SURVEY OF COWPEA VIRAL DISEASE SYMPTOMS AND DETECTION OF ASSOCIATED VIRUSES IN SELECTED COWPEA GROWING AREAS IN GHANA

BY

FULERATU ADAMS KARIM (BSC (HONS) AGRICULTURE)

JUNE, 2016

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI, GHANA

COLLEGE OF AGRICULTURE AND NATURAL RESOURCES SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF CROP AND SOIL SCIENCES

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A THESIS SUBMITTED TO THE DEPARTMENT OF CROP AND SOIL SCIENCES, FACULTY OF AGRICULTURE, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF PHILOSOPHY IN

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JUNE, 2016

DECLARATION

I certify that this thesis does not incorporate, without acknowledgement, any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief, does not contain any material previously published or written by another person, except where due reference has been made in the text.

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DEDICATION

I dedicate this work to Dr Charles Kwoseh and Prof. Richard Akromah for their immense contributions to my academic life. Their tireless efforts and prayers have guided me this far.

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TABLE OF CONTENTS

LIST OF

PAGE

TABLES

TABLE

LIST OF

PAGE

FIGURES

FIGURE

LIST OF

PAGE

LIST OF

PAGE

PLATES

PLATE

LIST OF

PAGE

ABBREVIATIONS

1X TAE – One-strength tris acetate EDTA

ACP-ELISA – Antigen-coated plate enzyme-linked immunosorbent assay

ALP – Alkaline phosphatase

BCMV-BICM – Bean common mosaic virus-blackeye cowpea mosaic

C.I – Cylindrical inclusions

CABMV – Cowpea aphid-borne mosaic virus

CCMV – Cowpea chlorotic mottle virus

CGMV – Cowpea golden mosaic virus

CMV – Cucumber mosaic virus

CP gene – Coat protein gene

CPMMV – Cowpea mild mottle virus

CPMoV – Cowpea mottle virus

CPMV – Cowpea mosaic virus

CPSMV – Cowpea severe mosaic virus

CTAB – Cetyl Trimethyl ammonium bromide

DAC-ELISA – Direct antigen coating enzyme-linked immunosorbent assay

DAS-ELISA – Double antibody sandwich enzyme-linked immunosorbent assay

DNA – Deoxyribonucleic acid

DNTPs – Deoxyribonucleotide triphosphate

DTBIA – Dot immunoblotting assay

ELISA	–	Enzyme-linked immunosorbent assay
IITA	–	International Institute of Tropical Agriculture
ISEM	–	Immunosorbent electron microscopy
ORF	–	Open reading frame
PBS	-	Tween 20 –Phosphate buffered saline with Tween 20
PCR	–	Polymerase chain reaction
PTA-ELISA	–	Plate trapped antigen-enzyme linked immunosorbent assay
RNA		Ribonucleic acid
RT-PCR	–	Reverse transcription-polymerase chain reaction
RYMV	–	Rice yellow mosaic virus
SBMV	–	Southern bean mosaic virus
SgRNA	–	Sub-genomic RNA
ss/ds DNA	–	Single stranded or double stranded DNA ss/ds
RNA	–	Single stranded or double stranded RNA
TAS-ELISA	–	Triple antibody sandwich-enzyme linked immunosorbent assay
UV	–	Ultraviolet light

ABSTRACT

A survey was conducted in 2014/2015 growing seasons covering 100 fields within four locations in Ghana. Cowpea (*Vigna unguiculata* (L.) Walp) plants showing leaf mosaic and other virus-like symptoms, were noticed during the 2014 growing season in fields located at Mampong, Ejura- Sekyeredumasi, Nkoranza (Humid forest zone) and Amantin-Atebubu (Derived savannah zone) in the Ashanti and Brong Ahafo Regions of Ghana. Incidence and severity of some viral symptoms as well as farmers' perception of viral diseases in the surveyed areas were obtained with the aid of survey sheets and questionnaires. Symptomatic leaf and mature seed samples were collected from each location for virus identification in the laboratory. Seed and Aphid transmission tests, Mechanical (sap) transmission, ACP-ELISA and RT-PCR were used for virus detection. The survey showed that farmers cultivate virus-infected cowpea seeds season after season, thus causing high incidence and severity of viral diseases. High incidence and severity of viral diseases were observed in the EjuraSekyeredumasi District where most farmers in the other districts obtained seeds for cultivation. Mosaic and mottling were the commonest symptoms observed. The highest incidence (81.6%) and mean severity (3.01) values of virus symptoms were observed in Ejura. Percent incidences (72.5 and 70.7%) and severities (2.7 each) recorded at Atebubu and Mampong, respectively were not significantly different ($P>0.01$). Nkoranza recorded the lowest incidence (46.7%) and severity (2.4) of cowpea virus symptoms. Viruses detected in the leaf and seed samples serologically were: *Cowpea Aphid Borne Mosaic Virus* (CABMV), *Bean Common Mosaic Virus* strain Blackeye Cowpea Mosaic Virus (BCMV-BICM), *Cowpea Mottle Virus*

(CPMoV), *Cowpea Mild Mottle Virus* (CPMMV), *Southern Bean Mosaic Virus*

(SBMV), *Cowpea Yellow Mosaic Virus* (CYMV) and *Cucumber Mosaic Virus* (CMV). The viruses were detected infecting cowpea plants in all the four districts surveyed. BCMV-BICM was detected to be seed borne with transmission rates between 0.8 and 27%. *Aphis craccivora* Koch. also transmitted BCMV-BICM in a non-persistent manner. In all cases, only symptomatic seedlings were found infected with the virus. Also, systemic infections were observed on mechanically inoculated 'Ife brown' cowpea plants. The study identified seven (7) cowpea viruses in the country of which six (6) are reported to be seed-borne. This, therefore, necessitates the need for the production and use of virus-free seeds, development of virus resistant genotypes and adoption of efficient seed certification systems.

CHAPTER ONE

1.0 INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is an essential food legume for human consumption in Africa. It originated from the semi-arid areas of West Africa and is a main source of vegetable protein for both humans and livestock in developing countries in Africa and Asia (Ahenkorah *et al.*, 1998). The crop has high amount of protein (25%), it's resistant to drought, adapted to different types of soils and intercropping systems and it is able to improve soil fertility and prevent erosion (IITA, 2009a).

Cowpea is commonly grown in many poor countries as it is a natural supplement to staple diets of cereals, roots and tubers due to its high protein and lysine contents (Adekola and Oluleye, 2007). Farmers also earn some income by selling stems and leaves as animal feed during dry seasons. According to the Food and Agriculture Organization (FAO) of the United Nations (2012), the average cowpea yield in West Africa was 483 kg/ha.

Cowpea diseases induced by pathogens such as higher parasitic plants, nematodes, bacteria, fungi, bacteria and viruses are a major constraint to commercial cowpea production (Hampton *et al.*, 1997). It is assumed that reduced photosynthesis due to infected leaves is the cause of low productivity of virus-infected cowpea plants (Chia and He, 1999). Several reports have been made on cowpea viruses present in Ghana. Southern bean mosaic virus (SBMV) and Cowpea aphid-borne mosaic virus (CABMV) were reported to be fairly prevalent and of moderate incidence on cultivated cowpeas in Ghana (Lamptey and Hamilton, 1974). Zettler and Evans (1973) reported that cowpea seed samples obtained from Ghana had more than 13% incidence of blackeye cowpea mosaic virus (BICMV). Cowpea mild mottle virus (CPMMV) was first reported as a minor virus in Ghana, but subsequently became

important on crops such as soybean in Nigeria (Jeyanandarajah and Brunt, 1993). CABMV is one of the major viruses that infect cowpea with devastating results, and total crop loss has been reported in some instances (Thottappilly and Rossel, 1992). The majority of viral diseases of cowpea produce symptoms such as leaf yellowing, mottling, mosaic, necrotic spots and blisters on leaves, green and yellow vein banding, leaf deformation, witches broom, defoliation, apical necrosis, and stunting or even plant death (Aliyu *et al.*, 2010).

Recently, severe yellowing on cowpea plants up to 100% incidence, especially in the forest and savannah agro-ecological zones of Ghana, has been observed in spite of farmers‟ use of calendar spraying to control virus-transmitting insects. Previous studies had shown that most viral infections on cowpea caused yellowing of the plant (Aliyu *et al.*, 2010). Viral diseases have disastrous effects on crop yields and threaten the food production potential of Africa (Manyangarirwa *et al.*, 2010). However, there is limited knowledge on detection and characterization of cowpea viruses in Ghana. Cowpea viruses are transmitted by insect (arthropod) vectors, fungi, nematodes and even humans and animals. They can also spread through seeds, grafting or vegetative propagation. Aphids, whiteflies and leafhoppers are among insects that transmit cowpea viruses (Kitajima *et al.*, 2008; Bashir *et al.*, 2002).

Although most farmers practice strict monitoring or calendar spraying with chemical insecticides to control these insects, they still observe severe yellowing on plants. It may be assumed that viruses responsible for the recent yellowing are not mainly insect-transmitted. Seed transmission has also been shown to provide a very effective means of introducing the virus into the crop at an early stage, thus, causing primary infections throughout the planting (Booker *et al.*, 2005). It is therefore suspected that the viruses associated with the recent yellowing observed in Ghana are seed-borne rather than

insect-transmitted due to 100% incidence of symptoms observed in most cases. Proper identification and characterization of cowpea viruses will enhance disease management and reduction in spread. The main objective of this study was to identify important viruses associated with recent yellowing of cowpea in some major growing areas in Ghana.

The specific objectives were to:

i. determine farmers‟ perception on cowpea virus diseases in some growing areas in Ghana; ii. determine the incidence and severity of cowpea virus diseases within the surveyed areas; iii. identify the viruses associated with the cowpea plants in these growing areas; and iv. establish the modes of transmission of the detected viruses.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Areas of Cowpea Cultivation in Ghana

The cultivation of cowpea in Ghana is carried out in the Transitional and northern Guinea Savannah zones of the Northern, Upper East and Upper West regions and the Forest and Transitional zones of the Brong-Ahafo and Ashanti regions (SARI, 2012). The areas surveyed have two main growing seasons in a year. The major season begins in April and ends in August and the minor season begins in September and ends in December. Farmers in the Mampong Municipal and Ejura-Sekyedumasi in Ashanti Region and Atebubu–Amantin and Nkoranza Districts in the Brong Ahafo Region grow cowpea mainly in the minor season. The most common varieties cultivated are the local types, namely "Mallam Yaya", "Pangaabu", "Alancash", "Yekoyenim", "Uganda", "Yaminu", "Burkina" and "Adamu akese".

2.2 Constraints to Cowpea Production

There are several constraints to cowpea production especially, in semi-arid tropical zones. These include biotic and abiotic factors. Abiotic constraints include poor soil fertility, heat, drought and acidity, among others. Pathogens such as fungi, bacteria and viruses are known biotic agents infecting the crop. Also, cowpea production is challenged by several insect pests such as aphids, thrips and weevils.

2.2.1 Abiotic Stress

The effects of the environment on plant growth may be divided into stress, caused by the environment and adaptive responses, controlled by the plant defences (Fitter and Hay, 1987). Damage, which may be expressed as death of the whole or part of the plant, or merely reduced growth rate due to physiological malfunction and thus reduced yield,

are common phenomena. Agents of abiotic stress include poor soil fertility, drought, heat, acidity and stresses due to intercropping with cereals (Singh and Tarawali, 1997). However, the most important environmental agent affecting plant growth in the Semi-arid Tropical zone is drought (Ibrahim, 2012). Cowpea fails to emerge when soil temperatures are below 19 °C (Fitter and Hay, 1987).

2.2.2 Biotic Stress

2.2.2.1 Insect Pests of Cowpea

Some of the major insect pests of cowpea are aphid (*Aphis craccivora* Koch.), legume pod borer (*Maruca testulalis* Geyer.) and flower thrips (*Megalurothrips sjostedti* Tryb.) which attack cowpea at the seedling, flowering and pod formation stages, respectively (Amoah, 2010). Cowpea weevil (*Callosobruchus maculatus* F.) is the major storage pests (Ibrahim, 2012). Losses due to pest attacks or diseases can be as high as 90 % (IITA, 2000).

2.2.2.2 Diseases of Cowpea

The average yield of cowpea is low due to complex of biotic and abiotic stresses (Ajeigbe *et al.*, 2008). Cowpea is susceptible to a wide variety of pests and pathogens that attack the crop at all stages of growth (Allen, 1983). The biotic factors include insect pests, parasitic plants, fungal, bacterial and viral diseases. For instance, cowpea wilt caused by *Fusarium oxysporium* Schlecht, cowpea root rot caused by nematode (*Meloidogyne* spp.) and cowpea bacterial blight caused by *Xanthomonas vignicola* Burkh. (Ibrahim, 2012). The two types of parasitic weeds that attack cowpea are *Striga gesnerioides* and *Alectra vogelii* (Onyibe *et al.*, 2006). Among the numerous pathogens infecting cowpea, viruses are known to infect cowpea either at one stage or throughout the life cycle of the plant. The effect of viral diseases can be devastating and they are

major constraints to large scale production. A yield loss estimate of 15- 87% was reported from Iran due to Cowpea aphid-borne mosaic virus infections on cowpea (Yadav, 2010). Taiwo *et al.* (2007) also reported yield losses between 20 and 100% on irrigated cowpea fields in northern Nigeria.

2.3 Cowpea Viruses

Viral diseases of cowpea have been reported to cause appreciable losses in yield if the plants are infected at early growth stages (Booker *et al.*, 2005). Over 140 viruses worldwide have been reported to attack cowpea and at least 11 of these occur in Africa (Hughes and Shoyinka, 2003). Hampton *et al.* (1997) listed nine viruses considered most damaging to cowpea in Africa, seven of which were reported as seed-borne; Bean common mosaic virus-Blackeye cowpea mosaic (BCMV-BICM), Cowpea aphid-borne mosaic virus (CABMV), Cucumber mosaic virus (CMV), Cowpea mosaic virus (CPMV), Cowpea severe mosaic virus (CPSMV), Southern bean mosaic virus (SBMV) and Cowpea mottle virus (CPMoV). The other seed- borne cowpea virus is Cowpea mild mottle virus (CPMMV) (Jeyanandarajah and Brunt, 1993). The two non-seed-borne viruses considered important by Hampton *et al.* (1997) are Cowpea golden mosaic Geminivirus (CGMV) and Cowpea chlorotic mottle virus (CCMV).

2.3.1 Cowpea Aphid-borne Mosaic Virus (CABMV)

Cowpea aphid-borne mosaic virus is a flexuous virus particle with an average size of 750 x12 nm (Damiri *et al.*, 2013). The virus is classified as a member of the *Potyvirus* genus. Its nucleic acid is a single stranded ribonucleic acid (ssRNA) which induces cytoplasmic cylindrical inclusions consisting of pinwheels and bundles associated with scrolls (Lima *et al.*, 1979). Among the many species of plant viruses infecting cowpea, CABMV is considered a significant and cosmopolitan cowpea virus because it causes

yield losses generally between 30 - 40% (Bashir *et al.*, 2002). Also, yield losses from this virus ranging between 87% and 100% were reported from Nigeria (Shoyinka *et al.*, 1997; Raheja and Leleji, 1974), 13-87% from Iran (Kaiser and Mossahebi, 1975) and 48-60% from Zambia (Kannaiyan and Haciwa, 1993). CABMV is readily transmitted by sap inoculation and non-persistently by aphids. *Aphis craccivora* Koch., *A. gossypii* Glov., *A. fabae* Scop., *Macrosiphum euphorbiae* Thos., *and Myzus persicae* Sulz. have all been reported as vectors (Kitajima *et al.*, 2008; Bashir *et al.*, 2002).

Bashir *et al.* (2002) postulated that CABMV had spread worldwide through the exchange of virus-infected germplasm as the virus is seed-borne. The nature and severity of the symptoms induced by CABMV vary with host cultivar, virus isolate, and the time of infection. Common symptoms observed on infected plants are mosaic, vein clearing, green vein banding, stunting, and distortion of the leaves (Damiri *et al.*, 2013). CABMV has been reported in many countries in different continents including Asia, Africa, Europe, North and South America, and Australia (Pio-Ribeiro *et al.*, 2000; Bashir and Hampton, 1996; Huguenot *et al.*, 1993; Mali and Kulthe, 1980; Behncken and Maleevsky, 1977). However, biological properties of CABMV may differ among isolates worldwide (Bashir *et al.*, 2002).

2.3.2 Bean Common Mosaic Virus-Blackeye Cowpea Mosaic (BCMV-BICM)

Blackeye cowpea mosaic virus was first reported on cowpea in the USA by Anderson (1955). The virus has been reported to be a type strain of the Bean common mosaic virus (BCMV) (Boxtel *et al.*, 2000; Shoyinka *et al.*, 1997; Mali *et al.*, 1983). The virus is characterized by flexuous particles with a modal length of 743-765 nm (Murphy *et al.*, 1984). BCMV-BICM is also considered as a member of the *Potyvirus* genus (Matthews, 1981; Hollings and Brunt, 1981). The infective nucleic acid is a single

stranded, positive sense RNA, with a molecular weight of 2.9 x 10^6 (Murphy *et al*., 1984). The virus has been reported to occur on cowpea in several countries including Ghana, Nigeria, Togo, South Africa, Tanzania, Saudi Arabia, Japan and Brazil (CABI/EPPO, 2010).

The type of symptom of and susceptibility to BCMV-BICM depends on the host species and cultivar and on viral strains being considered (Kuhn, 1990). BCMV- BICM produces both localized and systemic symptoms on cowpea. Localized symptoms include large reddish, often ring-like lesions which typically spread along the veins, forming a reddish-net pattern. Systemic symptoms include mottle, green vein banding often with interveinal chlorosis, stunting, and leaf distortion (Thottappilly and Rossel, 1985). Cytoplasmic cylindrical inclusions (pinwheels and scrolls) have also been detected in most tissues (Zhoa *et al*., 1991; Murphy *et al*.,

1984) of cowpea, *Crotalaria*, and other hosts. BCMV-BICM is seed-borne in cowpea (Orawu, 2007). Seed transmission is dependent on both cowpea cultivars and viral isolates. The virus is also readily transmitted mechanically and in a non-persistent manner by the aphids *A. craccivora, A. gossypii* and *Myzus persicae* (Orawu, 2007).

2.3.3 Cucumber Mosaic Virus (CMV) on cowpea

Cucumber mosaic virus is the type member of the genus *Cucumovirus*, family Bromoviridae, which infects over 800 plant species and causes economically important diseases of many crops worldwide (Palukaitis *et al.,* 1992). CMV is a linear positive-sense, single-stranded RNA virus with its genome consisting of three single stranded RNAs, each of which is enclosed inside a coat protein. It has a total genome size of 8.621 kb which is broken into three parts, the largest part is 3.389 kb; the second largest is 3.035 kb and the third largest is 2.197 kb (ICTV, 2006). The RNA is surrounded by

a protein coat consisting of 32 copies of a single structural protein which form isometric particles (ICTV, 2006).

Once inside the plant, the virus is able to inhibit the plant's ability to signal for gene silencing in other tissues. This allows the CMV to invade further into the plant. This virus, like many, replicates in the cytoplasm. Movement through the plasmodesmata occurs for cell-to-cell transfers but the phloem is utilized for long distance movement within the plant (Zitter and Murphy, 2009). CMV is transmitted by aphids in a non-persistent manner (Gray, 1996). The virus can be both acquired from and transmitted to a host within seconds to minutes after feeding. To accomplish this, the virus interacts with the anterior portion of the alimentary tract (food canal to foregut), from which it can be subsequently inoculated by egestion.

Unlike some of the other non-persistently transmitted plant viruses, CMV does not require helper proteins for transmission (Kaplan *et al.*, 1998). CMV is found worldwide, is very easily spread and causes severe damage to the host. There is often a loss of 10-20% of yield and even if harvested, crops are commonly found in poor condition (Zitter and Murphy, 2009). Local symptoms on inoculated leaves include poorly developed chlorotic areas or reddish necrotic rings; systemic symptoms are mild mottle and distortions. A few varieties develop severe mottle, distortions and considerable reddish vein necrosis. Symptoms in most varieties are mild and partial (Anderson *et al.*, 1994).

2.3.4 Southern Bean Mosaic Virus (SBMV)

Southern bean mosaic virus belongs to the *Sobemovirus* genus. It is made up of icosahedral particles of about 30 nm in diameter. Virions contain 21% nucleic acid, 79% protein and 0% lipid. The virions contain a single coat protein (approximately 30

kb in size), a genomic RNA, and one sub genomic RNA (sgRNA) molecule. The genomic RNA is a single-stranded messenger-sense molecule, approximately 4 to 4.5 kb in size (Hull, 1995). Replication does not depend on a helper virus. The virions are found in the mesophyll, cytoplasm and in the nuclei of host cells. Inclusions present in infected cells are unusual in shape. The virus is reported to have several strains including the cowpea strain (strain C), Ghana strain (strain G), severe bean mosaic strain or Mexican strain (strain M) (Tremaine and Hamilton, 1983).

The cowpea strain of SBMV is one of several viruses that cause important diseases of cowpea (Singh and Allen, 1979). The virus is transmitted by beetles in a semi-persistent manner, by seed**,** grafting, pollen to seed and also by pollen to the pollinated plant. SBMV causes mosaic and/or mottling, chlorotic spots, systemic vein clearing and banding, leaf deformation and stunting in cowpea.

2.3.5 Cowpea Severe Mosaic Virus (CPSMV)

Cowpea severe mosaic virus, a *Comovirus*, was first reported in *Vigna unguiculata* from Lousiana, Arkansas and Indiana in USA (Perez and Cortez-Monllar, 1970). It is considered as one of the most important viruses of cowpea (Umaharan, 1990). It causes chlorotic lesions, concentric ring spots often becoming necrotic, vein clearing then mosaic and malformation and blistering of younger leaves (Umaharan *et al.*, 1997). Cowpea severe mosaic virus is transmitted by Chrysomelid beetles, primarily *Ceratoma* spp. *Chenopodium amaranticolor* (Coste and Reyne) and *Phaseolus vulgaris* L. are effective diagnostic hosts (Rajnauth *et al.*, 1989).

The genome consists of a single-stranded RNA with total genomic size of 9.73kb. The virions are isometric, non-enveloped and 25nm in diameter with an angular profile. They contain 36, 25, or 0% nucleic acid and 64, 75 or 100% protein depending on the strain. Non-genomic nucleic acid is not found in the virions and its replication does not

depend on a helper virus. The virions are found in the mesophyll cells or cytoplasm of the host plant. Inclusions present in infected cells are irregular- shaped crystals in the cytoplasm and vacuolated structures adjacent to the nucleus (Brunt *et al.*, 1996).

2.3.6 Cowpea Mottle Virus (CPMoV)

Cowpea mottle virus belonging to the genus *Carmovirus* was first described from Nigeria as a causal agent of a serious disease of cowpea where a yield reduction of more than 75% was reported (Shoyinka *et al.*, 1997). It is readily transmitted by sap inoculation and by several Chrysomelid beetle vectors (Thottappilly and Rossel, 1992). The virus is transmitted in the seed of cowpea at a rate of 10% depending on the genotype or line of the crop and the time between infection and flowering (Bozarth and Shoyinka, 1979), but many lines produced no more than 0.4% infected seeds (Allen *et al.*, 1982). It is seed transmitted and has potentially serious effects once established in a growing area, making it of great importance in international exchange of *Vigna* germplasm. It causes mottling or mosaic in infected tissues. The genome of CPMoV is a positive sense single stranded RNA of 4,029 nucleotides with six major open reading frames (ORFs) (You *et al.*, 1995). *Vigna unguiculata*, *Chenopodium amaranticolor* and *C. quinoa* Willd. are effective diagnostic hosts of the virus. The virions are isometric, non-enveloped and 30nm in diameter with a rounded profile. They contain 20% nucleic acid, 80% protein and 0% lipid. CPMoV replication does not depend on a helper virus as well (Brunt *et al.*, 1996).

2.3.7 Cowpea Mild Mottle Virus (CPMMV)

Cowpea mild mottle virus was first reported on cowpea (*Vigna unguiculata*) in Ghana by Jeyanandarajah and Brunt (1993). Subsequently, it was reported from several tropical regions of Africa (Mink and Keswani, 1987), Asia (Reddy, 1991; Shahraeen, 1989), Brazil and Argentina (Laguna *et al.*, 2006; Almeida *et al.*, 2005) and from La

Cote d"ivoire in diverse range of plant species including leguminous and solanaceous food crops (Hartman *et al.*, 1999). CPMMV is reported to be transmitted by the whitefly, *Bemisia tabaci* Genn. in a non-persistent manner (Jeyanandarajah and Brunt, 1993).

CPMMV has filamentous particles of approximately 650 ×15 nm in size with a coat protein of 32-36 kDa (Demski and Kuhn, 1989). CPMMV is a member of the genus *Carlavirus* which has been classified under the plant virus family *Betaflexiviridae* (Giovanni *et al*., 2007). CPMMV causes mosaic, chlorosis, necrosis and distortion in a range of indicator host plants (Demski and Kuhn, 1989). Soybean (*Glycine max* (L.) Merill), groundnut (*Arachis hypogaea* L.), cowpea (*V. unguiculata*), broad bean (*Vicia faba* L.) and *Nicotiana clevelandii* Gray have been reported as diagnostichosts of CPMMV (Reddy, 1991; Demski and Kuhn, 1989). The CPMMV genome consists of a single-stranded RNA of size 2.5×10^6 with six open reading frames (ORF). The virions contain 5% nucleic acid, 95% protein and 0% lipid. They are found in the mesophyll, epidermis and palisade parenchyma of the cytoplasm of infected cells. CPMMV also leaves inclusion bodies which are brush-like in structure in infected cells (Brunt *et al.*, 1996). Mild to severe systemic symptom appearance is reported by CPMMV in different hosts (Tavassoli *et al.*, 2007; Laguna *et al*., 2006).

2.3.8 Cowpea Mosaic Virus (CPMV)

Cowpea mosaic virus is one of the most commonly reported virus diseases of cowpea, which causes chlorotic spots with diffuse borders in primary leaves, and trifoliate leaves develop bright yellow or light green mosaic in younger leaves (Pouwels *et al.*, 2002). CPMV is a member of the *Comovirus* genus (Wellink *et al.*, 2000). It is an RNA-containing virus with isometric particles about 28 nm in diameter. It is transmitted

mainly by the beetle *Ceratoma arcuata* (Rajnauth *et al.*, 1989) and readily by sap transmission.

Infected plants contain two kinds of nucleoprotein particles similar in size but differing in RNA content. The RNA species in different particle types represent separate parts of the viral genome. *Chenopodium amaranticolor* is reported as the diagnostic host (Kammen *et al.*, 2001). Yield reductions up to 95% have been reported and late infections had less effect on yield than early ones (Chant, 1960).

CPMV was reported in Africa from field-collected cowpea plants in Nigeria, Kenya, Tanzania, Togo, Mali and the Republic of Benin (Thottappilly, 1992). Gilmer *et al.* (1974) reported 1-5% seed transmission in cowpea in Nigeria, but Thottappilly and Rossel (1988) found no evidence of seed transmission using many seeds of different cowpea varieties. The host range has been shown to be rather limited, and few hosts are known outside the Leguminosae (Fulton and Allen, 1982).

2.4 Transmission of Cowpea Viruses

2.4.1 Sap Transmission

Mechanical inoculation involves the introduction of infective virus or viral RNA/DNA into a wound on the plant's surface. When the virus establishes itself successfully in the cell, infection occurs. However, sap transmission rarely happens in nature. Several cowpea viruses have been reported to be readily transmitted mechanically by sap. Kitajima *et al.* (2008) reported that cowpea aphid-borne mosaic virus (CABMV) is readily transmissible by sap in cowpea. Shilpashree (2006) successfully transmitted cowpea mosaic virus (CPMV) by sap inoculation and noted that symptoms were produced within five days after inoculation. Bashir (2000) also reported that CPMV was readily transmitted by artificial sap inoculation and symptoms

appeared seven to 10 days after sap inoculation to cowpea. BICMV has also been reported to be mechanically transmitted through sap in cowpea plants

(Oruwa, 2007). Anderson (1959) and Chenelu *et al.* (1968) reported on a strain of cucumber mosaic virus (CMV) to be sap transmissible. Thottappilly and Rossel (1992) reported the development of symptoms after sap inoculations of cowpea mottle virus (CPMoV) on cowpea plants.

2.4.2 Insect Transmission

In nature, insects form the most important agents of virus transmission. Most cowpea viruses reported from different regions in Africa are transmitted by aphid, beetle and whitefly vectors. Cowpea aphid borne mosaic virus is reported to be transmitted by several aphid species including *Aphis craccivora, A. gossypii, A. medicagenis, A. fabae, Macrosiphum euphorbaie, and Myzus persicae* (Kitajima *et al.*, 2008; Bashir *et al.*, 2002). Oruwa (2007) reported that blackeye cowpea mosaic virus is readily transmitted in a non-persistent manner by the aphids, *A. craccivora, A.gossypii, Macrosiphum solinifolii*, and *Myzus persicae*. Cucumber mosaic virus is transmitted by aphids in a non-persistent manner. The virus can be both acquired from and transmitted to a host within seconds to minutes after feeding (Gray, 1996).

Southern bean mosaic virus is transmitted by beetles in a semi-persistent manner

(Shepherd and Fulton, 1962). Cowpea severe mosaic virus is transmitted by Chrysomelid beetles, primarily *Ceratoma arcuata* (Rajnauth *et al.*, 1989). Cowpea chlorotic mottle virus is also transmitted by several Chrysomelid beetle vectors (Thottappilly and Rossel, 1992). Cowpea mild mottle virus is reported to be transmitted by the whitefly, *Bemisia tabaci,* in a non-persistent manner (Jeyanandarajah and Brunt, 1993) and Singh *et al.* (1984) reported that cowpea mosaic virus is transmitted by *Aphis*

craccivora, A. gossypii and M. persicae in a nonpersistent manner. It is also transmitted by beetles (Rajnauth *et al.*, 1989).

2.4.3 Seed Transmission

Seed transmission provides a very effective means of introducing the virus into a crop at an early stage, giving randomized foci of primary infection throughout the planting. Seed transmission may therefore, be of very considerable economic importance. Viruses may persist in seed for long periods, so commercial distribution of a seedborne virus over long distances may occur. Seed transmission rates vary from less than 1 to 100%, depending on the virus and host. Cowpea viruses, more importantly seed borne viruses have been reported to have devastating effect on cowpea production causing stunting and plant deformation in early growth stage and not allowing the plants to reach their full potential (Booker *et al.*, 2005).

Several cowpea viruses are reported to be seed borne. Bashir *et al.* (2002) postulated that cowpea aphid borne mosaic virus had spread worldwide through the exchange of virus-infected germplasm. Amayo *et al.* (2012) reported seed transmission levels of cucumber mosaic virus, cowpea mild mottle virus and cowpea aphid borne mosaic virus detected in seed samples from farmers‟ fields in Uganda to be 23, 20.3 and 16.4%, respectively. Seed transmission of cowpea mosaic virus depends on the type of cowpea cultivar and viral isolates (Orawu, 2007).

Zettler and Evans (1973) reported that cowpea seed samples obtained from Ghana had more than 13% incidence of BICMV. Southern bean mosaic virus is transmitted by seed**,** pollen to seed and also by pollen to the pollinated plant (Shepherd and Fulton, 1962). Cowpea chlorotic mottle virus is transmitted in the seed of cowpea at a rate up to 10% depending on the genotype of the line and the time between infection and flowering (Bozarth and Shoyinka, 1979). Fischer and Lockhart (1976) observed 26%

seed transmission of Cowpea mosaic virus in cowpea. Gilmer *et al.* (1974) also reported 1-5% seed transmission in cowpea in Nigeria.

2.5 Techniques for Virus Indexing

Formerly, initial diagnosis of plant viruses was achieved through bioassay, an indicator plant, determination of host range, symptomatology and virus particle morphology. The combination of several methods of virus detection may provide more reliable information on the detection of a virus (Naidu and Hughes, 2003). However, advances in molecular biology and immunology have led to the development of more accurate, rapid and less labour-intensive methods of virus detection. These include protein-based techniques such as precipitation/agglutination tests, enzyme-linked immunosorbent assay (ELISA), Immunosorbent electron microscopy (ISEM), dot immunoblotting assay (DTBIA) as well as viral nucleic acidbased techniques such as dot blot hybridization, polymerase chain reaction (PCR), nucleic acid hybridization and DNA/RNA probes.

2.5.1 Biological Detection

This method involves the use of diagnostic hosts of a virus for diagnosis. Disease symptoms on plants in the field are mostly inadequate to give correct identification, especially when several viruses are capable of causing similar symptoms. Indicator or diagnostic plants are species or varieties of host plants that can give clear, characteristic and consistent symptoms of the viruses being studied, usually under planthouse conditions. Many good indicator species including *Chenopodium amaranticolor*, *C. quinoa* and *Nicotiana benthamiana* have been used for cowpea virus studies (Kammen *et. al.*, 2001; Rajnauth *et. al.*, 1989). Biological methods for diagnosis and detection of viruses are more time consuming than most of the new methods now available. Wounds are created on the leaves of an indicator plant with the aid of Carborandum and sap

from infected plant material is introduced through the wounds. When the virus establishes itself successfully in the host cell, infection occurs. Several cowpea viruses have been successfully transmitted mechanically.

CABMV (Kitajima *et al.*, 2008), CPMV (Shilpashree, 2006; Bashir, 2000), BICMV (Orawu, 2007; Mali *et al.*, 1988), CMV (Chenelu *et al.*, 1968; Anderson, 1959) and CPMoV (Thottappilly and Rossel, 1992) have all been reported.

2.5.2 Serological Detection

ELISA and other modified forms, for example, direct antigen-coating enzyme-linked immunosorbent assay (DAC-ELISA), double antibody-sandwich ELISA (DASELISA), antigen-coated plate ELISA (ACP-ELISA), plate-trapped antigen ELISA (PTA-ELISA) and triple antibody-sandwich ELISA (TAS-ELISA) have been used extensively for the detection of CABMV, CMV and CPMoV from different parts of cowpea (Bashir and Hampton, 1996; Clark and Adams, 1977). ELISA proved sensitive and reliable for the detection of CMV in different plant tissues (Abdullahi *et al.*, 2001). Aliyu *et al.* (2010) detected CABMV, CYMV, BICMV and CPMoV infecting cowpea in Nigeria, using ACP-ELISA.

Factors such as quality of antibodies, preparation and storage of reagents, incubation time and temperature, quality of chemicals, selection of appropriate part of sample and the use of suitable extraction buffer influence the sensitivity and reliability of ELISA (McLaughlim *et al.*, 1981). ELISA is a very effective technique for the detection of seed-borne viruses (Bashir and Hampton, 1996). It is important that positive and negative controls be included in each assay to define a threshold for differentiating between infected and non-infected samples. Generally, a sample is regarded as positive if the absorbance value exceeds the mean value of a negative control by 2-3 standard deviations (Naidu and Hughes, 2003).

2.5.3 Polymerase Chain Reaction (PCR)

PCR is a molecular biology method for enzymatically copying target nucleic acid sequences using a thermostable DNA polymerase from *Thermus aquaticus* (Brock and Freeze) in which repeated replication of a given sequence forms millions of copies within a few hours (Akinjogunla *et al.*, 2008). The PCR process consists of a series of 20 or 30 cycles with each cycle consisting of three steps: heating of the double stranded DNA to 94–96 ^{o}C to separate the strands, lowering of the temperature to 45–60 ^{o}C so that the primers can attach themselves to the single DNA strand and extension of each primer, usually at 72 ^{o}C (Akinjogunla *et al.*, 2008).

Previous reports have shown that reverse transcription-polymerase chain reaction (RT-PCR) methods facilitate the accurate, rapid and less labour-intensive detection of a number of cowpea-infecting RNA viruses (Akinjogunla *et al.*, 2008; Gillaspie *et al.*, 1999; Bariana *et al.*, 1994). For instance, RT-PCR has successfully been used to detect CABMV in cowpea (Damiri *et al.*, 2013; Chalam *et al.*, 2008), as well as in other crops (Maciel *et al.*, 2009; Nascimento *et al.*, 2006; Gillaspie *et al.*, 2001; Pio-Ribeiro *et al.*, 2000). The method has been found to be more sensitive than DACELISA in detecting CPMoV since it gives no false positive reactions as is sometimes seen with ELISA (Akinjogunla *et al.*, 2008). A modified RT-PCR protocol (Gillaspie *et al.*, 2001) was used to confirm the presence of CMV, CPMMV and CABMV in cowpea following detection with ELISA (Amayo *et al.*, 2012). PCR can detect very small quantities of virus in plants where ELISA cannot and can also be used for further characterization of plant viruses (Vunch *et al.*, 1999). ELISA, rather than RT-PCR has been used widely in all previous cowpea virus detection studies. However, recent studies in cowpea virus detection combine both ELISA and RT-PCR.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Location

The survey and sample collection were carried out in cowpea growing areas: Ejura and Mampong in the Ashanti region and Atebubu and Nkoranza in the Brong Ahafo regions of Ghana. Identification of insect samples from the field was done at the Entomology laboratory of the Department of Crop and Soil Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Laboratory analysis for identification and characterization of cowpea viruses were conducted at the Virology and Molecular Diagnostics Laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The indexing of seeds from infected plants in the field for seed-borne viruses was done in the screen house of IITA.

3.2 Source of Antisera and other Reagents for ACP-ELISA and RT-PCR CABMV, BCMV-BICM, CMV, SBMV, CPMV, CYMV and CPMMV antisera used for the Antigen-coated plate enzyme-linked immunosorbent assay (ACP-ELISA) were obtained from the Virology and Molecular Diagnostics Laboratory of IITA. Antirabbit IgG (whole molecule) – Alkaline phosphatase as well as substrate salts were obtained from Sigma-Aldrich Company of Germany. Reagents used for total nucleic acid extraction, and cylindrical inclusions (CI) forward and reverse primers used for Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) were all obtained from IITA.

3.3 Characteristics of Surveyed Areas

Ejura-Sekyeredumasi District in the Ashanti Region of Ghana is one of the major cowpea producing belts in Ghana. The district is located within longitudes 1°5‟ W and

1°39‟ W and latitudes 7°9‟ N and 7°36‟ N. Ejura is located on an altitude of about 225 m. It covers a large land size of 1,782 km^2 making it the fifth largest of the 30 districts in the region (Ministry of Local Government, Rural Development and Environment, 2006). It is estimated that there are about 19,000 farmers in the district, of which 80% are cowpea farmers (SRID, 2007). The district is located between the transitional zone of the Semi-deciduous Forest and the Guinea Savannah zones and thus experiences both the savannah and forest climatic conditions. It is characterized by two rainfall patterns; the bi-modal pattern in the south and the uni-modal in the north with the main rainy season occurring from April to November. The annual rainfall varies between 1200 mm and 1500 mm. Relative humidity and solar radiation are very high during the rainy season and dry seasons, respectively (Ministry of Local Government, Rural Development and Environment, 2006).

Mampong Municipal is situated on the northern part of the Ashanti Region. It lies within longitudes 0.05° W and 1.30° W and latitudes 6.55° N and 7.30° N with a total land area of 2346 km^2. It is estimated that 80 % of land area is used for small- scale farming. Mampong has two rainy seasons and an annual rainfall of 1270 mm. The major season starts in March and peaks in May/June. There is a slight dip in July and a peak in August, ending in November. The period between December and February is usually dry, hot and dusty. The vegetation of the north of the municipality is reduced to savannah grassland (Ministry of Local Government, Rural Development and Environment, 2006).

Amantin-Atebubu District is located at latitude 7.5458° N and longitude -1.2007° W, covering a land area of 1,996 km^2 with an estimated population of 82,109 (Ministry of Local Government, Rural Development and Environment, 2006). Agriculture is the main economic activity of the Atebubu-Amantin district employing more than 50% of

the economically active population (MOFA, 2015). Farming in the district is commonly on small-scale basis. The average acreage cultivated ranges between 1.6 and 2.4 Ha for all crops. Major crops grown in the district are cowpea, yam, maize, groundnut, cassava, rice and vegetables (garden eggs, okro and pepper). The area cropped to cowpea is an estimated 490 Ha with yield of 1.30 Mt/Ha (MOFA, 2015).

Nkoranza District is one of the 27 districts in the Brong Ahafo Region of Ghana. It lies within longitudes 1°10' W and 1°55' W and Latitudes 7°20' N and 7°55' N, with a total area of 1,100 km^2. The district has a mean annual rainfall ranging between 8001200 mm. The major rainy season is from March to June and the minor in September to November. The dry season is from December to March, however, August experiences a short dry season. (Ministry of Local Government, Rural Development and Environment, 2006). Agriculture provides employment for about 70% of the district's population. A large number of households in the district are engaged in small-scale farming with average farm size ranging between 1.2 and 4.0 Ha for all crops. The main food crops produced are cowpea, maize, sorghum, yam, plantain, cocoyam, groundnut and tomato. Area cultivated to cowpea is 567 Ha with an output of 510 Mt (MOFA, 2015).

3.4 Survey: Assessment of Incidence, Severity and Farmers' Perception of Cowpea Viral Diseases in the Surveyed Areas

A survey was conducted to obtain information on cowpea viral disease symptoms and also to ascertain farmers' perception of cowpea viral diseases on 100 farms in one municipal and three districts in Ghana where cowpea is grown. This was done in the major cropping season (October/November) of 2014. Twenty-five farms separated by at least 0.5 km were randomly selected from various communities in each municipal or district; Amantin-Atebubu (Derived Savannah zone), Ejura- Sekyeredumasi, Mampong

and Nkoranza (Humid Forest zones). Information such as occurrence of virus through the degree of symptom development was obtained with the aid of structured survey sheets (Appendix 1) and questionnaires (Appendix 2).

Data of each field including the location details; longitude, latitude and altitude, size of the field, sowing and harvesting dates, crops in neighbouring fields, varieties in the field, planting design, intercrops, source of planting material, crops sown in previous season and treatments (application of fertilizer, insecticides and herbicides) were recorded. The survey was conducted five to six weeks after planting up to flowering of the cowpea. In each field, 30 plants in an „X" transect with 15 plants per diagonal axis were visually assessed for presence of viral symptoms. Presence or absence of fungal and bacterial diseases as well as arthropod pests (aphids, beetles and mites) was recorded. Viral symptoms such as leaf mosaic, mottling, necrosis, puckering, stunting, deformation and death were scored on a scale of 1-5 (Kumar, 2009) on each field. Scores were described as 1 (No visible symptom), 2 (Symptoms on 25% of the plant), 3 (Symptoms on 50% of the plant), 4 (Symptoms on entire plant but no stunting or deformation) and 5 (Deformation and death of the entire plant). Photographs of the fields and symptomatic plants were taken. Mean symptom severity (Gumedzoe *et al.*, 1997) and percent incidence (Madden and Hughes, 1999) were estimated as follows:

$$\text{Mean severity} = \frac{\text{Total score of plants with severity score 2,3,4 and 5}}{\text{Total number of plants with score 2,3,4 and 5}}$$

$$\text{Percent incidence} = \text{Number of symptomatic samples} \times \frac{100}{30}$$

3.5 Collection of Virus-infected Leaf and Seed Samples from the Field for Virus Indexing

Symptomatic leaf and seed samples were collected from all the 100 farms in the eight cowpea growing communities in the survey. Leaf and seed samples were collected

from local cowpea varieties, namely 'Alancash', 'Yekoyenim', 'Uganda', 'Yaminu', 'Burkina', 'Adamu akese', 'Mallam yaya' and 'Pangaabu'. In each community, 13 farms were visited. Five fresh leaf samples per field were collected from symptomatic plants. These plants represented all symptom types observed in the field. Leaf samples were pressed in newspapers with each sample in a separate sheet and labeled 1 to 100. Samples were separated by five sheets of paper to prevent crosscontamination.

One hundred mature seed samples; 25 from each of the four municipal and districts were also collected from farmers and labeled Ejura 1 - 25, Amantin 1 - 25, Mampong 1 - 25 and Nkoranza 1 - 25. Seed samples were kept in well labeled sample bags with naphthalene balls and kept on dry ice. Insect pests associated with cowpea plants in the field were collected in bottles containing 70% ethanol and preserved in the deep freezer at -20 ^{o}C till analysed. A GPS device (GPSMAP 62s, GARMIN, Taiwan) was used to measure the coordinates and altitudes of the field and market locations where leaf and seed were collected (Fig. 3.1). The sampleswere sent to the Virology and Molecular Diagnostics Laboratory, IITA under permit issued by Nigeria Plant Quarantine Service and phyotosanitary certificate by the Plant Protection and Regulatory Services Directorate of the Ministry of Food and Agriculture, Ghana for further analysis.

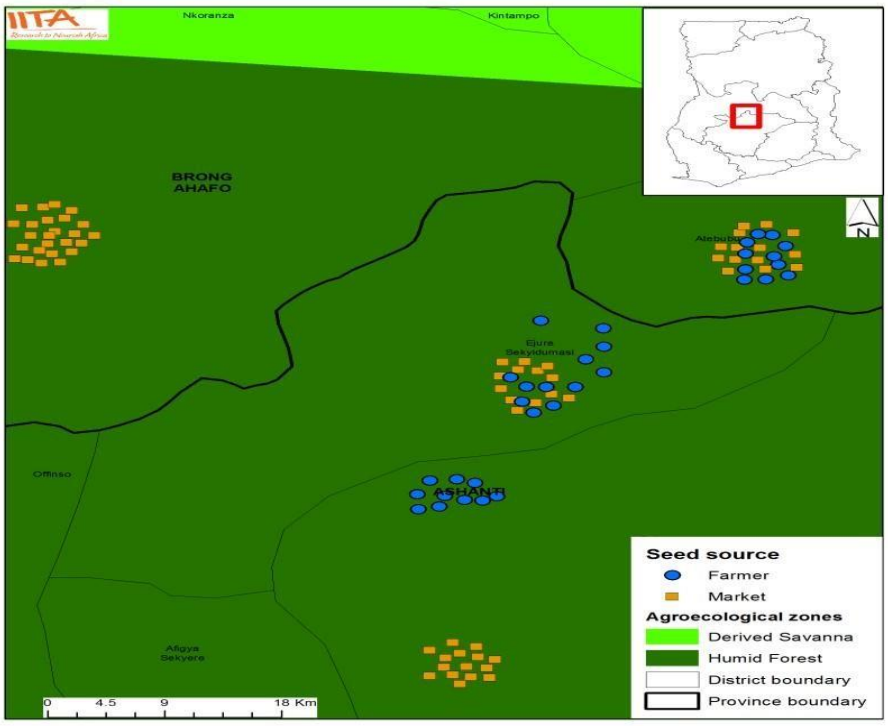

Fig. 3.1 Areas of Seed Collection in the Study

3.6 Screen house testing for seed transmission of viruses

Cowpea seeds obtained from farmers were planted in 60 x100 x 20cm sized plastic trays, each filled with two litre steam-sterilized top soil, in an insect-free screen house to determine the percent seed-borne viral infections. Eight each of the 25 seed lots collected from Ejura, Amantin and Mampong were randomly selected and grown in 24 separate trays. One hundred to two hundred (100-200) seeds depending on the total number of seeds available per seed lot were planted in each tray and labeled Ejura 18, Amantin 1-8 and Mampong 1-8. Watering was done as required. Cowpea seedlings (Plate 3.1) were examined visually, daily for symptoms and scored as described by Kumar (2009) above. At three weeks stage, number of cowpea plants in each tray was counted, symptomatic plants were removed from the tray and put in pots and then each plant tagged. The trays were labeled with the district or municipal where cowpea seeds were collected (Ejura, Amantin or Mampong), the tray number (1-8) and the plant tag

(A-X) (Appendices 8, 9, 10). Asymptomatic plants in each tray were put in groups of ten, tied together and each group tagged. Ten leaves were collected from each group, one from each plant, and all put together in one sample bag and labeled, accordingly. This was repeated for all 24 trays. Leaves were taken to the laboratory for virus indexing. With the aid of a sterile cork borer, 5mm diameter pieces each of all leaf samples collected were tested via Antigen-coated plate enzyme-linked immunosorbent assay (ACP-ELISA).

Plate 3.1 Cowpea plants used for seed transmission test in the screen house

3.7 Experiment 1: Aphid transmission of BCMV-BICM

Virus-free aphids (*Aphis craccivora*) tested via ELISA and reared on healthy but susceptible Ife brown cowpea variety obtained from Germplasm Health Unit of IITA were used for the test in the screen house. The aphids were starved for 60 min then allowed 5 min acquisition feeding on BICMV-infected cowpea leaves, followed by a brief inoculation feeding period of 3 min on four healthy Ife brown cowpea plants (five aphids per plant). The aphids were then killed by atomizing 2ml of cymethoate (cypermethrin + dimethoate) on each plant and the plants were examined daily for four

weeks for viral symptoms. Virus-free aphids fed on healthy Ife brown cowpea plants were used as negative control. Symptoms were observed and recorded daily, until leaves were collected from each of the four tests and control plants and tested via ACP-ELISA after four weeks.

3.8 Experiment 2: Mechanical (sap) Inoculation of Healthy Cowpea Seedlings for Seed Transmission of BCMV-BICM

Ife brown cowpea seeds were planted in an insect-proof screen house for the mechanical inoculation. At the two-leaf stage, leaves from the seedlings were tested via ACP-ELISA and confirmed to be virus-free before used for the mechanical inoculation. The original virus-infected leaf samples collected during the survey were not included in the test. Seedlings from seed transmission test above were confirmed to be BCMV-BICM-positive in ACP-ELISA before used to prepare the sap for transmission.

Sap was extracted from BCMV-BICM-infected cowpea leaves with 0.1 M phosphate buffer, pH 7.5. Two grammes each of the virus-infected cowpea leaves for each entry were macerated separately in chilled mortar and pestle with 2ml of phosphate buffer.

The upper surface of leaves was dusted with carborandum and then rubbed with the BCMV-BICM infected leaf sap using cotton gauze. Inoculated leaves were rinsed with sterile water to remove excess carborandum and sap. The mechanically inoculated cowpea plants were maintained in an insect-proof screen house and observed daily for symptoms development. Symptoms were scored as described by Kumar (2009) above. Leaves were collected from the mechanically inoculated plants and tested via ACP-ELISA after four weeks.

3.9 Experiment 3: Serological Detection of Cowpea Viruses using ACP-ELISA

3.9.1 Virus Indexing of Symptomatic Field Leaf Samples from the Survey Area

Virus indexing of the 100 leaf samples collected from fields in Ejura, Mampong, Amantin-Atebubu and Nkoranza during the survey was done serologically using single infection antigen-coated plate enzyme-linked immunosorbent assay (ACP- ELISA) described by Kumar (2009). The leaf samples were subjected to test using polyclonal antisera raised against *Cowpea Aphid Borne Mosaic Virus* (CABMV), *Bean Common Mosaic Virus* strain Blackeye Cowpea Mosaic Virus (BCMV-BICM), *Cowpea Mosaic Virus* (CMV), *Southern Bean Mosaic Virus* (SBMV), *Cowpea Mottle Virus* (CPMoV), *Cowpea Yellow Mosaic Virus* (CYMV) and *Cowpea Mild Mottle Virus* (CPMMV) in rabbit. One hundred micro litres (100 µl) of antigen, ground in carbonate coating buffer (0.015 M Na_2CO_3 and 0.0349 M $NAHCO_3$) with DIECA at a rate of 100 mg/ml buffer (1:10 w/v) was added to each well of a microtitre plate.

The controls used were blank (extraction buffer without plant sap), negative control (healthy Ife brown cowpea leaf sample) and positive control (leaf samples from infected-Ife brown cowpea plant). The microtitre plates were incubated in a humid chamber for one hour at 37 ^{o}C and then washed with three changes of Phosphate buffered saline with Tween 20 (PBS-Tween 20), allowing three minutes for each wash. Plates were emptied and tapped dry on a layer of paper towel. Each well was blocked with 200 µl of 3% dried skimmed milk in PBS-Tween 20. Plates were incubated at 37 ^{o}C for 30 min, and then tapped dry on a layer of paper towel.

Cowpea leaf extract (from healthy Ife brown plant) in PBS-TPO (1:10 w/v) was prepared for cross-adsorption of CABMV, BICMV, CYMV, CMV, SBMV, CPMoV and CPMMV polyclonal antisera. The polyclonal antisera were diluted with the leaf extract as follows: CABMV and BICMV at 1:5000 µl; CMV and CYMV at 1:3000 µl;

SBMV, CPMoV and CPMMV at 1:10000 µl and the mixture incubated at 37 ºC for 30 mins and shaked gently with the hand. One hundred microlitres of the cross- adsorbed antibody was dispensed in each well of the microtitre plate; the plate was covered and incubated at 37 ºC for one hour. Plates were washed with three changes of PBS-Tween, allowing 3 mins for each wash and then tapped dry. One hundred microlitres of goat anti-rabbit alkaline phosphatase (ALP) conjugate diluted in conjugate buffer (Ovalbumin, Polyvinyl Pyrrolidone and PBS-Tween 20) (1:15,000) was dispensed into each well and incubated for one hour at 37 ºC. Plates were washed with three changes of PBS-Tween 20, allowing 3 mins for each wash and then tapped dry after the third wash. One hundred microlitres of 0.5 mg/ml p- nitrophenyl phosphate substrate in substrate buffer (diethanolamine and distilled water) was added to each well and incubated in a dark room for one hour. Absorbance values were measured and plates were kept in a refrigerator at 4 ºC overnight. Quantitative measurements of the p-nitro phenyl substrate conversion resulting in yellow colour were made by determining the absorbance at 405 nm (A405) in an ELISA plate reader at one and six hours. The mean absorbance readings of negative controls were determined and twice the values were used as the positive thresholds.

3.9.2 Virus Indexing of Cowpea Seeds in the Screen House for Seed Transmission of Cowpea Viruses

ACP-ELISA (Kumar, 2009) was used for indexing of seed borne cowpea viruses in the screen house in seedlings raised from seeds collected during the survey. A total of 75 symptomatic plants from various trays, 14 from Amantin (Appendix 9), 20 from Ejura (Appendix 8) and 41 from Mampong (Appendix 10) were tested. Asymptomatic plants, 50 (five samples in groups of 10) each from Amantin, Ejura and Mampong were also tested. Two grammes of each leaf sample was ground separately in sterile mortar and pestle with coating buffer at a rate of 100 mg/ml. Sap from each sample was dispensed

into wells of microtitre plates with the aid of a 100ml pipette. Single infection tests with seven antisera raised against CABMV, BCMV-BICMV, CMV, SBMV, CPMoV, CYMV and CPMMV were performed. Absorbance values were measured and plates were kept in a refrigerator at 4 ^{o}C overnight. Quantitative measurements of the p-nitrophenyl substrate conversion resulting in yellow colour were made by determining the absorbance at 405 nm (A405) in an ELISA plate reader at one and six hours. The mean absorbance readings of negative controls were determined and twice the values were used as the positive thresholds.

3.10 Experiment 4: Reverse Transcription-Polymerase Chain Reaction (RT- PCR) analysis

3.10.1 Total Nucleic Acid Extraction from Field Cowpea Leaves and from Screen House Seedlings for Seed Transmission of Cowpea Viruses

Total nucleic acid was extracted from 46 symptomatic cowpea leaf samples (those that tested positive to BICMV in ACP-ELISA) from cowpea fields during the survey and from four (Amantin 2A and 2B, Ejura 2A and 2B) randomly selected symptomatic cowpea seedlings from the virus seed transmission test in the screenhouse. The four seedlings were selected from the 75 seedlings that tested positive to BICMV in ACP-ELISA above, to confirm the result. The Cetyl Trimethyle Ammonium Bromide (CTAB) method described by Dellaporta *et al.* (1983) was used.

The extraction buffer was prepared from 100 Mm tris-HCl, 20 Mm EDTA, 1.4 M NaCl, 0.2% β-Mercaptoethanol (v/v) and 2% CTAB powder (w/v). One hundred milligrammes of leaf was ground in 1000 µl of the extraction buffer in a sterile mortar. Sap was poured into sterile tubes and vortexed for 2 mins. Extracts in the tubes were incubated in a water bath at 60 ^{o}C for 10 min and brought to room temperature. Phenol, Chloroform and Iso-amyl alcohol at 25, 24, and 1 µl were mixed and 600 µl of the mixture was added to each extract. Extracts in the tubes were vortexed and centrifuged

at 12000 rpm for 10 min. Four hundred and fifty microlitres of the supernatant was put into new sterile tubes and 300 µl of ice-cold Isopropanol was added to each tube. Extracts in the tubes were mixed gently by inverting tubes 3-4 times and then incubated for 30 min in a freezer at -80 ^{o}C.

Extracts were centrifuged at 12000 rpm for 10 min to sediment the nucleic acid. The supernatant was discarded gently to ensure the pellets were not disturbed and 500 µl of ethanol was added to the pellets. Nucleic acids were centrifuged at 12000 rpm for 5 min to wash the pellets. The ethanol was discarded and nucleic acid was air-dried at room temperature. Pellets were suspended in 50 µl sterile distilled water and stored in a refrigerator at 4 ^{o}C for further use.

3.10.2 Integrity Test for Extracted Nucleic Acids

Integrity test (agarose gel electrophoresis) was performed to determine the quantity and concentration (quality) of extracted nucleic acids. Both nucleic acids extracted from symptomatic leaf samples (46) from the field and symptomatic leaf samples (4) from the screenhouse seedlings were tested. Nucleic acid fragments were separated according to size on 1% agarose gel prepared from 2 g agarose in 200 ml of one-strength tris-acetate EDTA (1X TAE) buffer in a beaker. The one-strength TAE buffer was prepared from 4.84 g of 0.04 M trizma base, 1.142 ml of acetic acid and 2.9224 g of 0.01 M EDTA. The solution was kept in an oven at 10 ^{o}C for 10 min until agarose dissolved totally in the TAE buffer. It was then cooled by pouring tap water flowing through a water hose at the base of the beaker containing TAE buffer and then 6 µl ethidium bromide was used to stain the gel to make the nucleic acids visible under ultra violet light after separation on the gel. The gel was poured into 200 ml electrophoresis tray and wells were made with combs by allowing it to polymerize first and then removing the comb. The tray containing the gel was placed in an electrophoretic tank

containing 1X TAE buffer to ensure that electric current flowed through the entire tank. Four microlitre loading dye was mixed with 4 µl nucleic acid template of each sample on a parafilm before loading into wells made on the gel. The loading dye helped to prevent nucleic acid from diffusing and also gave colour to the colourless nucleic acids. The gel was run at 120 V for 45 min for separation of nucleic acids. The gel was then viewed under UV trans-illuminator and sample bands were scored as present (+) or absent (-). Photographs of bands were taken.

3.11 RT-PCR Virus Confirmation for Field Cowpea Leaf Samples (BICMVpositive in ACP-ELISA)

A modified reverse-transcription polymerase chain reaction protocol (Gillaspie *et al.*, 2001) was set up to confirm the 46 samples that tested positive to blackeye cowpea mosaic virus (BICMV) in antigen-coated plate enzyme-linked immunosorbent assay.

Cylindrical inclusions forward (CI-F) and reverse (CI-R) primers with sequences 5‟-CGI VIG TIG GIW SIG GIA ART CIA C-3‟ and 5‟-ACI CCR TTY TCD ATD ATR TTI GTI GC-3‟ designed by Ha *et al.* (2008) to amplify the cylindrical inclusions coding region of Potyvirus genomes were used.

The reaction mixture was prepared from 6.38 µl sterile distilled water, 2.5 µl of 5X green GoTaq Flexi buffer, 0.25 µl deoxyribonucleotide triphosphate (dNTPs), 0.75 µl of $MgCl_2$, 0.25 µl of forward and reverse primers and 0.06 µl of both Taq polymerase and reverse-transcriptase per tube. Two microlitres of the extracted nucleic acid template was added to each tube to make a total reaction volume of 12.5 µl. One healthy, two positive controls and a blank (only buffer) were also included.

Samples were spanned down and reactions were run in an Eppendorf Master Cycler machine (Techne TC-512 Thermal cycler, Germany) under the following thermal cycling conditions: reverse transcription at 44 ^{o}C for 30 min followed by 35 cycles of

denaturation at 94 ºC for 60 s, primer annealing at 54 ºC for 120 s, extension at 72 ºC for 60 s and a final extension at 72 ºC for 3 min. PCR products were brought out after 1h and kept in a freezer at 4 ºC for further use.

3.11.1 Agarose gel Electrophoresis for RT-PCR Products

Amplified RT-PCR products (46 samples) from the field leaf samples were resolved on 1.5% agarose gel. The gel was prepared by mixing 3g of agarose with 200 ml of 1X TAE buffer in a beaker. The solution was kept in an oven at 10 ºC for 10 min until agarose totally dissolved in the buffer. The solution was allowed to cool and then 6 µl ethidium bromide was added as staining solution to aid viewing under ultra violet light. The gel was poured into a tray with combs and allowed to set. The PCR products, 12.5 µl each, were loaded into each well in an electrophoretic tank containing 1X TAE buffer and run at 120 volts for 45 min. Four microlitres of one hundred (100) base pair DNA marker was used as a ladder. The gel was removed after separation and viewed under a UV trans-illuminator. Photograph of gel was taken and sample DNA bands were scored as present (+) or absent (-) using the 100bp marker with respect to the expected amplicon size of 720bp.

3.12 RT-PCR Virus Confirmation for Four Symptomatic Screen House Cowpea Seedlings for Seed Transmission of Cowpea Viruses

RT-PCR assay was done to confirm the four randomly selected symptomatic screen house cowpea seedlings: Amantin 2A and 2B, Ejura 2A and 2B, that tested positive to BICMV in ACP-ELISA. The general Potyvirus CI F/R primers (5‟-CGI VIG TIG GIW SIG GIA ART CIA C-3‟ and 5‟-ACI CCR TTY TCD ATD ATR TTI GTI GC-3‟) were used. The reaction mix was prepared from 51.04 µl sterile distilled water, 20 µl of 5X green Go Taq Flexi buffer, 2 µl deoxyribonucleotide triphosphate (dNTPs), 6 µl of $MgCl_2$, 2 µl of forward and reverse primers and 0.48µl of both Taq polymerase

and Reverse-transcriptase in an eppendorf tube. The reaction mix (10.5µl) was put into eppendorf tubes containing 2 µl of the extracted nucleic acid template with the aid of a pippette. Samples were spanned down, and then loaded into the Eppendorf Master Cycler machine. Reactions were run under the following thermal cycling conditions: initial denaturation (Reverse-transcription) at 44 ^{o}C for 30 min followed by 35 cycles of denaturation at 94 ^{o}C for 60 s, primer annealing at

54 ^{o}C for 120 s, extension at 72 ^{o}C for 60 s and a final extension at 72 ^{o}C for 3 min.

PCR products were brought out after 1h and kept in the freezer at 4 ^{o}C.

3.12.1 Agarose gel electrophoresis for RT-PCR products

RT-PCR products (4 samples) from the screen house seedlings were separated on 1.5% agarose gel. The gel was prepared from 1.5g agarose in 100ml of 1X TAE buffer in a beaker. The solution was kept in an oven for 5 min until agarose totally dissolved in the buffer. The solution was allowed to cool and then 3 µl ethidium bromide was added to the prepared gel in the beaker to aid viewing under ultra violet light. The gel was poured into a 100 ml electrophoresis tray with combs and allowed to set. The PCR products (12.5 µl each) were loaded into each well in an electrophoretic tank containing 1X TAE buffer and the current switched on 120 volts for 40 min. Four microlitres of a 100 bp DNA marker was used as ladder. The gel was removed after separation of PCR products and viewed under a UVP-White/UV transilluminator. Photograph of the gel was taken and bands were scored as described

earlier.

3.14 Data Analysis

The Statistical Analysis Software (SAS) package (version 9.4, SAS Institute Inc., Cary, NC) was used to analyse the survey data to determine the incidence and severity of

cowpea viral disease symptoms in the surveyed areas. Statistical Package for the Social Sciences (SPSS) software (version 16.0) was used to analyse the data from the questionnaire to determine farmers' perception of cowpea viral diseases in the surveyed areas.

CHAPTER FOUR

4.0 RESULTS

4.1 Farmers' Perception of Cowpea Viral Diseases in the Surveyed Areas During the survey, it was revealed that majority (64%) of the farmers adopted the row method of planting cowpea, while 36% planted randomly on the field (Table 4.1). Weeds were observed on most of the fields on which random planting was done. Most fields in the surveyed areas had been cultivated to cowpea for over four years, yet their production (output) trends have remained the same (Table 4.1).

Farmers, especially those in Ejura-Sekyedumasi, keep their seeds from previous harvest for sowing during the next growing season. Those who sold out all their seeds due to lack of appropriate storage facilities obtained seeds mainly from local markets in Ejura-Sekyedumasi or within the municipal or districts where the seeds were produced, for sowing the next season. Most cowpea farmers in Amantin-Atebubu, Mampong and Nkoranza obtained seeds mainly from Ejura-Sekyedumasi for sowing (Table 4.2).

It was observed that most of the farmers preferred the local varieties Alancash and Uganda for their good taste and relatively greater yields, respectively (Table 4.1). However, the survey revealed that all the eight local cowpea varieties (Table 4.1) encountered during the study, especially the two preferred ones were highly susceptible to viral diseases. Further, the farmers revealed that severe yellowing of cowpea leaves had been occurring over the past six years and the trend had been increasing till date, although they were not aware of the causes. Farmers practiced strict calendar spraying of their fields with insecticides such as cymethoate (cypermethrin and dimethoate) and sunpyrifos (chlorpyrifos-ethyl) once or twice every two weeks, yet symptoms prevailed.

Table 4.1: Frequency of Responses to Questions by Farmers

Activity	Per cent frequency of respondents								Total
Planting method	Rows	Random							
	64	36							100
Period of cowpea cultivation	1-3 years	4-6 years	7-10 years	>10 years					
	14	36	25	25					100
Production trend	Increasing	Same	Decreasing						
	24	68	8						100
Seed source	Own	Agro-input	Market						
	49	1	50						100
Preferred variety	Alancash	Uganda	Yekoyenim	Burkina	Yaminu	Mallamyaya	Adamu	Pangaabu	
	28	31	10	4	8	17	1	1	100
Main reason of preference	No reason	Tasty	High yielding	Good	Disease				
	2	22	44	Market 20	tolerance 12				100
Major constraint of variety	None	Disease susceptibility	Row planting	Weediness	Poor growth				
	2	57	2	13	26				100
Period of virus disease problem	1-3 years	4-6 years	7-10 years	>10 years					
	42	43	10	5					100

Table 4.1cont'd: Frequency of responses to questions by farmers cont'd

Activity	**Per cent frequency of respondents**				Total
Cowpea virus disease trend	Decreasing	Same	Increasing		
	4	31	45		100
Yield loss due to *Aphis craccivora*	Low	Modest	High		
	53	11	36		100
Yield loss due to larva and adult *Pieris*	Low	Modest	High		
	58	4	38		100
Yield loss due to leaf yellowing	Low	Modest	High		
	19	54	27		100

Table 4.2 Market Locations where Cowpea Seeds are obtained by Farmers

	Market locations/Per cent respondents				
Locations	Ejura	Mampong	Atebubu	Nkoranza	Total
Ejura	100	0	0	0	100
Mampong	60	40	0	0	100
Atebubu	72	0	28	0	100
Nkoranza	72	20	0	8	100

Insects collected on the field were identified as aphid (*Aphis craccivora* Koch) (Plate 4.1), broad-bodied dragonfly (*Libellula depressa* L.), leaf-footed bug (*Leptoglossus phyllopus* L.), cotton stainer (*Dysdercus suturellus* H.-Schf) and larva and adult grasshopper (*Pieris brassicae* L.). Although the grasshoppers and aphids caused damage to plants on the field, yield loss due to these insects was reported to be low (Table 4.1). On the contrary, farmers revealed that severe leaf yellowing (mosaic and mottling) most often resulted in relatively higher yield losses (Table 4.1).

Plate 4.1 Aphids on the Stem and underside of cowpea leaves

4.2 Incidence and Severity of Cowpea Viral Disease Symptoms in the Surveyed Areas

Viral symptoms were observed in all field surveys across Mampong Municipal, Amantin-Atebubu, Ejura-Sekyedumasi and Nkoranza Districts. Fifty percent of the plants examined in Amantin-Atebubu, Ejura-Sekyedumasi, Mampong and Nkoranza each had symptom severity values ranging within 2.95 and 2.66, 3.16 and 2.19, 3.00 and 2.42, 2.57 and 2.09, respectively. This shows that the cowpea plants examined in Ejura-Sekyedumasi and Mampong had more severe viral symptoms (50% of total plants surveyed had symptoms on 50% on individual plants) as compared to AmantinAtebubu and Nkoranza Districts. There were no significant differences in cowpea viral disease severities recorded in Amantin-Atebubu District and Mampong Municipal (Boxes overlap with the same median).

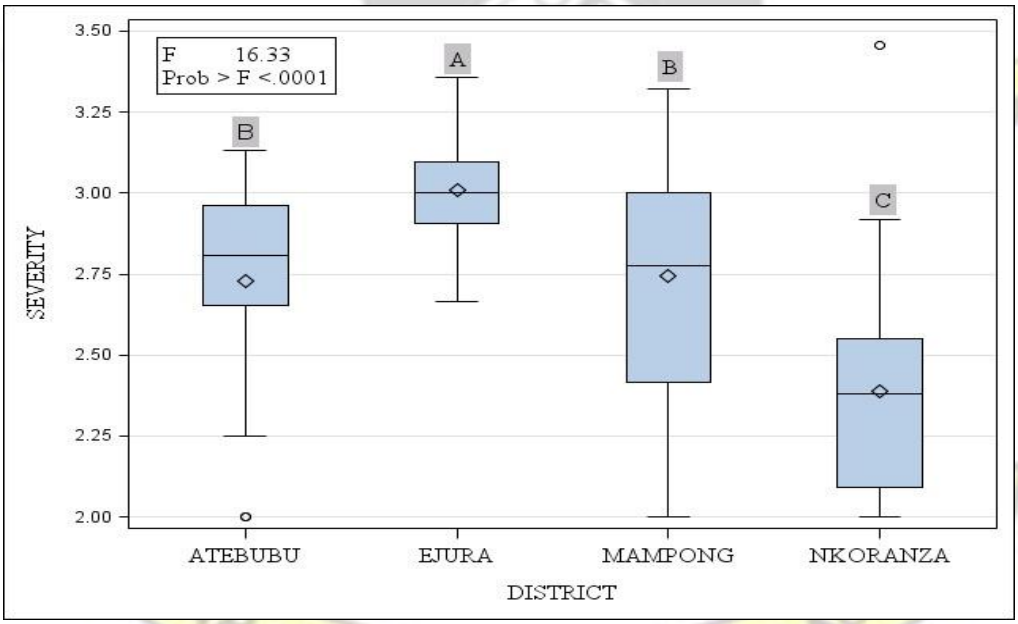

Fig.4.1. Box plots with Whiskers Showing Mean Severity Indices of Cowpea Viral Symptoms across Four Municipal and Districts in Ghana.

Severity indices of box plots with the same letter on whisker have no significant differences in symptom severities among them. However, the symptom severity observed in Ejura-Sekyedumasi was greater than that observed in Amantin-Atebubu and Mampong (Boxes

overlap but medians are different). Also, the symptom severity observed in Nkoranza was less than that observed in Amantin-Atebubu, Mampong and Ejura-Sekyedumasi (No overlap of boxes). The mean severity scores of symptoms observed in Amantin-Atebubu, Ejura-Sekyedumasi, Nkoranza Districts and Mampong Municipal were 2.72, 3.01, 2.38 and 2.74, respectively. The median values recorded were 2.80 in Amantin-Atebubu and Mampong, 3.00 in EjuraSekyedumasi and 2.36 in Nkoranza. The mean symptom severity scores observed in Amantin-Atebubu and Mampong were lower than their corresponding medians thus, suggesting a negative skewness in cowpea viral disease symptoms in both areas. Symptom severity scores of plants observed in Nkoranza were positively skewed whiles that observed in Ejura-Sekyedumasi was normally distributed. Mampong recorded the highest symptom severity range/variation (0.59), followed by Nkoranza (0.48), then Amantin-Atebubu (0.29). Symptom severities observed in EjuraSekyedumasi were not widely spread (0.25). Few outliers were observed in the Amantin-Atebubu and Nkoranza Districts.

Fig. 4.2 shows that 50% of the plants examined in Amantin-Atebubu, Ejura-Sekyedumasi, Mampong and Nkoranza each had percent incidences ranging within 83 and 63, 86 and 80, 83 and 63, 56 and 34%, respectively. This shows that Ejura-Sekyedumasi District had the highest and Nkoranza recorded the least incidence of cowpea viral infections. Percentage incidence of cowpea viral infections observed in Amantin-Atebubu District and Mampong Municipal had no significant differences between them (Boxes overlap with the same median). However, Ejura-Sekyedumasi recorded a percentage incidence greater than that observed in Amantin-Atebubu and Mampong (Boxes overlap but medians are different). Further, the percentage incidence of cowpea viral infections observed in Nkoranza was less than that observed in Amantin-Atebubu, Mampong and Ejura-Sekyedumasi (No overlap of boxes). The mean incidence of viral infections observed in Amantin-Atebubu, Ejura- Sekyedumasi, Nkoranza Districts and

Mampong Municipal were 72.5, 81.6, 46.7 and 70.7%, respectively. Also, the median values recorded were 73% in Amantin

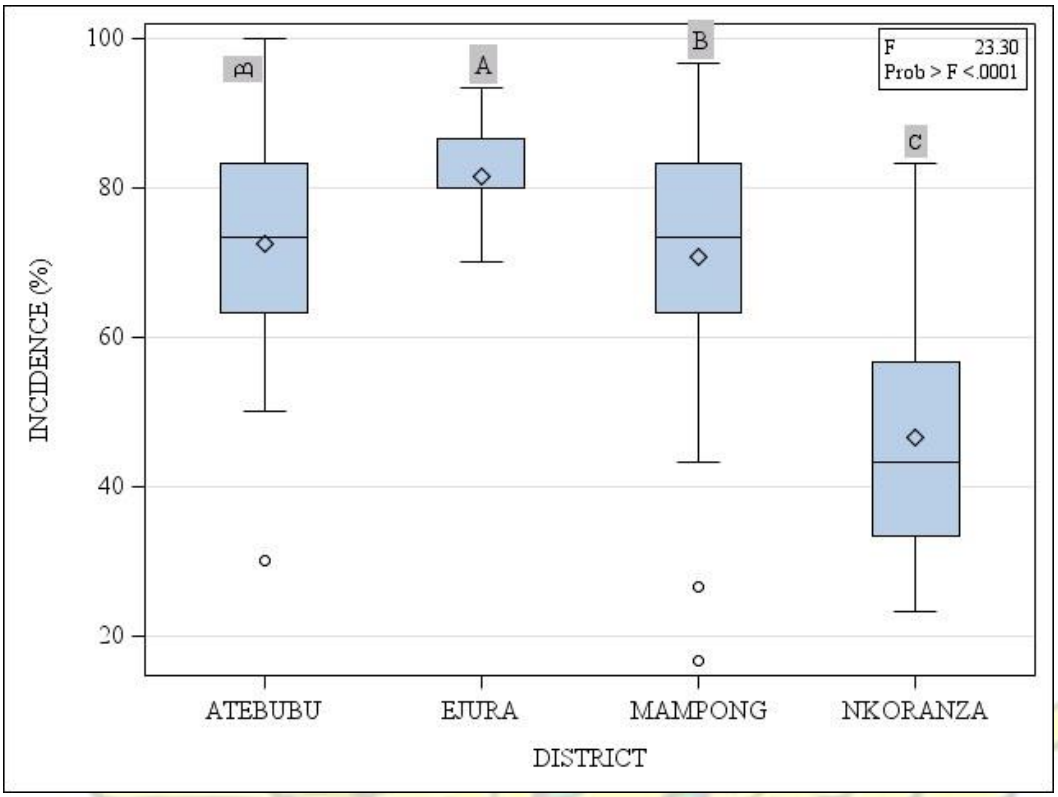

Fig.4.2 Box Plots with Whiskers showing the Percent incidence of Cowpea Viral Infection Observed across Four Municipal and Districts in Ghana.

Percent incidences of box plots with the same letter on whisker have no significant differences in incidences among them.

Atebubu and Mampong, 80% in Ejura and 43% in Nkoranza. Percent incidences of cowpea viral infections observed in Amantin-Atebubu and Mampong were negatively skewed, whiles that observed in Ejura-Sekyedumasi and Nkoranza were positively skewed. Amantin-Atebubu District and Mampong Municipal recorded the same level of variations/spread in disease incidence (20%). However, greater variation of incidence (22%) was observed in Nkoranza with Ejura recording the least variation

(6%) in cowpea viral infection incidence. Few outliers were observed in the AmantinAtebubu District and Mampong Municipal.

4.3 Prevalence of Characteristic Viral Disease Symptoms on the Surveyed Fields

Plate 4.2 shows the characteristic viral symptoms observed on cowpea plants during the survey. The result showed that mild leaf mosaic and mild mottling symptoms (Plate 4.2) were the most prevalent in all the locations surveyed.

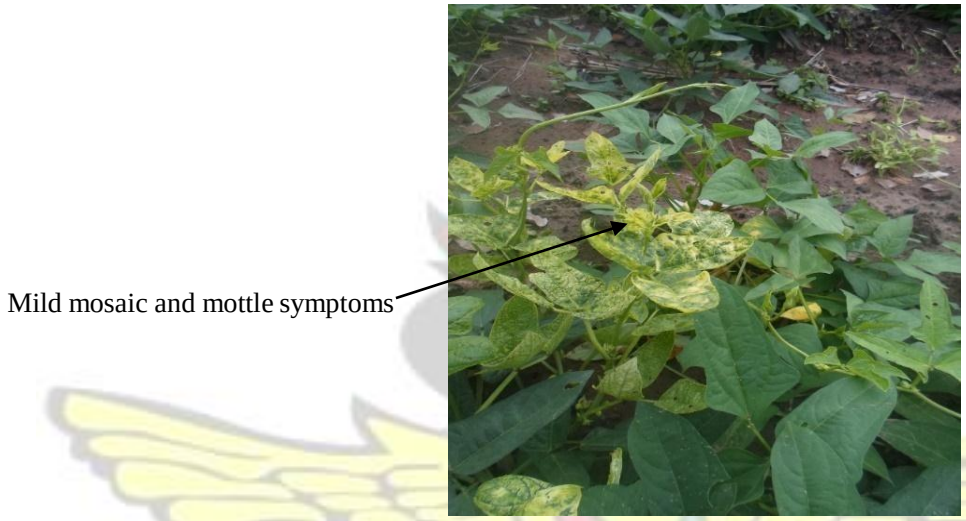

Plate 4.2 Leaf Mosaic and Mottling Symptoms on the Middle to Lower Portions of Cowpea Plant

Mampong had the highest prevalence of 36% followed by Atebubu (34.5%) and then Nkoranza (34.1%). Ejura recorded the least prevalence (28%) of mild mosaic and mottling symptoms. Severe leaf mosaic, mottling, puckering and necrosis (Plate 4.3) were the second most rampant viral symptoms observed.

Plate 4.3 Symptoms of Necrotic Lesions on Cowpea Plants

Table 4.3 Percentage Mean Incidence of Characteristic Virus Symptoms Observed on Cowpea during the Survey

	% mean incidence/viral symptom types/location				
Locations	1	2	3	4	5
Amantin-Atebubu	27.4	34.5	20.3	17.5	0.3
Ejura-Sekyedumasi	18.4	28.0	26.0	26.1	1.5
Mampong	29.3	36.0	14.9	18.0	1.8
Nkoranza	53.5	34.1	5.6	6.7	0.1

Symptoms key: 1=No visible symptom; 2=mild mosaic, mild mottle; 3=mosaic, mottle, puckering, necrosis; 4=severe mosaic, severe mottle, severe puckering, severe necrosis; 5=severe mosaic, severe mottle, severe necrosis, severe stunting, deformation, death fields in Ejura had the highest (26.1%) occurrence of these symptoms. Mampong and Atebubu recorded mean values of 18 and 17.5%,

respectively. Fields in Nkoranza had the least (6.7%) value for this symptom. Mosaic, mottle, puckering and necrosis were the third most recorded viral symptoms observed throughout the survey. The fields in Ejura recorded the highest value of 26% and Nkoranza the least value of 5.6% incidence. Atebubu and Mampong recorded

percentage means of 20.3 and 14.9%, respectively. Deformation and death (Plate 4.4 A and B) were the least observed of the viral symptoms on cowpea during the survey.

Plate 4.4 A and B. Cowpea Seedlings Displaying Mosaic, Mottling, Vein-Clearing and Vein Banding Symptoms Leading to Deformation and Death, due to BCMV-BlCM Infection on Field.

Fields in all municipal and districts recorded very low mean values of deformation and death symptoms of 1.8, 1.5, 0.3 and 0.1% for Mampong, Ejura, Atebubu and Nkoranza, respectively (Table 4.3).

4.4 Experiment 1: Aphid Transmission of BCMV-BICM

Aphis craccivora transmitted BCMV-BICM to the test (healthy) cowpea seedlings in a non-persistent manner. This was observed after the ACP-ELISA test. Vein clearing symptoms appeared on the test cowpea leaves two to four weeks after inoculation. Green vein banding with interveinal chlorosis, mottle, stunting and leaf distortion symptoms were also noted after further observation of infected cowpea seedlings. No symptoms were observed on control cowpea seedlings.

4.5 Experiment 2: Mechanical (sap) Inoculation of Healthy Cowpea Seedlings for Seed Transmission of BCMV-BICM

Leaves from mechanically inoculated cowpea plants tested positive to BCMV-BICM in ACP-ELISA. BCMV-BICM was readily transmitted mechanically, with cowpea leaves expressing necrotic ring-like localized lesions which spread along the veins.

These symptoms were observed at one week after inoculation. Further symptoms observed revealed after four weeks, were mottling, vein banding and stunting. No symptoms were observed on non-inoculated cowpea seedlings which served as negative control.

4.6 Experiment 3: Virus Indexing of Symptomatic Field Leaf Samples using ACP-ELISA

ACP-ELISA test indicated the presence of seven viruses in cowpea leaves sampled across all four municipal and districts. However, their distribution varied across the different fields. All seven viruses, namely BCMV-BICM, CpMoV, CPMMV, CABMV, SBMV, CMV and CYMV were detected in leaves sampled

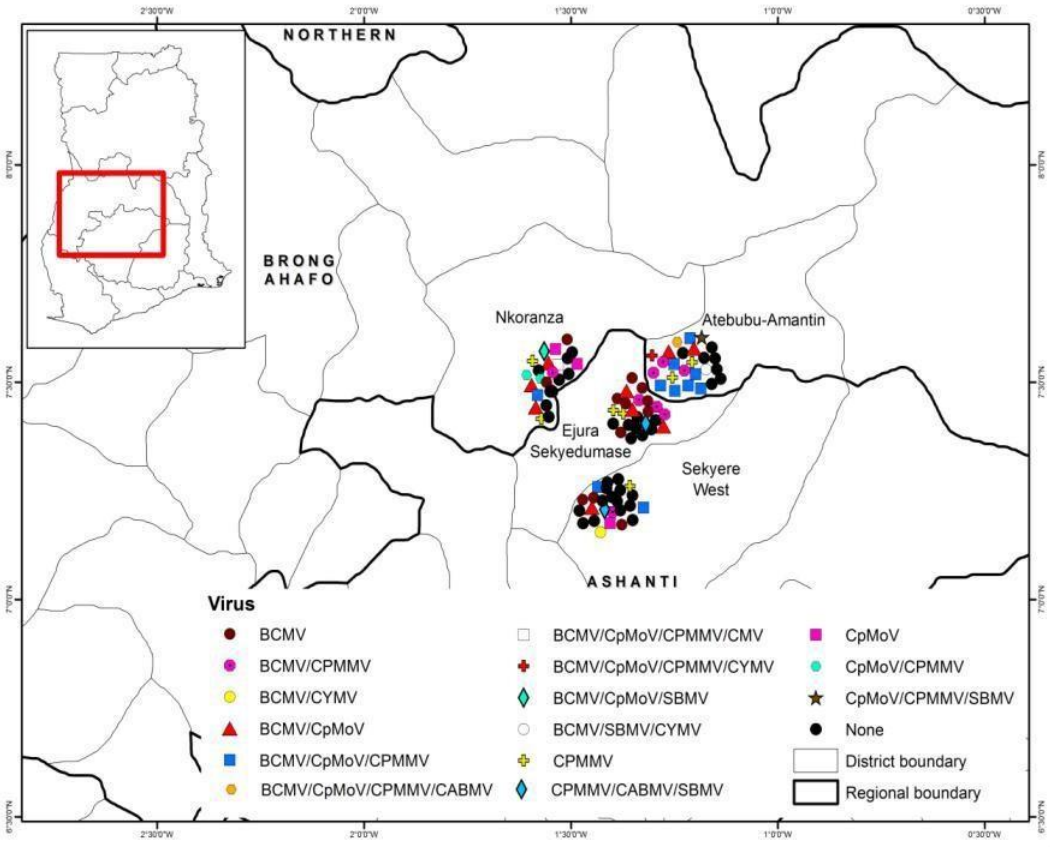

Figure 4.3 Areas Surveyed and the Viruses Detected in the Study

Table 4.4: Viruses Detected via ACP-ELISA in Field Leaf Samples of Cowpea Collected from Four Districts of Ghana

District/ Municipal	No. of fields	Total samples	Total	Total	Frequency of occurrence of viruses						
					CABM	BCMV					
Atebubu	25	25	25	18	1	15	12	2	15	0	2
Ejura	25	25	25	17	1	13	4	1	7	0	0
Mampong	25	25	25	11	1	8	4	1	5	0	1
Nkoranza	25	25	25	17	0	10	10	1	8	1	0
Total	100	100	100	63	3	46	30	5	35	1	3

From 63 out of 100 field locations (Table 4.4). The viruses occurred singly and in mixtures of two, three or four across the municipal and districts (Fig. 4.3). BICMV was

most prevalent occurring both singly and in mixtures with other viruses in a total of 46 field locations while CMV, occurring in only one location, was the least prevalent across all fields (Table 4.4). Multiple infections with all detected viruses were observed in 39 field locations. This notwithstanding, single infections with CpMoV, CPMMV and BICMV were also observed in a total of 24 field locations. No single infection with SBMV, CABMV, CMV and CYMV was observed across all locations. SBMV was detected in combination with CABMV, CMV and CYMV in five field locations across all the four municipal and districts surveyed. Further, CABMV was detected together with BICMV, CpMoV and CPMMV in the AmantinAtebubu District and with SBMV and CPMMV in the Ejura-Sekyeredumasi District and Mampong Municipal. CYMV was detected in the Amantin-Atebubu District and

Mampong Municipal while CMV occurred only in one field in the Nkoranza District. Frequent occurrence of mixtures of BICMV, CpMoV and CPMMV was observed in nine fields in the Atebubu District. Combination of four viruses (CPMMV+CMeV+CABMV+BICMV) was also detected from fields in the Atebubu

District (Appendices 3, 4, 5 and 6).

Eighteen out of 25 leaf samples tested positive to one or more combination of six viruses with frequencies of occurrence; BICMV (15), CpMoV(12), CPMMV(15),

CABMV(1), SBMV(2) and CYMV(2) in the Amantin-Atebubu district (Table 4.4).

Single infection with only CPMMV was detected from two fields (Appendix 3). However, mixed infections with all six viruses were the most common, with the viruses occurring in mixtures of two, three and four on the fields (Table 4.5).

Table 4.5 Mixed Virus Infections Detected from Symptomatic Cowpea Leaf Samples in the Mampong Municipal, Atebubu, Ejura and Nkoranza Districts.

	No. of field locations in

	Atebubu	Ejura	Mampong	Nkoranza
Two viruses				
BICMV+CpMoV	1	3	1	3
BICMV+CPMMV	3	3	1	1
CpMoV+CPMMV	0	1	0	2
BICMV+CYMV	0	0	1	0
Three viruses				
BICMV+CpMoV+CPMMV	7	0	2	1
CpMoV+CPMMV+SBMV	1	0	0	0
BICMV+SBMV+CYMV	1	0	0	0
CPMMV+CABMV+SBMV	0	1	1	0
BICMV+CpMoV+SBMV	0	0	0	1
Four viruses				
BICMV+CpMoV+CPMMV+CYMV	1	0	0	0
BICMV+CpMoV+CPMMV+CABMV	1	0	0	0
BICMV+CpMoV+CPMMV+CMV	0	0	0	1
Total	15	8	9	6

BICMV, CpMoV, CPMMV, CABMV and SBMV were detected in 13, 4, 7, 1 and 1 leaf samples, respectively, in the Ejura district (Table 4.4). Single infections with BICMV (7 samples) and CPMMV (2 samples) were observed (Appendix 4). Also, mixed infections with all five viruses occurring in combinations of two and three were observed (Table 4.5).

Eleven out of 25 leaf samples tested positive to one or more combination of six viruses with frequencies of occurrence; BICMV (8), CpMoV(4), CPMMV(5), CABMV(1), SBMV(1) and CYMV(1) in the Mampong municipal (Table 4.4). Single infection with BICMV (3 leaf samples), CpMoV (1 leaf sample) and CPMMV (1 leaf sample) were detected (Appendix 5). Mixed infections with all six viruses were also detected (Table 4.5).

BICMV, CpMoV, CPMMV, SBMV and CMV were detected in 10, 10, 8, 1 and 1 leaf samples, respectively, in the Nkoranza district (Table 4.4). Single infections with BICMV (3 samples), CpMoV (2 samples) and CPMMV (3 samples) were observed (Appendix 6). Mixed infections with all five viruses in various combinations were also observed (Table 4.5)

4.6.1 Virus Indexing of Cowpea Seeds by ACP-ELISA in the Screen House for Seed Transmission of Cowpea Viruses

Among the 24 seedlots of cowpea subjected to the growing-on test, seedlings in 17 seedlots made up of 75 symptomatic plants in all gave positive reactions to only BCMV-BICMV in ACP-ELISA (Appendix 7). Mottling and interveinal chlorosis (Plate 4.5) of leaves were observed on primary leaves of plants in six trays containing seedlings of seeds from Ejura and Amantin and five trays from Mampong. This was followed by mosaic, vein clearing, leaf puckering and distortion on the trifoliate leaves. BICMV transmission rates among growing-on test plants ranged from 0.8 to 27%. None of the 24 seedlots tested positive to CABMV, CYMV, CPMMV, SBMV, CpMoV and CMV (Appendix 7). Asymptomatic plants from all the trays tested negative to all seven antisera tested.

Plate 4.5 Mottle Symptoms on Cowpea Leaves caused by Seed-borne BICMV in the Screen house

4.7 Experiment 4: RT-PCR Virus confirmation from Field Cowpea Leaf Samples (BICMV-positive in ACP-ELISA)

Integrity test confirmed the presence of extracted nucleic acids from leaf samples. In order to obtain a more accurate identification, the 46 field samples that tested positive to blackeye cowpea mosaic virus (BICMV) in ACP-ELISA were tested in RT-PCR for CI. However, 19 out of the 46 samples amplified a 720bp amplicon, which confirmed preliminary results obtained using BICMV antiserum in ACP- ELISA (Table 4.6).

Table 4.6: RT-PCR for BICMV-infected Leaf Samples from the Field

Infection/Municipal/District/Sample

	Amantin-Atebubu										Ejura-Sekyedumasi										Mampong		Nkoranza			
Sample ID	2	3	5	7	8	19	25				37	38	41	42	46	50					53	58	76	84	94	95
BICMV	+	+	+	+	+		+	+			+	+		+	+	+	+				+	+	+	+	+	+

+ Positive samples

No amplification was observed in the other 27 samples after resolution of amplified PCR products (Plate 4.6), confirming the absence of BICMV in those samples.

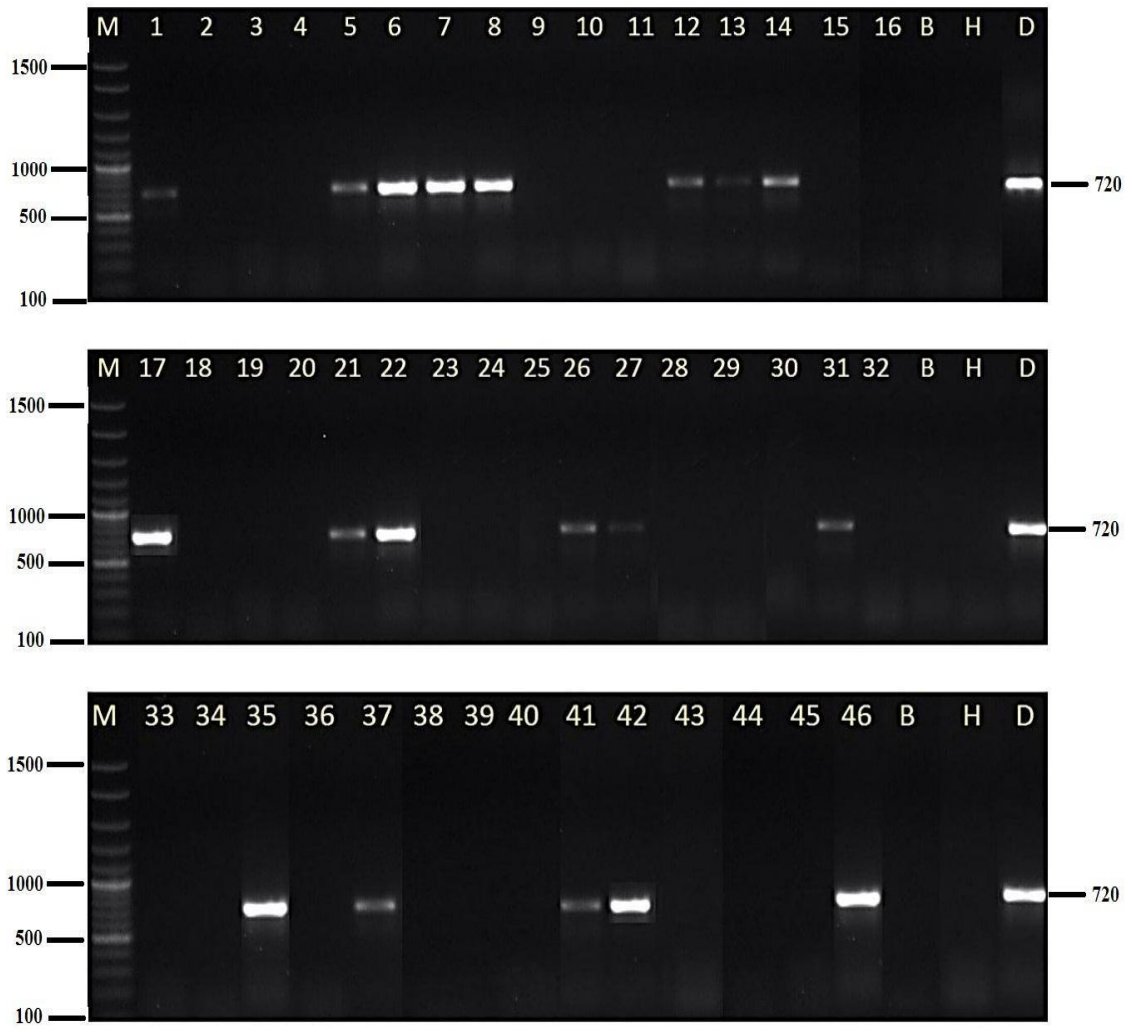

Plate 4.6 Gel showing Amplification of PCR Products

Key; M = DNA marker, H = Healthy control, B = Buffer, D = Positive control

4.8 RT-PCR Confirmation of Four Symptomatic Screen House Cowpea Seedlings for Seed Transmission of BCMV-BICM

All four samples tested from the growing-on test plants gave the expected amplicon size (720 bp) of BICMV in the cylindrical inclusions reverse transcriptase- polymerase chain reaction (CI RT-PCR) which confirmed results obtained in the antigen-coated plate-enzyme linked immunosorbent assay (Table 4.7).

Table 4.7: RT-PCR for Growing-on Test Plants from Screen House

Infection/District/Sample				
Sample ID	Ejura 2A	Ejura 2B	Amantin 2A	Amantin 2B
BICMV	+	+	+	+

+ Positive samples

CHAPTER FIVE

5.0 DISCUSSION

5.1 Farmers' Perception of Cowpea Viral Disease Symptoms in the Surveyed Areas

Thirty-six percent of farmers across all the four municipal and districts practiced random sowing of seeds on their fields exceeding the optimal plant density. Weeds such as *Tridax procumbens* and *Chromolaena odorata* were observed on such fields. However, weed control was hindered due to high plants stand. High viral infections observed on such fields may be attributed to such practices. This is in agreement with the findings of Isubikalu *et al*. (2000) who reported that high plant density may also be associated with high incidence of virus infection on the field. Sacristan *et al*. (2004) also reported that weeds can serve as alternative hosts to viruses on the field.

Some farmers kept own seeds from previous harvest whiles others obtained seeds from local markets within their vicinities to be used for the next growing season. Seeds sold in the local markets are likely to be produced from virus-infected plants within the town or village since most of the cowpea cultivated in the district or municipal are sold in local markets within the district or municipal where they were cultivated.

The farmers practiced strict calendar spraying of virus-transmitting insects with insecticides on their fields every growing season, yet viral symptoms prevailed. Proximity of the fields to each other may cause movement of virus inoculum from an infected to an uninfected field through the vectors (Dale, 2008). It was observed that most cowpea fields in Ejura, Atebubu and Mampong were relatively close to each other. Fields in Nkoranza were relatively farther apart. This could explain why fields in this district recorded the least incidence of infection although most farmers kept seeds for subsequent sowing as well. Further, within a district, planting time varied among

farmers. Consequently, pesticides are applied at different times within the growing season to control insect pests. Thus, insect pests may move to nearby unsprayed fields whiles other fields are being sprayed.

One possible explanation for the high incidence of cowpea viruses in the surveyed areas could be the highly susceptible local varieties that are preferentially grown (Orawu, 2002). Virus disease symptoms were observed in almost all the surveyed fields, and farmers reported that symptoms increased from season to season.

Most farmers prefer and therefore, cultivate two local cowpea varieties, namely Alancash and Uganda, because of their high market value and greater yields, respectively. According to the farmers, Alancash has high market value because it takes relatively shorter time to cook.

The haphazard manner in which infected plants appeared in the field confirmed virus infections instead of abiotic problems such as nutrient deficiencies and weather problems (Gilbertson, 2012). Since leaf yellowing was observed every season, some farmers in the surveyed areas considered yellowing as part of the plant's biology and not abnormal physiology, thus did not seek control measures. However, most farmers admitted that yellowing contributed to low yields. This is in agreement with Chia and He (1999) who reported that low yields of virus-infected cowpea plants is partly due to physiological stress that is associated with reduced photosynthesis as a result of infected leaves.

5.2 Incidence and Severity of Cowpea Viral Disease Symptoms in the Surveyed Areas

Generally, higher incidence of infection corresponded to higher severity of cowpea viral symptoms observed across all the four surveyed areas. This could be due to the additive effect of individual viruses or synergism among different viruses as reported in Uganda

(Amayo *et al*., 2012) because laboratory analysis of cowpea leaf samples from Ejura-Sekyedumasi and Mampong showed the presence of more than one virus.

Also, it could be attributed to the same local cowpea varieties cultivated in Ejura and Mampong and Amantin-Atebubu and Nkoranza Districts (Orawu, 2002). Farmers in Ejura-Sekyedumasi and Mampong cultivated mainly the same varieties which probably were more susceptible to the viruses than those cultivated by most farmers in Amantin-Atebubu and Nkoranza Districts.

There were no significant (*P>0.001*) differences in cowpea viral disease incidences and severities recorded in Amantin-Atebubu District and Mampong Municipal. This could be attributed to the initial source of viral inoculum in seeds used in Amantin- Atebubu and Mampong since farmers who do not keep own seeds obtained them mainly from Ejura for planting. The initial source of pathogen inoculum has an effect on disease incidence and severity later on the field (Agrios, 2005). Viral symptoms on seeds are not overtly seen. Thus recycling of farmers' own seeds from infected plants explains the high incidence and severity of symptoms observed. Booker *et al*. (2005) reported that seed transmission provides a very effective means of introducing the viruses into the crop at an early stage thus, causing primary infections throughout the planting. Also, high incidence and severity of cowpea viral infections observed could also be attributed to the highly susceptible local varieties that are preferentially grown by farmers in the districts. This is in agreement with report by Orawu in 2002. Another factor which could account for the high virus incidence and severity is the presence of weeds which serves as alternative hosts for the vectors (Sacristan *et al*., 2004). These hosts provide a favorable environment for the survival and perpetuation of the viruses as well as inoculum sources for the viruses. Further, high incidence and severity of viral symptoms in the districts could be due to the presence of cowpea insect pests,

particularly *Aphis craccivora* which is a known vector of most of the cowpea viruses (ICTVdB Management, 2006), observed on the fields during the survey.

Nkoranza recorded the least incidence and severity of cowpea viral symptoms. The survey revealed that most cowpea fields in Nkoranza as compared to the other three surveyed areas were farther apart. This could explain why fields in this district recorded the least incidence and severity of viral symptoms. This is in agreement with findings of Dale (2008) that proximity of fields to each other may cause movement of virus inoculum from an infected to an uninfected field through the vectors.

5.3 Prevalence of Characteristic Viral Disease Symptoms on the Surveyed Cowpea Fields

Symptoms such as leaf mosaic, mottling, necrosis, puckering, stunting and death that were observed have been reported previously elsewhere on cowpea infected by viral diseases (Aliyu *et al.*, 2012; Amayo *et al.*, 2012; Akinjogunla, 2005; Vanderborght and Baudoin, 2001). Higher incidence of infection corresponded to higher severity of cowpea viral symptoms observed across all four municipal and districts. In all the fields surveyed, mild mosaic and mottling were the most prevalent symptoms followed by severe mosaic, mottling, puckering and necrosis and then deformation and death of the entire plant.

The symptoms observed are indicative of the different viruses infecting cowpea in the surveyed areas. Most of the symptoms observed during the survey (mosaic, mottling, puckering, necrosis, stunting and death) were consistent with symptoms associated with infections by CABMV, BCMV-BICM, CpMoV, CPMMV and CYMV (Aliyu *et al.*, 2012; Bashir, 1992). The variations in symptoms observed on the field may be due to factors such as the type of viral strains, cowpea cultivar, the time of infection of the virus pathogen, mixed infections and/or the presence of unidentified viruses (Jones *et al.*, 1991).

5.4 Aphid Transmission of BCMV-BICM

Aphis craccivora transmitted BCMV-BICM to the Ife brown cowpea seedlings in a non-persistent manner, producing symptoms similar to those observed on the field.

This result confirms previous reports that BICMV is readily transmitted by *A. craccivora* in a non-persistent manner (Atiri *et al.*, 1986; Orawu, 2007; Damiri *et al.*, 2013). Also symptoms observed on cowpea in this study, including vein clearing, green vein banding with interveinal chlorosis, mottle, stunting and leaf distortion have been reported on BICMV-infected cowpea plants (Thottappilly and Rossel, 1985).

5.5 Mechanical (sap) Inoculation of Healthy Cowpea Seedlings for Seed Transmission of BCMV-BICM

BCMV-BICM has been reported to be mechanically transmitted through sap in cowpea plants (Orawu, 2007). Sap transmission of the BICMV isolates, with the expression of both localized and systemic symptoms in this study, confirms report by Orawu (2007). Localized symptoms included large reddish, often ring-like lesions which typically spread along the veins, forming a reddish-net pattern. Systemic symptoms included mottle, green vein banding often with interveinal chlorosis, stunting, and leaf distortion. These symptom descriptions were observed and reported by Thottappilly and Rossel (1985).

5.6 Virus Indexing of Symptomatic Field Cowpea Leaves and Seedlings in the Screen House using ACP-ELISA for Seed Transmission of Cowpea Viruses

This is the first comprehensive report on occurrence of viruses infecting cowpea in some growing areas in the Ashanti and Brong Ahafo regions of Ghana. The serological method used in this study was efficient in the detection of cowpea viruses in leaf samples. Aliyu *et al.* (2012) also detected CABMV, CYMV, BICMV and

CpMoV infecting cowpea in Nigeria, using the antigen-coated plate ELISA (ACP- ELISA).

Further, CPMMV has been reported on cowpea in Ghana (Jeyanandarajah and Brunt, 1993) while CpMoV, CYMV, CABMV and SBMV have been reported severally on cowpea in Nigeria (Aliyu *et al.*, 2012; Allen *et al.*, 1982), Senegal (Ndiaye *et al.*, 1993) and Pakistan (Bashir and Hampton, 1996) among other countries. Abdullahi *et al.* (2001) reported that ACP-ELISA was sensitive and reliable for the detection of CMV-infected cowpea in different tissues. Non-detection of viruses in the 37 locations could be due to low virus concentration in the cowpea leaf samples (Aliyu *et al*., 2012). This study further identified BCMV-BICM to be seedborne in cowpea collected from some farms in Amantin, Ejura and Mampong, with transmission rates ranging from 0.8 to 27%. None of the 24 seedlots screened were found to be infected with CpMoV, CABMV, SBMV, CPMMV, CYMV and CMV. Low transmission rates with BCMV-BICM observed in this study may be due to small number of samples taken (Ndiaye *et al*., 1993). However, Shanker *et al.* (2009) reported that sowing cowpea seeds even with low incidence of BCMV-BlCM (> 1) would result in
significant virus spread with a major influence on grain yield of cowpea. Puttaraju *et al.* (2004) also reported a 65- 100% BCMV-BlCM transmission resulting from sowing cowpea seeds with 4 - 10% infection rate. Thus, even with the relatively low seed transmission rates observed in the present study, there is a cause for concern. Threshold level below 2% infection for cowpea seeds has been reported to be suitable to avoid the risk of economic losses due to the spread of BCMV-BlCM in cowpea
(Shanker *et al.*, 2009). Further, seedborne cowpea viruses have detrimental effect on cowpea production causing stunting and plant deformation in early growth stage and not allowing the plants to reach their full potential (Booker *et al.*, 2005).

The number and distribution of viruses detected in this study are an indication of potential greater losses than is being observed currently on cowpea production in

Ghana. Also, the number of multiple virus infections detected in this study gives an indication of the presence of virus-complex infections within the study area and possibly within the country. Several reports have shown that multiple virus infections are usually associated with higher disease severity and yield reduction (Kareem and Taiwo, 2007; Taiwo and Akinjogunla, 2006). Leaf samples from 37 locations tested negative, although samples were symptomatic. This could be due to low virus concentrations in the leaf samples. It may also be due to the presence of serologically variable strains of the viruses and the non-availability of antibodies specific to them (Aliyu *et al.*, 2012).

5.7 RT-PCR Virus Confirmation for Symptomatic Field Cowpea Leaves and Seedlings (both BICMV-positive in ACP-ELISA) in the Screen House for Seed Transmission of BCMV-BICM

Although detection of BCMV-BICM in cowpea leaves and seeds have been reported previously from Ghana (Zettler and Evans, 1973), most of the previous detections were based on growing-on test, host range and reactivity to polyclonal antibodies. In the present study, presence of the most prevalent virus detected in ELISA, BICMV was assessed with RT-PCR for CI and the results confirmed BICMV from 19 out of 46 leaf samples that tested BICMV-positive in ACP-ELISA. Symptomatic screenhouse plants (growing-on test) were also confirmed to be BICMV-positive with RT- PCR for CI. The expected amplicon of 720 bp was observed in the positive samples.

However, the CI primer pair used failed to amplify BICMV in the remaining 27 field leaf samples, although positive reactions were observed in ACP-ELISA. BCMV-BICMV and CABMV are potyviruses which are considered serious pathogens on cowpea worldwide (Shanker *et al.*, 2009).

Field plants succumb to infections from virulent strains although some resistance has been introduced into breeding lines and commercial varieties (Hampton *et al.*, 1997). BICMV and CABMV are difficult to distinguish on cowpea based on symptoms alone (Dijkstra *et al.*, 1987; Taiwo *et al.*, 1982) and also with serological methods which employ the use of polyclonal antibodies. They can only be differentiated via serological methods which use monoclonal antibodies and molecular tools (Shanker *et al.*, 2009). The non-amplification of 27 field leaf samples in RT-PCR, although they gave positive reactions in ELISA, could possibly be due to cross-reactions in ACPELISA with polyclonal antibodies. Another likely reason for this observation could be that the viruses detected serologically may only be closely related to BICMV, thus suggesting the need for a more specific primer to identify the viruses.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

The study revealed farmers' perception of cowpea viral disease incidence and severity in the surveyed areas which influenced their activities. Farmers' activities which included high cropping density as a result of haphazard sowing methods, recycling of seeds from season to season, closeness of fields to each other with different planting and pesticide application periods as well as preference for and cultivation of susceptible cowpea cultivars, increased the incidence and severity of cowpea viruses.

Incidence and severity of cowpea viral disease symptoms were highest in the Ejura-Sekyedumasi District and least in the Nkoranza District. Incidence of infections and severity of cowpea viral disease symptoms in the Mampong Municipal and Atebubu-Amantin districts were not significantly different. Most viral symptoms observed on the field during the survey, which included mosaic, mottling, puckering, necrosis, deformation and death, were consistent with symptoms associated with cowpea viral diseases.

Aphis craccivora transmitted BCMV-BICMV in a non-persistent manner with the test plants expressing symptoms similar to those observed on the field. BCMV- BICMV was also transmitted mechanically through sap onto susceptible Ife brown cowpea test plants. The survey result provided a reliable confirmation of seven viruses on cowpea fields in the Ashanti and Brong Ahafo Regions of Ghana. It also presented for the first time at first hand, basic information about cowpea virus distribution in the areas that were examined. The viruses occurred in groups of two to four and were represented on the field by symptoms that were not specific to a particular virus.

BCMV-BICMV was found to be seedborne from infected cowpea fields. The number of multiple viral infections detected and the distribution of viruses observed in this study, suggest imminent greater losses in cowpea production in the country as multiple virus infections are usually associated with higher disease severity and yield reduction.

Serological detection (ACP-ELISA) of majority of symptomatic field leaf samples tested positive to one or more of seven viruses tested. However, some samples tested negative probably due to low virus concentrations or the presence of serologically different strains which could be detected only with antibodies specific to them when identified. Confirmation of ACP-ELISA results using RT-PCR showed that some BCMV-BICMV isolates detected in ACP-ELISA were perhaps only serologically related to BICMV and thus required a specific primer since the same cylindrical inclusion primer could not confirm all samples.

6.2 Recommendations

- Since the study covered only Ashanti and Brong Ahafo regions, a further extensive study covering other cowpea growing areas with the use of more specific antibodies and primers is required to identify species as well as other viruses present in the country.
- Mere identification of these viruses is not enough. Sequencing should be done for specific identification of the disease-causing agents.
- The use of resistant cultivars is the most effective method of managing cowpea viral diseases. There is the need therefore to study and identify virusresistant genes in cowpea varieties to enable breeders incorporate into preferred but susceptible varieties present in the surveyed areas.

- Also, it is necessary to produce virus-free seeds in order to prevent farmers from sowing infected seed stock to reduce infection.

REFERENCES

Abdullahi, I., Ikotun, T., Winter, S., Thottappilly, G. and Atiri, A. (2001). Investigation on seed transmission of cucumber mosaic virus in cowpea. *African Crop Science Journal*, 9(4): 677-684.

Adekola, F. O. and Oluleye, F. (2007). Influence of mutation induction on the chemical composition of cowpea, *Vigna unguiculata* (L.) Walp. *African Journal of Biotechnology*, 18 (6): 2143-2146.

Agrios, G. E. (2005). *Plant Pathology, fifth edition,* Academic press, New York. Pp. 952.

Ahenkorah, K., Adu Dapaah, H. K. and Agyeman, A. (1998). Selected nutritional components and sensory attributes of cowpea (*Vigna unguiculata* (L.) Walp) leaves. *Journal of Plant Foods for Human Nutrition,* 52: 221 – 229.

Ajeigbe, A. H., Singh, B. B. and Emechebe, M. A. (2008). Field evaluation of improved cowpea lines for resistance to bacterial blight, virus and striga under natural infestation in the West African Savannahs. *African Journal of Biotechnology*, 20 (7): 3563-3568.

Akinjogunla, O. J. (2005). Effects of single and mixed inoculation with viruses on symptomatology, growth, yield and nutritive content of cowpea: *MSc Thesis. University of Lagos, Nigeria.*

Akinjogunla, O. J., Taiwo, M. A. and Kareem, K. T. (2008). Immunological and molecular diagnostic methods for detection of viruses infecting cowpea (*Vigna unguiculata*). *African Journal of Biotechnology*, 1: 2099-2103.

Aliyu, T. H., Balogun, O. S. and Adeoti, O. M. (2010). Pathogenic responses of cowpea (*Vigna unguiculata* (L.) Walp) inoculated with cucumber mosaic virus to soil amendment with Neem leaf powder. *Agrosearch*, 11: 99 – 110.

Aliyu, T. H., Balogun, O. S. and Kumar, L. (2012). Survey of the symptoms and viruses associated with cowpea in the agro ecological zones of Kwara State, Nigeria. *Ethiopian Journal of Environmental Studies and Management EJESM*, 4(5): 2.

Allen, D. J. (1983). The pathology of tropical food legumes. John Wiley and Sons, Chichester.

Allen, D. J., Thottappilly, G. and Rossel, H. W. (1982). Cowpea mottle virus: Field resistance and seed transmission in virus-tolerant cowpea. *Annals of Applied Biology*, 100: 331-336.

Almeida, A. M. R., Piuga, F. F., Marin, S. R., Kitajima, E. W., Gaspar, J. O. and Moraes, T. G. (2005). Detection and partial characterization of a carlavirus causing stem necrosis of soybeans in Brazil. *Fitopatologia Brasilia*, 30: 191-194.

Amayo, R., Arinaitwe, A. B., Mukasa, S. B., Tusiime, G., Kyamanywa, S., Rubaihayo, P. R. and Edema, R. (2012). Prevalence of viruses infectingcowpea in Uganda and their molecular detection. *African Journal of Biotechnology*, 77 (11): 14132 – 14139.

Amoah, A. B. (2010). Efficacy of ethanolic extract of the *Vetia peruviana* (pers.) K. schum. (Milk bush) root in the control of major insect pests of cowpea. *MSc*

Thesis, Department of Crop and Soil Sciences, KNUST, Kumasi-Ghana, Pp. 8.

Anderson, C. W. (1955). *Vigna* and *Crotalaria* viruses in Florida, notations concerning cowpea mosaic virus (*Marmor vignae). Plant Disease Reporter*, 39:349-353.

Anderson, C. W. (1959). *Vigna* and *Crotalaria* viruses in Florida. Preliminary report on strain of cucumber mosaic virus obtained from cowpea plants. *Plant Disease Reporter,* 39: 346 – 348.

Anderson, E. J., Kline, A. S., Kim, K. S., Geoke, S. C. and Albriton, C. W. (1994). Identification of cowpea stunt disease in central Arkansas. *Arkansas Farm Research, 43: 14-15.*

Atiri, G.I., Enobakhare, D.A. and Thottappilly, G. (1986). The importance of colonizing and non-colonizing aphid vectors in the spread of Cowpea aphid-borne mosaic virus in cowpea (*Vigna unguiculata*). *Crop Protection*, 5: 406-410.

Bariana, H. S., Shannon, A. L., Chu, P. W. G. and Waterhouse, P. M. (1994). Detection of five seed borne legume viruses in one sensitive multiplex polymerase chain reaction test. *Phytopathology* 84, 1201–5.

Bashir, M. (1992). Serological and biological characterization of Blackeye Cowpea Mosaic and Cowpea Aphid-borne Mosaic Potyvirus isolates seed-borne in *Vigna unguiculata* (L.) Walp. *Ph.D. Thesis. Oregon State University, Corvallis, Oregon. USA.*

Bashir, M. (2000). Concept of host resistance to plant pathogens. *Science, Technology and Development*, 4: 1 – 6.

Bashir, M. and Hampton, R. O. (1993). Natural occurrence of five seed-borne cowpea viruses in Pakistan. *Plant Disease*, 77: 948- 951.

Bashir, M. and Hampton, R. O. (1996). Detection and identification of seed-borne viruses from cowpea (*Vigna unguiculata* (L.) Walp) germplasm. *Plant Pathology*, 45: 54-58.

Bashir, M., Ahmad, Z., Ghafoor, A. (2002). Cowpea aphid-borne mosaic potyvirus: A review. *International Journal of Pest Management*, 48: 155- 168.

Behncken, G. M. and Maleevsky, L. (1977). Detection of Cowpea aphid-borne mosaic virus in Queensland. *Australian Journal of Experimental Agriculture and Animal Husbandry*, 17: 674-678.

Booker, H. M., Umaharan, P. and McDavid, C. R. (2005). Effect of Cowpea Severe Mosaic Virus on crop growth characteristics and yield of cowpea. *Plant Disease*, 89: 515-520.

Boxtel, J., Singh, B. B., Thottappilly, G. and Maule, J. A. (2000). Resistance of cowpea breeding genotypes to Blackeye cowpea mosaic and Cowpea aphid-borne mosaic potyvirus isolates under experimental conditions. *Journal of Plant Diseases and Protection*, 107: 197-204.

Bozarth, R. F. and Shoyinka, S. A. (1979). Cowpea Mottle Virus. *CMI/AAB Descriptions of Plant Viruses*, No. 212. Commonwealth Mycological Institute/Association of Applied Biologist, Kew, England.

Brunt, A. A, Crabtree, K., Dallwitz, M. J., Gibbs, A. J., Watson, L. and Zurcher, E. J. (1996). Plant Viruses Online: *Descriptions and Lists from the VIDE Database*. Version 16th January 1997.

CAB International/European and Mediterranean Plant Protection Organization (CABI/EPPO) (2010). Cowpea aphid-borne mosaic virus [Distribution map]. *Distribution maps of plant diseases, Wallingford, UK: CABI*, Map 1075 (Edition 1).

Chalam, V. C., Parakh, D. B., Khetarpal, R. K., Maurya, A. K., Jain, A. and Singh, S. (2008). Interception of seed-transmitted viruses in cowpea and mungbean germplasm imported during 2003. *Indian Journal of Virology* 19 (1): 12-16.

Chant, S. R. (1960). The effect of infection with Tobacco mosaic and Cowpea yellow-mosaic viruses on the growth rate and yield of Cowpea in Nigeria. *Empire Journal of Experimental Agriculture*, 110(28): 114-120.

Chenelu, V., Sachchidananda, J. and Mehta, S. C. (1968). Studies on a mosaic disease of cowpea from India. *Phytopathologische Zeitschrift,* 63: 381 – 387.

Chia, T. F. and He, J. (1999). Photosynthesis capacity in *Oncidium* (Orchidaceae) plants after virus eradication. *Environmental and Experimental Botany*, 42: 11 – 16.

Clark, M. F. and Adams, A. N. (1977). Characteristic of the microplate methods of enzyme linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* 34: 475-483

Cucumber mosaic virus. *Advances in Virus Research,* 41: 281–348.

D. H. L., Carstens, E. B., Estes, M. K., Lemon, S. M., Maniloff, J., Mayo,

Dale, W. T. (2008). Observations on a virus disease of cowpea in trinidad. *Annals of Applied Biology*, 36: 327-333.

Damiri, B. V., Al-Shahwan, I. M., Al-Saleh, M. A., Abdalla, O. A. and Amer, M.

Dellaporta, S. L., Wood, J. and Hicks, J. B. (1983). A plant DNA minipreparation version II. *Plant Molecular Biology Reporter,* 1: 19-21.

Demski, J. W. and Kuhn, C. W. (1989). Cowpea mild mottle virus. In: *Compendium of soybean diseases (3rd edition). American Phytopathological Society,* St. Paul, USA, pp. 60-61.

Dijkstra, J., Bos, L., Bouromeester, H. J., Hadiastono, T. and Lohuis, H. (1987). Identification of Blackeye cowpea mosaic virus from germplasm of yard- long bean and from soybean, and the relationships between Blackeye cowpea mosaic and Cowpea aphid-borne mosaic viruses. *Netherlands Journal of Plant Pathology*, 93: 115-133.

Fischer, H. U. and Lockhart, B. E. (1976). A strain of cucumber mosaic virus isolated from cowpea in Morocco. *Phytopathologische Zeitschrift,* 85: 132-138.

Fitter, A. H. and Hay, R. K. M. (1987). *Environmental physiology of plants. (2nd Edition) Elsevier Academic Press,* London.

Fitter, A. H. and Hay, R. K. M. (1987). Environmental physiology of plants. Academic Press, London

Food and Agriculture Organization (2012). FAOSTAT Gateway http://faostat3.fao.org/faostat-gateway/go/to/download/Q/QC/E Accessed on 04/09/2014.

Fulton, J. P. and Allen, D. J. (1982). Identification of resistance to cowpea severe mosaic virus. *Tropical Agriculture,* 59: 66 – 68.

Gilbertson, R. L. (2014). Diagnosis of plant virus diseases and detection of plant viruses. IPM-CRSP tomato disease and insect pest diagnostics Workshop 2014. University of Ghana-Legon, Biotechnology Centre.

Gillaspie Jr. A. G., Pio-Ribeiro, G., Andrade, G. P and Pappu, H. R. (2001). RT-PCR Detection of Cowpea Aphid-borne Mosaic virus in Peanut from Brazil. *Phytopathology,* 91:2002.

Gillaspie, A. G., Mitchell, S. E., Stuart, G. W. and Bozarth, R. F. (1999). RTPCR method for detecting cowpea mottle carmovirus germplasm. *Plant Disease* 83: 639–643.

Gilmer, R. M., Whitney, W. K. and Williams, R. J. (1974). Epidemiology and control of cowpea mosaic in Western Nigeria. *In Proceedings of First IITA Grain Legume Workshop,* International Institute for Tropical Agriculture, Ibadan, Nigeria.

Giovanni, P., Martelli, P., Michael, J. A., Kreuze, J. F., Valerian, V. D. (2007). Family Flexiviridae: a case study in virion and genome plasticity. *Annual Review of Phytopathology,* 45: 73-100.

Gray, S. M. (1996). Plant virus proteins involved in natural vector transmission.

Gumedzoe, M. Y. D., Thottapily, G. and Asselin, A. (1997). Occurrence of Southern bean mosaic virus (SBMV) in Togo and its interaction with some cowpea cultivars. *African Crop Science Journal,* 5: 215-222.

Ha, C., Coombs, S., Revill, P. A., Harding, R. M., Vu, M. and Dale, J. L. (2008). Design and application of two novel degenerate primer pairs for the detection and complete genomic characterization of Potyviruses. *Archives of Virology*, 153: 25-36.

Hampton, R. O., Thottappilly, G. and Rossel, H. W. (1997). Viral diseases of cowpea and their control by resistance conferring genes. In: *Advances in Cowpea Research*, B. B. Singh, D. R. Mohan Raj, K. E. Dashiell and L. E.

Hartman, G. L., Sinclair, J. B. and Rupe, J. C. (1999). Compendium of soybean diseases (4rd edition). *American Phytopathological Society*, St. Paul, Minnesota, USA, pp. 182.

Hollings, M. and Brunt, A. A. (1981). Potyviruses. Pp.731-807. In: handbooks of plant virus infection and comparative diagnosis. ed. E. Kurstak, Elsevier/ North Holland. Biomedical Press Amsterdam, the Netherlands. Pp. 943.

Hughes, J. D. A., Shoyinka, S. A. (2003). Overview of viruses of legumes other than groundnut in Africa. In: *Plant virology in sub-Saharan Africa*. Proceedings of Plant Virology Workshop, IITA, Ibadan, Nigeria. eds. J. D. A. Hughes and B. Odu. pp. 553-568.

Huguenot, C., Furneaux, M. T., Thottapily, G., Rossel, H. W. and Hamilton, R.

Hull, R. (1995). Sobemovirus. In: Murphy, F. A., Fauquet, C. M., Bishop, D. H. L., Ghabrial, S. A., Jarvis, A. W., Martelli, G. P., Mayo, M. A. and Summers, M. D. Virus taxonomy: Classification and Nomenclature of viruses. Sixth report of the International Committee on Taxonomy of Viruses. Vienna, Austria. *Springer-Verlag*, pp. 376–378.

I. (1993). Evidence that Cowpea-aphid borne mosaic and blackeye cowpea mosaic viruses are different Potyviruses. *Journal of General Virology*, 74: 335-340.

Ibrahim, A. B. Y. (2012). Using morphological and physiological factors to evaluate six cowpea varieties for drought tolerance. MSc Plant breeding Thesis, Department of Crop and Soil Sciences, College of Agriculture and Renewable Natural Resource, KNUST, Kumasi-Ghana. pp. 116.

ICTVdB Management. (2006). Cowpea severe mosaic virus. In: *ICTVdB* – The Universal Virus Database, version 4. Buchen-Osmond C (ed), Columbia University, New York, USA.

International Institute of Tropical Agriculture (2000). *Crops and Farming Systems*. http://www.iita.org/crop/cowpea.htm.

International Institute of Tropical Agriculture (2009b). Cowpea. http://www.iita.org/cowpea. International Institute of Tropical Agriculture, Ibadan, Nigeria.

International Institute of Tropical Agriculture (IITA). (2009a). IITA Cereals and Legume Systems.www.iita.org/cms/details/ cowpea_project_details.asp Accessed on 04/09/2014.

Isubikalu, P., Erbaugh, J. M., Semana, A. R. and Adipala, E. (2000). The influence of farmer perception on pesticide usage for management of cowpea field pests in Eastern Uganda. *African Crop Science Journal*, 8:317- 325.

Jeyanandarajah, P. and Brunt, A. (1993). The natural occurrence, transmission, properties and possible affinities of cowpea mild mottle virus. *Journal of Phytopathology*, 137: 148-156.

Jones, J. B., Jones J. P., Stall, R. E. and Zitter T. A. (1991). Compendium of tomato diseases, Minnesota. *American Phytopathological Society*, p73.

Journal of Phytopathology, 134: 265-268.

Kaiser, W. J. and Mossahebi, G. H. (1975). Studies with Cowpea aphid-borne mosaic virus and its effect on cowpea in Iran. *FAO Plant Protection Bulletin*, 23: 33-39.

Kammen, V. A., Lent, V. J. and Wellink, J. (2001). Cowpea mosaic virus. Description of plant viruses. No. 378. *Commonwealth Mycological Institute and Association of Applied biologists.* Kew, Surrey, UK. 6 pp.

Kannaiyan, J. and Haciwa, H. C. (1993). Diseases of food legume crops for the scope of their management in Zambia. *FAO Plant Protection Bulletin*, 41:

73-90.

Kaplan, I. B., Zhang, L., Palukaitis, P. (1998). Characterization of cucumber mosaic virus, cell-to-cell movement requires capsid protein, but not virions. *Virology,* 246: 221–231.

Kareem, K. T. and Taiwo, M. A. (2007). Interactions of viruses in cowpea: effects on growth and yield parameters. *Virology Journal, 4*:15.

Kitajima, E.W., de Alcantara, B. K., Madureira P. M., AlfenasZerbini,P. J., Rezende, A. M. and Zerbini, F.M. (2008). A mosaic of beach bean (*Canavalia rosea*) caused by an isolate of Cowpea aphid-borne mosaic virus (CABMV) in Brazil. *Archives of Virology,* 153: 743-747.

Kuhn, C. W. (1990). Cowpea virus diseases in the United States: a status report. Pp 7-23. In: Cowpea Research, a US perspective. eds J. C. Miller, J. P. Miller and R. L. Ferry. MP 1639. Texas Agricultural Experimental Station. College station, TX, USA.

Kumar, L. (2009). Methods for the diagnosis of Plant Virus diseases, laboratory Manual. International Institute of Tropical Agriculture (IITA). p 94.

Laguna, I. G., Arneodo, J. D., Rodriguez-Pardina P. and Fiorona, M. (2006). Cowpea mild mottle virus infecting soybean crops in North-Western Argentina. *Fitopatologia Brasilia,* 31(3): 317.

Lamptey, R. N. L. and Hamilton, R. I. (1974). A new cowpea strain of Southern bean mosaic virus from Ghana. *Phytopathology,* 64: 110 – 114.

Lima, J. A. A., Purcifull, D. E. and Herbert, E. (1979). Purification, partial, characterization and serology of Blackeye cowpea mosaic virus. *Phytopathology,* 69: 1252-1258.

M. A., McGeoch, D. J., Pringle, C. R., Wickner, R. B.(Eds). *Seventh report of the International Committee on Taxonomy of Viruses*. *San Diego Academic Press*, Pp. 691-701.

Maciel, S. C., Nakano, D. H., Rezende, J. A. M. and Vieira, M. L. C. (2009). Screening of Passiflora species for reaction to Cowpea aphid-borne mosaic virus reveals an immune wild species. *Scientia Agricola* 66: 414-418

Madden, L. V. and Hughes, G. (1999). Sampling for plant disease incidence.

Mali, V. E., Mundhe, G. E., Patis, N. S. and Wathe, K. S. (1988). Detection and Identification of Blackeye Cowpea Mosaic and Cowpea Aphid-borne mosaic viruses in India. *International Journal of Tropical Plant Disease* 6: 159-173

Mali, V. R. and Kulthe, K. S. (1980). A seedborne potyvirus causing mosaic of cowpea in India. *Plant Disease,* 64:925-928.

Mali, V. R., Khalikar, P. V. and Gaushal, H. D. (1983). Seed transmission of potyvirus and cucumber mosaic virus in cowpea in India. *Indian Phytopathology* 36: 343.

Manyangarirwa, W., Sibiya, J. and Mortensen, C. N. (2010). Seed-borne viruses detected on farm-retained seeds from smallholder farmers in Zimbabwe, Burkina Faso, Bangladesh and Vietnam. Research application summary. *Second RUFORUM Biennial Meeting*, Entebbe, Uganda.

Matthews, R. E. F. (1981). The groups of plant viruses. In: *Plant Virology 2nd ed.* Pp. 715- 732. Academic press, New York. Pp. 897.

McLaughlim, M. R., Barnett, O. W., Burrows, P. M. and Bavm, R. H. (1981). Improved ELISA conditions for detection of plant viruses. *Journal of Virological Methods*, 3: 13-25.

Ministry of Food and Agriculture (MOFA) (2015). Districts in Ghana, Amantin- Atebubu. http://mofa.gov.gh/site/?page_id=1353 Accessed on 03/10/2015.

Ministry of Food and Agriculture (MOFA) (2015). Districts in Ghana, AmantinAtebubu.http://mofa.gov.gh/site/?page_id=1353 Accessed on 03/10/2015.

Ministry of Local Government, Rural Development and Environment (2006). *Districts in Ghana*: Ministry of Local Government, Rural Development and Environment.

Mink, G. I. and Keswani, C. L. (1987). First report of Cowpea mild mottle virus on bean and mungbean in Tanzania. *Plant Disease*, 71: 557.

Murphy, J. F., Barnett, O. W. and Witcher, W. (1984). A blackeye cowpea mosaic virus strain from South Carolina. *Phytopathology*, 74: 632.

N. Jackai (eds.). Co publication of International Institute of Tropical Agriculture (IITA) and Japan International Research Centre for Agriculture (JIRCAS), IITA, Ibadan, Nigeria, pp. 159 – 175.

Naidu, R. A. and Hughes, J. D. A. (2003). Methods for the detection of plant viral diseases in plant virology in sub-Saharan Africa, *Proceedings of plant virology Workshop*, IITA, Ibadan, Nigeria. Editors Hughes J. D. A., Odu, B, pp. 233-260.

Nascimento, A. V. S., Santana, E. N., Braz, A. S. K., Alfenas, P. F., Pio-Ribeiro, G., Andrade, G. P., Carvalho, M. G. and Zerbini, F. M. (2006). Cowpea aphid-borne mosaic virus (CABMV) is widespread in passion fruit in Brazil and causes passion fruit woodiness disease. *Archives of Virology* 151: 17971809.

Ndiaye, M., Bashir, M., Keller, K. E., and Hampton, R. O. (1993). Cowpea viruses in Senegal, West Africa: Identification, distribution, seed transmission, and sources of genetic resistance. *Plant Disease, 77*: 999-1003.

No. 274, pp. 6.

Onyibe, J. E., Kamara, A. Y. and Omoigui, L. O. (2006). Guide to cowpea production in Borno State, Nigeria. Promoting Sustainable Agriculture in Borno State (PROSAB). Ibadan, Nigeria. pp. 36.

Orawu, M. (2002). *Occurrence of cowpea diseases and insect pests under different management practices in Eastern Uganda*. MSc Thesis, pp. 85. Makerere University, Kampala Uganda.

Orawu, O. (2007). *Occurrence of Cowpea aphid-borne mosaic virus and prospects of improving resistance in local cowpea landraces in Uganda*. PhD thesis pp. 1-17. University of Makerere, Uganda.

Palukaitis, P., Roossinck, M. J., Dietzgen, R. G. and Francki, R. I. B. (1992).

Perez, J. E. and Cortez-Monllar, A. (1970). First Report on Cowpea severe mosaic virus. *Plant Disease Reporter*, 54: 212.

Phytopathology, 52:489-493.

Phytopathology, 89: 1088-1103.

Pio-Ribeiro, G., Pappu, S. S., Pappu, H. R., Andrade, G. P. and Reddy, D. V. R. (2000). Occurrence of Cowpea aphid-borne mosaic virus in peanut in Brazil. *Plant Disease*, 84: 760–766.

Pouwels, J., Carette, J. E., Lent, J. V. and Wellink, J. (2002). Cowpea mosaic virus: effect on host cell processes. *Molecular plant pathology*, 3: 411 – 418.

Puttaraju, H. R., Prakash, H. S. and Shetty, H. S. (2004). Seed infection by Blackeye cowpea mosaic potyvirus and yield loss in different cowpea varieties. *Journal of Mycology and Plant Pathology*, 34: 41-46.

R. (1991). Purification, serology and *in vitro* translation of an Alyce-clover isolate of Blackeye cowpea mosaic virus. *Plant disease*, 75:254-257.

Raheja, A. K. and Leleji, O. I. (1974). An aphid-borne mosaic disease of irrigated cowpeas in northern Nigeria. *Plant Disease*, 58: 1080-1084.

Rajnauth, G. L., Pegus, J. E. and Haque, S. Q. (1989). Major Field vector of cowpea severe mosaic virus in Trinidad. *Tropical Agriculture*, 66:221-224.

Reddy, D. V. R. (1991). Crop profile: Groundnut viruses and virus diseases: distribution, identification and control. *Revised Plant Pathology*, 70: 665- 678.

Sacristan, S., Fraile, A. and Garcia-Arenal, F. (2004). Population dynamics of Cucumber mosaic virus in melon crops and weeds in central Spain. *Phytopathology*, 94: 992-998.

Savanna Agricultural Research Institute (SARI) (2012). Production guide on cowpea (*Vigna unguiculata* L. Walp). csirsavannah.wordpress.com/2012/1 2/ 04/production-guide-on-cowpea-vigna-unguiculata-l-walp/ Accessed on 30/09/2015.

Shanker, U. C. A., Nayaka, S. C., Kumar, H. B., Shetty, H. S. and Prakash, S. H. (2009). Detection and identification of the Blackeye cowpea mosaic strain of Bean common mosaic virus in seeds of cowpea in Southern India. *Phytoparasitica*, 37: 283-293.

Shepherd, R. J. and Fulton, R. W. (1962). Identity of a seedborne virus of cowpea.

Shilpashree, K. (2006). Studies on blackeye cowpea mosaic viral diseases on cowpea (*Vigna unguiculata* (L) Walp). MSc thesis pp. 22-23, University of Agricultural Sciences, Dharwad.

Shoyinka, S. A., Thottappilly, G., Adebayo, G. G. and Anno-Nyako, F. O. (1997). Survey on cowpea virus incidence and distribution in Nigeria. *International Journal of Pest Management,* 43:127-132.

Singh, B. B. and Tarawali, S. A. (1997). Cowpea and its improvement: key to sustainable mixed crop/livestock farming systems in West Africa. In: Renard, C. (Ed.). *Crop Residues in Sustainable Mixed Crop/Livestock Farming Systems*. CAB in Association with ICRISAT and ILRI, Wallingford, UK, pp79–100.

Singh, B. B., Yadav, M. D. and Dingar, S. M. (1984). Studies on mosaic diseases of cowpea from Uttar Pradesh. *Science and Culture,* 50: 210-211.

Singh, S. R. and Allen, D. J. (1979). Cowpea pests and diseases. International Institute of Tropical Agriculture, Ibadan Nigeria. Manual series 2, pp. 113

Statistics, Research and Information Directorate (SRID). (2007). Crop production of Ejura/Sekyedumase District Report. pp. 10.

Statistics, Research and Information Directorate (SRID).(2007). Crop production of Ejura/Sekyeredumasi District Report. Pp. 10.

Taiwo, M. A. and Akinjogunla, O. J. (2006). Quantitative and qualitative effects of single and mixed viral infections. *African Journal of Biotechnology 5*:1749-1756.

Taiwo, M. A., Gonsalves, D., Provvidenti, R. and Thurston, H. D. (1982). Partial characterization and grouping of isolates of Blackeye cowpea mosaic and Cowpea aphid-borne mosaic viruses. *Phytopathology*, 72: 590-596.

Taiwo, M. A., Kareem, T. K., Nsa Y. I. and D'A Hughes, J. (2007). Cowpea viruses: Effect of single and mixed infections on symptomatology and virus concentration. *Virology Journal*, 4:95.

Tavassoli, M., Shahraeen, N., Ghorbani, S. H. and Hashemi, S. H. (2007). Identification of cowpea mild mottle virus (CPMMV) in soybean from Iran. *Proceedings of the 4th Iranian Virology Congress*, Medical University of Tehran, Iran, p. 342.

Thottappilly, G. (1992). Plant virus diseases of importance to African agriculture.

Thottappilly, G. and Rossel, H. W. (1985). World occurrence and distribution of virus diseases. In: cowpea research, production and utilization. S.R. Singh and K.O. Rachie. eds. John Wiley and sons, Chichester, London.

Thottappilly, G. and Rossel, H. W. (1988). Seed transmission of cowpea yellow mosaic virus unlikely in cowpea. *Tropical Grain Legume Bulletin*, 34: 27– 28.

Thottappilly, G. and Rossel, H. W. (1992). Virus diseases of cowpea in Tropical Africa. *Tropical Pest Management*, 38(4): 337-348.

Tremaine, J. H. and Hamilton, R. I. (1983). *CMI/AAB Description of Plant Viruses Trends in Microbiology*, 4: 259–264.

Umaharan, P. (1990). Genetics of resistance to cowpea severe mosaic virus (Trinidad Isolate) and some important agronomic traits in *Vigna unguiculata* (L.) Walp. PhD Thesis, The University of the West Indies, St. Augustine, Republic of Trinidad and Tobago.

Umaharan, P., Haque, S. Q., and Ariyananyagam, R. P. (1997). Identification of resistance to cowpea severe mosaic virus (Trinidad isolate) in cowpea [*Vigna unguiculata* (L.) Walp]. *Tropical Agriculture* (Trinidad), 74:324-328.

Vanderborght, T. and Baudoin, J. P. (2001). Grain legumes: Cowpea. In: Crop production in Tropical Africa. ed. R. Raemaeker. DGCI (Directorate Generale for International Cooperation), Brussels, Belgium, pp. 334-348.

Virus Taxonomy. In*:* van Regenmortel, M. H. V., Fauquet, C. M., Bishop,

Vunch, R. A., Rosner, A. and Stein, A. (1999). The use of the Polymerase Chain reaction (PCR) for detection of bean yellow mosaic virus in gladiolus. *Annals of Applied Biology,* 117: 561-569.

Wellink, J., Le Gall, O., Sanfacon, H., Ikegami, M. and Jones, A. T. (2000).

Yadav, A. (2010). Identification of a seedborne mosaic virus on cowpea (*Vigna sinensis* (L.) Savi). *Plant archives*, No. 2, 10: 793-795.

You, X. J., Kim, J. W., Stuart, G. W. and Bozarth, R. F. (1995). The nucleotide sequence of cowpea mottle virus and its assignment to the genus Carmovirus. *Journal of General Virology,* 76: 2841–2845.

Zettler, F. W. and Evans, I. R. (1973). Blackeye cowpea mosaic virus in Florida. Host range and incidence in certified cowpea seeds. *Proceedings of Florida State Horticultural society, 85*: 95-101.

Zhoa, G. S., Baltensperger, E., Herbert, E., Purcifull, D. E. and Edwardson, J.

Zitter, T. A. and Murphy, J. F. (2009). Cucumber mosaic virus. *Annals of Applied Biology,* 66: 381-386.

APPENDICES

Appendix 1: Ghana Cowpea Virus Disease Survey Sheet 2014

Sheet #		**Field size**	
Date/Time		**Age**	
Location name		**Seed source (farmers own/market or other farmers)**	
District / LGA			
Region		**Intercrops / crops around the field**	
Agro-ecology			
Latitude		**Varieties**	
Longitude		**Weediness**	
Altitude (m)		**Researcher (farmer)**	
Field summary:	**Percent infection:**	**Average severity:**	**Severity range:**

Plant #	**Symptoms[1]**	**Severity score[1]**	**Variety**	Photo ID (if any)	Vectors				Other diseases			
					Aphids	Thrips	Whitefly	Other				
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												

21												
22												
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24												
25												
26												
27												
28												
29												
30												
Comments:												

Appendix 2: Cowpea Virus Disease Survey Questionnaire

Questionnaire Identification Number: …….. PART

A: INTERVIEW BACKGROUND

Date of interview: Day: ……. Month: ……. Year: 20……

Locational Details

Country	State/ Regi	LGA/District	Village/Community	GPS readings of homestead			
				Location	Latitude	Longitude	Altitude
	on						

Respondent"s name: …….. 4. Gender: M or F (**Circle**); 5. Age: ……. (**In years**) 6. Level of participation: **0** (Non-participating); **1**(Participating)

7. Contact telephone number: ……

PART B: FARM INPUT & RESOURCE ALLOCATION

1. Land holding:

Land category	Holding **(Hectare)**	Cropping pattern and intercrop combinations: **1:Monocropping, 2: Intercropping, 3: Both**	Planting method **1:Rows 2:Random, 3: Ridges**
Total owned land			
Acreage under cowpea			

1. How long cowpea is being grown in that site? …….. (**In years**)
2. What is your main source of cowpea seed (**1=Own seed; 2=Agro-input dealer; 3=local market 4=Other, specify**: (……..…….)

1. If purchased from 3 above, from where? …… (**1=Inside this village; 2=Outside the village**) 2. Over the past five years, what is your cowpea production trend? …. (**1=Decreasing; 2=About the same; 3=Increasing**)

PART C: FARMERS' PREFERENCES

1. Farmer"s variety preferences

What are your preferred varieties?	Rank them according to the preference **(starting by 1= most preferred)**	Main reason of	Major constraints

PART D: FARM STRESSES

1. What are the major production constraints? Rank them in order of importance (**starting by 1=the most important**)

Cowpea production		Post harvest production	
Major constraints	Rank	Major constraints	Rank

1. What is the magnitude of yield loss associated with respective pests/diseases (Low, Modest, High)

Insect Pest/Disease	Low	Modest	High
1.			
2.			
3.			
4.			
5.			
6.			
8.			
9.			
10.			

1.From when has virus diseases (mosaic) become problem? ..... (indicate years)

2.Over the past five years, what is the trend of cowpea virus disease? ….
(1=Decreasing; 2=About the same; 3=Increasing)

Appendix 3: Detection of Viruses in Cowpea Leaf Samples from Selected Fields in the Atebubu District in the Brong Ahafo Region of Ghana.

S/N	Farm no.	Location/District Amantin-Atebubu	BICMV	CpMoV	CPMMV	CABMV	CMV	SBMV	CYMV
1	001	Amantin-Atebubu	0.416*	0.640*	OUT	0.208*	0.214*	0.391*	0.193*
2	002	Amantin-Atebubu	OUT	3.159	3.367	0.251*	0.304*	0.469*	0.252*
3	003	Amantin-Atebubu	3.170	3.834	3.071	0.239*	0.261*	0.396*	0.258*
4	004	Amantin-Atebubu	OUT	3.965	0.944*	0.240*	0.266*	0.345*	0.302*
5	005	Amantin-Atebubu	3.383	3.882	3.312	0.207*	0.244*	0.320*	0.288*
6	006	Amantin-Atebubu	3.851	3.828	3.579	0.210*	0.302*	0.449*	0.367*
7	007	Amantin-Atebubu	3.947	3.044	3.587	0.230*	0.284*	0.388*	0.349*
8	008	Amantin-Atebubu	3.532	0.100*	OUT	0.245*	0.257*	0.517*	0.305*
9	009	Amantin-Atebubu	OUT	3.554	3.748	0.303*	0.328*	0.684*	0.345*
10	010	Amantin-Atebubu	OUT	0.133*	3.303	0.332*	0.348*	0.526*	3.543
11	011	Amantin-Atebubu	3.587	3.152	3.980	0.256*	0.271*	0.603*	0.436*
12	012	Amantin-Atebubu	3.768	3.385	3.088	0.415*	0.316*	0.433*	0.409*
13	013	Amantin-Atebubu	OUT	0.935*	3.292	0.269*	0.360*	0.579*	0.304*
14	014	Amantin-Atebubu	3.393	3.300	3.410	3.886	0.490*	0.568*	0.367*
15	015	Amantin-Atebubu	0.962*	1.054*	1.013*	0.380*	0.491*	0.603*	0.335*
16	016	Amantin-Atebubu	0.626*	3.140	3.693	0.256*	0.314*	3.916	0.340*
17	017	Amantin-Atebubu	0.444*	0.593*	0.919*	0.304*	0.228*	0.471*	0.310*
18	018	Amantin-Atebubu	0.396*	0.614*	0.373*	0.257*	0.216*	0.328*	0.285*
19	019	Amantin-Atebubu	3.257	0.516*	0.510*	0.308*	0.390*	3.947	3.435
20	020	Amantin-Atebubu	0.635*	0.567*	3.120	0.251*	0.347*	0.469*	0.370*
21	021	Amantin-Atebubu	0.935*	0.440*	0.404*	0.451*	0.389*	0.445*	0.326*
22	022	Amantin-Atebubu	0.751*	0.483*	0.372*	0.463*	0.390*	0.456*	0.393*
23	023	Amantin-Atebubu	0.657*	0.612*	0.397*	0.137*	0.367*	0.445*	0.401*
24	024	Amantin-Atebubu	0.612*	0.677*	0.575*	0.276*	1.011*	0.410*	0.355*
25	025	Amantin-Atebubu	3.775	3.185	0.410*	0.409*	0.494*	0.450*	0.396*
	Diseased		3.050	3.491	3.263	3.290	OUT	OUT	3.100
	Healthy		0.540*	0.568*	0.518*	0.440*	0.572*	0.429*	0.206*

Appendix 4: Detection of

Buffer	0.435*	0.300*	0.419*	0.196*	0.322*	0.207*	0.270*	

*Absorbance value (A405 nm) is less than twice healthy control, thus negative; OUT indicates an out of range value, thus positive

Viruses in Cowpea Leaf Samples from Selected Fields in the Ejura District in the Ashanti Region of Ghana.

S/N	Farm no.	Location/District Ejura	BICMV	CpMoV	CPMMV	CABMV	CMV	SBMV	CYMV
26	026	Ejura	1.071*	0.541*	0.550*	0.275*	0.447*	0.488*	0.341*	
27	027	Ejura	0.043*	0.501*	0.588*	0.280*	0.422*	0.497*	0.328*	
28	028	Ejura	0.811*	0.431*	0.714*	0.312*	0.490*	0.558*	0.475*	
29	029	Ejura	1.002*	0.443*	0.565*	0.316*	0.321*	0.430*	0.351*	
30	030	Ejura	0.811*	0.353*	0.740*	0.257*	0.331*	0.628*	0.372*	
31	031	Ejura	0.632*	0.497*	0.631*	0.269*	0.331*	0.413*	0.315*	
32	032	Ejura	0.800*	0.720*	0.401*	0.354*	0.347*	0.369*	0.410*	
33	033	Ejura	0.668*	0.413*	3.990	0.259*	0.262*	0.379*	0.351*	
34	034	Ejura	0.764*	0.316*	3.769	0.251*	0.245*	0.353*	0.302*	
35	035	Hiawoanwu	0.841*	0.316*	0.705*	0.328*	0.247*	0.313*	0.312*	
36	036	Hiawoanwu	OUT	3.003	0.545*	0.365*	0.378*	0.539*	0.411*	
37	037	Hiawoanwu	3.288	0.787*	3.748	0.360*	0.341*	0.484*	0.361*	
38	038	Hiawoanwu	3.889	0.710*	0.632*	0.360*	0.313*	0.424*	0.323*	
39	039	Hiawoanwu	OUT	0.507*	0.391*	0.486*	0.413*	0.485*	0.309*	
40	040	Hiawoanwu	OUT	3.091	0.331*	0.442*	0.390*	0.400*	0.300*	
41	041	Hiawoanwu	3.845	0.501*	0.285*	0.530*	0.374*	0.556*	0.288*	
42	042	Hiawoanwu	3.037	0.849*	3.120	0.404*	0.311*	0.462*	0.314*	
43	043	Hiawoanwu	0.741*	0.803*	3.316	3.755	0.606*	OUT	0.378*	
44	044	Hiawoanwu	OUT	0.867*	0.487*	0.423*	0.456*	0.387*	0.309*	

Appendix 5: Detection of

45	045	Hiawoanwu	0.799*		3.070	3.196	0.398*		0.369*	0.518*	0.399*	
46	046	Hiawoanwu	3.334	0.799*		3.760	0.373*		0.349*	0.492*	0.369*	
47	047	Hiawoanwu	3.896	0.721*		0.643*		0.371*	0.319*	0.433*	0.329*	
48	048	Hiawoanwu	OUT	0.518*		0.399*		0.497*	0.421*	0.496*	0.317*	
49	049	Hiawoanwu	OUT	3.108	0.340*		0.446*		0.399*	0.410*	0.308*	
50	050	Hiawoanwu	OUT	0.509*		0.292*		0.524*	0.381*	0.562*	0.295*	
	Diseased				3.094	OUT		3.974	3.200	3.984	OUT	3.220
	Healthy				0.424*	0.450*		0.360*	0.357*	0.455*	0.603*	0.330*
	Buffer				0.317*	0.360*		0.269*	0.243*	0.292*	0.330*	0.262*

*Absorbance value (A405 nm) is less than twice healthy control, thus negative; OUT indicates an out of range value, thus positive

Viruses in Cowpea Leaf Samples from Selected Fields in the Mampong District in the Ashanti Region of Ghana.

S/N	Farm no.	Location/District Mampong	BICMV	CpMoV	CPMMV	CABMV	CMV	SBMV	CYMV
51	051	Atonsuagya	3.042	0.862*	3.136	0.408*	0.318*	0.471*	0.323*
52	052	Atonsuagya	0.650*	0.818*	3.337	3.770	0.465*	OUT	0.389*
53	053	Atonsuagya	OUT	3.883	0.497*	0.429*	0.369*	0.398*	0.318*
54	054	Atonsuagya	3.114	3.084	3.216	0.403*	0.378*	0.529*	0.385*
55	055	Atonsuagya	0.865*	0.488*	0.459*	0.240*	0.229*	0.313*	0.281*
56	056	Atonsuagya	0.536*		0.333*	0.478*	0.246*	0.253*	0.265*
57	057	Atonsuagya	3.853	0.527*		0.334*	0.245*	0.265*	0.302*
58	058	Atonsuagya	3.945	0.279*		0.283*	0.240*	0.234*	0.248*
59	059	Atonsuagya	3.112	0.274*		0.313*	0.219*	0.207*	0.274*
60	060	Atonsuagya	0.570*		0.326*	0.265*	0.231*	0.233*	0.271*
61	061	Atonsuagya	0.319*		0.209*	0.208*	0.160*	0.161*	0.183*
62	062	Atonsuagya	0.502*	0.221*	0.277*	0.261*	0.161*	0.189*	0.230*
63	063	Atonsuagya	0.747*	3.698	0.395*	0.267*	0.253*	0.240*	0.241*
64	064	Atonsuagya	0.301*	0.455*	0.397*	0.206*	0.215*	0.258*	0.258*

Appendix 6: Detection of

65	065	Atonsuagya	0.330*	0.219*	0.204*	0.182*	0.160*	0.210*	0.178*	66	066	Adidwan	0.505*
0.330*	0.341*	0.179*	0.160*	0.227*	0.216*								
67	067	Adidwan	0.580*	0.222*	0.326*	0.193*	0.147*	0.202*	0.215*				
68	068	Adidwan	0.515*	0.444*	0.388*	0.192*	0.149*	0.243*	0.214*				
69	069	Adidwan	0.529*	0.250*	0.423*	0.244*	0.160*	0.285*	0.229*				
70	070	Adidwan	0.780*	0.420*	0.438*	0.268*	0.166*	0.268*	0.241*	71	071	Adidwan	3.151
0.394*	0.246*	0.170*	0.147*	0.249*	0.234*	72	072	Adidwan	0.779*	0.253*	0.048*	0.250*	0.156*	0.240*	0.227*	73	073	Adidwan	3.506
3.827	3.652	0.268*	0.271*	0.363*	0.262*								
74	074	Adidwan	0.638*	0.315*	3.637	0.174*	0.159*	0.241*	0.305*				
75	075	Adidwan	0.672*	0.339*	0.178*	0.205*	0.139*	0.191*	0.271*				
	Diseased			3.962	3.783	3.799	3.871	OUT	3.400	3.700			
	Healthy			0.471*	0.313*	0.261*	0.175*	0.190*	0.295*	0.229*			
	Buffer			0.301*	0.231*	0.129*	0.170*	0.169*	0.225*	0.146*			

*Absorbance value (A405 nm) is less than twice healthy control, thus negative; OUT indicates an out of range value, thus positive

Viruses in Cowpea Leaf Samples from Selected Fields in the Nkoranza District in the Brong Ahafo Region of Ghana.

S/N	Farm no.	Location/District Nkoranza	BICMV	CpMoV	CPMMV	CABMV	CMV	SBMV	CYMV
76	076	Dasagba	OUT	0.251*	0.211*	0.320*	0.257*	0.179*	0.184*	77	077	Dasagba	0.220*
0.162*	0.415*	0.152*	0.152*	0.146*	0.173*	78	078	Camp	0.728*	3.584	0.284*	0.247*	0.144*	0.143*	0.187*
79	079	Dasagba	0.733*	3.704	0.296*	0.188*	0.170*	0.187*	0.241*	80	080	Bredi no. 1	0.820*
0.441*	0.245*	0.175*	0.167*	0.170*	0.182*	81	081	Bredi no. 1	3.231	3.693	0.453*	0.242*	0.329*	OUT	0.226*	82	082	Bredi no. 1	0.290*
0.279*	0.279*	0.214*	0.163*	0.290*	0.262*	83	083	Bredi no. 1	0.532*	0.322*	0.248*	0.309*	0.223*	0.442*	0.305*	84	084	Bredi no. 1	3.520
3.754	3.000	0.338*	OUT	0.577*	0.271*	85	085	Bredi no. 1	3.550	3.688	0.176*	0.325*	0.164*	0.283*	0.249*	86	086	Bredi no. 1	0.686*
0.410*	3.568	0.205*	0.148*	0.249*	0.195*														

Appendix 7: Detection of

87	087	Dasagba	3.356	0.500*	3.623	0.248*	0.169*	0.281*	0.250*				
88	088	Dasagba	3.731	3.621	0.192*	0.235*	0.159*	0.210*	0.217*	89	089	Bredi no. 1	0.759*
	3.707	3.751	0.392*	0.167*	0.230*	0.236*	90	090	Camp	0.637*	0.314*	0.420*	0.241*
	0.176*	0.182*	0.213*	91	091	Camp	0.831*	0.342*	3.510	0.186*	0.175*	0.287*	0.201*
	92	092	Camp	3.216	3.708	3.524	0.245*	0.185*	0.268*	0.252*	93	093	Camp
	0.698*	3.587	3.831	0.233*	0.168*	0.286*	0.244*	94	094	Camp	OUT	3.577	0.253*
	0.292*	0.168*	0.226*	0.228*	95	095	Bredi no. 1	3.501	0.445*	0.228*	0.221*	0.163*	0.228*
	0.206*	96	096	Camp	0.559*	0.383*	3.541	0.242*	0.245*	0.318*	0.278*	97	097
	Camp	0.572*	0.333*	0.379*	0.274*	0.192*	0.294*	0.239*	98	098	Camp	OUT	0.257*
	0.195*	0.459*	0.273*	0.299*	0.230*								
99	099	Camp	0.662*	0.394*	0.249*	0.237*	0.208*	0.259*	0.248*				
100	100	Camp	0.542*	0.516*	0.185*	0.235*	0.201*	0.259*	0.212*				
Diseased				3.690	3.603	3.955	3.949	OUT	3.808	3.678			
Healthy				0.417*	0.260*	0.229*	0.289*	0.161*	0.295*	0.256*			
Buffer				0.321*	0.213*	0.202*	0.249*	0.135*	0.271*	0.203*			

*Absorbance value (A405 nm) is less than twice healthy control, thus negative; OUT indicates an out of range value, thus positive

Appendix 7: BICMV Transmission Rates of Field Collected Cowpea Seeds

Cowpea Seedlots	Total seeds	Total germinated	Total symptomatic	Virus detected	Seed transmission
Amantin 1	200	125	0	None	0.0
Amantin 2	200	120	1	BICMV	0.8
Amantin 3	200	135	0	None	0.0
Amantin 4	100	78	2	BICMV	2.6
Amantin 5	100	94	3	BICMV	3.2
Amantin 6	100	96	2	BICMV	2.1
Amantin 7	100	100	4	BICMV	4.0
Amantin 8	100	30	1	BICMV	3.3
Ejura 1	158	103	0	None	0.0
Ejura 2	200	104	2	BICMV	1.9
Ejura 3	200	84	0	None	0.0
Ejura 4 100	38 7	BICMV	18.4 Ejura 5	100 69	4
BICMV	5.8				
Ejura 6	100	82	3	BICMV	3.7
Ejura 7	100	73	1	BICMV	1.4
Ejura 8	100	76	3	BICMV	4.0
Mampong 1	200	65	0	None	0.0
Mampong2	200	84	0	None	0.0
Mampong 3	200	124	0	None	0.0
Mampong 4	100	86	2	BICMV	2.3
Mampong 5	100	80	13	BICMV	16.3
Mampong 6	100	92	1	BICMV	1.1
Mampong 7	100	90	24	BICMV	27
Mampong 8	100	80	1	BICMV	1.3

*Local cowpea varieties: Amantin 1-8; Mixed, Mallamyaya, Adamu, Alancash, Alancash, Alancash, Mixed, Yekoyenim

Ejura 1-8; Mixed, Adamu, Mallamyaya, Adamu, Yaminu, Yekoyenim, Mallamyaya, Alancash

Mampong 1-8; Mallamyaya, Allancash, Mixed, Yaminu, Alancash, Mallamyaya, Mallamyaya, Mallamyaya

Appendix 8: Single Infection ACP-ELISA Result for Seed Transmission Test

	Viruses	CABMV	BICMV	CMV	SBMV	CMeV	CYMV	CPMMV
	Ejura 2A	0.382	OUT*	0.513	0.452	0.402	0.389	0.367
	Ejura 2B	0.432	2.658*	0.420	0.442	0.424	0.390	0.450
	Ejura 4A	0.339	3.240*	0.218	0.247	0.327	0.187	0.279
	Ejura 4B	0.269	3.370*	0.209	0.218	0.223	0.188	0.315
	Ejura 4C	0.163	3.303*	0.177	0.242	0.351	0.171	0.246
	Ejura 4D	0.165	2.826*	0.254	0.217	0.263	0.188	0.213
	Ejura 4E	0.195	3.396*	0.202	0.201	0.212	0.171	0.319
	Ejura 4F	0.228	3.457*	0.196	0.196	0.281	0.200	0.303
	Ejura 4G	0.227	3.285*	0.225	0.196	0.303	0.209	0.305
Cowpea								
	EjuraEjura 55AB	0.3020.294	3.413*3.343*	0.2390.232	0.2450.247	0.2780.289	0.1730.209	0.2870.204
	Ejura 5C	0.213	3.267*	0.188	0.281	0.334	0.205	0.345
	Ejura 5D	0.228	3.322*	0.197	0.249	0.293	0.240	0.282
	Ejura 6A	0.257	2.638*	0.180	0.255	0.194	0.236	0.281
	Ejura 6B	0.298	2.594*	0.189	0.249	0.310	0.257	0.224
	Ejura 6C	0.206	3.258*	0.178	0.195	0.306	0.225	0.282
	Ejura 7A	0.237	3.248*	0.175	0.237	0.382	0.240	0.283
	Ejura 8A	0.357	3.234*	0.174	0.535	0.266	0.255	0.232
	Ejura 8B	0.206	2.994*	0.201	0.304	0.296	0.233	0.331
	Ejura 8C	0.322	3.467*	0.203	0.233	0.268	0.224	0.271
Controls	Positive	0.783*	OUT	OUT	OUT	OUT	0.780*	2.251*
	Negative	0.330	0.360	0.500	0.440	0.390	0.386	0.380
	Buffer	0.190	0.280	0.200	0.210	0.210	0.200	0.210

*Absorbance value (A405 nm) is greater than twice negative control, thus positive;

OUT indicates an out of range value, thus positive

Appendix 9: Single Infection ACP-ELISA Result for Seed Transmission Test

	Viruses	CABMV	BICMV	CMV	SBMV	CMeV	CYMV	CPM
	Amantin 2A	0.382	OUT*	0.513	0.452	0.402	0.389	0.3
	Amantin 2B	0.432	2.658*	0.420	0.442	0.424	0.390	0.4
	Amantin 4A	0.208	3.416*	0.348	0.526	0.133	0.219	0.3
	Amantin 4B	0.251	2.710*	0.271	0.603	0.327	0.324	0.1
	Amantin 5A	0.239	3.383*	0.316	0.433	0.185	0.345	0.2
	Amantin 5B	0.303	2.851*	0.360	0.579	0.325	0.305	0.2
Cowpea								
	AmantinAmantin 56CA	0.2450.207	2.947*2.532*	0.4100.419	0.5680.316	0.2470.416	0.3490.367	0.447
	Amantin 6B	0.240	3.396*	0.314	0.471	0.373	0.288	0.3
	Amantin 7A	0.239	2.962*	0.228	0.449	0.185	0.193	0.3
	Amantin 7B	0.210	3.000*	0.216	0.716	0.444	0.252	0.2
	Amantin 7C	0.354	3.176*	0.348	0.328	0.396	0.258	0.2
	Amantin 7D	0.332	3.228*	0.124	0.156	0.560	0.302	0.3
	Amantin 8A	0.415	3.140*	0.244	0.503	0.176	0.288	0.4
Controls	Positive	0.783*	OUT	OUT	OUT	OUT	0.780*	2.25
	Negative	0.330	0.360	0.500	0.440	0.390	0.386	0.3
	Buffer	0.190	0.280	0.200	0.210	0.210	0.200	0.2

*Absorbance value (A405 nm) is greater than twice negative control, thus positive;

OUT indicates an out of range value, thus positive

Appendix 10: Single Infection ACP-ELISA Result for Seed Transmission Test

Viruses	CABMV	BICMV		SBMV		CYMV	CPMMV
Mampong 4A	0.196	3.175*	0.224	0.241	0.338	0.296	0.460
Mampong 4B	0.220	3.077*	0.213	0.221	0.392	0.253	0.517
Mampong 5A	0.234	3.370*	0.195	0.213	0.413	0.246	0.333
Mampong 5B	0.253	2.943*	0.219	0.239	0.543	0.270	0.430

				CMV		CMeV		
	Mampong 5C	0.225	3.438*	0.186	0.375	0.245	0.260	0.226
	Mampong 5D	0.222	3.446*	0.271	0.385	0.250	0.246	0.235
	Mampong 5E	0.229	3.645*	0.288	0.214	0.438	0.242	0.406
	Mampong 5F	0.251	3.445*	0.280	0.371	0.405	0.249	0.312
	Mampong 5G	0.257	3.631*	0.286	0.398	0.327	0.253	0.362
Cowpea	Mampong 5H	0.248	3.326*	0.250	0.861	0.616	0.218	0.517
	Mampong 5I	0.247	3.249*	0.256	0.270	0.285	0.205	0.193
	Mampong 5J	0.206	3.174*	0.147	0.360	0.161	0.139	0.251
	Mampong 5K	0.255	3.381*	0.268	0.312	0.402	0.249	0.208
	Mampong 5L	0.181	3.275*	0.217	0.219	0.513	0.257	0.425
	Mampong 5M	0.208	3.300*	0.243	0.261	0.385	0.193	0.330
	Mampong 6A	0.219	3.301*	0.206	0.233	0.315	0.267	0.201
	Mampong 7A	0.215	3.376*	0.244	0.233	0.317	0.199	0.251
	Mampong 7B	0.218	3.485*	0.222	0.244	0.335	0.281	0.268
	Mampong 7C	0.228	3.618*	0.213	0.208	0.362	0.188	0.250
Controls	Positive	2.783*	OUT		OUT	OUT	3.780*	2.251*
	Negative	0.260	0.268	0.301	0.356	0.382	0.236	0.317

*Absorbance value (A405 nm) is greater than twice negative control, thus positive;

OUT indicates an out of range value, thus positive

Appendix 10 cont'd

	Viruses	CABMV	BICMV	CMV	SBMV	CMeV	CYMV	CPMMV
	Mampong 7D	0.243	0.899*	0.223	0.252	0.431	0.206	0.336
	Mampong 7E	0.228	0.701*	0.213	0.224	0.387	0.232	0.284
	Mampong 7F	0.274	0.695*	0.324	0.509	0.555	0.235	0.448
	Mampong 7G	0.301	0.820*	0.312	0.371	0.329	0.237	0.273
	Mampong 7H	0.275	0.708*	0.283	0.177	0.196	0.163	0.168
	Mampong 7I	0.231	0.823*	0.261	0.170	0.249	0.141	0.250
	Mampong 7J	0.238	0.822*	0.229	0.142	0.169	0.135	0.187
	Mampong 7K	0.235	0.604*	0.231	0.149	0.239	0.126	0.240
	Mampong 7L	0.265	0.545*	0.255	0.138	0.228	0.142	0.217

Cowpea	Mampong 7M	0.267	0.626*	0.291	0.287	0.493	0.148	0.220
	Mampong 7N	0.285	0.828*	0.264	0.246	0.228	0.144	0.222
	Mampong 7O	0.264	0.945*	0.280	0.250	0.219	0.162	0.256
	Mampong 7P	0.268	0.851*	0.268	0.259	0.234	0.153	0.340
	Mampong 7Q	0.268	0.636*	0.276	0.253	0.309	0.148	0.325
	Mampong 7R	0.204	0.645*	0.244	0.626	0.440	0.179	0.388
	Mampong 7S	0.221	0.731*	0.237	0.230	0.216	0.161	0.194
	Mampong 7T	0.259	0.871*	0.262	0.252	0.175	0.127	0.153
	Mampong 7U	0.284	0.668*	0.249	0.272	0.278	0.153	0.260
	Mampong 7V	0.281	0.959*	0.222	0.252	0.226	0.142	0.311
	Mampong 7W	0.275	0.702*	0.264	0.247	0.316	0.151	0.260
	Mampong 7X	0.260	0.572*	0.246	0.243	0.230	0.136	0.249
	Mampong 8A	0.200	0.568*	0.273	0.276	0.320	0.148	0.574
Controls	Positive	2.783*	OUT	OUT	OUT	OUT	3.780*	2.251*
	Negative	0.260	0.268	0.301	0.456	0.382	0.236	0.357
	Buffer	0.181	0.220	0.200	0.210	0.196	0.200	0.210

*Absorbance value (A405 nm) is greater than twice negative control, thus positive;

OUT indicates an out of range value, thus positive