag. The samples were preserved in a iced chest box and transported to the laboratory for analysis.

DNA Extraction

Genomic DNA was extracted from pooled leaf samples per farm using a modified CTAB protocol by Doyle and Doyle (1990). About 200 mg of leaf tissue was ground with Kontes Microtube pellet pestle rods in a 1.5 ml microfuge tube containing 500 µl of CTAB buffer. The mixture was transferred into a fresh microcentrifuge tube and incubated for 15 minutes at 55°C in a recirculating water bath. The mixture was centrifuged at 12000 g for 5 minutes to separate cell debris and the supernatant transferred to a clean microfuge tube with 250 µL of chloroform (Iso Amyl Alcohol, 24:1). The solution was mixed by inversion and centrifuged at 13000 rpm for 1 minute. The upper aqueous phase, which contains DNA, was transferred to a clean microfuge tube and 50 µL of 7.5 M Ammonium Acetate was added. This was followed by the addition of 500 µL of ice-cold absolute ethanol. The tubes were inverted slowly to precipitate the DNA. The tubes were centrifuged at 13000 rpm for 1 minute to pellet the DNA. The supernatant was removed, and the DNA pellet dried for 15 minutes at room temperature and re-suspension in 100 µL sterile DNase-free water. The quality of the DNA was determined by running 10 µL aliquot on 1% agarose gel for 30 minutes at 100 V.

PCR amplification

All 90 samples were screened for ACMV and EACMV by PCR using primers UV AL1/F and ARO/R and primers EAB555/F and EAB555/R respectively (Zhou *et al.*, 1997; Fondong *et. al.*, 2000a; Ndunguru *et al.*, 2005) (Table 5.1).

Table 5.1: List of primers used for PCR detection of ACMV and EACMV

Primers	Sequence 5'-3'	Virus	Target	Expected size
ACMV-AL1/F	GCGGAATCCCTAACATTATC	ACMV	AL1	1030 bp
ACMV-AR0/R	GCTCGTATGTATCCTCTAAGGCCTG			
EAB555/F	TACATCGGCCTTTGAGTCGCATGG	EACMV	BC1/CR	540–560 kbp
EAB555/R	CTTATTAACGCCTATATAAACACC			
References	Zhou *et al.*, 1997; Ndunguru *et al.*, 2005.			

PCR reaction conditions were initiated at 94 °C for 2 minutes, followed by 45 cycles at 94 °C for 1 minute, 55 °C for 1.5 minutes. There was an extension of 2 min at 72 °C and a final extension of 72 °C for 10 minutes and held for 4 °C. The reaction was performed in Applied Biosystems 2720 thermal cycler PCR machine using 20 µL reaction mixture. After PCR, 12 µL of the reaction mixture was loaded on 1.2 % agarose gel prepared with TAE (Tris base, acetic acid and EDTA) buffer for 35 minutes at 100V for DNA amplification.

Rolling Circle Amplification (RCA)

Samples were pooled according to districts (six samples) for the RCA. "Illustra TempliPhi 100 Amplification Kit" (GE Healthcare) protocol was used according to the manufacturer's recommendations. Specifically, 5 µL of sample buffer was mixed with 1 µL of the DNA from each district and denatured at a temperature of 95°C. The mixture was cooled on ice for 2 to 3 minutes after which 5 µL reaction buffer and 0.2 µL of enzyme mix were added. The mixture was incubated for 16-18 hours at a temperature of 30 °C. Water was used as the negative control.

Preparation of nextera libraries for Illumina MiSeq sequencing

Good quality RCA product with purity indices equal to or greater than 1.8 to 2.0 ng/µl were selected and pooled for the three agro ecological zones (coastal savannah, forest zone and guinea savanah) samples for next generation sequencing. The RCA-DNA libraries were prepared from total RCA-DNA extract concentration ranging from 1.67 to 1.69 ng/µl using the Illumina nextera DNA Sample Preparation kit™ according to the manufacturer's instructions (Illumina, San Diego, California). The first step involved DNA fragmentation followed by the addition of adapter sequences to the ends to allow for amplification by PCR. Addition of indexes and enrichment was done. The final size and concentration of each library was estimated using a Bioanalyzer (Agilent, Santa Clara, CA, USA) and the Qubit (Invitrogen, Carlsbad, CA, USA), respectively. Library pools of 2 nM were prepared by mixing the libraries from each sample to achieve an equal molar concentration of each. Libraries were normalized, pooled and sequenced using a 2×300 -cycle PE V3 Illumina kit. Paired end reads were generated using the Illumina MiSeq System at the Inqaba Biotechnical Industries Limited in Pretoria, South Africa.

Illumina Miseq short sequence assembly and analysis

The reads were first trimmed before being assembled into contigs in Geneious 8.1.8 by using de novo assembly function. Contigs were sorted according to length (minimum length 500), and the longest contigs subjected to BLAST searches (Altschul, Gish, Miller, Myers & Lipman (1990). Furthermore, the reads were mapped to reference genomes derived from GenBank as variants of the same virus. Mapping was performed with minimum overlap of 10%, minimum overlap identity of 80%, allow gaps of 10% and fine

tuning set to iterate up to 10 times (Kehoe, Coutts, Buirchell & Jones 2014). By aligning with Clustal W, a consensus between the relevant contigs and mapping was produced in Geneious. The sequences that were produced were compared to the reference sequences used during the mapping procedure.

Results

Molecular characterisation of ACMV and EACMV species in farmers' fields in the Volta Region

Molecular results obtained from PCR detection of Cassava Mosaic Geminiviruses in farmers' fields are presented in Figures 5.1 to 5.4. The detection of ACMV is shown in Figures 5.1 and 5.2 and EACMV shown in Figures 5.3 and 5.4. In Figures 5.1 and 5.3, lanes 1-15 denote cassava samples from Krachi Nchumuru district (Chinderi (1-5), Lumbusu (6-10) and Boreae (11-15). 16-30 represent samples from Krachi East district (Kparipari (16-20), Anyanbor (21-25) and Yariga (26-30) while 31-45 represent samples from Hohoe municipality (Santrokofi (31-35), Fodome (36-40), Gbi Woebe (41-45).

In Figures 5.2 and 5.4, lanes 46-60 denote samples from Adaklu districts (Kpeleho (46-50), Hilhavi (51-55) and Adzoedukope (56-60). 61-75 were samples from Afadjato south district (Have (61-65), Goviefe Kowu (66-70) Logba (71-75) and samples 76-90 represent that of Akatsi south districts (Atsidzive (76-80), Gefia (81-85), Abedrafor, (86-90).

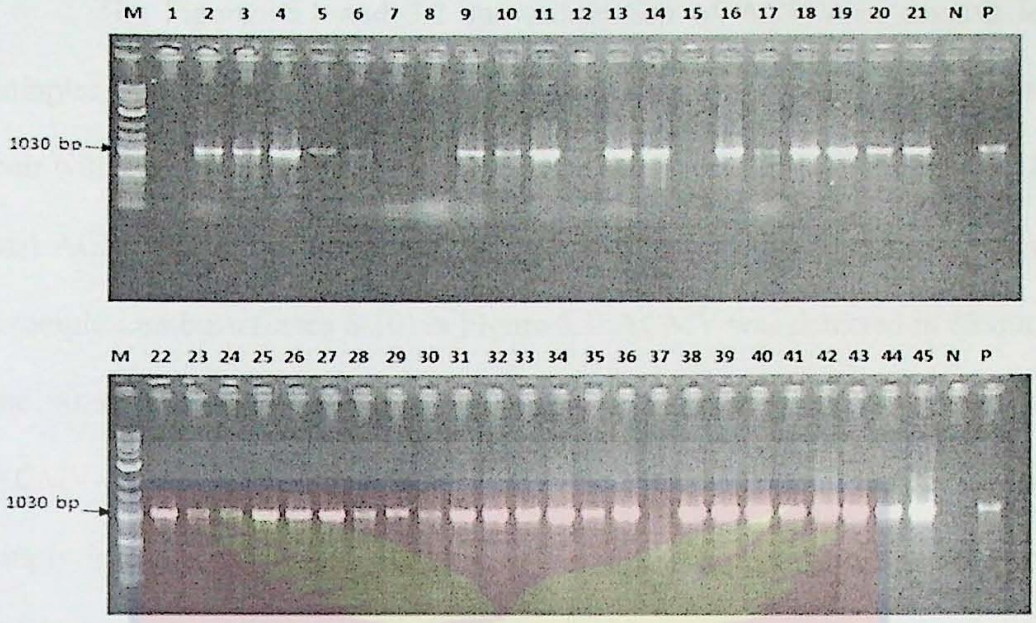


Figure 5.1. Amplicon of ACMV obtained from cassava leaves from farmers' fields using ACMV-AL1/F and ACMV-AR0/R primer pairs of size 1000 bp-1030 bp, lanes 1-15 (Krachi Nchumuru (Chinderi (1-5), Lumbusu (6-10), Boreae (11-15)), 16-30 (Krachi East (Kparipari (16-20), Anyanbor (21-25) Yariga (26-30)), 31-45 (Santrokofi (31-35), Fodome (36-40), Gbi Woebe (41-45)). N and P denote the negative and positive control respectively while M denotes 1 kb DNA Ladder

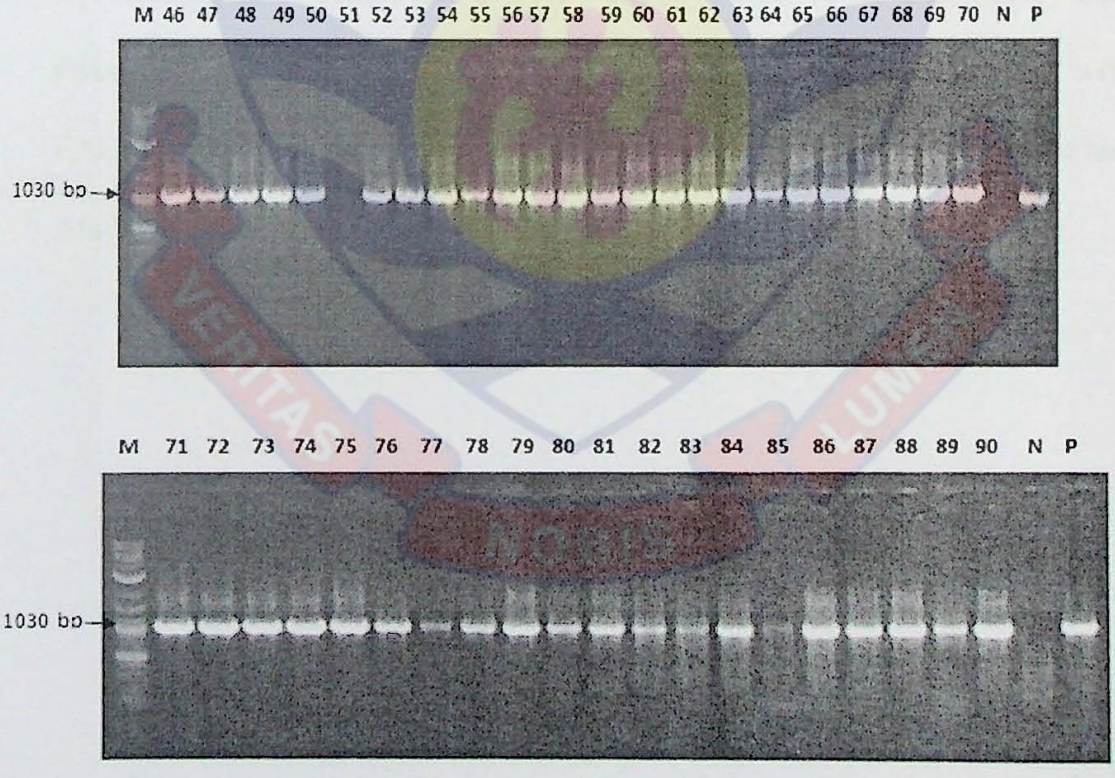


Figure 5.2:Amplicon of ACMV obtained from cassava leaves from farmers' fields using ACMV-AL1/F and ACMV-AR0/R primer pairs of size 1000 -1030 bp, lanes 46-60 (Adaklu (Kpeleho (46-50), Hilhavi (51-55), Adzoedukope (56-60)), 61-75 (Afadjato south (Have (61-65), Goviefe Kowu (66-70) Logba (71-75)), 76-90 (Akatsi south Atsidzive (76-80), Gefia (81-85), Abedrafor, (86-90)). N and P denote the negative and positive control respectively while M denotes 1 kb DNA Ladder.

The Figures 5.1 and 5.2 show detection of ACMV in cassava leaf samples from farmers' fields using ACMV-AL1/F and ACMV-AR0/R primer pair with expected band size 1030 bp. From the figures, primer ACMV AL1/F and ACMV-AR0/R detected the ACMV in all the districts but not all farms example Lumbusu (lanes 6-10) in Figure 5.1. ACMV was detected in 83 out of the 90 samples representing 92.2% infection rate using ACMV-AL1/F and ACMV-AR0/R primer pairs. Out of this, 71 samples representing 78.9% were singly infected with ACMV with 12 (13.3%) being in mixed infection with EACMV.

Figures 5.3 and 5.4 show detection of EACMV in cassava leaf samples from farmers' fields using EAB555/F and EAB555/R primer pair with expected band size of 540 bp. EACMV DNA was detected in 19 out of 90 samples, representing an infection rate of 21.1 %. Out of this, 18 samples (20%) were mixed infection with ACMV and 1 (1.1%) sample was singly infected with EACMV. EACMV was not detected in any of the samples from Adaklu and Afadjato South using the primer pair EAB555/F and EAB555/R.

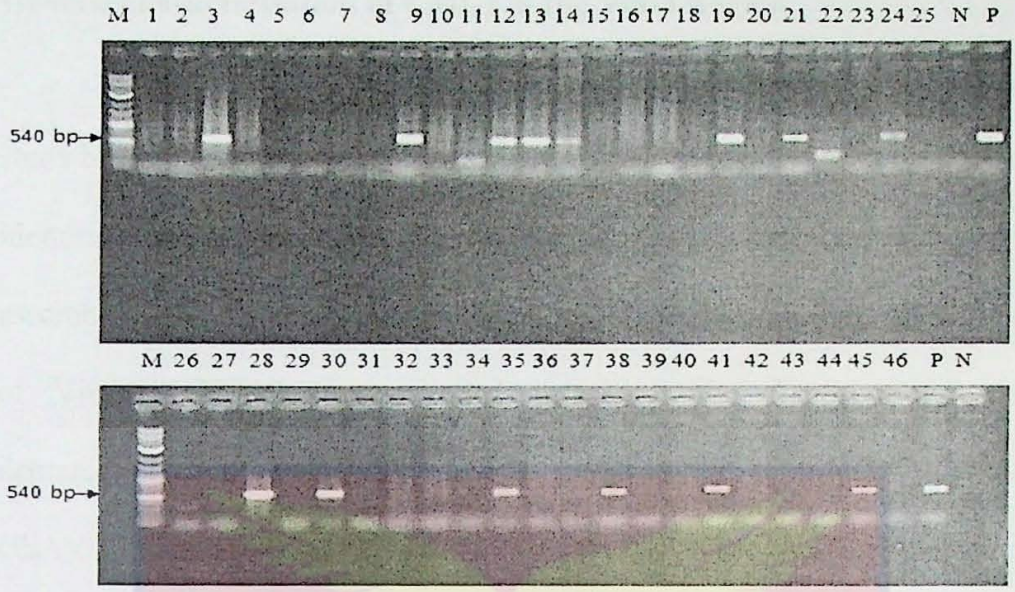


Figure 5.3 Amplicon of EACMV obtained from cassava leaves from farmers fields using EAB555/F and EAB555/R primer pairs of size 540 bp, lanes 1-15 (Krachi Nchumuru (Chinderi (1-5), Lumbusu (6-10), Boreae (11-15)), 16-30 (Krachi East (Kparipari (16-20), Anyanbor (21-25) Yariga (26-30)), 31-45 (Santrokofi (31-35), Fodome (36-40), Gbi Woebe (41-45)). N and P denote the negative and positive control respectively while M denotes 1 kb DNA Ladder.

Figure 5.4. Amplicon of EACMV obtained from cassava leaves from farmers fields using EAB555/F and EAB555/R primer pairs of size 540 bp, lanes 46-60 (Adaklu (Kpeleho (46-50), Hilhavi (51-55), Adzoedukope (56-60)), 61-75 (Afadjato south (Have (61-65), Goviefe Kowu (66-70) Logba (71-75)), 76-90 (Akatsi south (Atsidzive (76-80), Gefia (81-85), Abedrafor, (86-90)). N and P denote the negative and positive control respectively while M denotes 1 kb DNA Ladder.

Genetic characterisation of CMGs in the Volta Region

A total of twelve full length genome components were assembled in this study based on sequence alignment (BLAST) search results, pairwise nucleotide identities, and phylogenetic analysis. Taxonomic classification of the newly-assembled genome components using International Committee on Taxonomy of Viruses (ICTV) recommended thresholds for *Begomovirus* species demarcation identified six genome components (GhAVGS, GhAVFZ, GhAVCS, GhAEVRGS, GhAEVRFZ and GhAEVRCS highly similar (95.52 – 99.01%) to ACMV (DNA-A) isolates from Nigeria (X17095.1) and Ghana (MG250089.1). The variant GhAVCS from the Coastal Savanah displayed high nucleotide sequence identity (96.73% - 98.95%) with another ACMV variant from Ghana (MG250089.1) (Figure 5.5).

Based on blast search results, the remaining six newly assembled genomes showed high nucleotide sequence identities to DNA-B of ACMV (GhBVCS, GhBVGS, GhBVFZ) and DNA-B of EACMV (GhBEVRGS, GhEAVRFZ and GhBEVRCS) isolates from Ghana, Nigeria, or Cameroon (Figure 5.6).

The genome component's, GhBEVRGS, GhEAVRFZ and GhBEVRCS were highly similar to EACMV Cameroon virus or EACMV Cameroon virus-Ghana isolate (AF112355.1 and JN165087.1).

Genome components GhBVCS, GhBVGS and GhBVFZ were isolated from the coastal savannah, guinea savannah and the forest zones respectively.

The three genome components identified in this study that showed high nucleotide sequence identity to DNA-B of the EACMV species were identified

in the guinea savannah (GhBEVRGS), forest (GhEAVRFZ) and coastal savannah (GhBEVRCS) zones (Figure 5.6).

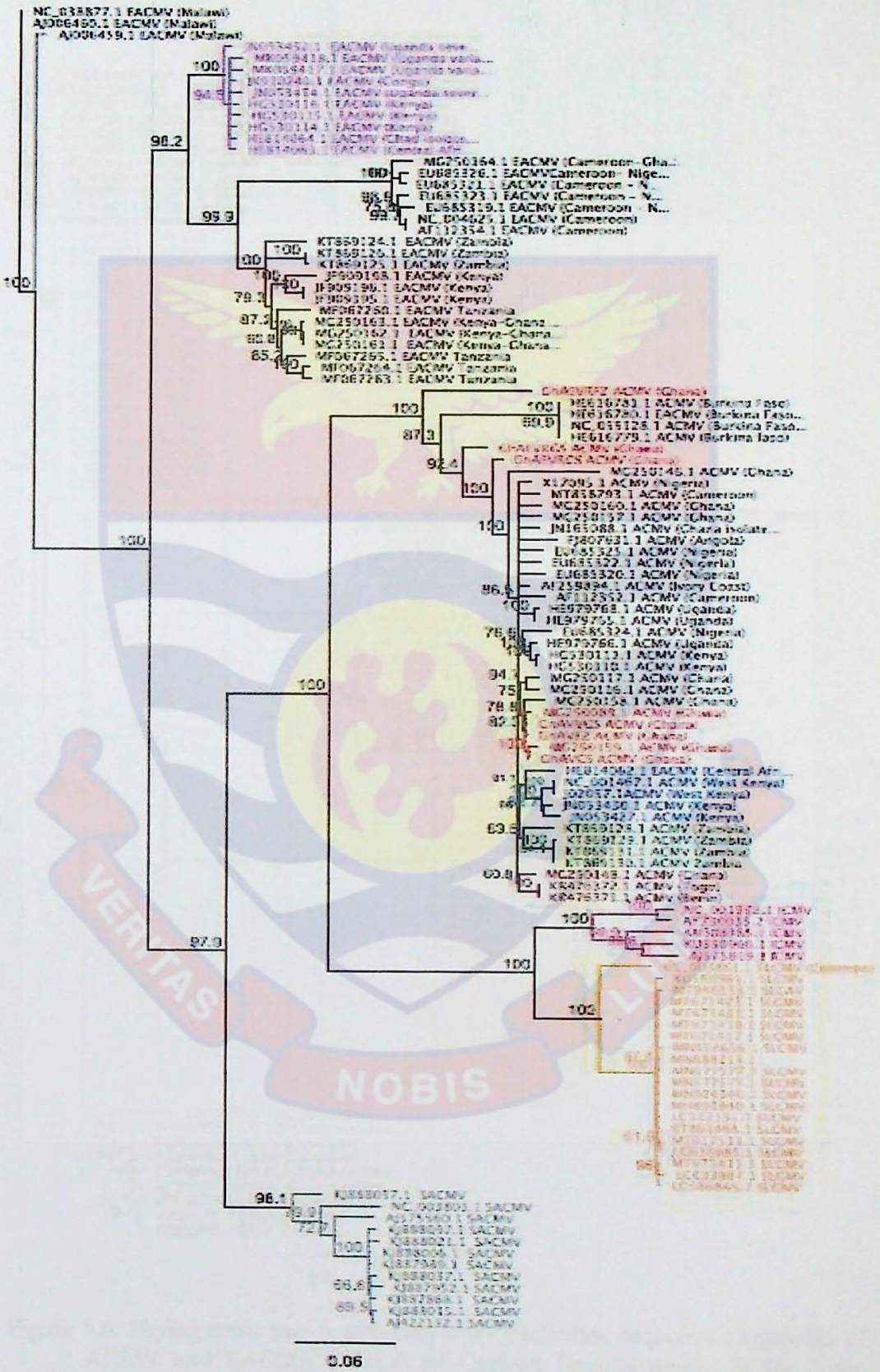


Figure 5.5. Phylogenetic tree constructed from nucleotide sequence alignments of ACMV DNA-A of Cassava Begomoviruses in the Volta Region of Ghana.

All the three isolates identified in this study aligned closely (93.83%, 93.87% and 92.87%) to Ghanaian isolate (JN165087.1).

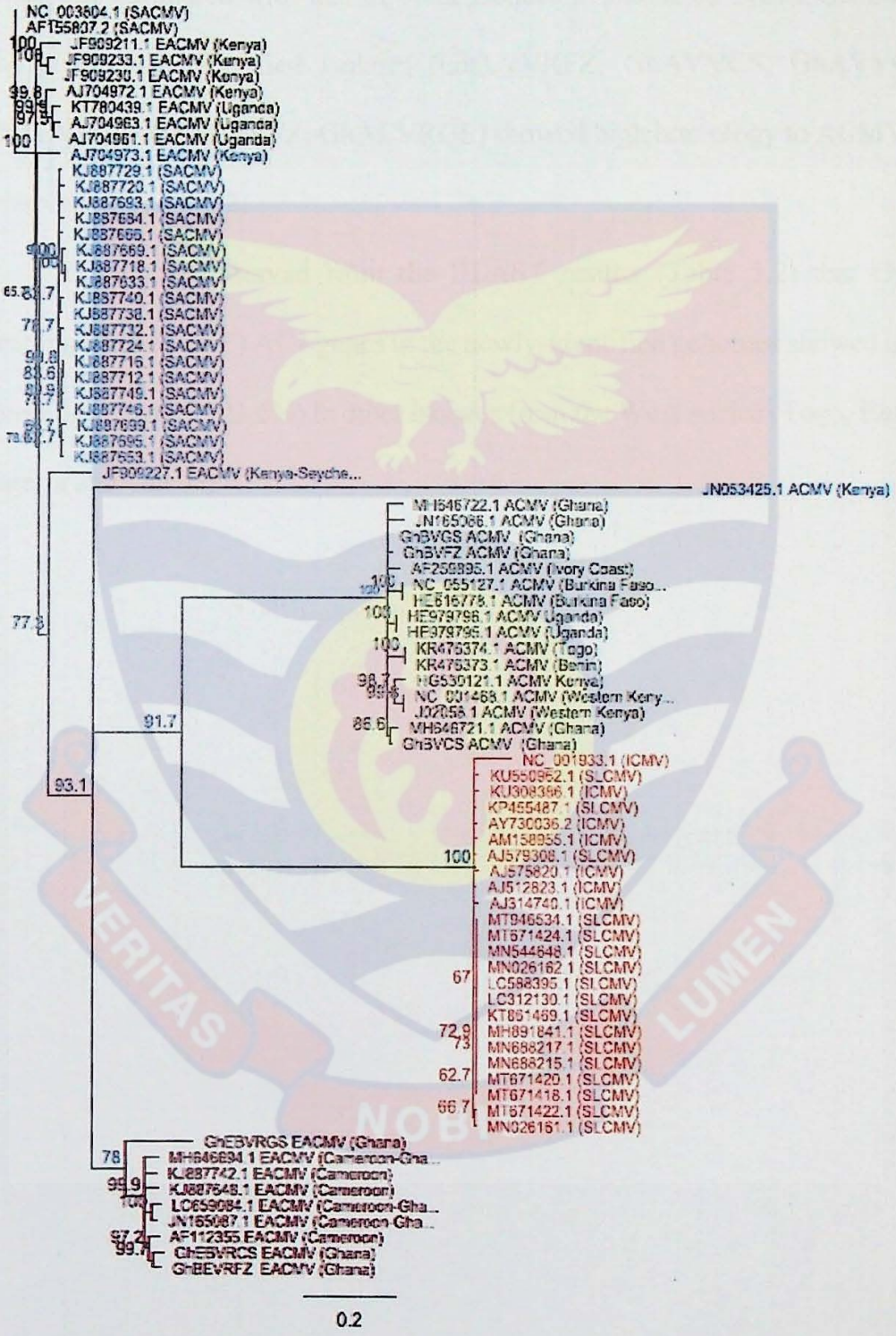


Figure 5.6. Phylogenetic tree constructed from nucleotide sequence alignments of ACMV and EACMV DNA-B of Cassava Begomoviruses in the Volta Region of Ghana.

Genetic diversity of genes on the DNA-A genome of newly identified CMGs

To determine genetic diversity of genes on the DNA-A genomes of ACMV species identified in this study nucleotide sequences of the individual genes was compared with that of other isolates available on NCBI GenBank. The six newly identified isolates (GhAVVRFZ, GhAVVCS, GhAVVGS, GhAEVRCS, GhAEVRFZ, GhAEVRGS) showed high homology to ACMV as observed in Table 5.2.

It can be observed from the BLAST results (Table 5.2) that Open Reading Frame (ORF) AC1 genes in the newly-identified genomes showed high homology (92.0% - 93.6%) to other isolates from the West Africa (Togo, Benin, Nigeria and Ghana).

Table 5.2: Nucleotide sequence identities (%) of the AC1 genes of ACMV and EACMV isolates identified in this study compared to other selected isolates on NCBI

		New isolates from the study					
Isolates from NCBI	Country of Origin	GhAVVRFZ ACMV	GhAVVCS ACMV	GhAVVGS ACMV	GhAEVRCS ACMV	GhAEVRFZ ACMV	GhAEVRGS ACMV
NC_038877.1 EACMV	Malawi	92.8	94.2	92.8	87	61.6	76.4
AJ575560.1	SACMV	53.4	53.3	52.9	53.4	53.7	56.5
JX910240.1 EACMV	Congo	44.3	44.6	43.8	47.4	70.8	57.5
HE814064.1 EACMV	Chad	42.7	42.9	42.7	49.1	75.8	60.5
HE814063.1 EACMV	C.A.Republic	53.8	54.1	54.1	50.2	41	48.3
MK059418.1 EACMV	Uganda	42.7	42.6	41.9	46.3	70.2	56.4
KR476372.1 ACMV	Togo	92.5	93.6	92.6	87	62.7	77
KR476371.1 ACMV	Benin	92.5	93.6	92.6	87	62.7	77
AF259894.1 ACMV	Ivory Coast	87	88.3	87	81.4	57.4	71.1
X17095.1 ACMV	Nigeria	92	93	92.3	86.1	61.6	75.9
MG250160.1 ACMV	Ghana	92	93	92.3	86.1	61.6	75.9
MG250160.1:1214-1621 EACMV	Ghana	42.4	42.4	41.6	46.6	71.4	57.2
HE979765.1 ACMV	Uganda	93.1	94.4	92.8	87.5	62.2	76.1
KT869131.1 ACMV	Zambia	42.1	42.1	41.3	46	70.8	56.4
FJ807631.1 ACMV	Angola	41.8	41.8	41	46.3	71.1	56.6
NC_055128.1 ACMV	B. Faso	87.8	89.4	88.1	82.8	59.4	72.2
NC_004625.1 EACMV	Cameroon	48.1	48.1	47.6	47.9	46.1	49.5
MT671423.1 SLCMV	S. Lanka	52.7	52.9	51.9	53.3	46.6	55
AJ575819.1 ICMV	India	93.6	95	94.2	87.8	62.4	77.5

For the AC2 genes, the six newly-identified ACMV genomes from Ghana showed highest homology (95.6 – 97%) to other ACMV genomes identified in Togo, Benin and Uganda (Table 5.3).

Similarly, for the AC3 genes, the six newly-identified ACMV genomes from Ghana showed highest homology (94 – 95.6%) to other ACMV species from Ghana and Uganda (Table 5.4).

Table 5.3. Nucleotide sequence identities (%) of the AC2 genes of ACMV and EACMV isolates identified in this study compared to other selected isolates on NCBI

		New Isolates from the study					
Isolate from NCBI	Country of Origin	GhAVVRFZ ACMV	GhAVVCS ACMV	GhAVVGS ACMV	GhAEVRCS ACMV	GhAEVRFZ ACMV	GhAEVRGS ACMV
NC_038877.1 EACMV	Malawi	60	60	60.1	60	60	60.1
AJ575560.1	SACMV	63	63	63	63	63	63
JX910240.1 EACMV	Congo	60.7	60	60.8	60	60.7	60.8
HE814064.1 EACMV	Chad	60.7	60	60.8	60	60.7	60.8
HE814063.1 EACMV	C. A. Republic	60.7	60	60.8	60	60.7	60.8
MK059418.1 EACMV	Uganda	62.2	61.5	62.3	61.5	62.2	62.3
KR476372.1 ACMV	Togo	97	96.3	95.6	96.3	97	95.6
KR476371.1 ACMV	Benin	97	96.3	95.6	96.3	97	95.6
AF259894.1 ACMV	Ivory Coast	97	96.3	95.6	96.3	97	95.6
X17095.1 ACMV	Nigeria	97	96.3	95.6	96.3	97	95.6
MG250160.1 ACMV	Ghana	94.8	94.1	93.4	94.1	94.8	93.4
MG250160.1:1214-1621	Ghana	94.8	94.1	93.4	94.1	94.8	93.4
HE979765.1 ACMV	Uganda	97	96.3	95.6	96.3	97	95.6
KT869131.1 ACMV	Zambia	94.1	93.3	94.1	93.3	94.1	94.1
FJ807631.1 ACMV	Angola	91.9	91.1	91.1	91.1	91.9	91.1
NC_055128.1 ACMV	Burkina Faso	92.6	91.9	91.1	91.9	92.6	91.1
NC_004625.1 EACMV	Cameroon	55.6	54.8	55.6	54.8	55.6	55.6
MT671423.1 SLCMV	SLCMV	55.6	54.8	54.8	54.8	55.6	54.8
AJ575819.1 ICMV	ICMV	41.3	40.6	41.4	40.6	41.3	41.4

Table 5.4: Nucleotide sequence identities (%) of the AC3 genes of ACMV and EACMV isolates identified in this study compared to other selected isolates on NCBI

		New Isolates from the study					
Isolate from NCBI	Country of Origin	GhAVVGS ACMV	GhAVVCS ACMV	GhAVVRFZ ACMV	GhAEVRCS ACMV	GhAEVRFZ ACMV	GhAEVRGS ACMV
NC_038877.1 EACMV	Malawi	56.4	54.9	57.1	54.9	57.1	56.4
AJ575560.1	S. Africa	57.1	55.7	57.8	55.7	57.8	57.1
JX910240.1 EACMV	Congo	57.1	55.7	57.8	55.7	57.8	57.1
HE814064.1 EACMV	Chad	57.1	55.7	57.8	55.7	57.8	57.1
HE814063.1 EACMV	C.A. Republic	60.1	59.4	60.8	59.4	60.8	60.1
MK059418.1 EACMV	Uganda	58.6	57.9	59.3	57.9	59.3	58.6
KR476372.1 ACMV	Togo	94.2	94.2	94.9	94.2	94.9	94.2
KR476371.1 ACMV	Benin	92	92	92.7	92	92.7	92
AF259894.1 ACMV	Ivory Coast	93.4	92	93.4	92	93.4	93.4
X17095.1 ACMV	Nigeria	92	92	92.7	92	92.7	92
MG250160.1 ACMV	Ghana	92	92	92.7	92	92.7	92
MG250160.1:1214-1621	Ghana	94.9	94.2	95.6	94.2	95.6	94.9
HE979765.1 ACMV	Uganda	94.9	94.2	95.6	94.2	95.6	94.9
KT869131.1 ACMV	Zambia	93.4	92.7	94.1	92.7	94.1	93.4
FJ807631.1 ACMV	Angola	92	91.2	91.9	91.2	91.9	92
NC_055128.1 ACMV	Burkina Faso	89	88.3	89	88.3	89	89
NC_004625.1 EACMV	Cameroon	44.5	44.6	46	44.6	46	44.5
MT671423.1 SLCMV	SLCMV	45.3	45.3	46.7	45.3	46.7	45.3
AJ575819.1 ICMV	ICMV	44.5	44.6	45.3	44.6	45.3	44.5

Table 5.5 compares nucleotide sequences of selected ACMV and EACMV isolates on GenBank with the newly identified genomes from Ghana. All the AC4 genes showed high homology (91.5 to 92.9%) to isolates from Benin, Togo, Ivory Coast and Uganda with the exception of the AC4 gene of the ACMV isolate (GhAEVRFZ), which showed highest homology (97.9%) to EACMV Cameroon isolate.

Table 5.5: Nucleotide sequence identities (%) of the AC4 genes of ACMV and EACMV isolates identified in this study compared to other selected isolates on NCBI

Isolates from NCBI	Country of Origin	New Isolates from the study GhAVVRFZ ACMV	GhAVVCS ACMV	GhAVVGS ACMV	GhAEVRCS ACMV	GhAEVRFZ ACMV	GhAEVRGS ACMV
NC_055128.1 ACMV	B. Faso	28.2	28.9	28.9	26.2	34.5	37.5
AJ575819.1	India	42.1	42.9	42.9	38.8	37.1	45.8
AJ575560.1	S. Africa	40.8	40.9	40.2	38.3	32.4	45.9
KR476372.1 ACMV	Togo	92.2	92.9	92.9	80.2	23.6	52.3
KR476371.1 ACMV	Benin	92.2	92.9	92.9	80.2	23.6	52.3
MG250160.1 ACMV	Ghana	89.3	90	90	78.1	22.9	50.1
MG250160.1:2150-2572	Ghana	89.3	90	90	78.1	22.9	50.1
FJ807631.1 ACMV	Angola	89.3	90.7	90	78.8	25	50.8
KT869131.1 ACMV	Zambia	90	90.7	90.1	79.5	22.1	51.6
AF259894.1 ACMV	Ivory Coast	92.2	92.9	92.2	80.9	22.1	50.8
HE979765.1 ACMV	Uganda	91.5	92.2	90.7	80.2	22.9	49.4
MT671423.1	S. Lanka	50	50.7	50.8	45.2	25	36.6
NC_004625.1 EACMV	Cameroon	22.9	23.6	24.4	24.5	97.9	68
JX910240.1 EACMV	Congo	23.6	23.6	24.4	23.1	85.7	62.3
HE814063.1 EACMV	C.A. Republic	23.6	23.6	24.4	23.8	86.4	63.7
HE814064.1 EACMV	Chad	25	25	25.8	24.5	86.4	63
MK059418.1 EACMV	Uganda	23.6	23.6	24.4	23.8	85.7	63.7
NC_038877.1 EACMV	Malawi	22.9	23.6	23.6	22.4	87.1	60.8

Genetic diversity of genes on the DNA-B genomes of newly-identified CMGs

To characterise diversity on the DNA-B genome, nucleotide sequences of the BV1 and BC1 genes of the newly- identified genomes from Ghana was compared with other reported isolates on NCBI GenBank. Table 5.6 shows the comparison of the BV1 gene of the newly-identified isolates with selected CMGs from elsewhere in Africa and Asia. The BV1 gene of three of the newly-identified ACMV genomes (GhBVFZ, GhBVCS, GhBVGS) showed high homology (93.0% - 95.7%) to the BV1 gene of ACMV isolates from West Africa (Ghana, Ivory Coast and Burkina Faso). In contrast, the BV1 gene of the three newly identified EACMV genomes (GhBEVRCS, GhBEVRFZ, GhBEVRGS) showed high homology (91.1% - 97.3%) to EACMV DNA-B of isolates from East Africa (Uganda, Kenya and West Kenya) (Table 5.6).

Similarly, comparison of the BC1 gene of the newly-identified isolates to other isolates from Africa and Asia on the NCBI GenBank revealed high homology (88.1% - 94.9%) between isolates GhBVFZ, GhBVCS and GhBVGS and BC1 of ACMV isolates from West Africa (Ghana, Ivory Coast and Burkina Faso) (Table 5.7). For isolates GhBEVRCS, GhBEVRFZ, GhBEVRGS, the BC1 gene showed high homology (85.7% - 96.9%) to EACMV isolates from East Africa (Uganda, Kenya and West Kenya) (Table 5.7).

Table 5.6. Nucleotide sequence identities (%) of the BV1 genes of ACMV isolates identified in this study compared to other selected isolates on NCBI

		New Isolates from this study					
Isolates from NCBI	Country of Origin	GhBVCS ACMV	GhBVGS ACMV	GhBVFZ ACMV	GhEBVRFZ EACMV	GhEBVRCS EACMV	GhEBVRGS EACMV
AF112355.EACMV	Cameroon	35.2	35.2	35.4	23.9	84.3	53
MH646694.1 EACMV	Ghana	21.8	21.8	21.9	27.4	62.2	55
HE979796.1 ACMV	Uganda	96.5	97.3	93.4	12.3	33.2	26.9
MH646722.1:434-1204	Ghana	93.7	94.5	92.2	12.7	32.4	26.9
AF259895.1 ACMV	Ivory Coast	95.3	95.7	93.4	12.3	33.2	26.9
NC_055127.1 ACMV	B. Faso	95.7	95.3	93	12.3	34	27.7
HG530121.1 ACMV	Kenya	94.1	93.3	92.2	11.5	32.8	26.5
J02058.1 ACMV	W. Kenya	92.5	92.5	91.1	12.7	33.6	27.3
MH646723.1 ACMV	S. Lanka	90.5	91.3	89.9	11.5	33.2	28.1
KT861469.1 (SLCMV)	S. Lanka	28.4	28.8	29	10.7	20.9	15
LC588395.1 (SLCMV)	S. Lanka	28.4	28.8	29	10.7	20.9	15
NC_001933.1 (ICMV)	India	28.4	28.8	29	10.7	20.9	15
AY730036.2 (ICMV)	Kenya	28	28.4	28.2	11.1	21.3	14.2
JF909233.1 EACMV	Uganda	36.5	36.4	36.6	17.4	54.5	34.8
KT780439.1 EACMV	S. Africa	35.7	35.6	35.8	17.4	54.2	34.8
KJ887740.1 (SACMV)	S. Africa	36.1	36	36.2	17.4	55.7	34.8
KJ887749.1 (SACMV)	S. Africa	36.1	36	36.2	17.4	55.7	34.8

Table 5.7: Nucleotide sequence identities (%) of the BC1 genes of ACMV isolates identified in this study compared to other selected isolates on NCBI

Isolates	Country of Origin	GhBVFZ ACMV	GhBVGS ACMV	GhBVCS ACMV	GhEBVRCS EACMV	GhBEVRFZ EACMV	GhEBVRGS EACMV
MH646723.1 ACMV	Ghana	92.2	92.9	94.9	17.3	18.5	9.6
HE979796.1 ACMV	Uganda	91.9	95.3	96.9	18	18.8	9.6
NC_055127.1 ACMV	B.Faso	88.1	90.5	92.5	18.3	19.5	9.9
AF259895.1 ACMV	I.Coast	88.5	90.5	92.1	18.6	18.5	9.2
MH646722.1:1213-2109	Ghana	87.4	90.1	92.5	17.6	17.8	9.2
HG530121.1 ACMV	Kenya	85.7	88.8	89.7	17.6	18.2	8.9
J02058.1 ACMV	W.Kenya	88.5	90.5	92.5	19.3	19.2	10.6
JF909233.1 EACMV	Kenya	21.8	22	22.3	52.6	58.4	7.7
AF112355.EACMV	Cameroon	7.1	7.3	7.2	20.6	13.4	48.7
MH646694.1 EACMV	Ghana/C.roon	7.7	7.9	7.9	21.8	14.1	50
KJ887740.1	S. Africa	7.1	7.3	7.2	11.7	8.3	42.1
KJ887749.1	S. Africa	6.7	7	6.9	12	8.7	42.1
KT780439.1 EACMV	Uganda	6.7	7	6.9	9.2	7.1	42.4
KT861469.1	S.Lanka	7.2	7.2	7.4	8.1	5.6	18.5
LC588395.1	S.Lanka	7.2	7.2	7.4	8.1	5.6	18.5
AY730036.2	India	7.2	7.5	7.7	7.4	5.9	18.1
NC_001933.1	India	6.9	7.2	7.4	7.8	5.9	19.5

Discussion

Molecular characterisation of ACMV and EACMV species present in farmers' fields in the Volta Region

Cassava Mosaic Geminiviruses have become a major constraint to cassava production mainly in regions where the crop is a major source of carbohydrate and income for farmers (Chikoti *et al.*, 2019). The intensive nature of cassava cultivation on the continent has facilitated the movement of cassava planting materials across different geographical areas resulting in the introduction of *begomovirus* diseases into previously unaffected regions. This situation has led to an increase in prevalence and more severe symptoms of Cassava Mosaic Disease (CMD) in farmers' fields as observed in this study. From the PCR results, ACMV was detected in 78.9 % of samples and EACMV in 1.1% of samples screened with mixed infections (ACMV and EACMV) detected in 20% of samples. The high occurrence of ACMV infections in farmers' fields supports earlier reports (Legg and Fauquet, 2004, Fondong *et al.*, 2000a) that ACMV is the dominant CMG species in West Africa. Comparatively, the prevalence of EACMV in single infection in farmers' fields in the Volta Region is low (1.1%) as observed in this study. Offei *et al.*, (1999) and Torkpo *et al.*, (2017) earlier reported the predominance of ACMV infections compared to EACMV in most cassava growing areas in Ghana.

In the present study, high incidence of mixed infections with ACMV and EACMV was recorded, which concurs with Torkpo *et al* (2017) who observed high rates of mixed infections in field samples. Mixed infections of cassava with ACMV and EACMV species has been reported in other West African countries such as Burkina Faso, Cameroon, Ivory Coast and Nigeria

(Pita *et al.*, 2001b; Ariyo *et al.*, 2005; Ogbe *et al.*, 2003 & Fondong *et al.*, 2000a). The high rate of mixed infection and EACMV in farmers fields warrants a more comprehensive control strategy to reduce losses due to CMGs. Furthermore, the situation of mixed infection increase the possibility of EACMV displacing ACMV as the dominant species. A similar observation was seen in Uganda where mixed infections led to the Ugandan variant (UgV) displacing ACMV as the predominant variant resulting in a severe epidemic in cassava growing areas (Were, 2001).

Geographically, it was observed that most of the EACMV infections were detected in samples from the guinea and coastal savannah agro-ecological zones. This could be due to the cultivation of CMD-susceptible varieties by farmers in these areas. Torkpo *et al.*, (2017) reported high CMD prevalence in less common cultivars, reaching 100% in most instances. Another factor which may have accounted for the presence of EACMV in the guinea and coastal savannah zones is the closer proximity of the two agroecological zones to the borders of Burkina Faso and Togo respectively. These regions are characterised by high movement of people possibly leading to introduction of infected planting materials across borders into Ghana. In Zambia, Chikoti *et al.*, (2014) reported that the movement of planting materials resulted in the introduction of more severe CMGs from Mozambique, Malawi and Tanzania. This practice, thus increases the incidence of mixed infection thus increasing the risk of recombination between CMG species which can lead to evolution of new strains of CMGs (Fondong *et al.*, 2000a; Legg & Fauquet, 2004 & Mabasa 2007).

Genetic diversity of of Begomoviruses infecting cassava in the Volta Region

Currently, about nine CMG species have been linked to Cassava Mosaic Disease (CMD) in Africa with several strains identified in the field (Brown *et al*., 2015; De Bruyn *et al*., 2016). Until now, these species were location specific, with ACMV being common in West, Central, and Southern Africa whereas EAMV was prevalent in East Africa, and rarely found in West Africa (Zhou *et al*.1997; Deng *et al*., 1997).

In this study, majority of CMG DNA-A genomes identified were ACMV species. Sequence analysis of the complete genomes (DNA A and DNA B) suggested that the isolates identified in this study are highly similar to earlier reported CMG isolates found in West Africa.

Similarly, the DNA-B genomes identified in this study shared close nucleotide sequence identity with other isolates from Nigeria, Burkina Faso and other countries from West Africa (Figure 5.5).

Interestingly, we identified three EACMV DNA-B genomes without the corresponding EACMV DNA-A genome components. This observation suggests that cassava in farmers' fields is infected with ACMV and EACMV species, increasing the chance for recombination or pseudo recombination. In cassava fields in Uganda, pseudo-recombination was reported whereby the DNA-A of ACMV co-occurs with and trans-replicates the DNA B of EACMV (Legg & Fauquet, 2004, Patil *et al*., 2001; 2009). The non-detection of EACMV DNA-A genomes from sequence data generated from leaf samples collected from farmer fields strongly suggests that DNA-A of ACMV trans-replicates DNA-B of EACMV in cassava in the Volta Region.

Unlike the DNA-A, the DNA-B of the EACMV of the isolates identified in this study had close sequence identity with previously published isolates in West Africa but dissimilar to those from the East Africa. All the three isolates of EACMV DNA-B aligned closely to the Cameroon isolates (AF112355.1; LC659084.1;KJ887667.1;KJ887742.1), Cameroon-Ghana isolate (JN165 087.1), Cameroon-Ivory Coast (AF259897.1) and Ghana isolate of EACMV DNA-B (MH646694.1) (Figure 5.6). For EACMV, genetic diversity is common among isolates identified in different countries and are prone to variation compared to ACMV species (Patil & Fauquet 2009).

From this study, we did not detect the Uganda variant of EACMV-Ug in Ghana as indicated in earlier studies (Torkpo *et al.*, 2017). The EACMV-Ug variant has been implicated in widespread epidemics of severe CMD in East Africa (Gibson, Legg & Otim-Nape 1996; Otim-Nape, Thresh & Shaw 1994). It is thus imperative that stricter measures be maintained to prevent the introduction of this variant into the country.

Conclusion

In conclusion, ACMV was the most dominant species identified in farmers' fields in the Volta Region of Ghana. Although, EACMV was identified in some fields in the coastal and Guinea savannah agro-eological zones, the prevalence was far lower (1.1% out of the total samples screened) compared to ACMV. Therefore, breeding local cassava varieties resistant to the ACMV species is of high importance in Ghana. However, the presence of EACMV in some cassava fields warrants attention due to the increased risk of recombination between ACMV and EACMV species under mixed infection conditions. In East Africa, severe CMD epidemic was reported in cassava fields

as a result of recombination between ACMV and EACMV species leading to a recombinant EACMV-Ugandan (Legg *et al.*, 2002; Patil & Fauquet, 2009).

From the twelve gemome components identified in the study, six were ACMV DNA-A genomes, three ACMV DNA-B genomes and three EACMV DNA-B genomes.

The assembly of EACMV DNA-B genomes without the corresponding EACMV DNA-A genomes suggest that ACMV DNA-A genomes can co-occur and trans replicate the EACMV DNA-B genomes.

The identification of EACMV species in cassava fields in the Volta Region confirms the increasing geographic spread of EACMV across cassava fields in Ghana. This warrants the implementation of effective measures to minimise spread of CMGs in Ghana.

CHAPTER SIX

CASSAVA MOSAIC DISEASE (CMD) RESISTANCE EVALUATION IN SELECTED IMPROVED CASSAVA VARIETIES (ICVs) IN GHANA USING SEROLOGICAL AND MOLECULAR APPROACHES

Introduction

Cassava is a staple food crop for more than 800 million people living in the tropics (FAO, 2013). It is a good source of energy for both human and industrial use with about 70 % of farmers in Ghana engaged in cassava production ((Jarvis *et al.*, 2012; GrowAfrica, 2015). Given these benefits, cassava is considered a critical weapon in the fight against poverty through food security and marketing (FAO, 2013). Despite its importance, the yield of cassava is low,18.6 mt/ha as of 2014, far below the actual potential yield of 38 mt/ha (Agriculture Global Practice, 2017). Many factors, including susceptibility of widely grown varieties to diseases and pests, mainly Cassava Mosaic Disease (CMD) account for the low average yields in Africa and Ghana (Moses *et al.*, 2015; Uzokwe *et al.*,2016).

Since the first report of CMD in 1930 in Ghana, the government of Ghana and other organizations have put in place interventions that have led to a greater emphasis on research and development resulting in the release of around 40 improved cassava varieties (ICVs) (Ofori *et al.*, 1997; Catalogue of Crop Varieties Released & Registered in Ghana 2019); GrowAfrica, 2015). Findings have shown that these ICVs are among the strategies for increasing crop production, contributing about 50-90 % of the global increase in crop yields (World Bank, 2007; Bruins, 2009). Several of the released varieties (*Capevars, TEK, Ampong, Sika, Afisiafi, Bankye Hemaa, IFAD, Nkabom*, etc) are known to

be resistant and tolerant to CMD with yields of more than 35 t/ha (Ofori *et al.*, 1997; Adjekum & Ofori, 2000; RTIP, 2002; Adjekum, 2006). Observations made at Ohawu Agricultural College and Asuansi cassava multiplication centre for some of the ICVs and engagement with a cassava farmer (personal communication with Mr. Thomas Baagmae, 0208026434) suggest that some of these ICVs do exhibit CMD symptoms that are very severe. An earlier study by Gibson (2006) confirmed this observation and led to the rejection of the improved cassava varieties by farmers. The breakdown of resistance observed in the improved varieties occurs over time under different environments (Koike *et al.*, 2000). Therefore, it is important to take into consideration the environment during resistance screening of new or improved varieties since this affects the persistence of the resistance developed (Chellappan *et al.*, 2005; Velásquez *et al.*, 2018).

Several studies have assessed the resistance of improved cassava varieties to CMD over time (Okogbenin *et al.*, 2007; Bi *et al.*, 2010). Asare *et al.*, (2014) observed that most of these studies were carried out under natural conditions or on farmers fields which tends to either underestimate or overestimate the infestation pressure. According to Bi *et al.* (2010) and Rwegasira *et al.* (2015), variation in infestation pressure is due to the differences in agronomic practices, spatio-temporal variation in whitefly activity and variations in plant age at the time of infection.

In the glass or screen house, the establishment of an infection source, as well as inoculum dose-response effects on resistance levels in cassava cultivars, can be assessed (Ambang *et al.*, 2009; Bi *et al.*, 2010). However, other approaches such as molecular and grafting techniques to determine resistance

should be explored in the re-evaluation of ICVs years after their existence or release. Mechanical transmission of CMD through grafting has been reported to be reliable for screening cassava for resistance to virus diseases (Fregene *et al.*, 2001; Akano *et al.*, 2002; Okogbenin *et al.*, 2012; Wagaba *et al.*, 2013).

Other types of grafting such as side grafting and bud grafting methods have allowed for broad screening of cassava plants (Yadav *et al.*, 2011; Wagaba *et al.*, 2013; Anjanappa *et al.*, 2016). However, both methods show lower infection rates compared to top grafting, which normally attains infection rates of 100% (Moreno *et al.*, 2011; Vanderschuren *et al.*, 2012; Anjanappa *et al.*, 2016; Elegba *et al.*, 2020).

In view of several observations of CMD symptoms on improved cassava varieties across cassava multiplication centres (Ohawu Agricultural College and Asuansi cassava multiplication centre) and confirmation from farmers and other stakeholders, it is important to assess the level of resistance of ICVs to CMD in the field. Assessing CMD resistance levels of ICVs based on phenotypic symptoms can be misleading, thus, use of more robust virus indexing methods such as ELISA (enzyme-linked immunosorbent assay) (serology) and PCR screening (molecular) were employed in this study. The goal of this current study was to assess the resistance or tolerance of 21 improved cassava varieties to CMD after about 10 to 15 years of their release under natural field conditions and artificial inoculation (grafting).

Materials and Methods

CMD resistance evaluation of Improved Cassava varieties under two agroecological zones

Planting of ICVs in the coastal savannah and forest ecological zones

The planting of 21 selected ICVs to assess their resistance against CMD infection was carried out under the coastal savannah (Ohawu Agricultural College) and forest ecological zones (Logba-Alakpeti) during the 2018/2019 and 2019/2020 cropping seasons. Assessment were done on the incidence and severity of CMD in the 21 ICVs and a local variety at each ecology. Infected leaves at 3 month after planting were collected from each of the ICVs under the two ecologies for two years, and then indexed via serological and molecular methods.

Ohawu Agricultural College is located in the coastal savannah zone of Ketu North district in the Volta Region of Ghana and approximately 158 km East from Accra and 8 km from Abor. Ohawu primarily shares borders with Tadzewu, Kporkuve, Manu, and Torkanu. Geographically, Ohawu lies between 6°7'60" N latitude and 0°54'0 E longitude. The area experiences two rainfall seasons: starting from April to July and the second from September to October. The main soils identified in the district vary from sandy to clay loam, with sandy loam and clay loam being the major soils supporting most crops like maize, groundnut, cowpea, cassava, rice, plantain, and most vegetables (MoFA, 2019-2020).

The ecology of Logba-Alakpeti, has been described under Afadjato South Districts (Chapter 3).

Planting materials

A total of 21 ICVs were sourced from Wenchi and Asuansi Cassava Multiplication centres and the Teaching and Research Farm of the School of Agriculture, University of Cape Coast, Cape Coast. These varieties include "Afisiafi, Abasafita, Bronyi, Dodze, AGRA, Amansan, Botan, Abrabopa, Sika, IFAD, Nkabom, Esam, Hemaa, Lamesese, Ampong, Doku Duade, TEK, Duade Kpakpa, Otuhia, Abelifia and Capevars". In addition to these, two farmer-preferred varieties, "Hushivi" from the coastal savannah ecology and "Biafra" obtained from the forest zone were used as control.

Experimental design and field layout

The 21 ICVs and the 2 farmer varieties were planted under rainfed conditions in the coastal and forest ecologies. They were planted in a 2*11 Alpha Lattice Design with three replicates (Appendix D). A total land area of approximately 2489 m^2 (19 m x 131 m) was ploughed, harrowed, and partitioned into blocks and plots. There was 2 m spacing between each block and 1m spacing between the plots with each plot measuring 4 m x 3 m (Appendix D).

Cultural Practices

Cassava cuttings that did not sprout after three weeks of planting were replaced. Weeding was manually done with a hoe on the 1st, 3rd, and 4th months after planting. No fertilizer or insecticide were applied. Serological evaluation of ICVs for resistance to CMD were carried out in the two ecologies was done from 2018-2020.

Each experimental plot had twenty-five (25) plants, out of which, five (5) test plants from the inner row of each plot (ICVs and local varieties) were randomly selected, tagged and monitored for their responses to CMD for 2 consecutive years. At 4 Month After Planting (MAP), all the ICVs and the local varieties at the two location showed symptoms of CMD. CMD symptomatic leaves from all the ICVs and the local varieties were collected at 4 MAP for serological evaluation.

In the first year (2018/2019), 3month old symptomatic fresh leaf samples were picked from all 660 (5*3*22*2) test plants per ecology at 3 MAP. Each variety consisting of 15 samples was pooled into one sample per ecology. In total, 22 (330/15) samples per ecology were used for serological evaluation. The fresh leaves were initially kept in labelled Ziploc bags and placed on ice before transfer to a -20C freezer until they were worked on. All 44 samples (from both ecologies) were screened using the TAS ELISA procedure as described in Chapter 4 for the detection of CMGs.

Molecular evaluation of ICVs for resistance to CMD in coastal savannah and forest ecologies

In the second year (2019 /2020), the same number of samples used in the first year were collected in the 4th MAP for molecular evaluation. DNA was extracted from all 44 (22samples * 2 ecologies) samples and used for the molecular detection of CMGs in each ICV. All 44 samples were screened by PCR for the presence of ACMV and EACMV using primers listed in Table 5.1. Positive samples were confirmed by gel electrophoresis and viewed under the UV gel documentation system (VWR International bvba, VWRS3/1046, Leuven, UK).

Morphological and Molecular Evaluation of ICVs against CMD Infection after Indexing

The performance of selected ICVs was confirmed through indexing using grafting. Five plants of each ICV were planted in pots and kept in the screen house (to prevent insect attack) for 6 weeks before grafting. In total, 105 test plants were used for the experiment. A CMD-susceptible variety '*Wenchi*' was used as a rootstock for the graft challenge of the selected ICVs. It should be noted that all the ICVs were tested using PCR to determine the absence of ACMV and EACMV. The same was done for potted susceptible varieties to detect presence of the virus (ACMV and EACMV). Leaf samples collected per variety at 3 MAP from all five test and pooled into one sample (totalling 21 leaves) and screened for ACMV and EACMV. The same procedure (pooling and screening) was repeated for all ICVs challenged with CMD-infected rootstock.

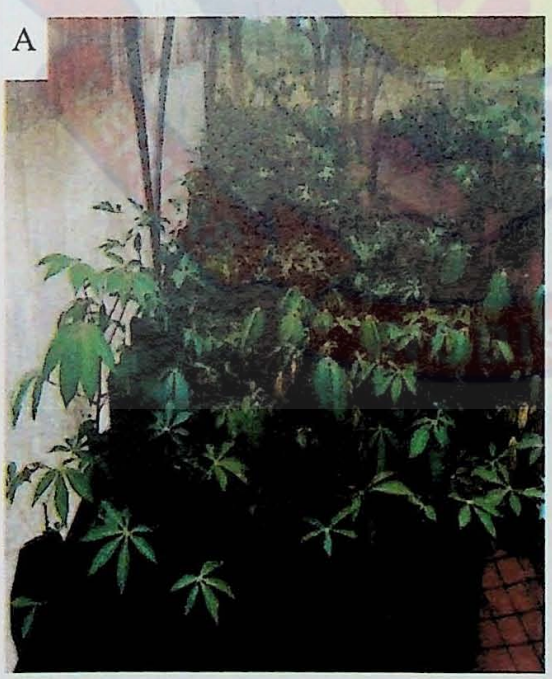


Figure 6.1A: Pots containing plants from 21 ICVs used as scion for grafting at 4 weeks old

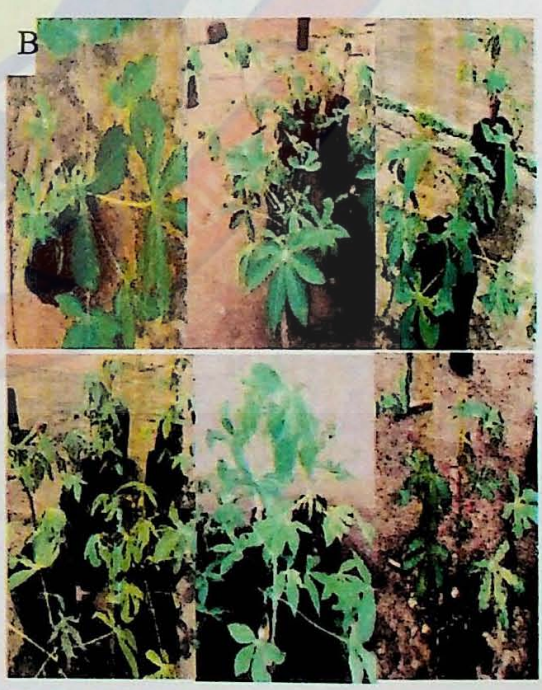


Figure 6.1B. CMD symptomatic plants of Wenchi used as rootstock for graft-challenge of ICVs

The side-cleft method of grafting was used as described by Wagaba *et al.* (2013). In this approach, a tangential cleft is made in the main stem of the rootstock close to a leaf node. Axillary buds of 3 mm to 6 mm in length were excised from virus-free plants with the petiole and leaf attached. Using parafilm, the grafted joint were securely tied and covered with a transparent rubber to facilitate union and avoid desiccation. The grafts were kept in a humid environment for 4 -7 days after which they were transferred to the screen house for observation and data collection. It should be noted that both scions and rootstocks were about 6 to 8 weeks old before grafting. Treatment consisted of four "healthy scions on diseased rootstock" and one healthy scion on healthy rootstock. All 105 samples (5plants*22varieties) were grafted for the study. Grafted samples were numbered from 1 to 105 with every fifth plant as the healthy scion on healthy rootstock.

A

Figure 6.2A. Side cleft grafted cassava

B

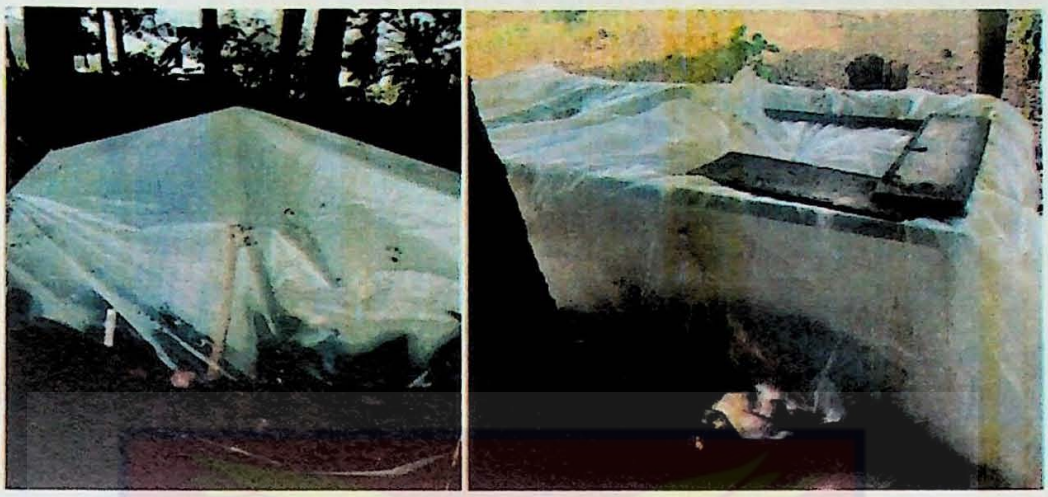


Figure 6.2B. Artificial humidity chamber used for curing after grafting

Data Collection

Serological evaluation of ICVs against CMD in two ecologies in coastal savannah and forest ecologies

Based on the ELISA absorbance values, samples were either declared positive or negative for the virus. Samples with an absorbance value more than twice that of the negative control were considered positive for the virus (Charoenvilaisiri *et al.*, 2021). The various absorbances values obtained for each of the samples for TAS-ELISA tests were recorded.

Morphological and Molecular Evaluation of ICVs against CMD Infection after Indexing

Leaves of grafted ICVs were visually assessed for CMD incidence and severity. This was done from 1 to 6 weeks after grafting (WAG) using standard severity key by Hahn (1980), IITA (1990), and Ariyo *et al.*, (2005). A total number of 105 leaf samples (from grafted ICVs) were picked for molecular assessment using PCR. The primers used were JPS1/JSP2 for detecting ACMV and JSP1/JSP3 for detecting EACMV (Houngue *et al.*, 2019) (Table 6.1).

Table 6.1: List of primers used in the amplification of CMGs in grafted samples

Primers	Sequence 5'-3'	Virus	Expected size (bp)
JPS1/JSP2	ATGTCGAAGCGAGGAGAT	ACMV	770
	TGTTTATTAATTGCCAATACT		
JSP1/JSP3	ATGTCGAAGCGACCACCAGGAGAT	EACMV	770
	CCTTTATTAATTTGTCACTGC		
Reference	Houngue *et al.*, 2019.		

Data Analysis

Serological evaluation of ICVs for resistance to CMD infection in coastal savannah and forest ecologies

Absorbance values obtained from the TAS-ELISA test for the various samples were analysed as described earlier in Chapters 4 and 5 respectively.

Molecular evaluation of ICVs for resistance to CMD in coastal savannah and forest ecologies

The number of grafted plants that showed CMD symptoms and became infected were expressed as a percentage of the total number of grafted plants (Figure 6.3). Average severities of the individual grafted plants were used to describe the response of each ICV to CMD. The positives and negative results from the molecular screening were recorded and presented in percentages.

PCR thermocycling (Applied Biosystems 2720 thermal cycler) conditions were initiated at 94 ° C for 2 minutes, followed by 4 cycles at 94 ° C for 1 minute, 55° C for 1.5 minutes. There was an extension for 2 minutes at 72 ° C and a final extension of 72 ° C for 10 minutes and held for 4 ° C. The PCR was done in 20 µL volume and 12 µL of PCR product loaded on a 1.2 % agarose

gel in TAE buffer and viewing under ultraviolet light for confirmation of the presence of ACMV and EACMV.

Figure 6.3: Leaves of some ICVs showing CMD symptoms 30 days after grafting. Where a.-Abrabopa, b-Afisiasi, c-AGRA, d-Amansan, e-Ampong, f-Botan, g-Capevars, h-Esam, i-IFAD, j-Sika, k-Lamesese, l-Kpakpa, m-TEK, n-Doku duade, o-Bronyi, p-dodze

Results

Serological evaluation of ICVs for resistance to CMD in coastal savannah and forest ecologies in 2018/2019

Figures 6.4A and 6.4B show a summary of TAS-ELISA detection of ACMV in the 21 ICVs using control varieties from the coastal savannah and forest zones respectively. From the results, ACMV was detected in all the ICVs and the local variety from the coastal savannah except in varieties Hemaa and AGRA. However, all the samples from the forest zone tested positive for the virus (Figure 6.4B)

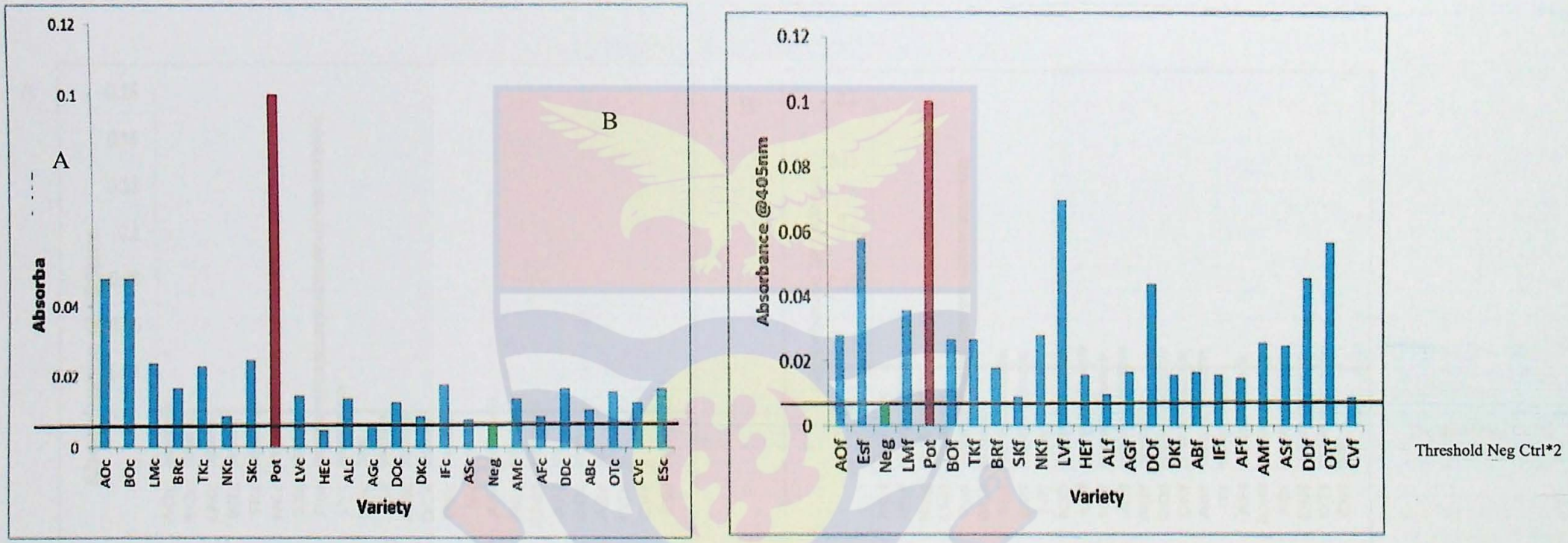


Figure 6.4: TAS-ELISA detection of ACMV in 21 ICVs and a control variety from (A) the coastal savannah and (B) the forest zone

* AO-Ampong *BO- Botan *LV-Local *Duade Kpakpa *AS-Abasafitaa *ES-Essam *TK-TEK *HE-Hemaa *AB-Abrabopa *DD-Doku duade * Neg –Negative *BR-Bronyi *AL-Abelefia *IF-IFAD *OT-Otuhia *LM-Lamesese *SK-Sika *AG-AGRA *AF-Afisiafi *CV- Cape vars * Pot-Positive * NK-Nkabom *DO-Dodze *AM-Amansan

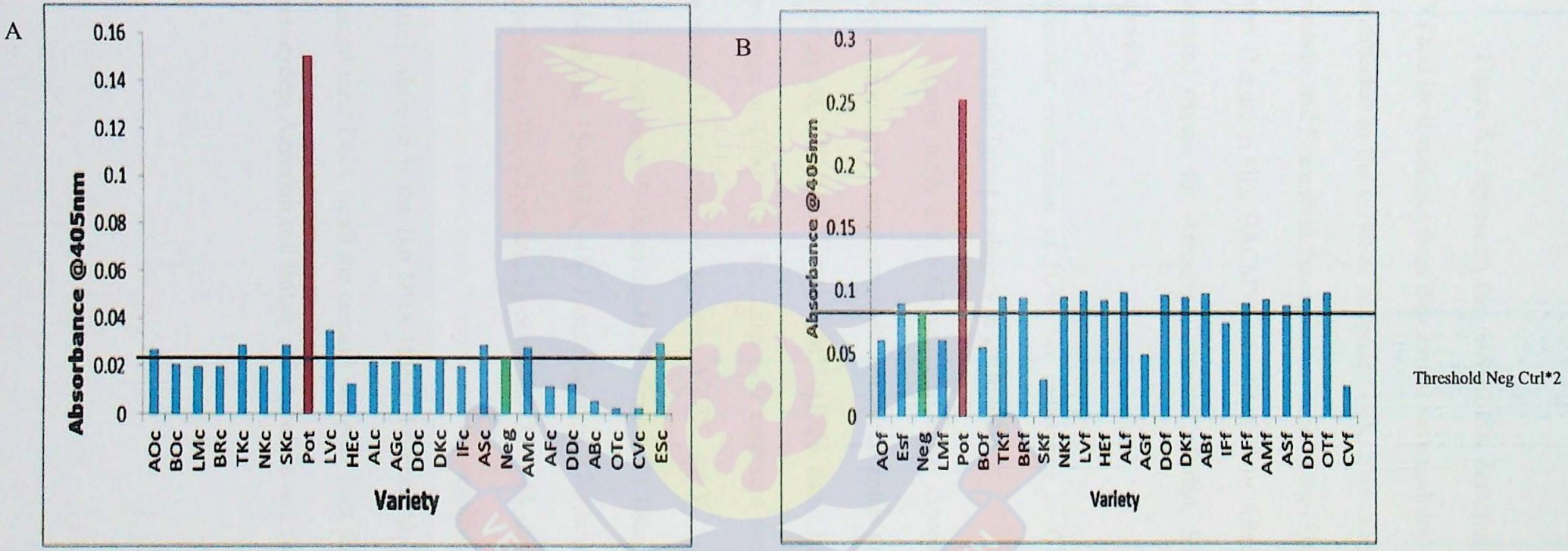


Figure 6.5:TAS-ELISA detection of EACMV in 21 ICVs and a control variety from (A) the coastal savannah and (B) the forest zone

* AO-Ampong *BO- Botan *LV-Local *Duade Kpakpa *AS-Abasafitaa *ES-Essam *TK-TEK *HE-Hemaa *AB-Abrabopa *DD-Doku duade* Neg –Negative *BR-Bronyi *AL-Abelefia *IF-IFAD *OT-Otuhia *LM-Lamesese *SK-Sika *AG-AGRA *AF-Afisiafi *CV- Cape vars * Pot-Positive * NK-Nkabom *DO-Dodze *AM-Amansan

Figure 6.5 represents the TAS-ELISA detection of EACMV in the 21 ICVs and local varieties from the Coastal Savannah and Forest zones. EACMV was detected in the varieties Ampong, TEK, Sika, Local variety, Abasafitaa, Amansan, and Esam from the coastal savannah zone (Figure 6.4A). In the forest zones (Figure 6.4B), EACMV was detected in fifteen out of 21 varieties evaluated except for Ampong, Lamesese, Botan, Sika, AGR, IFAD, and Capevars.

Molecular evaluation of ICVs for resistance to CMD under in coastal savannah and forest ecologies in 2019/2020

Figures 6.6A and 6.6B summarize the detection of ACMV in leaf samples from the Coastal savannah and Forest zones respectively. Primers for detecting ACMV, AL1/F and ACMV-AR0/R show bands with the expected size range of 1000 -1030 bp. Lane 1-22 represent samples from the Coastal savannah (1-Ampong, 2-Lamesese, 3-TEK, 4-LV, 5-Agbelifia, 6-Dodzi, 7-IFAD, 8-Amansan, 9-Doku duade, 10-Otuhia, 11-Esam, 12-Botan, 13-Bronyi, 14-Nkabom, 15-AGRA, 16- Duade Kpakpa 17-Abrabopa, 18- Afisiafi, 19-Abasaafitaa, 20- Capevars, 21-Sika and 22 - 44 denote the corresponding samples from the forest zone. N represents the negative control, P is a positive control while M is the 1kb DNA ladder. Primers AL1/F and ACMV-AR0/R detected viral DNA in all the cassava samples from the coastal savannah and forest except Amansan and Botan, and Agbelifia and Doku duade respectively.

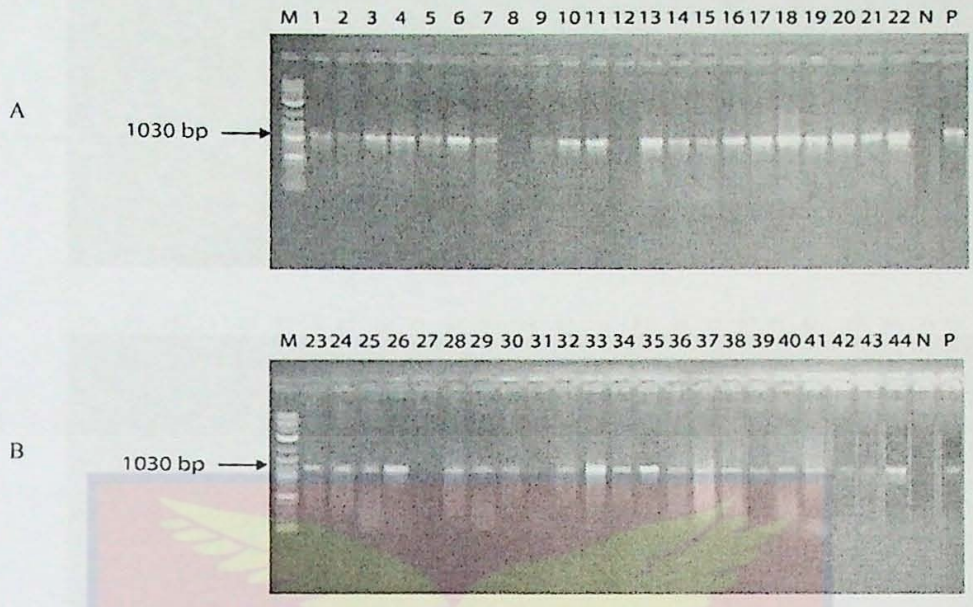


Figure 6.6 (A) Amplicon of ACMV obtained from cassava leaves from coastal savannah using ACMV-AL1/F and ACMV-AR0/R primer pairs of size 1030 bp. Lanes 1-22 denote coastal savannah (1-Ampong, 2-Lamesese, 3-TEK 4-LV, 5-Abelefia, 6-Dodzi, 7-IFAD, 8-Amansan, 9-D. duade, 10-Otuhia, 11- Esam, 12- Botan, 13-Bronyi, 14- Nkabom, 15-Hemaa, 16-AGRA, 17-D. kpakpa, 18-Abrabopa, 19-Afisiafi, 20-Abasafita, 21-Capevars, 22-Sika) 6.6 (B) Amplicon of ACMV obtained from cassava leaves from forest zone using ACMV-AL1/F and ACMV-AR0/R primer pairs of size 1030 bp. Lanes 23-44 denote Forest zone (23-Ampong, 24-Lamesese, 25-TEK 26-LV, 27-Abelefia, 28-Dodzi, 29-IFAD, 30-Amansan, 31-D. duade, 32-Otuhia, 33- Esam, 34-Botan, 35-Bronyi, 36- Nkabom, 37-Hemaa, 38-AGRA, 39-D. kpakpa, 40-Abrabopa, 41-Afisiafi, 42-Abasafita, 43-Capevars, 44-Sika) and N denote the negative control, P denote positive while M is 1 kb DNA Ladder

The amplicon of EACMV obtained from cassava leaves using EAB555/F and EAB555/R primer pairs of band size 540 bp is presented in Figures 6.7. Lane 1-22 represents samples from the Coastal savannah (1-Ampong, 2-Lamesese, 3-TEK, 4-LV, 5-Abelefia, 6-Dodzi, 7-IFAD, 8-Amansan, 9-Doku duade, 10-Otuhia, 11-Esam, 12-Botan, 13-Bronyi, 14-Nkabom, 15-AGRA, 16-Duade Kpakpa 17-Abrabopa, 18- Afisiafi, 19-Abasaafita, 20- Capevars, 21-Sika and 22-44 denote the corresponding samples from the Forest zone. N represents the negative control, P is a positive control while M is a 1kb DNA ladder. Primer EAB555/F and EAB555/R did not amplify any of the samples from the two locations.

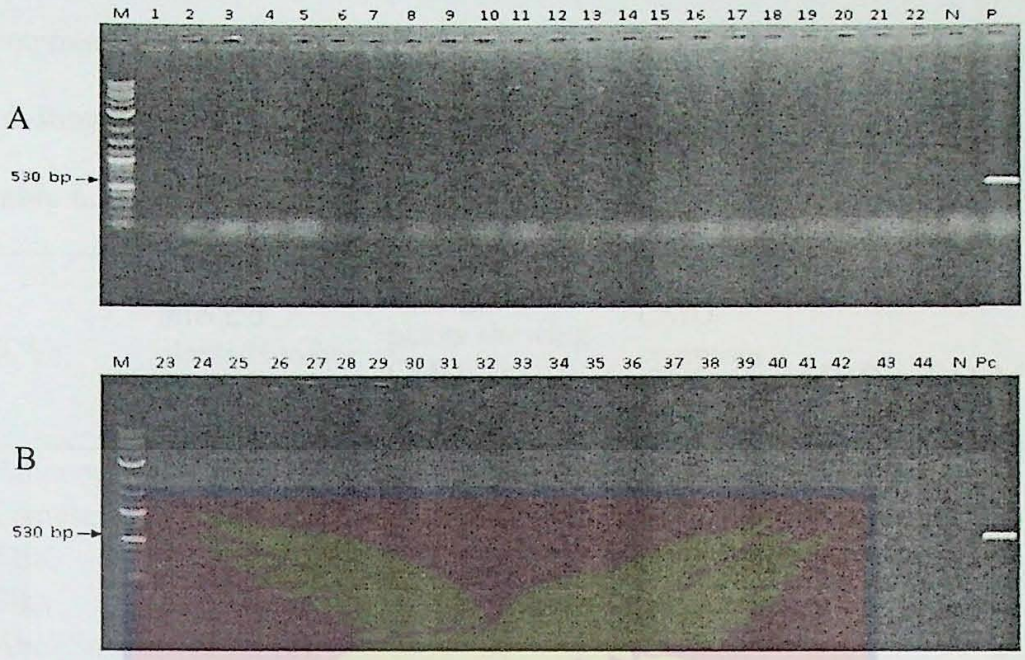


Figure 6.7 (A) Amplicon of EACMV obtained from cassava leaves from coastal savannah and forest zone using EAB555/F and EAB555/R primer pairs of size 540 bp. Lanes 1-22 denote coastal savannah (1-Ampong, 2-Lamesese, 3-TEK 4-LV, 5-Abelefia, 6-Dodzi, 7-IFAD, 8-Amansan, 9-D. duade, 10-Otuhia, 11- Esam, 12- Botan, 13-Bronyi, 14- Nkabom, 15-Hemaa, 16-AGRA, 17-D.kpakpa, 18-Abrabopa, 19-Afisiafi, 20-Abasafita, 21-Capevars, 22-Sika) 6.7 (B) Amplicon of EACMV obtained from cassava leaves from forest zone using EAB555/F and EAB555/R primer pairs of size 540 bp. Lanes 23-44 denote coastal savannah (23-Ampong, 24-Lamesese, 25-TEK 26-LV, 27-Abelefia, 28-Dodzi, 29-IFAD, 30-Amansan, 31-D. duade, 32-Otuhia, 33- Esam, 34- Botan, 35-Bronyi, 36- Nkabom, 37-Hemaa, 38-AGRA, 39-D. kpakpa, 40-Abrabopa, 41-Afisiafi, 42-Abasafita, 43-Capevars, 44-Sika) and N denote the negative control, P denotes positive while M is 1 kb DNA Ladder

Morphological and Molecular Evaluation of ICVs for resistance to CMD Infection by grafting

All the ICVs were graft-challenged with CMD infected rootstocks of Wenchi and showed symptoms of CMD with varying infection percentages and responses after 6-8 weeks. It was observed from Table 6.2 that, Ampong, Lamesese, TEK bankye, Abelifia, Dodzi, Amansan, Doku duade, Otuhia, Esam, Botan, and Nkabom had three out of the five grafted plants showing symptoms. They however responded differently with TEK bankye and Amansan having moderate severity (3) and the remaining having mild infections. Ten ICVs (Sika, IFAD, Bronyi, Hemaa, Duade Kpakpa, Abrabopa, Afisiafi, Abasafita, Capevars, and AGRA) had two out of five grafted plants showing CMD

symptoms with all responding mildly to the disease except Duade and AGRA that showed moderate infection (3).

Table 6.2: Side-cleft graft transmission of CMV to 21 improved cassava varieties in Ghana

ICVs	Number of infected plants/Number of grafted plants	% of grafted plants showing CMD symptoms/ICVs	CMD symptom severity	PCR Results	
				ACMV	EACMV
Ampong	3/ 5 (3)	60	2	+	-
Lamesese	3/ 5 (3)	60	2	+	-
TEK	3 / 5 (3)	60	3	+	-
Sika	2/ 5 (2)	40	2	+	-
Abelifia	3 / 5 (2)	60	2	+	-
Dodzi	3 / 5 (2)	60	2	+	-
IFAD	2 / 5 (2)	40	2	+	+
Amansan	3 / 5 (3)	60	3	+	+
Doku Duade	3 / 5 (3)	60	2	+	-
Otuhia	3 / 5 (3)	60	2	+	-
Esam	3 / 5 (2)	60	2	+	-
Botan	3/ 5 (3)	60	2	+	-
Bronyi	2 / 5 (2)	40	2	+	-
Nkabom	3 / 5 (3)	60	2	+	-
Hemaa	2 / 5 (2)	40	2	+	-
Duade Kpakpa	2 / 5 (2)	40	3	+	-
Abrabopa	2 / 5 (2)	40	2	+	-
Afisiafi	2 / 5 (2)	40	2	+	-
Abasafita	2 / 5 (2)	40	2	+	-
Capevars	2 /5 (2)	40	2	+	-
AGRA	2/5 (2)	40	3	+	+
Total plants assessed	**105**				

1=No symptoms, 2 = Mild chlorotic pattern on entire leaflets or mild distortion at base of leaflets, 3 = Strong mosaic pattern on the entire leaf, and narrowing cum distortion of lower 1/3 of leaflets, 4 =Severe mosaic distortion of 2/3 of leaflets, and general reduction of leaf size and 5 =Severe mosaic distortion of 4/5 or more of leaflets, twisted and misshapen leaves

PCR screening of leaf samples collected from scions of graft-challenged plants with primers JPS1/JSP2 (ACMV) and JSP1/JSP3 (EACMV) are presented in Figures 6.8 (A-D). Samples in every five sets of wells represent a

variety: 1-5 represent Ampong, 6-10 is Lamesese, 11-15 is TEK bankye, 16-20 represent Abelefia, 26-30 is for Dodze, 31-35 for IFAD, 36-40 is Amansan, 41-45 is Doku duade and 46-50 for Otuhia. Walls 51-55 represent Esam, 56-60 is Botan, 61-65 is for Bronyi, 66-70 represent Nkabom while 71-75, 76-80, 81-85, 86-90, 91-95, 96-100 and 101-105 represent Heman. Kpakpa, Abrabopa, Afisiafi Abasafitaa, Capevars and AGRA respectively. N denotes negative control and P is a positive control for all the plates. Generally, the PCR results confirmed the presence of ACMV/EACMV in some of the improved cassava varieties graft-challenged with CMD-infected rootstocks except in the controls (the fifth plant).

For CMD screening, except for Esam (51-55) and Afisiafi (86-90), primers JPS1/JSP2 detected viral DNA in 33 out of 105 samples of all the ICVs. ACMV DNA was amplified in each sample of Ampong (1-5), Lamesese (6-10), Sika (16-20), Abelefia (21-25), and Dodzi (26-30). TEK (11-15) and IFAD (31-35) had three and two of their samples being positive for ACMV respectively (Figure 6.8).

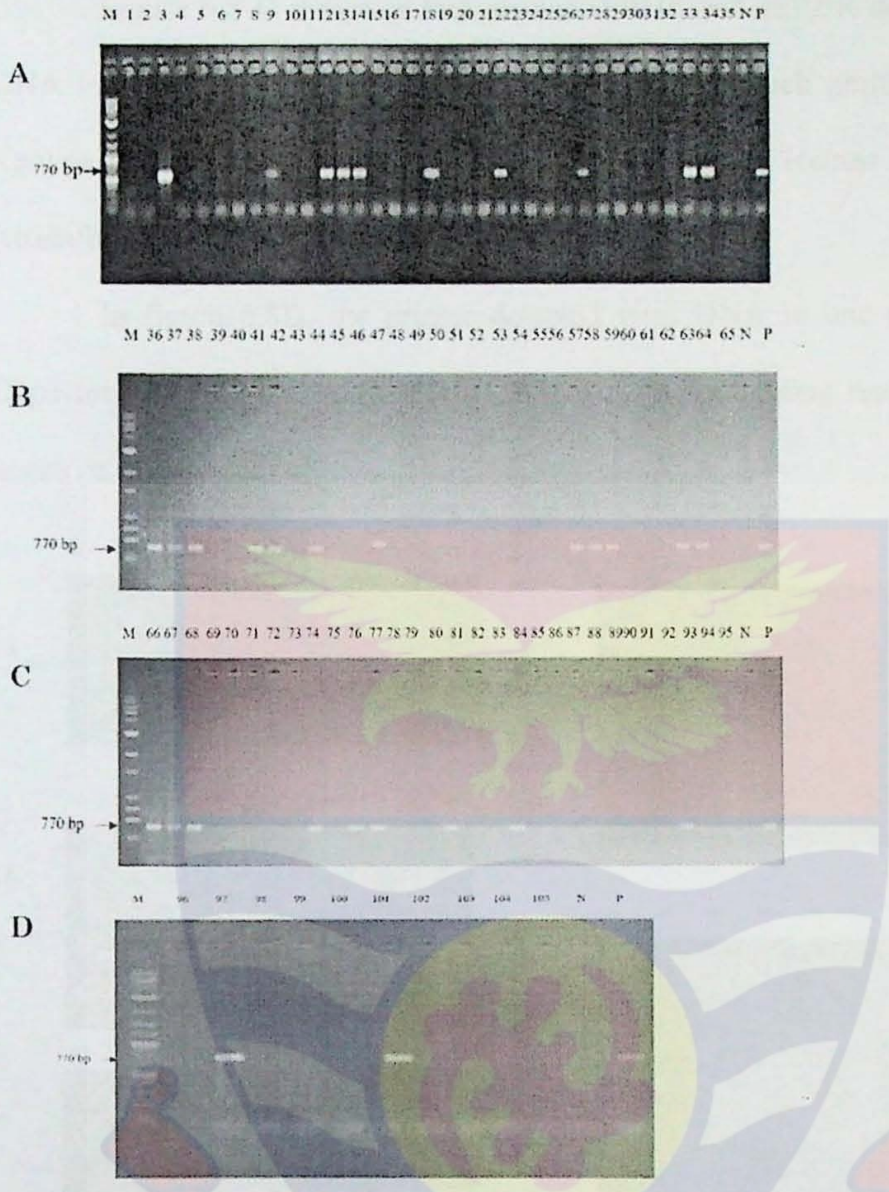


Figure 6.8: PCR screening of graft-challenged ICVs for ACMV using JSP1/F and JSP2/R primer pairs of size 770 bp. Lanes 1-5 denote Ampong, 6-10 is Lamesese, 11-15 for TEK, 16-20 is Sika, 21-25 is Abelefia, 26-30 is for Dodzi, 31-35 is for IFAD, 36-40 denote Amansan, 41-45 is Doku duade, 46-50 for Otuhia, 51-55 is Esam, 56-60 is Botan, 61-65 is for Bronyi, 66-70 denote Nkabom, 71-75 is Hemaa, 76-80 for Kpakpa, 81-85 is Abrabopa, 86-90 is Afisiafi, 91-95 is for Abasafitaa, 96-100 denote Cape vars, 101-105 is AGRA and N denote the negative control, P denote positive while M is 1 kb DNA Ladder.

From Figure 6.8 B, the primer pair JSP1/F/JSP2/R, detected one ACMV DNA in Otuhia (46-50) and two in Bronyi (61-65). Varieties Amansan (36-40), Duade, (41-45) and Botan (56-60) had three of their grafted samples testing positive to the ACMV. Variety Esam (56-60) however had all its samples testing negative to the virus.

Figure 6.8 C indicates that primer JSP1/F and JSP2/R detected viral DNA in three test plants of Nkabom (66-70), two in each grafted plants of Kpakpa (76-80) and Abrabopa (81-85), one in each of Hemaa (71-75) and Abasafitaa (91-95) and none in Afisiafi (86-90).

In figure 6.8D, the primer detected viral DNA in one test plant of Capevars (96-100) and AGRA (101-105) with the remaining test plant being negative.

Figure 6.9: PCR screening of graft-challenged ICVs for EACMV using JSP1/F and JSP3/R primer pairs of size 770 bp. Lanes 1-5 denote Ampong, 6-10 is Lamesese, 11-15 for TEK, 16-20 is Sika, 21-25 is Abelefia, 26-30 is for Dodzi, 31-35 is for IFAD, 36-40 denote Amansan, 41-45 is Doku duade, 46-50 for Otuhia, 51-55 is Esam, 56-60 is Botan, 61-65 is for Bronyi, 66-70 denote Nkabom, 71-75 is Hemaa, 76-80 for Kpakpa, 81-85 is Abrabopa, 86-90 is Afisiafi, 91-95 is for Abasafitaa, 96-100 denote Cape vars, 101-105 is AGRA and N denote the negative control, P denote positive while M is 1 kb DNA Ladder.

Figure 6.9 indicates amplicons of CMD-EACMV obtained from grafted ICVs using JPS1/JSP3 primer pair of band size 770 bp. In all the 105 grafted plants, only three ICVs had one of their test plants testing positive to EACMV. These three include Amansan (36-40) (Figure 6.9 B), Abrabopa (81-85) (Figure

6.9 D), and AGRA (101-105) (Figure 6.9 E). The rest of the test plant tested negative to EACMV.

Discussion

Serological Evaluation of Improved Cassava Varieties for resistance to CMD in two ecologies in year 1 - 2018/2019

Evaluation of disease tolerance or susceptibility in cassava cultivars based on visual symptoms can be misleading because CMD symptom expression is influenced by the environment, genotype, plant age, and viral isolates (Hillocks & Jennings 2003). The use of serological and molecular methods in addition to symptom monitoring presents a robust approach to screen cassava cultivars for resistance to CMD. Majority of the 21 improved varieties used in the present study are reported to exhibit resistance to CMD in Ghana (Catalogue of Crop Varieties Released & Registered in Ghana 2019). However, evaluation of their resistance to CMD using serology revealed that about 85% of the improved varieties were susceptible (tested positive) to ACMV antibody whiles about 50% of all the samples tested positive to the antibody of EACMV. This confirms other reports that ACMV species is the most prevalent in most West African countries (Fondong *et al.*, 2000a; Pita *et al.*, 2001a; Ogbe *et al.*, 1997, 2003). In Ghana, Torkpo *et al.*, (2017) has also concerted that ACMV is the most common CMGs found in farmers' fields. The 50% detection of EACMV in improved varieties indicates that, although ACMV is prevalent, EACMV is becoming widespread in farmers' fields in Ghana as seen in the study.

Generally, the number of samples that tested positive for ACMV and EACMV from the forest zones was more than that of the coastal savannah. This observation could be a result of, high temperature and high rainfall that was observed in the forest zone during the fifth month (5MAP) of 2018 when the leaves were collected for testing (Appendix C). Though the temperature was high (26.0 °C), the high rainfall lowered the temperature leading to increased infection and severity as observed by Gerik *et al.* (1990) and Chellappan *et al.* (2005).

Among the improved varieties, only "Hemaa" obtained from the Coastal savannah tested negative for both ACMV and EACMV. This may be due to low virus titres that might have prevented ELISA detection. This observation does not indicate resistance in Hemaa since the variety tested positive in the subsequent experiments in the second year (2019). Low virus titres in leaf tissues can lead to non-detection of CMGs in cassava plants as observed in other studies by Were, Winter & Maiss (2004), Fargette *et al.* (1987) and Fargette *et al.*(1994).

Molecular Evaluation of ICVs for resistance to CMD in two Ecologies in year 2 - 2019/2020

In confirming the performance of all the improved varieties, a more specific, reliable, and sensitive detection technique thus, PCR was used in the second year (2019) to detect virus DNA at both locations. Specifically, primers ACMV-AL1/F, ACMV-ARO/R and EAB555/F, EAB555/R which detect only ACMV and EACMV were used (Zhou *et al.*, 1997; Chikoti, Tembo, Chisola, Ntawuruhungu, Ndunguru, 2015). PCR screening in the second year detected CMGs in 20 (90%) out of 22 improved cassava varieties and the local variety

(landrace). Single infection of ACMV was the most common in the improved varieties and the landrace tested similar to other studies conducted in Africa (Ogbe *et al.*, 1997, Chikoti *et al.*, 2015,). Although all 21 varieties tested have been reported to be resistant to CMD during release, no variety devoid of CMD was observed, indicating that CMD resistance has likely broken down. None of the samples from the two locations (Ohawu and Logba-Alakpeti) tested positive for EACMV (Figure 6.7). This may be due to the absence of EACMV or low accumulation of EACMV DNA in the other ICV samples tested. It was noticed that PCR screening of samples did not detect EACMV in some improved varieties that had tested positive to EACMV using ELISA in the previous year (2018). The primer used for detection of EACMV in this study, EAB555/F, EAB555/R amplifies the DNA-B component of EACMV, thus, the absence of EACMV DNA-A in field samples might be misleading. Similarly, a study by Mabasa (2007) in South Africa failed to detect a new strain of ACMV. However, pseudo-recombination, where the DNA-A component of one species, co-occurs and trans-replicates the DNA-B component of another virus of the same species can occur in mixed infections of ACMV and EACMV. These are common in the field (Pita *et al.*, 2001; Bull *et al.*, 2007; Patil & Fauquet , 2009).

Morphological (Indexing) and Molecular Evaluation of ICVs for resistance to CMD Infection

Several authors have investigated the resistance of cassava varieties to CMD under natural occurrence (Okogbenin, Porto, Egesi, Mba, Espinosa, Santos…2007; Bi, Aileni & Zhang, 2010). A major limitation of this approach is the low level of infection because only whiteflies transmit the disease under field conditions besides the use of infected planting materials/cuttings

(Houngue, Pita, Ngalle, Zandjanakou-Tachin, Kuate, Cacaï…2019; Asare, Galyuon, Asare, B., Sarfo, Tetteh, 2014). It has been reported by Rwegasira and Chrissie (2015) that, CMD transmission by whiteflies is limited by their population at the experimental site and this affects incidence and severity. Furthermore, the age of the plant at infection and the environmental conditions on the prevailing field also affect CMD severity (Fargette, Fauquet & Thouvenel 1988).

In assessing the resistance levels of ICVs to CMD several years after their release (About 10 year (Torkpo *et al.* 2017), they were put to test by grafting them onto rootstocks carrying mixed CMG infection. Within a period of four weeks after grafting (4WAG) all the ICVs showed CMD symptoms but at varying percentages confirming Wagaba, Beyene, Trembley, Alicai, Fauquet & Taylor (2013) and Fondong (2017) that most cassava varieties show CMD between 9 days and 3 months after grafting. Phenotypically, most of the ICVs showed mild infection to CMD at the 4WAG. TEK, Amansan, Abrabopa, and AGRA varieties showed moderate infection to CMD. However, according to the molecular analysis using PCR, none of the improved varieties were completely resistant to CMD except Afisiafi and Esam.

All the ICVs tested positive to ACMV except the two varieties: Afisiafi and Esam indicating the prevalence of ACMV in Ghana as reported by Fondong *et al.*, (2000a), Pita *et al.*, (2001a), Ogbe *et al.*, (1997, 2003) and also confirmed by Torkpo *et al.*,(2017). It should be noted that all the ICVs used in this study are supposed to be resistant to CMD (Asare *et al.*, 2014; Appiah, 2015). Apart from Afisiafi and Esam, each of the ICVs had either one or more of their replicate showing mild or moderate infection and testing positive to ACMV,

therefore susceptible to ACMV. However, none of the ICVs tested positive to EACMV except Amansan, Abrabopa, and AGRA which had one of their replicates testing positive to EACMV and therefore showed moderate infection. This is in line with a study by Fondong (1999) and Fondong (2017), where three IITA improved varieties showed mild infection to CMD and moderate to severe infection under mixed infection with ACMV and EACMV. The non-detection of EACMV in the ICVs tested could be due to the low concentration of EACMV DNA in these varieties. Reports by Patil and Fauquet (2015a) and Kuria, Ilyas, Ateka, Miano, Onguso, Carrington, and Taylor (2017) concluded that improved varieties accumulate fewer viral particles compared to susceptible ones. Therefore, apart from the three ICVs: Amansan, Abrabopa, and AGRA, the remaining ICVs may be considered resistant to EACMV.

Conclusion

Based on serological evaluation of the 21 varieties, ACMV was not detected in only 2 varieties: Hemaa and AGRA in the coastal savannah. For EACMV, 7 out of 21 ICVs (Ampong, TEK, Sika, Lamesese, Abasafita, Amansan, and Esam) tested positive in the coastal savannah compared to 14 out of 21 testing positive to EACMV in the forest zone. Thus, most of the ICVs tested in this study are susceptible to both ACMV and EACMV with susceptibility increasing under the Forest conditions.

PCR screening of the ICVs for ACMV and EACMV in the second year confirmed the susceptibility of all the 21 ICVs except Amansan and Botan to ACMV in the coastal savannah and Abelefia and Doku duade in the forest zone. However, the absence of EACMV in all the ICVs except Amansan, Botan, Abelefia, and Doku duade based on PCR screening suggests that most of the

ICVs are resistant to EACMV. Therefore, the development of ACMV resistance in improved cassava varieties that show tolerance or resistance to EACMV can extend their cultivation and use as food by farmers in Ghana and Africa. Furthermore, cultivation of varieties with resistance against both ACMV and EACMV will reduce yield loss and possibly increase farmers' incomes.

Lastly, results from this study highlight the need to carry out periodic surveillance of plant diseases especially virus diseases on cassava in farmers fields across the country. The evaluation of the level of resistance of the improved varieties should be carried out regularly to prevent the total breakdown of developed resistance in varieties cultivated by farmers in the field.

CHAPTER SEVEN

SUMMARY, GENERAL CONCLUSION AND RECOMMENDATIONS

Summary

The first goal of this study was to determine farmer awareness of Cassava Mosaic Disease (CMD), management practices, knowledge and use of released improved/resistant cassava varieties by farmers in six districts (two districts in the coastal (Akatsi South and Adaklu), forest (Afadjato south and Hohoe) and Guinea savannah zones (Krachi East and Krachi Nchumuru)) in the Volta Region of Ghana. A structured questionnaire was used for data collection. All the 180 farmer respondents in the study area had knowledge of CMD but did not know the cause (51 farmers) and ways to manage (24 out of 51 farmer) the disease on their fields. Furthermore, farmers' were aware of the existence of ICVs but did not cultivate the ICVs due to availability and some undesirable traits such as woody tubers, palatability issues, unsure about the yield and disease and colour issues.

The second objective of the study was to assess the prevalence and spatial distribution of CMD, the abundance of whitefly population in farmers' fields, and associated Cassava Mosaic Geminiviruses (CMGs) in the Volta Region. A field survey was carried out to assess the incidence and severity of CMD in cassava farms in six districts in the Volta Region. The abundance of whitefly populations in farmers' fields was estimated. TAS-ELISA was used to determine the associated CMGs in 2700 leaf samples (30*90 farms: 2250 symptomatic and 450 asymptomatic). CMD incidence was endemic, almost reaching 100% in most of the fields surveyed with severe symptoms. Widespread use and exchange of local varieties which are susceptible to CMD

and high whitefly populations in the study area might account for the high incidence of CMD in farmers' fields. TAS-ELISA screening of leaf material revealed a high incidence of ACMV and EACMV with a high incidence of co-infection with ACMV and EACMV.

The use of PCR, RCA and illumine sequencing to characterise the genetic diversity of CMGs in the six districts in the Volta Region revealed high levels of single ACMV infection and 20% of mixed infection with ACMV and EACMV. Mixed infection with ACMV and EACMV was common in the two Savannah agro-ecological zones. This could be due to the susceptibility of cassava cultivars grown by farmers in those areas. Full genome sequencing resulted in the detection of twelve isolates, with three on each DNA-A and DNA-B of both ACMV and EACMV. The isolates had a close identity to isolates from other West African countries (Nigeria, Cameroon, Burkina Faso and Ghana isolates).

Lastly, the final goal of the study was to assess the performance of 21 ICVs to CMD several years after their cultivation by farmers. The 21 ICVs including a local variety were planted (2018–2019) in the coastal and forest zones and their CMD resistance was evaluated serologically using ELISA. The same 21 ICVs including a local variety were evaluated and biologically indexed in the second year (2019-2020). Leaf samples from the evaluation and biologically indexed plants were molecularly assessed. Samples from biologically indexed plants were subjected to a serological test (TAS ELISA). In the first and second years, only Botan tested negative for ACMV and EACMV in TAS ELISA, PCR and biological indexing indicating resistance to CMD.

General Conclusions

The key findings from the research are summarised below

From the study, it was found that the majority of farmers had either heard of CMD or experienced the disease on their fields, thus, they could recognize the symptoms on cassava in their farms. However, the farmers' inability to adopt management or control strategies to reduce CMD on their farmland might have resulted in the high prevalence and incidence of CMD recorded in the surveyed districts in this study. The low percentage of farmers planting improved cassava varieties on their farms due to either undesirable traits or scarcity of planting materials meant that the majority of farmers planted local CMD-susceptible varieties exacerbating the prevalence of CMD in the region. This result highlights the need for researchers and farmers to work together during the breeding stages in selecting improved lines or varieties for release. This approach might lead to higher adoption rates and acceptability of recently released improved cassava varieties.

A field survey of cassava farms in six districts in the Volta Region revealed a high incidence and severity of CMD, as well as a high whitefly population. Both CMD symptomatic and asymptomatic leaves tested positive for ACMV or EACMV with high levels of coinfection with both species.

Molecular characterisation of the ACMV and EACMV species using the Illumina MiSeq platform identified twelve full length genomes which showed high similarity to ACMV and EACMV species from West Africa.

To manage CMD in Ghana, a number of improved varieties have been released and have been cultivated by farmers. Assessing the resistance/tolerance levels of a selection of twenty-one of these improved cassava varieties to CMD

in the coastal and forest agro-ecological zones and screen house showed varying tolerance levels to CMD. None of the ICVs consistently tested negative for ACMV and EACMV in the first and second years using three different screening techniques (TAS-ELISA, PCR and graft challenge with infected rootstocks). Based on this result, most of the ICVs are showing mild to moderate CMD infection.

Recommendations

Although all the cassava farmers interviewed in the study had experienced CMD on their farms, about 74.4% did not implement any management practices. Stakeholders should invest in regular education of farmers on how to implement CMD management or control practices on their farms.

The number of multiplication centres for improved cassava varieties/seed in the region should be increased to ensure the availability of planting material for farmers all year round. A yearly audit of supplied planting material should be carried out by MoFA, Research institutions and other stake holders to monitor quality and identify off-types.

Since the prevalence of CMD was high in the studied areas, it is recommended that epidemiological surveys for CMD be conducted regularly (every 2 years) to detect new isolates or species present in cassava fields. This will prevent outbreaks that threaten the food security of smallholder farmers who depend on the crop for sustenance.

Quarantine laws should be enforced at the borders to reduce the influx and exchange of cassava planting materials across neighbouring countries without proper sanitation checks. In addition, this will prevent the import of other CMG strains into Ghana leading to pandemics that affect food production.

Stakeholders should patronise the use of clean planting materials that can be scaled through the use of plant tissue culture. Research institutions with capacity (human and infrastructure) such as Biotechnology and Nuclear Research Institutions (BNARI) and others should be engaged regularly to produce planting materials for farmers in Ghana.

All these efforts in combination with education can reduce the incidence of CMD and other viral diseases in Ghana.

REFERENCES

Adams, M. J., Lefkowitz, E. J., King, A. M., Harrach, B., Harrison, R. L., Knowles, N. J., Kropinski, A. M., Krupovic, M., Kuhn, J. H., Mushegian, A. R., Nibert, M., Sabanadzovic, S., Sanfacon, H., Siddell, S. G., Simmonds, P., Varsani, A., Zerbini, F. M., Gorbalenya, A. E. & Davison, A. J (2017) Ratification vote on taxonomic proposals to the International Committee on Taxonomy of Viruses. *Archives of Virology 161*:2921–2949

Acheampong, P., Owusu, V. & Gyiele K. N. (2013). Farmers Preferences for Cassava Variety Traits: Empirical Evidence from Ghana. In Fourth International Conference of the African Association of Agricultural Economists (pp. 1–21). Hammamet, Tunisia.

Acheampong P. P.& Owusu, V (2015). Impact of improved cassava varieties' adoption on farmers' incomes in Rural Ghana. Retrieved on 18/10/2019 from https://ageconsearch.umn.edu/record/210875/

Adekayode, F. O & Adeola, O. F (2009). The response of cassava to potassium fertilizer treatments. *International journal of food, agriculture and environment 7*: 279-282.

Adjei-Nsiah, S. & Sakyi-Dawson, O. (2012). Promoting Cassava as an Industrial Crop in Ghana: Effects on Soil Fertility and Farming System Sustainability. *Applied and Environmental Soil Science 8*: doi:10.1155/2012/940954

Adjekum, A. A. (2006). Cassava processing and marketing: Experiences from Ghana. Paper Presented at IFAD Regional Initiative Workshop. Accra

Adjekum, A & Ofori, F.(2000). Promotion of root and tuber crops in Ghana. In Potential of Root Crops for Food and Industrial Resources. 12th Symposium of the International Society for Tropical Root Crops (ISTRC) 10-16 September 2000, Tsukuba Japan

Adriko, J., Sserubombwe, W. S., Adipala, E., Bua, A., Thresh, J. M. & Edema, R. (2011). Response of improved cassava varieties in Uganda to Cassava Mosaic Disease (CMD) and their inherent resistance mechanisms. *African Journal of Agricultural Research. 6*(3), 521-531

Adriko, J., Sserubombwe, W., Adipala, E., Bua, A. Thresh, J. M. & Edema, R. (2012). Response of Local Cassava Varieties in Uganda to Cassava Mosaic Virus Disease. *American Journal of Experimental Agriculture 2*. 111-132.

Afolami, C. A., Obayelu, A. E., & Vaughan, I. I. (2015). Welfare Impact of adoption of improved cassava varieties by rural households in South Western Nigeria. *Agricultural and Food Economics, 3*(1), 1–7.

AgSSIP (Agricultural Services Sub-sector Investment Project) (2007). Implementation completion and results report. A World Bank document presented to the Republic of Ghana. Washington, D.C.: World Bank.

Agriculture Global Practice (2017) Transforming Agriculture for Economic Growth, Job Creation and Food Security. Retrieved on 15/9/2020 from http://documents1.worldbank.org/curated/pt/3365415059269

020/pdf/119753-PN-P133833-PUBLIC-Ghana-Policy-Note-Ag-Sector-Review.pdf

Ahmed, H. & Anang, B. T (2019). Does Farmer Group Membership Enchance TECHNOLOGY Adoption? Empirical Evidence from Tolon District of Ghana. *Review of Agricultural and Applied Economics 22*(2)

Akano, A. O. Dixon, A. G. O. Mba, C. Barrera, E. Fregene, M. (2002). Genetic mapping of a dominant gene conferring resistance to Cassava Mosaic Disease, *Theoretical Applied Genetics 105* 521–525.

Akorade, M. A. (2004). Cassava industrial revolution in Nigeria: the potential for a new industrial agriculture. *Journal of Agriculture, 1*(1), 40-43.

Akumatey, S. (2017). Volta Region is becoming a cassava processing hub. Retrieved from https://www.ghananewsagency.org/features/volta-region-is-becoming-cassava-processing-hub-124666 on 9 March 2019

Akyeampong, K. (2009). Revisiting Free Compulsory Universal Basic Education (FCUBE) in Ghana. *Comparative Education 45* (2), 175-195

Alabi, O. J., Kumar, P. L. & Naidu, R. A. (2011). Cassava Mosaic Disease: A curse to food security in Sub-Saharan Africa. Retrieved from https://www.apsnet.org/edcenter/apsnet features/Pages/cassava.aspx

Alene, A D., Khataza, R., Chibwana, C., Ntawuruhunga, P. & Moyo, C (2013) Economic impacts of cassava and extension in Malawi and Zambia. *Journal of Development Agricultural Economics 5*:457–469

Alexandratos, N., & Bruinsma, J. (2012). World agriculture towards 2030/2050: The 2012 Revision (ESA Working Paper No. 12-03). Rome: Food and Agriculture Organization of the United Nations (FAO), Agricultural

Development Economics Division. Retrieved on 14/9/2020 from http://www.fao.org/3/ap106e/ap106e.pdf

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., Lipman, D. J (1990). Basic local alignment search tool. *Journal of Molecular Biology*, 215:403–410

Amernope, G., Asare-Bediako E., Tetteh, J. P., Asare, P. A., Taah, K. J., & Van der puijie, G. *et al*. (2019). Evaluation report on cassava elite lines proposed for release, presented to National Varietal Release Committee. Department of Crop Science, School of Agriculture, University of Cape Coast. Ghana.

Ambang, Z. Amougou, A. Ndongo, B. Nantia, J. Nyobe, L. Ongono, Y.S.B. (2007). Tolérance de quelques cultivars de manioc (M. esculenta) et de l'espèce sauvage (M. glaziovii) à la mosaïque virale africaine et à la Cercosporiose. *Tropicultura, 25*, 140-145

Ambang, Z., Amougou, A., Ndongo, B., Nantia, J. & Chewachong, G. M. (2009) Re´sistance à la mosaïque virale de *Manihot glaziovii* par greffage sur *M. esculenta*. *Tropicultura 27*: 8–14.

Angelucci F (2013). Analysis of incentives and disincentives for cassava in Ghana. Technical notes series, Monitoring African Food and Agricultural Policies project (MAFAP), FAO, Rome.

Anjanappa, R. B., Mehta, D., Maruthi, M. N., Kanju, E., Gruissem, W., Vanderschuren, H. (2016). Characterization of Brown Streak Virus–Resistant Cassava. *Molecular Plant-Microbe Interactions, 29*, 527–534.

Anyanwu, S. O., Kalio, A. E., Manilla, H. A., & Ojumba, T. P. (2012). Cassava commercialization and its determinants in River State, Nigeria. *Journal of Agricultural Science,* India (in press)

Alves, A. A. C. (2002) Cassava botany and physiology. In: R. J. Hillocks., J. M. Thresh, & A.C. Bellotti (eds) Cassava: biology, production and utilization. CABI, Wallingford, UK.

Appiah, P. (2015). Six disease-resistant cassava hybrid varieties developed in Ghana. Retrieved from https://www.myjoyonline.com/business/2015/November-13th/five-disease-resistant-cassava-hybrid-varieties-developed-in-ghana.php on 22/01/2020

Ariyo, O. A., Dixon, A. G. O., Atiri, G. I. (2003). The relative resistance of cassava cultivars to African Cassava Mosaic Disease (ACMD) as determined by two methods: rank-sum and the area under the disease progress curve. *Archives. Phytopathology and Plant Protection 35*, 23-30.

Ariyo, O. A., Dixon, A. G. O. & Atiri, G. I. (2005). Whitefly *Bemisia tabaci* (Homoptera Aleyrodidae) infestation on cassava grown at different ecozones in Nigeria. *Journal of economic entomology, 98*(2), 611-617.

Asare, P. A., Galyuon, I. K. A., Asare-Bediako, E., Sarfo, J. K., & Tetteh, J. P. (2014). Phenotypic and molecular screening of cassava (*Manihot esculentum* Crantz) genotypes for resistance to Cassava Mosaic Disease. *Journal of General and Molecular Virology, 6*(2), 6-18.

Asare-Bediako, E., Wonkyi-Mensah, D., van der Puije, G., Amenorpe, G. & Osei, M., (2017) Variation in the susceptibility of tomato (Lycopersicon solanum L.) genotypes to tomato yellow leaf curl virus (TYCVD) infections at coastal savannah and forest zones of Ghana. *Australian Journal of Crop Science 11*, 373-381 doil: 10.21475/ajcs.17.11.04.pne124

Asgedom, S., Struik, P. C., Heuvelink, E. P., & Araia, W. (2011). Opportunities and constraints of tomato production in Eritrea. *Africa Journal of Agriculture and Research, 6*(4)

Atieno, E., Ogendo, J & Midatharahally, N & Hillocks R., Mulwa, R. M. & Arama, P. (2016). Occurrence and estimated losses caused by cassava viruses in Migori County, Kenya. African *Journal of Agricultural Research 11* (24) 2064-2074.

Bampoe, E. A. (2015). Cassava Farmers' Perceived Impact of the West Africa Agricultural Productivity Programme on their Livelihood Systems in Brong-Ahafo Region, Ghana. (Master of Philosophy thesis) University of Cape Coast, Cape Coast, Ghana. Retrieved on 7/8/2019 from https://ir.ucc.edu.gh/xmlui/bitstream/ handle/12345 6789/2593 /BAMPOE%202015.pdf?sequence=1&isAllowed=y

Bayitse, R., Tornyie, F., & Bjerre, A. (2017). Cassava cultivation, processing and potential uses in Ghana. Retrieved from https:// www.researchgate.net/publication/320234151_Cassava_cultivation _processing_and_potential_uses_in_Ghana/citation/download

Bentley, J. W., & Thiele, G. (1999). Farmer knowledge and management of crop disease. *Agriculture and Human Values 16,* 75-81.

Bentley, J., Olanrewaju, A. Madu, T. Olaosebikan, O. Abdoulaye, T.Wossen, T. Manyong, V. Kulakow, P. Ayedun, B. Ojide, M. Girma, G. Rabbi, I. Asumugha, G. & Tokula, M. (2017). Cassava farmers' preferences for varieties and seed dissemination system in Nigeria: Gender and regional perspective. IITA Monograph, IITA, Ibadan, Nigeria. ISBN 978-978-8444-82-4. 90pp.

Berrie, L. C., Palmer, K. E., Rybicki, E. P., Rey, M. E. (1998). Molecular characterisation of a distinct South African cassava infecting geminivirus. *Archives of Virology 143*(11):2253-60. doi: 10.1007/ s007050050457. PMID: 9856107.

Berry, S. D. & Rey, M. E. C. (2001). Molecular evidence for diverse populations of cassava infecting begomoviruses in southern Africa. *Archives of Virology 146*, 1795-1802

Bi, M. Aileni, H. & Zhang, P. (2010). Evaluation of cassava varieties for Cassava Mosaic Disease resistance jointly by agro-inoculation screening and molecular markers. *Africa Journal of Plant Science 4* 330–338.

Bock, K. R. & Woods, R. D. (1983). Etiology of African Cassava Mosaic Disease. *Plant Disease 67*, 994-995

Briddon, R. W., Liu, S., Pinner, M. S. & Markham, P.G. (1998). Infectivity of African cassava mosaic virus clones to cassava by biolistic inoculation. *Archives of Virology 143*, 2487-2492.

Brown, J. K., Frohlich, D. R. & Rosell, R. C. (1995). The sweetpotato or silverleaf whiteflies biotypes of Bemisia tabaci or a species complex? *Annual Review of Entomology, 40*, 511-534.

Brown, K. K., Idris, A. M., Torres-Jerez, I., Banks, G. K. & Wyatt, S. D. (2001). The core region of the coat protein gene is highly useful for establishing the provisional identification on and classification of begomoviruses. *Archives of Virology 146*, 1581- 1598.

Bruins, M. (2009). The evolution and contribution of plant breeding to global agriculture, in: UPOV, responding to the challenges of a changing world: The role of new plant varieties and high quality seed in agriculture. In Proceedings of the Second World Seed Conference held at the FAO Headquarters, Rome (pp. 18–29). September 8-10, 2009. Geneva: UPOV.

Bull, S. E., Briddon, R. W., Sserubombwe, W.S., Ngugi K., Markham, P. G. & Stanley J. (2006). Genetic diversity and phylogeography of cassava mosaic viruses in Kenya. *Journal of General Virology 87*:3053–3065.

CABI (2020). Cassava Mosaic Disease (African Cassava Disease) Retrieved from https://www.cabi.org/isc/datasheet/2747 on 22th March 2020

Catalogue of Crop Varieties Released & Registered in Ghana (2019). Root and Tubers. Retrieved on 29/10/2021 from https://nastag.org/docx/resources/2019%20NATIONAL%20CROP%20VARIETY%20CATOGUE.pdf

Campbell, C. L., & Benson, D. M., eds. (1994). Epidemiology and management of root diseases. Springer-Verlag, Berlin

Carrington, J. C., Kasschau, K. D., Mahajan, S. K. & Schaad, M. C. (1996). Cell to cell and long-distance transport of viruses in plants. *The Plant Cell 8*, 1669-1681.

Ceballos, H., Iglesias, C. A., Pérez, J. C. & Dixon, A. G. O. (2004). Cassava breeding: opportunities and challenges. Plant molecular biology., 56(4), 503-516.

Changadeya, W., Kamowa-Mbewe, W., Kumar, L., Ntawuruhunga, P., Legg, J. P., Fauquet, C. M. & Mkumbira, J (2016). The prevalence of Cassava Mosaic Geminiviruses in Malawi. *International Journal of Research in Social Sciences 6* (1)

Charoenvilaisiri, S , Seepiban, C. Kumpoosiri, M. Rukpratanporn, S. Warin, N. Phuangrat, B. Chitchuea, P. Siripaitoon, S. Chatchawankanphanich O. & Gajanandana, O. (2021). Development of a triple antibody sandwich enzyme-linked immunosorbent assay for Cassava Mosaic Disease detection using a monoclonal antibody to Sri Lankan cassava mosaic virus. *Virology Journal (18)* 100

Chellappan, P., Vanitharani, R., Ogbe, F. & Fauquet, C. M (2005). Effect of temperature on geminivirus-induced RNA silencing in plants. *Plant Physiology 138*:1828– 841.

Chernoh, E (2014). Cassava Green mite. Retrieved from http://africasoilhealth.cabi.org/wpcms/wp-content/uploads/2015/02/28-tubers-cassava-greenmite.pdf on on 18 November 2019.

Chikoti, P. C., Ndunguru, J., Melis, R., Tairo, F., Shanahan, P. & Sseruwagi, P. (2013a). Cassava Mosaic Disease and associated viruses in Zambia, occurrence and distribution. *International Journal Pest Management 59*:63–72

Chikoti, P. C., Shirima R., & Legg, J. P. (2013b). First report of super-abundant *Bemisia tabaci* on cassava in Zambia. First International Whitefly Symposium, 20-24th May, 2013. Poster. Kolymbari, Crete, Greece.

Chikoti, P. C., Tembo, M., Chisola, M., Ntawuruhunga, P. & Ndunguru, J (2015) Status of Cassava Mosaic Disease and whitefly population in Zambia. *Africa Journal of Biotechnology 14*:2539–2546

Chikoti, P. C., Melis, R., & Shanahan, P. (2016). Farmer's Perception of Cassava Mosaic Disease, Preferences and Constraints in Lapaula Province of Zambia. *American Journal of Plant Sciences, 7*, 1129-1138

Chikoti, P. C., Mulenga, R. M., Tembo, M. & Sseruwagi, P. (2019). Cassava Mosaic Disease: a review of a threat to cassava production in Zambia. *Journal of Plant Pathology 101*, 467–477

Chomdej, O., Chatchawankanpanich, O., Kositratana, W., & Chunwongse, J (2007). Response of resistant breeding lines of tomato germplasm and their progenies with seedto Tomato yellow leaf curl virus, Thailand isolate (TYLCTHV-[2]). *Songklanakarin Journal of Science Technology, 29*(6), 1469-1477

CIAT, (2005). Whitefly and whitefly-borne viruses in the tropics: Building a knowledge base for global action. Retrieved on 30/8/2019 from http://ciat-library.ciat.cgiar.org/Articulos_CIAT/9586940748.pdf

Cochran, W. G. (1977). Sampling techniques (3rd edn). New York, John Wiley & Sons

Colvin, J, Otim-Nape, G. W., Holt J, Omongo C, Seal S, Stevenson P, Gibson G. I, Cooter, R J., Thresh, J. M, (1999). Symbiotic interactions drive whitefly-borne plant virus epidemic disease in Uganda. In: Cooter, R. J., Otim-Nape, G. W., Bua, A., Thresh, J. M., eds. Cassava Mosaic Disease Management in Smallholder Cropping Systems. Chatham, UK: Natural Resources Institute/NARO, 76–86.

Colvin, J., Nagaraju, N., Moreno-Leguizamon, C., Govindappa, R. M., Reddy, T. B. M., Padmaja, S. A., Joshi, N., Hanson, P. M., Seal, S. E., Muniyappa, V. (2012). Socioeconomic and scientific impact created by whitefly-transmitted, plant-virus disease resistant tomato varieties in southern India. *Journal Integrative Agriculture 11* (2): 337-345.

Cossa, N. S. (2011). Epidemiology of Cassava Mosaic Disease in Mozambique. Unpublished Ph.D. Thesis, Johannesburg, South Africa: University of Witwatersrand,

Cours, G., (1951). Le Manioc à Madagascar. Mémoires de l'Institut Scientifique de Madagascar, Série. *B, Biologie Végétale, 3*, 203–400.

Cudjoe, A., Gyamenah, J. & Braima, J. (2005) Whiteflies and whitefly-borne viruses in the tropics: Building a knowledge base for global action. P.K. Anderson, F.J. Morales, A.L. Jones and R.H. Markham (eds). Arte Libro Impresores, Cali. Colombia. pp. 24 – 25.

CUSABIO TECHNOLOGY(2007-2020). Four types of ELIZA. Retrieved on 27/7/2020 from https://www.cusabio.com/c-20659.html

Danso-Abbeam, G. Antwi Bosiako, J. Ehiakpor D. S. & Nantui M. F. (2017) Adoption of improved maize variety among farm households in the northern region of Ghana. *Cogent Economics & Finance, 5*:1, 1416896

De Bruyn, A., Harimalala, M., Zinga, I., Mabvakure, B. M., Hoareau, M., Ravigné, V., Walters, M., Reynaud, B., Varsani, A., Harkins, G. W., Martin, D. P., Lett, J. M., & Lefeuvre, P. (2016). Divergent evolutionary and epidemiological dynamics of Cassava Mosaic Geminiviruses in Madagascar.*BMC evolutionary biology*,*16* (1), 182. https://doi.org/10.1186/s12862-016-0749-2

Doyle, J. J.& Doyle, J. L. (1990). Isolation of Plant DNA from Fresh Tissue. *Focus, 12*, 1, , 13-15

Dubern, J. (1994). Transmission of African cassava mosaic geminivirus by the whitefly (*Bemisia tabaci*). *Tropical Science 34*:82-91.

Denkyirah, E. K., Okoffo, E. D., Adu, D. T., Aziz, A. A. & Ofori, (2016). Modeling Ghanaian cocoa farmers' decision to use pesticide and frequency of application: The case of Brong-Ahafo region. *SpringerPlus, 5*(1), 1–13

Deng, D., McGrath., P. F. Robinson, D. J. & Harrison, B. D. (1994). Detection and differentiation of whitefly-transmitted *geminiviruses* in plants and vector insects by the polymerase reaction with degenerate primers. *Annals of Applied Biology 125*: 327-336.

Deng, D., Otim-Nape, W. G., Sangaré, A., Ogwal, S., Beach, R. N. & Fauquet, C. M. (1997). Presence of a new virus closely related to East African cassava mosaic geminivirus, associated with cassava mosaic outbreak in Uganda. *African Journal of Root and Tuber Crops 2*, 23-28.

Dade, H. A. (1930). "Cassava Mosaic." Paper No. XXVIII. Yearbook. pp. 245–247. Department of Agriculture, Gold Coast.

Diiro, M. G., Ker, A. P., & Sam, A. G. (2015). The role of gender in fertilizer adoption in Uganda. *African Journal of Agricultural and Resource Economics, 10*(2), 117–130.

Doku, E. V. (1966). Root crops in Ghana. *Ghana Journal of Science 6*: 15-36.

Dubern, J. (1994). Transmission of African cassava mosaic geminivirus by the whitefly Bemisia tabaci. *Tropical Science 34*, 82–91.

Duffy, S., & Holmes, E.C. (2008). Phylogenetic evidence for rapid rates of molecular evolution in the single-stranded DNA begomovirus tomato yellow leaf curl virus. *Journal of Virology* . *82*(2):957-65.

Dunne, L. (2004). The Development of immunoassays for the detection of bovine brucellosis and aflatoxin Bt. Retrieved on 27/7/2020 from http://doras.dcu.ie/17344/1/lynsey_ dunne_RR.pdf

Echodu, R., Edema, H., Wokorach, G., Zawedde, C., Otim, G., Luambano, N., Ateka, E. M., & Asiimwe, T. (2019). Farmers' practices and their knowledge of biotic constraints to sweetpotato production in East Africa. *Physiological and molecular plant pathology, 105*, 3–16.

Elegba, W., Gruissem, W., & Vanderschuren, H. (2020). Screening for Resistance in Farmer-Preferred Cassava Cultivars from Ghana to a Mixed Infection of CBSV and UCBSV. *Plants, 9*(8), 1026. doi.org/10.3390/plants9081026

Elegba, W., Appiah, S. A., Azu, E., Afful, N., Agbemavor, W., Agyei-Amponsah, J., Owureku-Asare, M., Quaye, B. & Danso, K. (2013). African Journal of Biotechnology Effect of mosaic virus diseases on

dry matter content and starch yield of five cassava (*Manihot esculenta Crantz*) accessions in Ghana. *Africa Journal of Biotechnology 12*(27), 4310-4316

Elegba, W., Gruissem W., & Hervé V. (2018). CBSD susceptibility of farmer-preferred cassava cultivars from Ghana to mixed CBSV and UCBSV infections. (PhD Thesis) ETH Zurich, Zürich, Switzerland.

El-Sharkawy, M. A. (2004). Cassava biology and physiology. *Plant Molecular Biology, 56*, 481-501

El-Sharkawy, M.A. (2006). International research on cassava photosynthesis, productivity, eco-physiology, and responses to environmental stresses in the tropics. *Photosynthetica 44*: 481- 512

El-Sharkawy, M. A (2007). Physiological characteristics of cassava tolerance to prolonged drought in the tropics: implications for breeding cultivars adapted to seasonally dry and semiarid environments. *Brazilian Journal of Plant Physiology 19*: 257-286.

Ennin, S. A., Asafu-Agyei, J. N., Dapaah, H. K. & Ekyem, S. A. (2001). Cowpea rotation with maize in cassava-maize intercropping systems. *Tropical Agriculture 78*. 218-225.

Ennin, S. A., Clegg, M. D. & Francis, C. A. (2002). Resource utilization in soybean/maize intercrops. *African Crop Science Journal, 10*:251-261.

Etessami P., Callis R., Ellwood S., Stanley J. (1988). Delimitation of essential genes of cassava latent virus DNA 2. *Nucleic Acids Research. 16* 4811–4829 doi:10.1093/nar/16.11.4811

Fanou, A.; Valerien Z. Wydra, K.& Erfurt F. (2018). Cassava Bacterial Blight: A Devastating Disease of Cassava. Retrieved from http://www. intechopen.com/books/cassava on 14/5/2020

FAOSTAT. (2016). *FAO Statistical Yearbook*. Food and Agricultural Organization of the United Nations. Rome, Italy.

Fargette, D., Fauquet, C. & Thouvenel, J. C. (1985). Field studies on spread of African cassava mosaic. *Annals of Applied Biology 106*: 285-294.

Fargette D., Thouvenel. J. C & Fauquet, C. (1987). Virus content of leaves of cassava infected by Africa cassava mosaic virus. *Annals of Applied Biology 110*:65-73

Fargette, D., Fauquet, C & Thouvenel, J-C, (1988). Yield losses induced by African cassava mosaic virus in relation to the mode and the date of infection. *Tropical Pest Management 34*, 89–91.

Fauquet, C. & Fargette, D (1990). African Cassava Mosaic Virus: Etiology, Epidemiology, and Control. *Plant Disease, 74* (6). Retrieved from www.apsnet.org/publications/plantdisease/backissues/Documents1990/Articles/PlantDisease74n06_404.pdf

Fargette D, Jeger M, Fauquet C, Fishpool, L. D. C. (1994). Analysis of temporal disease progress of African cassava mosaic virus. *Phytopathology 84*, 378– 82.

Fargette, D., Colon, L. T., Bouveau, R. & Fauquet , C. (1996). Components of resistance of cassava to African cassava mosaic virus. *European Journal of Plant Pathology 102*, 645–654

Fauquet, C. M. & Stanley, J. (2003). Geminivirus classification and nomenclature: progress and problems. *Annals of Applied Biology. 142*: 165–189.

Fauquet, C. M., Briddon, R. W. Brown., J. K. Moriones., E. Stanley., J. Zerbini, M.& Zhou. X. (2008). Geminivirus strain demarcation and nomenclature. *Archives of Virology 153*:783–821.

Fisher, M., & Carr, E. R. (2015). The influence of gendered roles and responsibilities on the adoption of technologies that mitigate drought risk: The case of drought-tolerant maize seed in eastern Uganda. *Global Environmental Change, 35*, 82–92.

Fermont, A M., Van Asten, P., Tittonell, P., Van Wijk, M. & Giller, K (2009). Closing the cassava yield gap: An analysis from smallholder farms in East Africa. *Field Crops Research 112*: 24-36.

Fondong, V. N. (1999). Viruses Associated with the Cassava Mosaic Disease in Cameroon: Spread and Molecular Characterization. Ph.D. thesis, University of the Witwatersrand, Johannesburg, 152.

Fondong, V. N., Pita, S. T., Rey, M. E. C., de Kochko, A., Beachy, R. N. & Fauquet, C. M. (2000a). Evidence of synergism between African cassava mosaic virus and a new double recombinant geminivirus infecting cassava in Cameroon. *Journal of General Virology 81*, 287-297.

Fondong, V. N, Thresh, J. M, Fauquet, C, (2000b). Field experiments in Cameroon on cassava mosaic virus disease and the reversion phenomenon in susceptible and resistant cassava cultivars. *International Journal of Pest Management 46*, 211–7.

Fondong, V. N., Thresh, J. M. & Zok, S, (2002). Spatial and temporal spread of cassava mosaic virus disease in cassava grown alone and when intercropped with maize and/or cowpea. *Journal of Phytopathology 150*, 365–74.

Fondong, V. N. (2017). The Search for Resistance to Cassava Mosaic Geminiviruses: How Much We Have Accomplished, and What Lies Ahead. *Frontiers in Plant Science 8*, 408

FAO, (2013). Save and Grow Cassava: A guide to sustainable production intensification. Retrieved on 6/9/2020 on http://www.fao.org /3/i3278e/i3278e.pdf

FAO (2013). FAOSTAT statistical database. Retrieved on 20/9/2020 from http://faostat.fao.org.

FAO (2015). Food and Agriculture Organization of the United Nations, Country Fact Sheets on Food and Agriculture Policy Trends. Ghana: Food and Agriculture Policy Development Analysis.

Food and Agriculture Organization (FAO) (2019). Protecting Cassava, a neglected crop from pests and disease. retrieved from http://www.fao.org/3/ca7117en/ca7117en.pdf on 3 February 2020.

Fregene, M. Okogbenin, E. Mba, C. Angel, F. Suarez, M.C. Guitierez, J. Chavarriaga, P. Roca, W. Bonierbale, M. Tohme, J. (2001). Genome mapping in cassava improvement: challenges, achievements and opportunities. *Euphytica 120* 159–165.

García-Arenal, F., & McDonald, B. A. (2003). An analysis of the durability of resistance to plant viruses. Phytopathology, 93, 941-952.

Gebresilassie, L., & Bekele, A. (2015). Factors determining allocation of land for improved wheat variety by smallholder farmers of Northern Ethiopia. *Journal of Development and Agricultural Economics, 7*(3), 105–112.

Gerik, J. S., Duffus, J., E., Perry, R., Stenger, D. C. & Van Maren, A. F. (1990) Etiology of tomato plant decline in the California desert. *Phytopathology, 80*, 1352–1356

Gerling, D., Alomar, O., & Arno, J. (2001). Biological control of *Bemisia tabaci* (s.) using predators and parasitoids. *Crop Protection 20*, 779-799

Ghana Districts (2009). Krachi Nchumuru. Retrieved on 15 may 2019 from http://www.ghanadistricts. com/Home/District/187

Ghana Statistical Services. (2016). Revised 2015 annual gross domestic product (GDP). Ghana: Ghana Statisitcal Service.

Gibson, R. W (2006). Crop protection programme: Extending control of sweet potato diseases in East Africa. Retrieved on 1/9/20 from http://www.sweetpotatoknowledge.org/wp-contentg/uploads/2016/02/ Extending-control-of-sweerpotato-virus-disease-R8457_FTTR.pdt

Gorfe, H.A. (2004). The comparative influence of intervening variables in the adoption behaviour of maize and dairy farmers in Shashemene and Debrezeit Ethiopia. Retrieved on 18/5/2019 from http://www. semanticscholar.org/paper/The-comparative-influence-of-intervening-variable-Gorfe/d42a12694bdf486a015483f1fef3192494fdeddc

Growafrica (2015) Market opportunities for commercial cassava in Ghana, Mozambique, and Nigeria. Retrieved on 14/9/2021 from https://www.growafrica.com/sites/default/files/Market%20Opportuni

ties%20forCommercial%20Cassava%20in%20Ghanan%2C%20Mozambique%20and%20Nigeria_0pdf

Guthrie J, (1990). Controlling African Cassava Mosaic Disease. Wageningen, the Netherlands: CTA.

Gutierrez, G. (2000). DNA replication and cell cycle in plants: learning from geminiviruses. *The EMBO Journal 19*, 792-799.

Hahn, S. K., Terry, E. R, Leuschner K. (1980). Breeding cassava for resistance to Cassava Mosaic Disease. *Euphytica 29*(3):673–83.

Haible, D, Kober, S & Jeske, H. (2006). Rolling circle amplification revolutionizes diagnosis and genomics of *geminiviruses*. *Journal of Virological Methods 135*(1):9-16. doi: 10.1016/j.jviromet. 2006.01.017. PubMed PMID: 16513183.

Hanley-Bowdoin, L., Settlage, S. B., Orozco, B. M., Nagar, S., & Robertson, D. (1999). Geminiviruses: models for plant DNA replication, transcription, and cell cycle regulation. *Critical Reviews in Plant Sciences, 18*(1), 71-106.

Hanley-Bowdoin, L., Settlage, S. B. & Robertson, D. (2004). Reprogramming plant gene expression: A prerequisite to geminivirus DNA replication. Molecular Plant Pathology 5, 149-156.

Harrison, B. D. & Robinson, D. J. (1999). Natural genomic and antigenic variation in whitefly-transmitted geminiviruses (begomoviruses). Annu. Rev. Phytopathol. 37, 369–398

Harimalala, M., Chiroleu, F., Giraud-Carrier, C., Hoareau, M., Zinga, Randriamampianina J. A., Velombola, S. Ranomenjanahary, S. Andrianjaka, A. Reynaud, B. Lefeuvre P &. Lett J. M (2015).

Molecular epidemiology of Cassava Mosaic Disease in Madagascar. *Plant Pathology 64*, 501-507 doil 10.1111/ppa.12277

Herren, H. R. & Neuenschwander, P. (1991). Biological control of cassava pests in Africa. *Annual Review of Entomology, 36*:25-73

Hershey, C. (2012). Cassava genetic improvement: theory and practice. In Hillocks, R. J., Thresh, J. M., & Bellotti, A. (Eds.). (2002). *Cassava: biology, production and utilization*. CABI Publishing. New York. USA.

Heyraud, F., Matzeit, V., Kamman, M., Schaefer, S., Schell, J. & Gronenborn, B. (1993a). Identification of the initiation sequence for viral-strand DNA synthesis of wheat dwarf virus. *EMBO Journal 12*, 4445-4452.

Hillocks, R. J., Raya, M. D. & Thresh, J. M. (1996). The association between root necrosis and aboveground symptoms of brown streak virus infection of cassava in southern Tanzania. *International Journal of Pest Management 42*, 285-289.

Hillocks, R. J., & Thresh, J. M. (2000). "Cassava Mosaic and Cassava Brown Streak Virus Diseases in Africa: A Comparative Guide to Symptoms and Etiologies," *Roots 7*, (1)1-8.

Hillocks, R. J., Raya, M., Mtunda, K. & Kiozia, H. (2001). Effects of brown streak virus disease on yield and quality of cassava in Tanzania. *Journal of Phytopathology 149*, 1-6.

Hillocks, R. J (2002). Cassava in Africa. In: RJ Hillocks, J. M Thresh, A. C Bellotti (eds) Cassava: biology, production and utilization. CABI, Wallingford, UK.

Hillocks, R. J. & Jennings, D. L. (2003): Cassava brown streak disease: A review of present knowledge and research needs. International *Journal of Pest Management 49*, 225 – 234.

Hong, Y. G., Robinson, D. J. & Harrison, B. D. (1993). Nucleotide sequence evidence for the occurrence of three distinct whitefly-transmitted geminiviruses in cassava. *Journal of General Virology 74*, 2437-244.

Horowitz, R. A., Antignus. Y. & Gerling, D. (2015). Management of *Bemisia tabasi* Whiteflies. Springer: Netherlands doi: 10.1007/978-94-007-1524-0_11

Hou, Y-M. & Gilbertson, R. L. (1996). Increased pathogenicity in a pseudorecombinant bipartite geminivirus correlates with intermolecular recombination. *Journal of Virology 70*, 5430-5436.

Houngue, J. A., Pita, J. S., Cacaï, G. H. T., Zandjanakou-Tachin, M., Abidjo, E. A. E., & Ahanhanzo, C. (2018). Survey of farmers' knowledge of Cassava Mosaic Disease and their preferences for cassava cultivars in three agro-ecological zones in Benin. *Journal of Ethnobiology and Ethnomedicine, 14*(1), 29. doi:10.1186/s13002-018-0228-5

Houngue, J. A, Pita, J. S, Ngalle, H. B, Zandjanakou-Tachin, M, Kuate, A. F, Cacaï, G. H. T., Bell, J. M., Ahanhanzo, C. (2019) Response of cassava cultivars to African cassava mosaic virus infection across a range of inoculum doses and plant ages. *PLoS ONE 14*(12)

Howeler, R.L., Thomas, N., Holst Sanjuán, K., Sanjuán, K.H., Quirós, H., Isebrands, J., Martínez Oliva, E., Lagos Molina, S.M., Echeverri Rodríguez, J.H., Corrales González, V.G., (2013). Save and Grow Cassava: A Guide to Sustainable Production Intensification. FAO,

Roma (Italia). Retrieved on from 24/10/22 http://www.fao.org/3/a-i3278e.pdf

Hull, R. (2009). Comparative Virology (2nd ed). Norwich, United Kingdom: Elsevier Academic Press

Huson, D. H., Beier, S., Flade, I., Gorska, A., El-Hadidi, M., Mitra, S., Ruscheweyh, H-J., Tappu, R (2016) MEGAN community edition interactive exploration and analysis of large-scale microbiome sequencing data. *PLOS Computational Biology, 12* (6)

IFAD, (2012). Convergence on value-chain enhancement for rural growth and empowerment. Retrieved on 9/7/19 from

International Finance Co operation (IFC). (2013). Working with Smallholders: A hand for Firms Building Sustainable Supply Chains. Washington D.C: International Finance Corporation, World Bank Group. Retrieved on 14/9/2020 from https://www.ifc.org/wps/wcm/connect/647f85fc-6ad7-4315-aad8-4967075a304b/Handbook+-+Working+with+Smallholders.pdf?MOD=AJPERES&CVID=ka-TX8j

Imran, M., Shakeel, A., Azhar, F. M., Farooq, J., Saleem, M. F., Saeed, A., Nazeer, W., Riaz, M., Naeem, M., & Javaid, A. (2012). Combining ability analysis for within-boll yield components in upland cotton (*Gossypium hirsutum* L.). *Genetics and Molecular Research, 11*(3), 2790-2800

Inoue-Nagata, A K, Albuquerque, L. C, Rocha, W. B, Nagata T (2004). A simple method for cloning the complete begomovirus genome using the bacteriophage φ29 DNA polymerase. *Journal of Virological Methods 116*(2):209-11. doi: 10.1016/j.jviromet.2003.11.015

International Institute of Tropical Agriculture (IITA) (1990). Annual Reports, IITA, Ibadan, Nigeria

IITA/SARRNET (2007). Cassava transformation in southern Africa (CATISA) Project Malawi Report-2007. Lilongwe

IITA (1990). *Cassava in Tropical Africa*: A reference manual. International Institute of Tropical Agriculture, Ibadan. Nigeria. pp. 176.

Islam. W (2017). Management of plant virus diseases; farmer's knowledge and our suggestions. *Hosts and Viruses 4*(2): 28-33 doi: http://doi.org /10.17582/journal.bjv/2017/4.2.28.33

Jarvis, A., Ramirez-Villegas, J., Herrera Campo, B.V., Navarro-Racines, C., (2012). Is Cassava the answer to African climate change adaptation? *Tropical Plant Biology 5*,9–29.

Jeremiah, S. C., Ndyetabula, I. L., Mkamilo, G. S., Haji, S., Muhanna, M. M., Chuwa, C., Kasele, S., Ijumba, J., Legg, J. P. (2015). The dynamics and environmental influence on interactions between cassava brown streak virus disease and the whitefly, *Bemisia tabaci*. *Phytopathology,105*, 646–655.

Jericho, C., G. J. Thompson, U. Gerntholtz, & J. C. Viljoen. (1999). Occurrence and distribution of cassava diseases in South Africa. In: Akoroda, M. O., Teri, J. M. (Eds), Proceedings of the Scientific Workshop of the

Southern African Root Crops Research Network (SARRNET), Lusaka, Zambia, 17-19August 1998. 252-262.

Jones, R. A. C. (2014). Plant virus ecology and epidemiology: historical perspectives, recent progress and future prospects. *Annals of Applied Biology 164*: 320–347.

Kaliba, A., Verkuijl, H., & Mwangi, W. (2000). Factors affecting adoption of improved maize seeds and use of inorganic fertilizer for maize production in the intermediate and 39 Lowland Zones of Tanzania. *Journal of Agricultural and Applied Economics, 32*(1), 35–47.

Kehoe, M. A., Coutts, B. A., Buirchell, B. J., Jones, R. A. C. (2014). Plant Virology and NextGeneration Sequencing: Experiences with a Potyvirus. PLoS ONE 9 (8):e104580.doi:10.1371/ journal.pone. 0104580

Kiros-Meles, A., Abang, M. M.(2008). Farmers' knowledge of crop diseases and control strategies in the Regional State of Tigrai, northern Ethiopia: implications for farmer–researcher collaboration in disease management. *Agriculture and Human Values 25*, 433

Korang-Amoako, S., Cudjoe, R. A. & Adjakloe R. K. (1987). Biological Control of Cassava Pests in Ghana. *International Journal of Tropical Insect Science, 8* (905-907)

Kleih, U., Phillips, D., Wordey, M. T., Komlaga, G. (2013) 'Cassava Market and Value Chain Analysis, Ghana Case Study'. Final Report January 2013.Retrieved on 28/4/2020 from http://www.valuechains.org/dyn/bds /859/GhanaCassavaMarketStudyFinalFebruary2013_anoym.pdf

Khan, M. R., Ghani, I. A., Ghaffar, A., & Tamkeen, A. (2011). Host plant selection and oviposition behaviour of whitefly Bemisia tabaci (Gennadius) in a mono and simulated polyculture crop habitat. *African Journal of Biotechnology, 10*(8), 1467-1472.

Koike, S. T., Cintas, N. A. & Bull, C. T. (2000). Bacterial blight, a new disease of broccoli caused by *Pseudomonas syringae* in California. *Plant Disease 84:* 370

Kumar, A., & Khan, J. A. (2019). Geminivirus Resistance Strategies. Research Gate 197-218 doi: 10.1007/978-3-030-183248-9_11

Kuria, P., Ilyas, M., Ateka, E., Miano, D., Onguso, J., Carrington, J. C., Taylor, N. J. (2017). Differential response of cassava genotypes to infection by Cassava Mosaic Geminiviruses. Virus Research .227, 69–81. doi: 10.1016/j.virusres.2016.09.022

Lamptey, J. N. L., Okoli, O. O. & Frimpong-Manso, P. P (1998). Incidence and severity of African Cassava Mosaic Disease (ACMD) and cassava bacterial blight (CBB) on some local and exotic cassava varieties in different ecological zones of Ghana. *Ghana Journal Agricultural. Science. 31 (1)*:35-43.

Lefeuvre, P., Lett J-M., Reynaud, B., & Martin, D. P. (2007) Avoidance of Protein Fold Disruption in Natural Virus Recombinants. *PLoS Pathogens 3*(11):181

Legg. J. P. & Ogwal, S. (1998). Changes in the incidence of African cassava mosaic virus disease and the abundance of its whitefly vector along south-north transacts in Uganda. *Journal of Applied Entomology 122*: 169-178.

Legg, J.P. & Raya, M.D. (1998) Survey of cassava virus diseases in Tanzania. *International Journal of Pest Management 44*, 17–23.

Legg, J P., Okao-Okuja, G., Mayala, R. & Muhinyuza, J. B, (2001). Spread into Rwanda of the severe cassava mosaic virus disease pandemic and the associated Uganda variant of East African cassava mosaic virus (EACMV-UG). *Plant Pathology 50*, 796.

Legg, J. P., French, R., Rogan, D., Okao-Okuja, G., Brown, J. K. (2002). A distinct, *Bemisia tabaci* (Gennadius) Hemiptera: Sternorrhyncha: Aleyrodidae) genotype cluster is associated with the epidermic of severe cassava mosaic virus disease in Uganda. *Molecular Ecology. 11*, 1219-1229

Legg, J. P. & Thresh, J. M. (2003). Cassava virus diseases in Africa. In: Proceedings of the First International Conference on Plant Virology in Sub-Saharan Africa (4–8 June 2001, Ibadan, Nigeria), IITA, Ibadan, Nigeria, pp. 517–522.

Legg, P. J & Fauquet, M. C. (2004). Cassava Mosaic Geminiviruses in Africa. *Plant Molecular Biology 56*(4):585-99 doi: 10.1007/s11103-004-1651-7

Legg, J., Jeremiah, S., Obiero, H., Maruthi, M., Ndyetabula, I., OkaoOkuja, G. & Gashaka, G. (2011). Comparing the regional epidemiology of the *cassava mosaic* and *cassava brown streak virus* pandemics in Africa. *Virus Research, 159*, 161–170.

Legg, J. P., Owor, B., Sseruwagi, P., & Ndunguru, J., (2006). Cassava mosaic virus disease in East and Central Africa: epidemiology and management of a regional pandemic. *Advances in Virus Research 67*: 355-418.

Legg, J. P., Sseruwagi, P, Boniface, S., Okao-Okuja, G., Shirima, R., & Bigirimana, S. (2013). Spatio-temporal patterns of genetic change amongst populations of cassava Bemisia tabaci whiteflies driving virus pandemics in East and Central Africa. *Virus Research Journal 186,* 61–75. doi: 10.1016/j.virusres

Legg, J., Kumar, L., Makeshkumar, T., Tripathi, L., Ferguson, M., Kanju, E., & Ntawuruhunga, P. & Cuellar, W.. (2015). Cassava Virus Diseases: Biology, Epidemiology, and Management. *Advances in Virus Research 91*(1)

Lister, R. M., (1959). Mechanical Transmission of Cassava brown streak virus. Nature 183, 1588-1589

Liu, Q., Liu, J., Peng, Z., He, S. (2014). Root and Tuber Crops. *Encyclopedia of Agriculture and Food System 5* doi: 10.1016/B978-0-444-52512-3.00151-0.

Lopez-Bueno A, Tamames J, Velazquez D, Moya A, Quesada A, Alcami A (2009). High diversity of the viral community from an Antarctic lake. *Science 326*(5954):858- 61

Mabasa, K. G. (2007). Epidemiology of Cassava Mosaic Disease and molecular characterization of cassava mosaic viruses and their associated whitefly (*Bemisia tabaci*) vector in South Africa. (Unpublished MSc. Thesis) University of the Witwatersrand, Johannesburg.

Mansaray, A., Sundufu, A., Samura, A., Massaquoi, F., Quee, D., Fomba, N., Moseray, M. (2012). Cassava Genotype Evaluation for Grasshopper Zonocerus variegatus (L.) (Orthoptera: *Pyrgomorphidae*) Susceptibility

in Southern Sierra Leone. *International Journal of Agriculture and Forestry 2.* 294-299. 10.5923/j.ijaf.20120206.05.

Manu-Aduening, J. A., Lamboll, R. I., Mensah, G. A., & Gibson, R. (2007). Farmers' perceptions and knowledge of cassava pests and diseases and their approach to germplasm selection for resistance in Ghana. *Annals of Applied Biology, 151*, 189-198.

Manyong, V, Dixon, A., Makinde, K., Bokanga, M., & Whyte, J. (2000). The Contribution of IITA-Improved Cassava to Food Security in Sub-Saharan Africa: An Impact Study.

Maruthi, M. N., Colvin, J., Seal, S. & Thresh, J. M. (2002a). First report of a distinct begomovirus infecting cassava from Zanzibar. *Plant Disease 86*, 187

Maruthi, M. N., Colvin, J., Seal, S., Gibson, G. & Cooper, J. (2002b) Co-adaptation between Cassava Mosaic Geminiviruses and their local vector populations. *Virus Research 86*, 71–85. doi: 10.1016/s0168-1702(02)00051-5.

Maruthi, G., Susan, S., Colvin, J. Briddon, R., & Simon, B. E. (2005). East African cassava mosaic Zanzibar virus - A recombinant begomovirus species with a mild phenotype. *Archives of virology. 149.* 2365-77. 10.1007/s00705-004-0380-1.

Maruthi, G., Jeremiah, S., Mohammed, I. & Legg, J. (2017). The role of the whitefly, *Bemisia tabaci* (Gennadius), and farmer practices in the spread of cassava brown streak ipomoviruses. *Journal of Phytopathology. 165*. 10.1111/jph.12609.

Mbanzibwa, D. R., Tian, Y. P., Tugume, A. K., Mukasa, S. B., Tairo, F., Kyamanywa, S., Kullaya, A. & Valkonen, J. P. T. (2009). Genetically distinct strains of Cassava brown streak virus in the Lake Victoria basin and the Indian Ocean coastal area of East Africa. Arch Virol 154, 353-359

Mbewe, W., Kumar, P. L., Changadeya, W., Ntawuruhunga, P. & Legg, J. (2015). Diversity, distribution and effects on cassava cultivars of cassava brown streak viruses in Malawi. *Journal of Phytopathology 163*, 433–443. doi: 10.1111/jph.12339

McQuaid, C. F., Gilligan, C. A., & Bosch, F. van den. (2017). Considering behaviour to ensure the success of a disease control strategy. *Royal Society Open Science, 4*(12), 170721. doi:10.1098/rsos.170721

Mmbando, F. E., & Baiyegunhi, L. J. (2016). Socio-economic and institutional factors influencing adoption of improved maize varieties in Hai District, Tanzania. *Journal of Human Ecology, 53*(1), 49–56.

Michelle, K. J. (2005). Technology adoption in West Africa: Adoption and disadoption of soybeans on the Togo-Benin Border. Dissertation for Award of MSc Degree at North Carolina State University, Releigh, NC. 175pp.

Million, T. & Belay, K. (2004). Determinants of fertilizer use in Gununo area, Ethiopia. In Tesfaye Zegeye, Legesse Dadi & Dawit Alemu (eds) in *Proceedings of agricultural technology evaluation adoption and marketing*. Workshop held to discuss results of 1998-2002, August 6-8, 2002. Pp. 21-31.

Ministry of Food and Agriculture, (n. d). A Country Case Study of Cassava Development in Ghana Retrieved from https://www.fao.org/3/a0154e/a0154e07.htm

Ministry of Food and Agriculture (1997). *Weekly Market Price Information, January to December, Credit and Marketing Service Division*. Accra-Ghana.

Ministry of Food and Agriculture (2009). Annual Progress Report 2009 – Volume 1. Retrieved on 5/11/21https://new- ndp cstatic1.s3. amazonaws.com/CACHES/PUBLICATIONS /2016/04/16/MOFA_2009+APR.pdf

MoFA-SRID. (2014). Agriculture in Ghana (Facts and Figures). Ministry of Food and Agriculture. Retrieved on 20/9/2020 from http://facts/mofa. gov.gh/site/wp-content/uploads//mofafactsandfigures_pdf.

Ministry of Food and Agriculture, (2019-2020). Ketu North. Retrieved on 17/9/2020 from https://mofa.gov.gh/site/ sports/district-directorates/ volta-region/287-ketu-north

Ministry of Food and Agriculture (2020). Akatsi District. Retrieved http://mofa.gov.gh/site/sports/district-directorates/volta-region/276-akatsi

Morales, F. J. & Jones, P. G. (2004). The ecology and epidemiology of whitefly-transmitted viruses in Latin America. *Virus Research 100*, 57–65. doi:10.1016/j.virusres.2003.12.014

Moreno, I. Gruissem, W. & Vanderschuren, H. (2011). Reference genes for reliable potyvirus quantitation in cassava and analysis of Cassava brown

streak virus load in host varieties. *Journal Virological Methods*, *177*, 49–54.

Morse, S. & Buhler, W. (1997). Integrated pest management: Ideals and Realities in Developing Countries. Lynne Riener Publishers, London, 171 pp.

Moses, E., Asafo-Agyei, J. N., Adubofuor, K. & Adusei, A. (2008). Guide to the identification and control of cassava diseases. Ashmetro Prints, Kumasi, Ghana. pp. 1-7.

Moses, E. (2009). Development of appropriate strategies to control cassava diseases in Ghana. *Plant Pathology in the 21st Century 3*:11-24.

Moses, E., Oppong, A. & Lamptey, J. N. L. (2015). Reaction of local accessions of cassava to diseases in Southern Ghana. *African Crop Science Journal, 23* (1) 27 – 34

Muhammad-Lawal, A., Salau, S., & Ajayi, S. A. (2012). Economics of Improved and Local Varieties of Cassava among Farmers in Oyo State, Nigeria. *Ethiopian Journal of Environmental Studies and Management 5*(2)

National Crop Variety Catalogue (2019). Catalogue of Crop Varieties Released and Registered in Ghana. Retrieved on 24/10/22 from https://nastag.org/docx/resources/2019%20NATIONAL%20CROP%20VARIETY%20CATALOGUE.pdf

Nag, O. S. (2017). Top Cassava Producing Countries in the World. Retrieved on 6/9/21 from https://www. worldatlas.com/articles/top-cassava-producing-countries-in-the-world.html

Nakabonge, G., Samukoya, C. & Baguma, Y. (2018) Local varieties of cassava: conservation, cultivation and use in Uganda.*Environment, Development and Sustainability 20*, 2427–2445.

Navas-Castillo, J., Fiallo-Olive, E. & Sanchez-Campos, S. (2011). Emerging virus diseases transmitted by whiteflies. *Annual. Review of Phytopathology. 49*, 219–248.

Ndunguru, J., Taylor, N. J., Yadav, J., Aly, H., Legg, J. P., Aveling, T., Thompson, G. & Fauquet, C. M. (2005). Application of FTA technology for sampling, recovery and molecular characterization of viral pathogens and virus-derived transgenes from plant tissues. *Virology Journal, 2*(45)

Ngobisa A. I. C. N, Djidjou , P K. , Ntsefong, G. N., Mbenoun, M., Zok, S., & Fontem, D. (2015). Isolation and identification of some pathogenic fungi associated with cassava (*Manihot esculenta* Crantz) root rot disease in Cameroon. *African Journal of Agricultural Research 10*(50), 4538-4542 doi:10.5897/AJAR2015.10272

Night, G., Asiimwe, P., Gashaka, G. Nkezabahizi, A. D. Legg J. P., Okao-Okuja G., Obonyo R. Nyirahorana C., Mukakanyana C., Mukase F., Munyabarenzi. & Mutumwinka, M. (2011). Occurrence and distribution of cassava pests and diseases in Rwanda. *Agriculture, Ecosystems and Environment 140* 492–497

Njoku, D. (2012*). Improving beta-carotene content in farmers preferred cassava cultivars in Nigeria.* Unpublished doctoral dissertation. University of Ghana. Legon. Accra. Ghana

Nmadu, J. N., Sallawu, H., & Omojeso, B. V. (2015). Socioeconomic factors affecting adoption of innovations by cocoa farmers in Ondo State, Nigeria. *European Journal of Business, Economics and Accountancy, 3*(2), 58–66.

Norman, M. C. (2017). Concern Worldwide. Crop Weather and Climate Vulnerability Profile. Retrieved on 24/10/22 from https://admin. concern.net/sites/default/files/media/migrated/crop_weather_and_cli mate_vulnerability_profile_pdf

Nsiah Frimpong, B., Oppong, A., Prempeh, R., Appiah-Kubi, Z., Abrokwah, L. A., B. Mochiah, M., N. Lamptey, J., Manu-Aduening, J., & Pita, J. (2021). Farmers' knowledge, attitudes and practices towards management of cassava pests and diseases in forest transition and Guinea savannah agro-ecological zones of Ghana. *Gates Open Research, 4*, 101.

Ntawuruhunga P, Legg J, Okidi J, Okao-Okuja G, Tadu G, Remington T. (2007). Southern Sudan Equatorial Region. Cassava baseline survey technical report, IITA, Ibadan, Nigeria; p. 65

Ntui, V. O., Kong, K., Khan, R. S., Igawa, T., Janvi, G. J. J., Rabindran R., Nakamura, I & Mii, M. (2015). Resistance to Sri Lankan Cassava Mosaic Virus (SLCMV) in Genetically Engineered Cassava cv. KU50 through RNA Silencing. *PLoS ONE 10*(4) doi: 10.1371/ journal.pone.0120551

Nwabueze, T. U., & Odunsi, F. O. (2007). Optimization of process conditions for cassava (*Manihot esculenta*) production. *African Journal of Biotechnology, 6*(5), 603-611

Nweke, F., Dunstan, I., Spencer, S. C., & Lynam. J. K. (2001). The Cassava Transformation: Sub-Saharan Africa's Best Kept Secret. Michigan State University Press, East Lansing.

Nweke, .F. I., Spencer, D. S. C., Lynam, J. K., (2002). *The Cassava Transformation*. East Lansing, MI, USA: Michigan State University Press.

Offei, S. K., Owuna-Kwakye, M. & Thottappilly, G. (1999). First Report of East African Cassava Mosaic Begomovirus in Ghana. *Plant Disease 83* (9):877.

Ofori, F., AI-Hassan, R., Afuakwa J.J., & Noamesi R.K., (1997) A Case Study of Cassava Development in Ghana; A report submitted to the International Fund for Agricultural Development (IFAD), Rome, Italy.

Ogbe, F. O., Legg, J. P., Raya, M. D., Muimba-Kankalongo, A., Theu, M. P., Kaitisha, G., Phiri, N. A. & Chalwe, A (1997). Diagnostic survey of cassava mosaic viruses in Tanzania, Malawi and Zambia. *Roots 4*, 12-15

Ogbe, F. O.,Thottappilly, G., Dixon, A. G. O. & Mignouna, H. D. (2003). Variants of *East `African cassava mosaic virus* and its distribution in double infections with *African cassava mosaic virus* in Nigeria. *Plant Disease* 87, 229-232.

Ogundari K, & Ojo, S. O. (2006). An Examination of Technical, Economic and Allocative Efficiency of Small Farms: The Case Study of Cassava Farmers in Osun State of Nigeria. *Journal of Central European Agriculture 7*, 3 (423-432)

O'Hair, S. K., (1995).Cassava: New Crop Factsheet. Center for New Crop & Plant Product. Retrieved from http://www.hort.purdue.edu/newcrop/c .....ropfactsheets/cassava.html#Ecol#Ecol

Okogbenin, E., Egesi, C. & Fregene, M. (2006). Marker Aided Introgression of CMD Resistance in Latin American Germplasm for the broadening of the Genetic base of cassava in Nigeria. NRCRI, Umudike. pp. 2-6.

Okogbenin, E. Porto, M.C.M. Egesi, C. Mba, C. Ospinosa, E. Santos, L.G. Ospina, C. Marin, J. Barera, E. Gutierrez, J. E kanayake, .I. Iglesias, C. & Fregene, M. (2007). Marker assisted introgression of CMD resistance in Latin American germplasm for genetic improvement of cassava in Africa, *Crop Science 47*, 1895–1904.

Okogbenin, E. Egesi, C.N. Olasanmi, B. Ogundapo, O. Kahya, S. Hurtado, P. Marin, J. Akinbo, A.O. Mba, C. Gomez, H. de Vicente, C. Baiyeri, S. Uguru, M. Ewa, F. Fregene, M. (2012). Molecular marker analysis and validation of resistance to Cassava Mosaic Disease in elite cassava cultivars in Nigeria. *Crop Science 52* 2576–2586

Okogbenin, E., Setter, T. L., Ferguson, M., Mutegi, R., Ceballos, H., Olasanmi, B., & Fregene, M. (2013). Phenotypic approaches to drought in cassava: review. *Frontiers in physiology*,*4*, 93.

Onyemauwa, C.S. (2012). Analysis of Women Participation in Cassava Production and Processing in Imo State, Southeast Nigeria. *Journal of Economics and Sustainable, 3* (5), 81-90.

Oparinde, A., Abdoulaye, T., Manyong, V., Birol, E., Asare-Marfo, D., Kulakow, P., & Ilona, P. (2016). A technical review of modern cassava technology adoption in Nigeria (1985-2013): Trends, Challenges and Opportunities. HarvestPlus Working Paper (3). Retrieved from https://books.google.com.gh/books?id=kcT_ CwAAQBAJ &printsec= frontcover&source=gbs_ge_summary_r&cad=0#v=onepage&q&f=fals e

Oslen, K. M. & Schaal, B. A. (1999). Evidence on the origin of cassava: Phylogeography of Manihot esculenta. Proceeding of the Otim-Nape G.W. & Bua, A. *African Crop Science Journal*, *2*, 479-495.

Oteng-Frimpong R., Levyd, Y., Torkpo, S. K., Danquah, E.Y., Offei, S. K., Gafni, Y. (2012). Isolation, characterization and analysis of two causative viruses of Cassava Mosaic Disease in Ghana. *Acta virologica 56*. 305-14 doi: 10.4149/av_2012_04_305

Otim-Nape, G. W., Shaw, M. W. & Thresh, J. M. (1994). The effects of African cassava mosaic geminivirus on the growth and yield of cassava in Uganda. *Tropical. Science 34*, 43 –54

Otim-Nape, G. W., A. Bua., J. M. Thresh., Y. Baguma., S. Ogwal., G. N. Semakula., G. Acola., B. Byabakama & Martin A. (1997). Cassava mosaic virus disease in Uganda: The current pandemic and approaches to control. Natural Resources Institute, Chatham, UK. Pp 65.

Otim-Nape, G. W., Bua, A., Thresh, J. M., Baguma, Y., Ogwal, S., Ssemakula, G. N., Acola, G., Byabakama, B. A, Colvin, J., Cooter, R. J & Martin, A. (2000). The Current Pandemic of Cassava Mosaic Virus Disease in East Africa and its Control. Chatham, UK: NARO/NRI/DFID.

Ouma, J. O., & De Groote, H. (2011). Determinants of improved maize seed and fertilizer adoption in Kenya. *Journal of Development and Agricultural Economics, 3*(11), 529–536.

Oyegbami, A., Oboh, G., & Omueti, O (2010). Cassava Processors' Awareness of Occupational and Environmental Hazards Associated with Cassava Processing in South-Western Nigeria. *African Journal of Food, Agriculture, Nutrition and Development 10* 2.

Pacumbaba, R. P. (1987). A method for selection of tolerance to African cassava mosaic virus. *Journal of Phytopathology 118* (4) 312-316

Padidam, M., Beachy, R. N. & Fauquet, C. M. (1995). Tomato leaf curl geminivirus from India has a bipartite genome and coat protein is not essential for infectivity. *Journal of General Virology 76*, 25-35.

Padidam, M., Beachy, R. N. & Fauquet, M. (1999). A phage single-stranded DNA (ssDNA) binding protein complements ssDNA-Accumulation of geminivirus and interfers with viral movement. *Journal of Virology 73*, 1609-1616

Palaniswami, M. S., Nair, R. R., Pillai, K. S., & Thankappan, M. (1996). Whiteflies on cassava and its role as vector of cassava mosaic in India. *Journal of Root Crops 22*:1-8

Palaniswami, M. S., & Henneberry, T. J. (2011). Bemisia tabaci (Genn.): Biotypes and Cassava Mosaic Virus in India. In The Whitefly, Bemisia tabaci (Homoptera: Aleyrodidae) Interaction with Geminivirus-Infected Host Plants; Springer: Houten, The Netherlands, pp. 121–163.

Parsa, S & Winotai, A. (2012). The cassava mealy bug (Phenacoccus manihot) in Asia: First Records, Potential Distribution, and Identification Key. *PLOS One 7* (10)

Pita, S., Fondong, V. N., Sangare, Otim-Nape, G. W., Ogwal, S. & Fauquet, C. M. (2001a). Recombination, pseudorecombination and synergism of geminiviruses are determinant keys to the epidemic of severe Cassava Mosaic Disease in Uganda. *Journal of General Virology 82*, 655-665.

Patil, B. L. & Fauquet, C. M (2015a). Light intensity and temperature affect systemic spread of silencing signal in transient agroinfiltration studies. *Molecular Plant Pathology, 16*(5):484-494 doi: 10.1111/mpp.12205

Pita, J. P., Fondong, V. N., Sangare, A., Kokora, R. N. N. & Fauquet, C. M. (2001b). Genomic and biological diversity of the African cassava geminiviruses. *Euphytica 120*, 115-125.

Patil, B., Legg, J. P.& Kanju E. E (2014). Cassava brown streak disease: A threat to food security in Africa. *Journal of General Virology 5* 96

Patil, B. L., & Fauquet, C. M. (2009). Cassava Mosaic Geminiviruses: actual knowledge and perspectives. *Molecular Plant Pathology 10*, 685–701.

Perring, T. (2001). The *Bemisia tabaci* complex. *Crop Protection. 20*. 725-737.

Plantwise, (2014). Whiteflies in Cassava. Retreived from https://www.cab i.org/ISC/Full TextPDF/2014/20147801181

Polston, J. E., De Barro, P. & Boykin, L. M. (2014). Transmission specificities of plant viruses with the newly identified species of the *Bemisia tabaci* species complex. *Pest Management Science 70*, 1547–1552. doi: 10.1002/ps.3738

Posada, D. & Crandall, K. A. (2001). Evaluation of methods for detecting recombination from DNA sequences: Computer simulations. *Proceedings of the Natural Academic Sciences of USA* 98, 13758-13762

Preiss W, & Jeske H. (2003) Multitasking in replication is common among geminiviruses. *Journal of Virology.;77*:2972–80

Reeves, T. G. & Cassaday, K. (2002). History and past achievements of plant breeding. *Australian Journalof Agricultural Research, 53*,851–863.

Rector, A., Tachezy, R. & Van Ranst, M. (2004). A Sequence-Independent Strategy for Detection and Cloning of Circular DNA Virus Genomes by Using Multiply Primed Rolling-Circle Amplification. *Journal of Virology, 78* 10. 4993–4998. doi: 10.1128/JVI.78.10.4993–4998.2004

Rojas, M. R., Gilbertson, R. L., Russell, D. R. & Maxwell, D. P. (1993). Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted *geminivirus*. *Plant Disease 77*, 340-347.

RTIP, (2002). Varietal Characteristics of four improved cassava varieties in Ghana. RTIP Factsheet

Rwegasira, G. M. & Chrissie, M. E. R. (2015). Efficiency of non-vector methods of cassava brown streak virus transmission to susceptible cassava plants. *Africa Journal of Food, Agriculture, Nutrition and Development 15* 1684–5374.

Rybicki, E. P. (1994). A phylogenetic and evolutionary justification for three genera of Geminiviridae. *Archives of Virology* 139: 49-77.

Saeed, S.T. & Samad, A (2017) Emerging threats of begomoviruses to the cultivation of medicinal and aromatic crops and their management strategies. *Virus Disease, 28*(1):1–17 doi 10.1007/s13337-016-0358-0

Salasya, B., Mwangi, W. M., Mwabu, D., & Diallo, A. (2007). Factors influencing adoption of stress-tolerant maize hybrid (WH 502) in western Kenya. *African Journal of Agricultural Research, 2*(10), 544–551.

Salifu, A., Francesconi, G.N., Kolavalli, S., (2010). A review of collective action in rural Ghana. IFPRI Discussion Paper 00998, International Food Policy Research Institute (IFPRI), Washington DC.

Salifu, A. Funk, R. L., Keefe M. & Kolavalli, S. (2012). Performance of Farmer-based Organizations in Ghana. Retrieved on 12/7/2020 from https://citeseerx.ist.psu.edu/view doc/download?doi=10.1.1.278.7883&rep=rep1&type=pdf

Salum, K. A. (2016). Factors influencing adoption of improved cassava varieties in increasing farm yield. A Case of Magharibi District, Zanzibar, Tanzania . (Master of Science dissertation). University of Agriculture.Morogoro, Tanzania Retrieved from http://www. suaire.suanet.ac.tz:8080/xmlui/bitstream/handle/123456789/1463/Ali%20Kassim %20Salum.pdf?sequence=1&isAllowed=y

Sánchez, T., Salcedo E., Ceballos H., Dufour D., Mafla G., Morante N., Jaramillo G. (2009). Screening of starch quality traits in cassava (Manihot esculenta Crantz) *Starch Staerke 61*(1):12–19.

Sanginga, N. & Mbabu, A. (2015) "Root and Tuber Crops (Cassava, Yam, Potato and Sweet Potato), Feeding Africa, Background Paper on Action

Plan for African Agricultural Transformation", Abdou Diouf International Conference Center, Dakar, Senegal;

Saranraj, P.,Behera, S. & Ray, R., (2019). "Traditional Foods from Tropical Root and Tuber Crops: Innovations and Challenges". Innovations in Traditional Foods. 159-191. 9.

Satija, J., Punjabi, N., Mishra, D. & Mukherji S. (2016). Plasmonic-ELISA: expanding horizons. *RSC Advances, 6*, 85440-85456 Retrieved on 9/8/2020 from https://pubs.rsc.org/ en/content/ articlelanding /2016/ra/c6ra16750k#!divAbstract

Saunders, K., Lucy, A. & Stanley, J. (1992). RNA-primed complementary-sense DNA synthesis of the geminivirus African cassava mosaic virus. *Nucleic Acid Research 20*, 6311-6315.

Schreinemachers, P., Balasubramaniam, S., Boopathi, N. M., Viet, Ha. C., Kenyon, L., Praneetvatakul, S., Sirijinda, A., Tuan, Le. N., Srinivasan, R., & Wu, M. H. (2015). Farmers' perceptions and management of plant viruses in vegetables and legumes in tropical and subtropical Asia. *Crop Protrotection Journal 75*: 115-123

Sedano J. C. S., Moreno, M. R. E., Boby, M., Jens, L., Cano, F. A. G., Agim, B. & Carrascal, L.C. E. (2017). Major Novel QTL for Resistance to Cassava Bacterial Blight Identified through a Multi-Environmental Analysis. *Frontiers in Plant Science 8* (1160) doi: 10.3389/fpls.2017.01169

Seepiban, C., Charoenvilaisiri, S., Warin, N. Bhunchoth, A., Phironrit, N. Phuangrat, B., Chatchawankanphanich, O., Attathom, S. & Gajanandana, O. (2017). Development and application of triple

antibody sandwich enzyme-linked immunosorbent assays for begomovirus detection using monoclonal antibodies against Tomato yellow leaf curl Thailand virus .*Virology Journal 14*, 99.

Sequeira, J. C. & Harrison, B. D. (1982). Serological studies on cassava latent virus. *Annals of Applied Biology* 101: 33-42.

Shackelton, L. A., Parrish, C. R., Truyen, U, & Holmes, E. C (2005). High rate of viral evolution associated with the emergence of carnivore parvovirus. *PubMed 102*(2):379-84. doi: 10.1073/pnas.0406765102. PubMed PMID: PMC544290.

Sherwood, S. G. (1997). Little things mean a lot: Working with Central American farmers to address the mystery of plant disease. *Agriculture and Human Values 14*: 181–189

Shipman, M. (2017). Studying a disease in order to prevent starvation. Retrieved on 6/9/2020 from https://news.ncsu.edu/2017/07/cassava-mosaic-disease-field-2017/

Sodjinou, E., Glin, C. L., Nicolay, G., Tovignan, L., & Hinvi, J. (2015). Socioeconomic determinants of organic cotton adoption in Benin, West Africa. *Agricultural and Food Economics, 3*(1), 12.

Stanley, J. & Gay, M. R. (1983). Nucleotide sequence of cassava latent virus DNA. *Nature 301*, 260-362.

Stanley, J. & Townsend, R. (1986). Infectious mutants of cassava latent virus generated in vivo from intact recombinant DNA clones containing single-copies of the genome. *Nulceic Acid Research 14*, 5981-5998.

Stanley, J. (1995). Analysis of African cassava mosaic virus recombinants suggests strand nicking occurs with the conserved motif during the initiation of rolling circle DNA replication. *Virology 206*, 707-712.

Stanley, J., Bisaro, D. M., Briddon, R. W., Brown, J K., Fauquet, C. M., Harrison, B. D., Rybicki, E. P., Stenger, D, C. (2005) Geminiviridae. In: Fauquet, C. M, Mayo, M. A, Maniloff, J., Desselberger, U., Ball, L. A (eds) Virus Taxonomy, 8th report of the ICTV. Elsevier/Academic Press, London, pp 301–326

Stanturf, J. A., Melvin, L., Warren, Jr., Charnley, S.,. Polasky, S. C. Goodrick, S. F Armah, L &. Atuahene Nyako, Y (2011). Ghana climate change vulnerability and adaptation assessment. Retrieved from https://www.climatelinks.org/file/2113/download? tok en=Q2oNtsPe

Stenger, D. C., Revington, G. N., Stevenson, M. C. & Bisaro, D. M. (1991). Replicational releaseof geminivirus genomes from tandemly repeated copies: evidence for rolling-circle replication of a plant viral DNA. *Proceedings of Natural Academic Science of USA 88*, 8029-8033.

Storey, H. H. & Nichols, R. F. W. (1938). Studies of the mosaic disease of cassava. *Annals of Applied Biology 25*: 790-806.

Smith, H A., Seijo, E. T., Vallad, E. G., Peres, A. N. & Druffel, L. K. (2015). Evaluating Weeds as Hosts of Tomato yellow leaf curl virus. *Environmental Entomology 44*, (4): 1101–1107.

Sogbedji, J. M., Agboyi, L. K., Detchinli, K. S, Atchoglo, R. & Mazinagou, M (2015) Sustaining improved cassava production on West African Ferralsols through appropriate varieties and optimal potassium

fertilization schemes. *Journal of Plant Sciences 3*: 111-122. doi: 10.11648/j.jps.20150303.12.

Storey, H. H. (1936). Virus diseases of East African Plants. VI. A progress report on studies of the diseases of cassava. *East African Agricultural Journal 2*, 34-39.

Sseruwagi, P., Otim-Nape, G. W., Osiru, D. S. O. & Thresh, J. M. (2003) Influence of NPK fertiliser on populations of the whitefly vector and incidence of cassava mosaic virus disease. *African Crop Science Journal 11*, 171–179

Sseruwagi, P, Sserubombwe, W. S., Legg, J. P, Ndunguru, J. & Thresh, J. M. (2004). Methods of surveying the incidence and severity of Cassava Mosaic Disease and whitefly vector populations on cassava in Africa: a review. *Virus Research 100*,129–42.

Sserubombwe, W. S. , Briddon, R. W. , Baguma, Y. K., Ssemakula, G. N., Bull, S. E., Bua, A., Alicai, T. Omongo, C. Otim-Nape, G. W., & Stanley, J. (2008). Diversity of begomoviruses associated with mosaic disease of cultivated cassava (Manihot esculenta Crantz) and its wild relative (*Manihot glaziovii* Mull. Arg.) in Uganda. *Journal of General Virology 89*, 1759–1769 doi: 10.1099/vir.0.83637-0

Swanson, M. M. & Harrison, B. D. (1994). Properties, relationships and distribution of Cassava Mosaic Geminiviruses. *Tropical Science 34*, 15-25.

Swanson, B. & Rajalahti, R. (2010). Strengthening agricultural extension and advisory systems: Procedures for assessing, transforming, and

evaluating extension systems. Agriculture and Rural Development Discussion Paper 44. ARD, Washington, DC, USA: World Bank.

Szyniszewska, A. M., Chikoti, P. C., Tembo, M., Mulenga, R., Gilligan, C. A., van den Bosch, F., & McQuaid, C. F. (2021). Smallholder Cassava Planting Material Movement and Grower Behavior in Zambia: Implications for the Management of Cassava Virus Diseases. *Phytopathology, 111*(11), 1952–1962.

Tembo, M. (2016). Epidemiological and molecular characterization of cassava mosaic geminiviruses and their effect on yield of cassava. M.Sc. Thesis, University of Zambia

Theu, M. P. K. J. & Sseruwagi, P. (2003). Whiteflies as a vector of plant viruses in cassava and sweetpotato in Africa. In "Whiteflies and Whitefly-Born Viruses in the Tropics: Building a Knowledge Base for Global Action" (Anderson, P.K. and Markham, R.H. eds.), pp. 68-71. Centro Internacional de Agricultura Tropical, Cali, Colombia

Thompson, R. (2013). Genetic analysis of postharvest physiological deterioration in cassava (Manihot esculenta crantz) storage roots. PhD Thesis. West Africa Centre for Crop Improvement. University of Ghana. Legon. Accra. Ghana

Thomson, K., Gelson, T., & Elias, K. (2014). Adoption of improved maize varieties in Southern Zambia. *Asian Journal of Agricultural Sciences, 6*(1), 33–39.

Thresh, J. M., Otim-Nape G. W. & Nichols R. F. W. (1994). Strategies for controlling Africa cassava mosaic geminivirus. Adv. Dis. Vector Res. 10:215-236

Thresh, J. M., Otim-Nape G. W., Legg, J. P. & Fargette, D (1997). *Africa cassava mosaic virus disease*: The magnitude of the problem. *Africa Journal of Root Tuber Crop 2*: 13-18

Thresh, J. M., Otim-Nape, G. W. & Fargette, D (1998). The components and deployment of resistance to cassava mosaic virus disease. *Integrated Pest Management Reviews* 3, 209–24.

Thresh J. M, (2003). Control of plant virus diseases in sub-Saharan Africa: the possibility and feasibility of an integrated approach. *African Crop Science Journal 11*, 199–223.

Thresh, J. M. &. Cooter, J . R. (2005). Strategies for controlling cassava mosaic virus disease in Africa. *Plant Pathology 54*, 587–614 doi: 10.1111/j.1365-3059.2005.01282.x

Theu, M. P. K. J. & Sseruwagi, P. (2003). Whiteflies as a vector of plant viruses in cassava and sweetpotato in Africa. In: Anderson, P.K. and Markham, R.H. (eds.)

Tiendrebeogo, Lefeuvre, Hoareau, Harimalala, De Bruyn, Villemot, Traoré, Konaté, Traore, Barro, Reynaud, Traore, & Lett, (2012). Evolution of *African cassava mosaic virus* by recombination between bipartite and monopartite begomoviruses. *Virology Journal 9*, 67 (2012).

Torkpo, S. K, & Offei, S. K (2007). Status of Cassava Mosaic Disease in farmers' fields. Book of Abstracts of the 25th Biennial Conference of the Ghana Science Association held at Bunso and Tafo in the Eastern Region between August 5-10, 2007.

Torkpo, S. K, Gafni, Y., Danquah, E. Y., Danquah, E. Y. & Offei, S. K (2017). Farmers' Perception of Cassava Mosaic Disease and Cassava Cultivar Attributes Preferences in Ghana. *Innovative Techniques in Agriculture* 2(1):294–300.

Torkpo, S. K. Gafni, Y. Danquah, E. Y. & Offei, S. K. (2018). Incidence and Severity of Cassava Mosaic Disease in Farmer's Fields in Ghana. Ghana *Journal of Agricultural Science. 53*, 61-71.

Tsai, W. S., Hu, C J., Shung, D. P., Lee, L. M., Wang, J. T., Kenyon, L., (2011). First report of Squash leaf curl Philippines virus Infecting Chayote (*Sechium edule*) in Taiwan. *Plant Dis. 95*:1197.

Torvikey, G. D. (2017). Programmes to increase cassava production. Retrieved on 20/9/2020 from https://r4d-demeter.info/2017/09/research -notes-the-cassava-production-craze-inghana-the-story-ofcommercialization Commercialization-and-the-exclusionofwomen

Undie, U. L., Uwah, D .F. & Attoe, E. E. (2012) Effect of intercropping and crop arrangement on yield and crop productivity of late season maize/soybean mixtures in the humid environment of south south Nigeria. *Journal of Agricultural Science 4* (4), 37 – 50)

Uzokwe, V. N. E., Mlay, D. P., Masunga, H. R., Kanju, E., Odeh, I. O. A., Onyeka, J., (2016). Combating viral mosaic disease of cassava in the Lake Zone of Tanzania by intercropping with legumes. *Crop Protect. 84*, 69–80.

Vanderschuren, H. Moreno, I. Anjanappa, R. B. Zainuddin, I. M. & Gruissem, W. (2012). Exploiting the combination of natural and genetically engineered resistance to cassava mosaic and cassava brown streak viruses impacting cassava production in Africa. *PLoS ONE, 7*,

Van Mele, P., (2000). Farmers' knowledge, perceptions and practices in mango pest management in the Mekong Delta, Vietnam. Retrieved on 9/6/2020 from https://library.wur.nl/WebQuery/wurpubs/66715

Van den Bosch, F., Jeger., M. J. & Gilligan, C. A. (2007). Disease control and its selection for damaging plant virus strains in vegetatively propagated staple food crops; a theoretical assessment. *Proceedings Biological Science 274* (1606): 11–18.

Velásquez, A. C., Castroverde, C. D. M. & He S. Y. (2018). Plant and pathogen warfare under changing climate conditions. *Current Biology 28*(10)

Wagaba, H. Beyene, G. Trembley, C. Alicai, T. Fauquet, C. M. Taylor, N. J. (2013). Efficient transmission of Cassava brown streak disease viral pathogens by chip bud grafting, *BMC Reserch Notes 6*, 516.

Warburg, O. (1894). Die kulturpflanzen usambaras. *Mittcilungenaus den Deutschen Schutzgebieten, 7*, 131.

Wenbo C., Wosulab, E N. Hasegawac, K. D Casingad, C. Shirimab, R. R., Fiaboee, K. K. M., Hannae, R., Fostoe, A Goergenf, G., Tamòf, M., Mahukub, G., Murithib, H. M., Tripathig, L., Mwareg, B., Kumarh, L. P., Ntawuruhungai, P. Moyoj, C., Yomenik, M., Boahenl, S., Edetm, M., Awoyalem, W., Wintermantel, W. M., Ling, K.S., Legg, J. P.& Feia, Z. (2019). Genome of the African cassava whitefly Bemisia tabaci and

distribution and genetic diversity of cassava-colonizing whiteflies in Africa. *Insect Biochemistry and Molecular Biology 110* (112-120).

Were H. K. (2001). Serological and a Molecular Characterization of Begomoviruses infection cassava in Ghana Retrieved from https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.632.9677&rep=rep1&type=pdf

Were, H. K., Winter, S & Maiss, E. (2004) Occurrence and distribution of cassava begomoviruse in Kenya. *Annals of Applied Biology 145* (2), 175 – 184.

Winter, S, Koerbler, M, Stein, B, Pietruszka, A, Paape, M & Butgereitt, A, (2010). Analysis of cassava brown streak viruses reveals the presence of distinct virus species causing cassava brown streak disease in East Africa. *Journal of General Virology, 91*(5):1365-1372

World Bank (2007). World development report 2008: Agriculture for development. Washington, DC: Author.

Wydra K, & Verdier V. (2002) Occurrence of cassava diseases *in relation to environmental, agronomic and plant characteristics. Agriculture, Ecosystems and Environment.: 93*:211-226

Yadav, J. S.; Ogwok, E.; Wagaba, H.; Patil, B. L.; Bagewadi, B.; Alicai, T.; Gaitan-Solis, E.; Taylor, N. J.; Fauquet, C. M. (2011). RNAi-mediated resistance to Cassava brown streak Uganda virus in transgenic cassava. *Molecular Plant Pathology 12*, 677–687.

Yajima, M., Chiwona-Karltun, L., Kambewa, P., Huis, A.V. & Jiggins, J. (2005). Learning about cassava in an HIV.AIDs affected area of Malawi, 8th Oxford Conference on Learning and Development: 13-15 September 2005, Oxford. UK

Yu-sheng, W. Hu, T., Fang-hao1, W & Gui-fen, Z. (2019). Species-specific COI primers for rapid identification of a globally significant invasive pest, the cassava mealybug Phenacoccusmanihoti Matile-Ferrero. *Journal of Integrative Agriculture 18*(5): 1042–1049

Zhou, X., Liu, Y., Calvert, L., Munoz, C., Otim-Nape, G. W., Robinson, D. J. & Harrison, B.D (1997). Evidence that DNA-A of a geminivirus associated with severe Cassava Mosaic Disease in Uganda has risen by interspecific recombination. Journal of General Virology 78, 2101-2111

Zhou, X., Robinson, D. J. & Harrison, B. D. (1998). Types of variation in DNA-A among isolates of East African cassava mosaic virus from Kenya, Malawi and Tanzania. *Journal of General Virology 79*, 2835-2840.

APPENDICES

APPENDIX A: SURVEY QUESTIONNAIRE

UNIVERSITY OF CAPE COAST
SCHOOL OF AGRICULTURE
CROP SCIENCE DEPARTMENT

This interview schedule is designed to solicit information about your encounter with the CMV disease Cassava fields. I would therefore ask you few questions about your cassava production practices especially disease control measure.

District.....AEZ..... COMMUNITY ..... No.....

Please provide information about your cassava management practices

1. Gender
   a) Male [ ]
   b) Female [ ]
2. Age at last birthday ..... years
3. What is your highest level of Education?
   a) No formal education [ ]
   b) Non formal [ ] if Non formal go to question 4
   c) Forman education [ ] if Formal go to question 5
4. Non formal education
   Artisanal training [ ]
   Numeracy training [ ]
   Literacy training [ ]
5. Formal education
   a) Primary [ ]
   b) JHS/MLSC [ ]
   c) SSS/SHS [ ]
   d) Tertiary [ ]
6. How many years have you been planting Cassava? ..... years
7. What is the size of your land? ..... acres
8. What variety of cassava do you use in planting?
   a) Local [ ]
   b) Improved [ ]
   c) Both [ ]
   (d) Others [ ]
9. If local what is the name.....
10. Do you intercrop
    a) Yes [ ]
    b) No [ ]
11. What do you intercrop?
    a). Maize [ ]
    b). Cowpea [ ]
    c). Groundnut [ ]

.d) Potato [ ]

12. What do you plant cassava for?
    a) Fufu [ ]
    b). Dough [ ]
    c). Gari [ ]
    d). Multipurpose [ ]
13. Have you ever had any disease(s) attacking your farm?
    a)Yes [ ]
    b) No [ ]
14. Rate the following symptoms into less common or most common experienced in your field

Most seen	**1(Less common)**	**2 (Most common)**
Mosaic		
Yellowing		
Curling		
Rotten tubers		
Whitish substance/insects		
Shedding of leaves		
Necrosis		
Stunted		

Knowledge of Cassava Mosaic Virus Disease

Explain the symptoms to the farmer and show pictures of symptoms to the farmer

15. Have you heard of Cassava Mosaic Virus Disease before?
    a) Yes [ ]
    b) No [ ]
16. Do you know the causes of the disease?
    a) Yes [ ]
    b) No [ ]
17. If yes, what are the causes?

Causes of disease	**Choose as many as applicable**
White flies	
Nature of plant	
Soil borne	

Spraying Chemical

Untimely weeding

18. Do you know the name of the disease(s)?

a) Yes [ ]
b) No [ ] (if no answer 11)

19. What name do you call this disease in this community?

a) Ayifle [ ]
b) Dayo [ ]
c) Kafu [ ]
d)Kwata [ ]
e) Mosaic [ ]
f) Nkuko [ ]
g) Nsonso [ ]
h) Zongolachichi [ ]
i) Others [ ]
j) No idea [ ]

20. At what month do you encounter the disease?

a) first 3 month after planting [ ]
b) 3-6 months after planting [ ]
c) 7-9 months after planting [ ]
d) others [ ] specify
.....

Management of CMVD

21. Have you been controlling CMVD

a)Yes [ ]
b)No [ ]

22. If no, why not?

a) High cost of pesticide [ ]
b) No effect after use of pesticide [ ]
c) Recovers [ ]
d) No idea controlling [ ]

23. If yes, how have you been controlling the disease?

a)Pesticide application [ ]
b) Removal of infected plant [ ]
c) Use of improved variety [ ]
d) Detopping [ ]

24. Did the control measure work?
    a) Yes [ ]
    b) No [ ]
25. Have you received training on the management of CMVD in your farms?
    a). Yes [ ]
    b). No [ ]
26. If yes, who gave you the training?
    a). MoFA [ ]
    b). NGO [ ]
    c). Crop Research Institute (CRI) [ ]
    d). Universities [ ]
    e). Others…… .[ ]

Knowledge on Improved Varieties

27. Are you aware there are improved/resistant varieties to control the disease?
    a)Yes [ ]
    b) No [ ]
28. Mention the improved varieties received/ you have heard of or know ……
29. Have you ever planted any of those before?
    a) Yes [ ]
    b) No [ ]
30. If yes which of the following varieties have you planted before?
    a) CAPEVARS [ ]
    b)Ampong [ ]
    c) Bankye botan [ ]
    d) Sika [ ]
    e) Ankra [ ]
    f) Others [ ], please state……
31. Did you record an increase in yield when you planted improved variety (ies)?
    a) Yes [ ]
    b) No [ ]
    c) Do not know [ ]
32. Did you notice any disease symptoms when you planted the improved varieties
    a) Yes [ ]
    b) No [ ]
33. Which of the improved varieties is/are more susceptible to CMD?
    a) Ankrah [ ]

b) Ampong [ ]
c) Sika [ ]
d) Afisiafi [ ]
e) Bosom esia [ ]
f) Others [ ]

34. Do you still plant the variety?
a) Yes [ ]
b) No [ ]

35. If No why are you not using/planting it?
a) It is expensive [ ]
b) Not tasty [ ]
c)Too white [ ]
d) Too yellow [ ]
e) The texture is less elastic [ ]
f) Difficult accessing it [ ]
g) Woody [ ]
h) Others [ ]

Source and Selection of Planting Materials

36. Where do you get your planting material from?
a. NGO [ ]
b. Own farm [ ]
c. Family and Friends [ ]
d. MoFA [ ]
e. Research institutions [ ]
f. Universities [ ]

37. How do you select your planting material?
a.) Select disease free [ ]
b) Appearance/ healthy looking [ ]
c) High yielding [ ]
d) Availability [ ]
e) Others [ ]

38. Have you ever been trained on how to select healthy cassava planting material
a) Yes [ ]
b) No [ ]

39. If yes by who?
a) MoFA [ ]
b) NGO [ ]
c) CRI [ ]
d. Universities [ ]
e. Others [ ].....

40. If No, would you be interested to receive such training?

a) Yes [ ]
b)No [ ]

41. In selecting the material from the farm, do you consider CMD-free material?
a) Yes [ ]
b) No [ ]

42. Give reasons for your answer?
a. Drought [ ]
b. Soil borne [ ]
c. Shortage of planting materials [ ]
d. Recovers [ ]
e. Chemicals [ ]

Assess to Extension services/FBO membership

43. Do you get extension services
a) Yes [ ]
b) No [ ]

44. How often do you get extension officer visiting?
a) Once a week [ ]
b) More than twice a week [ ]
c) Once in a month [ ]
d) Once in a while [ ]

45. Are you part of any FBO
a) Yes [ ]
b) No [ ]

46. What do you do as an FBO
a) Sharing cassava production ideas [ ]
b) Exchanging or sharing planting materials for members [ ]
c) Marketing of food products [ ]

APPENDIX B

i).

Table 3.3. Farm characteristics and access to extension services

Variable	Frequency f	Percentage %
a) Years in cassava production		
1-5	30	16.7
6-10	65	36.1
11-15	39	21.7
16-20	27	15
Above 20	19	10.5
Total	180	100
b) Land size		
1-2.9	59	32.8
3.0-4.9	109	60.5
Above 5	12	6.8
Total	180	100
c) Varieties planted		
Local Varieties (LV)	135	75
Improved Varieties (IV)	0.0	0.0
Both Varieties (BV)	45	25
Total	180	100
d) Names of IV planted with LV		
Abasafita	2	4.8
Ampong	14	34.5
Bosom esia	6	14.6
Ankra	25	70
Sika	19	46.3
Afisiafi	9	22

Total ** multiple responses	75	145.9
e) Intercropping		
Yes	102	56.7
No	78	43.3
Total	180	100
f) Crops intercropped		
Maize	65	63.7
Cowpea	14	13.7
Groundnut	14	13.7
Vegetable	9	8.8
Total	102	100
g) Purpose of planting cassava		
Dough	3	1.7
Gari	32	17.8
Multipurpose	145	80.6
Total	180	100

ii).

Fig. 3.2. Respondents who had Knowledge/experience CMD

iii.

Table 3.6. Crosstab of most common local name of CMD

	Districts						
	Akatsi South	Adaklu	Hohoe	Afadjato south	Krachi east	Nchumuru	Total
Local name of CMD							
Ayifle	0	16	0	0	0	3	19
Dayo	0	0	4	0	0	0	4
Kwafo	0	0	0	0	8	0	8
Kwata	0	0	4	0	14	0	18
Mosaic	3	1	1	1	1	0	7
Nsoson	0	0	0	0	0	7	7
Zongolachichi	25	3	2	6	2	2	40
Kokobi	0	0	0	0	2	0	2
Quateri	0	0	0	0	3	0	3
Edzekpo	0	4	0	7	0	0	11
Total	28	24	11	14	30	12	119

iv)Table 3.7. Crosstab between Highesteducation*whethertheymanage

Highesteducation**whethertheymanage Haveyoubeenmanaging?			
	Yes	No	Total
Highest education			
Non-formal	10	74	84
Formal	36	60	96
Total	46	134	180

v)Table 3.8. Crosstab between Haveyoubeenmanaging*Formaleducation or Management of disease*Formal education

	Haveyoubeenmanaging?		
	Yes	No	Total
Formal education			
Primary	15	41	56
JHS/MLSC	9	16	25
SHS	8	3	11
Tertiary	4	0	4
Total	36	60	96

vi)Table 3.9. Crostab management practices and their result

	Didthectrlwork		
Ifyeshow	No	Yes	Total
Pesticide	3	0	3
Roguing	1	17	18
De-toping	26	4	30
****Multiple responses			

vii. Table 3.13. Reasons respondents consider or not consider CMD in selecting PM

		Give reason for your answer			
In selecting planting material do you consider CMD	Shortage of planting material	Drought	Soil borne	Recovers	High yield
Yes	0	0	0	0	43
No	100	4	4	29	0
Total	100	4	4	29	43

APPENDIX C

Climatic Data

i)

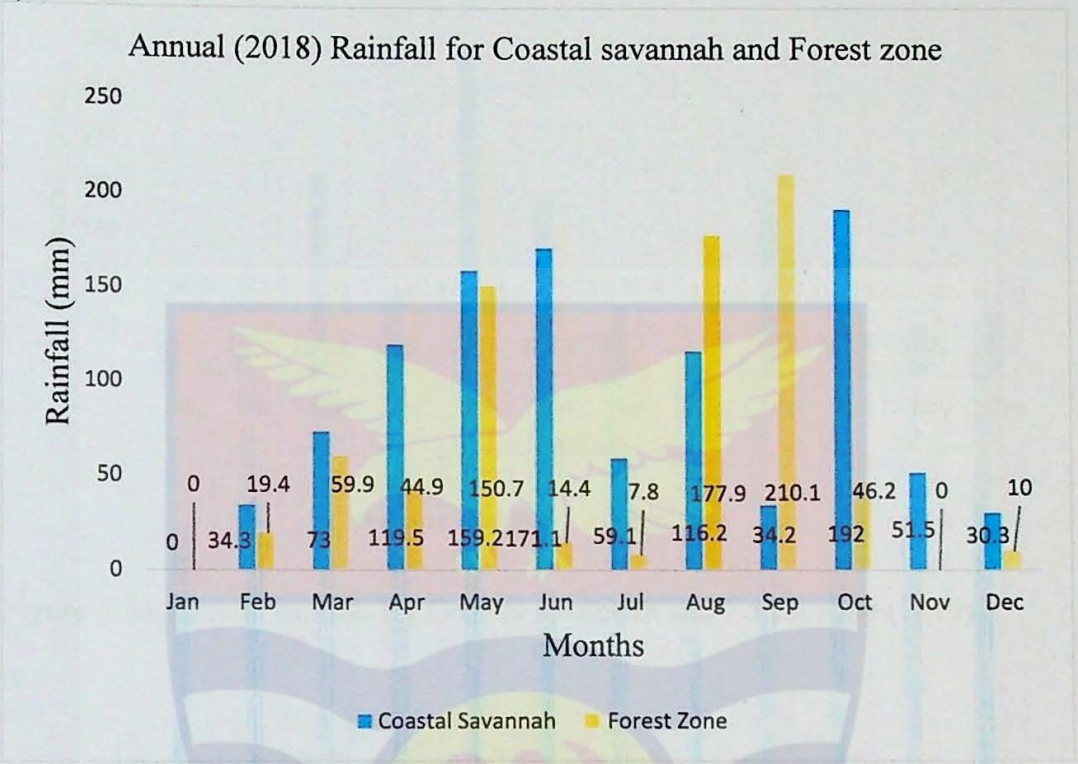


Figure 6.22. Annual rainfall for Coastal savannah and Forest zone (2018)

ii)

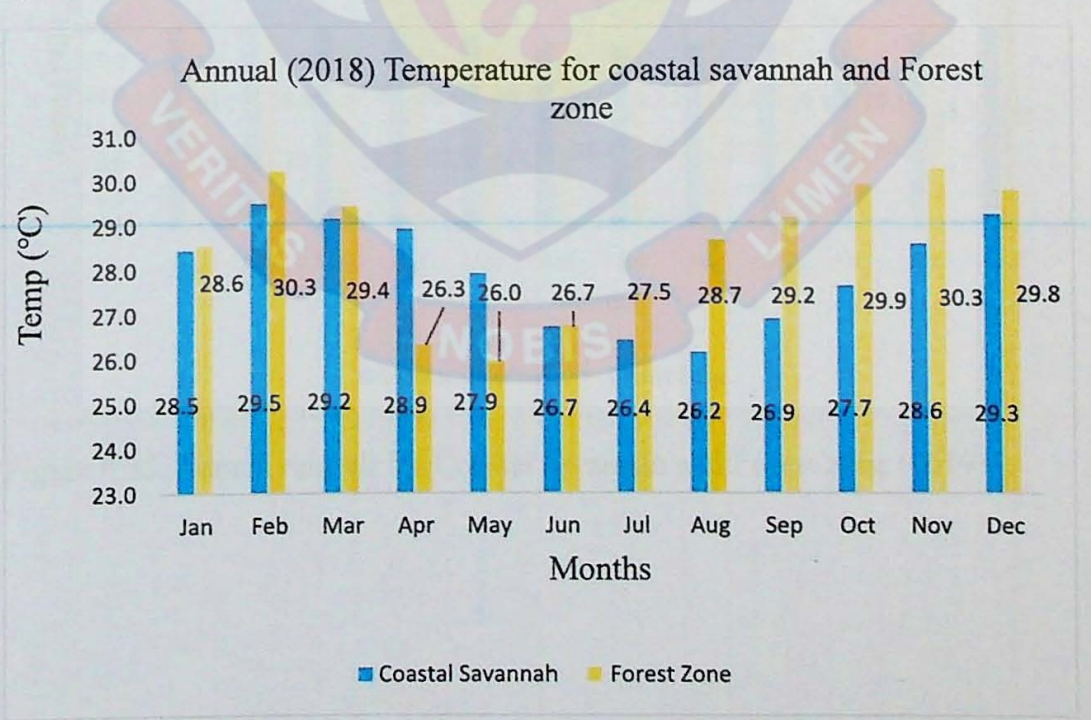


Figure 6.23. Annual Temperature for Coastal savannah and Forest zone (2018)

iii.

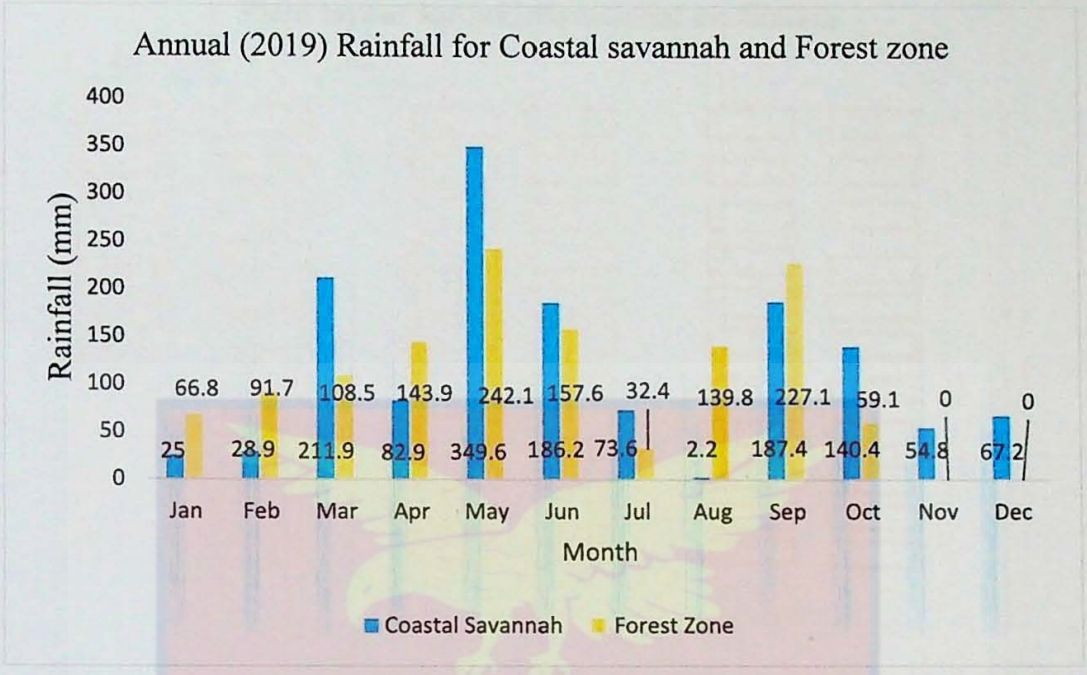


Figure 6.24. Annual rainfall for Coastal savannah and Forest zone (2019)

iv.

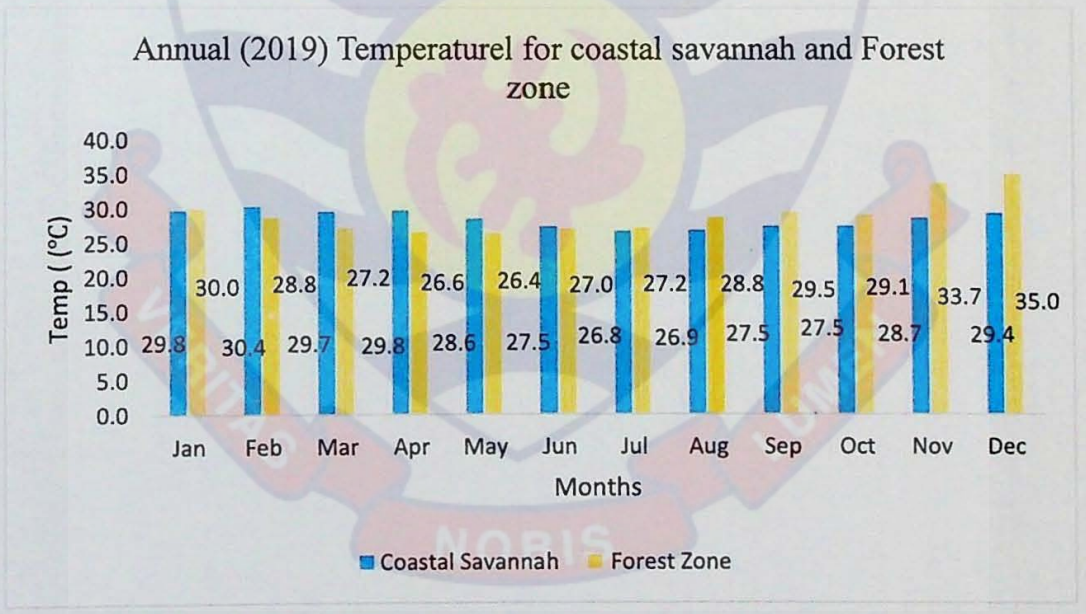


Figure 6.25. Annual rainfall for Coastal savannah and Forest zone (2019)

APPENDIX D

Field layout for multilocational evaluation