

MAKERERE



UNIVERSITY

**IDENTIFICATION OF COWPEA VIRUSES AND SOURCES
OF RESISTANCE AMONG COWPEA GERMPLASM IN
UGANDA**

BY

MBEYAGALA K EMMANUEL

BSc. AGRIC (MAK), MSc. CROP SCIENCE (MAK)

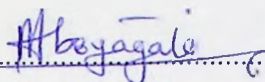
REG. NO: 2010/HD02/18151U

**A THESIS SUBMITTED TO THE DIRECTORATE OF RESEARCH AND
GRADUATE TRAINING IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN
AGRICULTURE (CROP SCIENCE) OF MAKERERE UNIVERSITY**

FEBRUARY 2020

DECLARATION

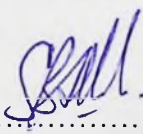
I declare that the work presented in this thesis was carried out in accordance with the requirements of the university and it has not been submitted for any other academic award/degree in any university. Any views expressed in the thesis are those of the author.

Signature  Date 24/2/2020

Mbeyagala K Emmanuel

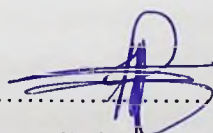
PhD candidate, School of Agricultural Sciences, Makerere University

This thesis has been submitted for examination with our approval as Makerere University supervisors.

Signature  Date 24/02/2020

Associate Professor Settumba B Mukasa,

School of Agricultural Sciences, Makerere University

Signature  Date 24/02/2020

Professor Phinehas Tukamuhabwa,

School of Agricultural Sciences, Makerere University

ABSTRACT

Cowpea (*Vigna unguiculata* L. Walp) grain constitutes an important source of protein for several households in Africa. In Uganda, cowpea is the third most important legume crop after common beans and groundnuts with production mainly concentrated in the eastern and northern regions of the country. However, productivity of cowpea is still very low, estimated at less 500 kg ha⁻¹. Widespread occurrence of viral diseases contributes greatly to the low productivity of the crop. Viral infections also reduce protein content of the grain thus affecting its nutritional value. The use of resistant varieties is the most feasible, effective and environmentally friendly approach for management of viral infections. However, success of this approach requires a better understanding of the pathogens, availability of reliable diagnostic assays and good sources of resistance to guide design of appropriate breeding programs. The objectives of this study were; (i) to identify and characterize viruses infecting cowpea in Uganda; (ii) to develop a molecular diagnostic assay for *Cowpea aphid-borne mosaic virus* (CABMV) in Uganda; and (iii) to determine the level of virus resistance among cowpea genotypes under field conditions. Next-generation sequencing (NGS) using illumina platform identified four viruses; *Cowpea aphid-borne mosaic virus* (CABMV; genus *Potyvirus*), *Peanut mottle virus* (PeMoV; genus *Potyvirus*), *Sugarcane mosaic virus* (SCMV; genus *Potyvirus*) and *Maize chlorotic mottle virus* (MCMV; genus *Machlomovirus*) from symptomatic leaf samples collected from farmer's fields. With the exception of CABMV, the other viruses had never been reported on cowpea or known to infect cowpea in Uganda. This is also the first report of the occurrence of SCMV and MCMV on a leguminous crop like cowpea. NGS also led to the assembly of the first complete genome from a Ugandan isolate of CABMV. The Ugandan isolates of CABMV clustered separately from isolates from other African countries indicating phylogenetic affinity to geographic origin. Ugandan isolates of CABMV also showed high nucleotide percentage identity (89.3 to 94.3%) indicating that they belong to the same strain. A new reverse transcriptase polymerase chain reaction (RT-PCR) diagnostic assay based on CABFF1(5'-GGT AAC AAY AGT GGR CAA CC-3')/CABRR1(5'-CTG AGC ACT CMA ACC GGG-3') was developed and tested for detection of CABMV. The assay accurately detected CABMV in infected leaf samples yielding a fragment of ~1642 bp. The assay is recommended for use in rapid screening of segregating progeny in cowpea breeding programs, quarantine and epidemiological studies of CABMV.

105 cowpea genotypes were evaluated in 2012 for two consecutive seasons (2012A and 2012B) in Serere, Budaka and Tororo in a 10 x 11 alpha lattice design to assess the level of resistance to virus infestation under natural conditions. The landraces had low virus infection levels compared to the introduced cowpea genotypes. Cowpea genotypes WC48, NE43, NE15, WC35A, WC39, WC33, WC35C and WC18 showed broad resistance to virus infestation and could be used as donor parents in developing virus resistant varieties. With regard to yield and AUDPC, Serere, Budaka and Tororo formed a single mega-environment. However, with regard to incidence, two mega-environments were formed, the first mega-environment comprised of Serere and Tororo while the second one comprised of Budaka. WC51, NE48, MU20, WC32, NE51, NE37, WC2, WC42, NE15, WC53, SECOW2W and NE50 were high yielding and stable.

TABLE OF CONTENTS

DECLARATION	ii
ABSTRACT	iii
TABLE OF CONTENTS	v
LIST OF FIGURES.....	x
LIST OF TABLES	xiii
LIST OF APPENDICES	xiii
DOCTORAL COMMITTEE MEMBERS.....	xiv
DEDICATION	xv
ACKNOWLEDGEMENTS	xvi
PUBLICATIONS FROM THIS THESIS	xviii
CHAPTER ONE	1
GENERAL INTRODUCTION	1
1.1 Origin, distribution and domestication of cowpea.....	1
1.2 Taxonomy of cultivated cowpea.....	2
1.3 Importance of cowpea	3
1.4 Cowpea production status.....	4
1.5 Cowpea production constraints	5
1.6 Problem Statement.....	6
1.7 Justification.....	7
1.8 Objectives of the study	7

1.8.1	Main objective.....	7
1.8.2	Specific objectives.....	7
1.8.3	Hypotheses	8
CHAPTER TWO.....		9
LITERATURE REVIEW.....		9
2.1	Taxonomy of viruses	9
2.2	Viruses infecting cowpea and their transmission	10
2.2.1	<i>Cowpea aphid-borne mosaic virus</i> (CABMV)	11
2.2.2	<i>Bean common mosaic virus</i> - blackeye cowpea mosaic strain (BCMV-BICM)	12
2.2.3	<i>Cucumber mosaic virus</i> (CMV)	14
2.2.4	<i>Cowpea mosaic virus</i> (CPMV)	15
2.2.5	<i>Cowpea severe mosaic virus</i> (CPSMV)	16
2.2.5	<i>Southern bean mosaic sobemovirus</i> (SBMV)	17
2.2.6	<i>Cowpea mottle virus</i> (CPMoV)	18
2.2.7	<i>Cowpea mild mottle carlavirus</i> (CPMMV).....	19
2.3	Methods of detecting and classifying viruses.....	20
2.3.1	Symptomatology	21
2.3.2	Transmission tests	21
2.3.3	Electron microscopy (EM)	21
2.3.4	Serological methods	22
2.3.4.1	Immunosorbent electron microscopy (ISEM)	22
2.3.4.2	Enzyme-linked immunosorbent assay (ELISA)	22
2.3.5	Molecular methods	23

2.3.5.1	Nucleic acid hybridization assays	23
2.3.5.2	Polymerase Chain Reaction (PCR)	24
2.3.6	Next-generation sequencing (NGS)	24
2.4	Management of cowpea viral diseases	25
2.4.1	Cultural measures	26
2.4.2	Chemical control	26
2.4.3	Host resistance.....	27
2.4.4	Integrated approaches.....	27
CHAPTER THREE.....		29
MOLECULAR CHARACTERIZATION OF VIRUSES INFECTING COWPEA IN UGANDA USING NEXT-GENERATION SEQUENCING		29
3. 1	Introduction	29
3. 2	Materials and methods.....	29
3. 2. 1	Sample collection and preservation.....	29
3. 2. 2	RNA extraction	30
3. 2. 3	Preparation of cDNA libraries and sequencing.....	31
3. 2. 4	De novo assembly and sequence analysis	31
3. 2. 5	Phylogenetic analysis	32
3. 2. 6	Pair wise comparison of Ugandan cowpea virus isolates with other isolates ...	32
3.3	Results	32
3. 3. 1	Sequence analysis and de novo assembly	32
3. 3. 2	Annotation of CABMV isolate (Serere1) genome sequence	37
3. 3. 3	Comparison of proteins in Serere1 with other CABMV isolates.....	38

3.3.4	Phylogeny of CABMV Serere1 with other isolates	42
3.4	Discussion.....	44
CHAPTER FOUR		47
DEVELOPMENT OF A MOLECULAR DIAGNOSTIC ASSAY FOR COWPEA APHID- BORNE MOSAIC VIRUS.....		47
4.1	Introduction	47
4.2	Materials and methods.....	48
4.2.1	Plant samples.....	48
4.2.2	Nitrocellulose membrane based ELISA (NCM-ELISA)	48
4.2.3	RNA extraction and cDNA synthesis.....	49
4.2.4	Primer sequences used	49
4.2.5	Polymerase chain reaction (PCR) conditions.....	50
4.2.6	Sanger sequencing and sequence analysis	51
4.3	Results	51
4.4	Discussion.....	56
CHAPTER FIVE.....		59
LEVELS OF FIELD RESISTANCE TO NATURAL VIRUS INFESTATION AMONG COWPEA GENOTYPES.....		59
5.1	Introduction	59
5.2	Materials and Methods	60
5.2.1	Description of study sites	60
5.2.2	Plant materials	60
5.2.3	Layout of experiment	60

5.2.4	Data collection.....	61
5.2.5	Data analysis	62
5.3	Results	63
5.3.1	Viral symptom expression among genotypes.....	63
5.3.2	Variation among cowpea genotypes for AUDPC, incidence and other traits...	64
5.3.2	Correlation matrix for AUDPC, incidence and grain yield.....	66
5.3.3	Response to virus infection and yield potential	66
5.3.4	Biplot analysis of disease indexes and yield data	68
5.3.4.1	Biplot analysis based on AUDPC.....	68
5.3.4.2	Biplot analysis based on incidence.....	70
5.3.4.3	Biplot analysis based on yield	72
5.4	Discussion.....	74
CHAPTER SIX		77
GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS		77
6.1	General discussion.....	77
6.2	General conclusions.....	79
6.3	Recommendations	79
REFERENCES.....		80

LIST OF FIGURES

Figure 1: Genomic map of <i>Cowpea aphid-borne mosaic potyvirus</i> . The open bar represents the ORF and UTRs are indicated by a single line. Vertical lines indicate putative cleavage sites within the polyprotein. Amino acids on either side of each cleavage site are shown above the site. Ten mature proteins are predicted from the polyprotein. Numbers below the map indicate the position of the first coding nucleotide of each gene. An: poly (A) tail; VPg: genome linked protein. Source: Mlotshwa <i>et al.</i> (2002).	12
Figure 2: Genome structure of potyviruses. Source: Hulo <i>et al.</i> (2011).	14
Figure 3: Genome organization of <i>Cucumber mosaic virus</i> . Source: Hulo <i>et al.</i> (2011).	15
Figure 4: Particle morphology (A) and genome organization (B) of comoviruses. Source: Hulo <i>et al.</i> (2011).	16
Figure 5: Particle morphology (A) and genome organization (B) of <i>Southern bean mosaic virus</i> . Source: Hulo <i>et al.</i> (2011).	18
Figure 6: Particle morphology (A) and genome organization (B) of <i>Cowpea mottle virus</i> . Source: Hulo <i>et al.</i> (2011).	19
Figure 7: Genome of organization of a Carlavirus. Source: Hulo <i>et al.</i> (2011).	20
Figure 8: NCBI BLAST of the sequence data for the NMF1 isolate (594 bp) under accession number KT970618. The sequence was identified as a replicase gene of MCMV in the database.	33
Figure 9: NCBI BLAST of the sequence data for the SELKB2 isolate (2,021 bp) under accession number KT970619. The sequence was identified as a polyprotein of PeMoV in the database.	34
Figure 10: NCBI BLAST of the sequence data for the NMF3 isolate (227 bp) under accession number KT970621. The sequence was identified as a coat protein of MCMV in the database.	34

Figure 11: NCBI BLAST of the sequence data for the NMF6 isolate (230 bp) under accession number KT970622. The sequence was identified as a coat protein of MCMV in the database.	35
Figure 12: NCBI BLAST of the sequence data for the LVIC1 isolate (833 bp) under accession number KT970623. The sequence was identified as a polyprotein of SCMV in the database.	35
Figure 13: NCBI BLAST of the sequence data for the LVIC2 isolate (659 bp) under accession number KT970624. The sequence was identified as coat protein of SCMV in the database. .	36
Figure 14: NCBI BLAST of the sequence data for the LVIC3 isolate (833 bp) under accession number KT970625. The sequence was identified as polyprotein of SCMV in the database...	36
Figure 15: NCBI BLAST of the sequence data for the Serere1 isolate (9,904 bp) under accession number KT726938. The sequence was identified as a complete genome of CABMV in the database.	37
Figure 16: Schematic representation of the genome of Serere1 isolate. The length of nucleotide and amino acid sequences of mature proteins are indicated. The proteolytic cleavage sites for the 10 proteins identified in this isolate were as follows; P1/HC-Pro(VQHYS), HC-Pro/P3(YKVG/G), P3/6K1(VETQ/A), 6K1/CI(VRMQ/S), CI/6K2(VCLQ/S), 6K2/VPg (VITQ/G), VPg/Nla(VGVE/S), Nla/Nib(VATQ/S), Nib/CP (VMLQ/S).	38
Figure 17: Translation of <i>Cowpea aphid-borne mosaic virus</i> genome with cleavages sites (yellow) and conserved motifs (blue) highlighted.	40
Figure 18: Maximum Likelihood method tree for complete genomes (A) and coding sequences (B) of CABMV isolates.	43
Figure 19: Maximum Likelihood tree for coat protein sequences of CABMV isolates.	43
Figure 20: RT-PCR product for CABMV resulting from CABFFI/CABRR1 primer pair: Lane 1: Negative control, lanes 2-6: Samples, lane 7: Positive control.....	52
Figure 21: RT-PCR product for CABMV for GF/GR primer pair: M is 100 bp ladder, lane 1-5: Samples, lane 6: Negative control.....	53

Figure 22: NCBI BLAST of the sequence data for the RT-PCR fragment from CABFF1/CABRR1 primer.	53
Figure 23: Phylogenetic analysis of complete CP encoding nucleotide sequences of three CABMV isolates from Uganda and other countries. The tree was rooted on the sequence of <i>Barley yellow dwarf virus</i>	56
Figure 24: Virus symptoms on infected cowpea leaves: (A) Vein banding and leaf curling, (B) vein banding and leaf distortion, (C) leaf mottling, (D) mild mosaic, (E) intense mosaic, (F) severe mosaic, (G) Mosaic and necrotic spots, (H) Necrotic spots and (I) purpling.	63
Figure 25: Mega-environment and most responsive genotypes based on AUDPC among cowpea genotypes across three locations in Uganda using a genotype focused biplot.....	69
Figure 26: Ranking of genotypes in relation to the “most susceptible” to viral infection based on AUDPC values using a genotype focused biplot.	70
Figure 27: Mega-environments and most responsive genotypes for virus incidence in cowpea genotypes across locations in Uganda using a genotype focused biplot.....	71
Figure 28: Ranking of cowpea genotypes based on virus incidence using a genotype focused concentric circle biplot.	72
Figure 29: Biplot view of mega-environments and winning genotypes for grain yield using a genotype focused biplot.	73
Figure 30: Ranking of cowpea genotypes relative to the ideal genotype for grain yield using a genotype focused concentric circle biplot.	74

LIST OF TABLES

Table 1: Sequence identity (%) comparison of mature proteins of CABMV obtained in this study with five genome sequences	41
Table 2: Primers used for detection of CABMV in Ugandan cowpea samples	50
Table 3: Pairwise sequence comparison matrix for complete coat protein nucleotide sequences of three Ugandan isolates and other isolates of CABMV ^a	55
Table 4: List and source of cowpea genotypes used in the study	61
Table 5: Mean squares for area under disease progress curve, incidence, yield and other agronomic traits	65
Table 6: Correlation of AUDPC, incidence and yield	66
Table 7: Mean values of AUDPC, incidence and yield across three locations	68

LIST OF APPENDICES

Appendix 1: Mean values of AUDPC, severity, incidence (%) and yield (kg ha ⁻¹) for 105 cowpea genotypes evaluated in 2012	99
---	----

DOCTORAL COMMITTEE MEMBERS

Assoc. Prof. Settumba B. Mukasa, School of Agricultural Sciences, Makerere University

Prof. Phinehas Tukamuhabwa, School of Agricultural Sciences, Makerere University

Dr. Jenipher Bisikwa, School of Agricultural Sciences, Makerere University

Dr. James Ssebuliba, School of Agricultural Sciences Makerere University

Prof. Samuel Kyamanywa, School of Agricultural Sciences, Makerere University

Dr. Denis Mpairwe, School of Agricultural Sciences, Makerere University

DEDICATION

This work is dedicated to my dear wife, *Anita* and our wonderful children, *Enoch, Mishael, Tendo and Caleb* for their prayers, love and support during the period of my studies.

"Praise the LORD, my soul, and forget not all his benefits -Psalm 103:2"

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to Assoc. Prof. Settumba Mukasa and Prof. Phinehas Tukamuhabwa, my academic supervisors, for their invaluable advise, counsel, friendship and guidance during the course of my studies. Appreciation is extended to other members of my doctoral committee; Dr. Jennipher Bisikwa, Dr. Denis Mpairwe, Prof. Samuel Kyamanywa and Dr. James Ssebuliba, for their helpful advice and suggestions during research and preparation of this thesis. I also wish to thank Dr. Valentine Aritua (formerly at CIAT, Kawanda) for granting me access to CLC Genomics Workbench software to analyze sequence data. The invaluable advise offered by Dr. Arthur Tugume, from College of Natural Sciences (CoNAS), Makerere University and Dr Deusdedith Mbanzibwa from Mikocheni Agricultural Research Institute (MARI), Tanzania is duly acknowledged.

I wish to thank my sponsors the McKnight Foundation through the Collaborative Crops Research Program (CCRP). The support of the CCRP regional team (Linnet Gohole, and Rebecca Nelson) and the research methods team led by Richard Coe is duly acknowledged. I also acknowledge partial funding from Carnegie Corporation of New York through the Regional Universities Forum for Capacity Building in Agriculture (RUFORUM). Support for laboratory work received from Biosciences eastern and central Africa-International Livestock Research Institute (BecA-ILRI Hub) through Africa Biosciences Challenge Fund (ABCF) fellowship program is greatly acknowledged. The support extended to me by the National Agricultural Research Organization (NARO) through the National Semi-Arid Resources Research Institute (NaSARRI) and the National Agricultural Research Laboratories (NARL) is duly acknowledged.

My thanks also go to the administrative and scientific staff at BecA-ILRI Hub in Nairobi for providing a conducive environment during my fellowship program. I particularly thank Mercy Macharia, Solomon Maina and Dr. Timothy Holton for the support and guidance during laboratory work. I thank Moses Njahira, Valerian Aloo and Rob Skilton for coordinating the necessary logistical support.

I am grateful for the support extended to me by Warren Arinaitwe and Allan Male Sekamatte from CIAT-Kawanda while conducting laboratory experiments at National Agricultural Research Laboratories (NARL), Kawanda.

Finally, I appreciate my family especially my wife, *Anita* and our sons for giving me the much needed support through prayer and encouraging words during this study. It is my prayer that God richly rewards you. The sacrifices of my late mother, Thereza Tolofaina Kabale who took pride in every academic success I made is greatly appreciated. It is a pity that you did not live to witness this great achievement!

PUBLICATIONS FROM THIS THESIS

Some of the results from this thesis have been published as research articles/conference papers under the following titles:

Mbeyagala, E.K., Tukamuhabwa, P., Bisikwa, J., Holton, T. and Mukasa, S.B. 2018a. Next-generation sequencing reveals the first complete genome sequence of *Cowpea aphid-borne mosaic virus* from Uganda. *Genome Announcements* 6: e01491-17. <https://doi.org/10.1128/genomeA.01491-17>.

Mbeyagala, E.K., Bisikwa, J., Tukamuhabwa, P. and Mukasa, S.B. 2018b. Trait association and stability of virus resistance among cowpea genotypes in Uganda. *African Crop Science Journal* 26(2):259-275. <http://dx.doi.org/10.4314/acsj.v26i2.8>.

Mbeyagala, E.K., Tukamuhabwa, P. and Mukasa, S.B. 2018c. Detection of *Cowpea aphid-borne mosaic virus* (CABMV) in cowpea by reverse transcription polymerase chain reaction. *African Crop Science Journal* 26(3):377-387. <http://dx.doi.org/10.4314/acsj.v26i3.4>.

Mbeyagala, E.K., Mukasa, S.B., Holton, T., Aritua, V., Katari, S. M., Tukamuhabwa, P. and Bisikwa, J. 2016. Application of next generation sequencing in identification of cowpea viruses. *RUFORUM Working Document Series* 14(1):653-660. ISSN 1607-9345

Mbeyagala, E.K., Mukasa, S.B., Tukamuhabwa, P. and Bisikwa, J. 2014. Evaluation of cowpea genotypes for virus resistance under natural conditions in Uganda. *Journal of Agricultural Science* 6(10):176–187. <http://dx.doi.org/10.5539/jas.v6n10p176>.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Origin, distribution and domestication of cowpea

Cowpea (*Vigna unguiculata*) originated in Africa due to the abundance of wild and weedy forms but there are contrasting reports regarding the precise location for its origin or domestication. According to Padulosi and Ng (1997) and Madamba *et al.* (2006), cowpea originated in southern Africa due to the presence of highest genetic diversity and most primitive forms of wild *Vigna unguiculata*. This region encompasses Namibia, Botswana, Zambia, Zimbabwe, Mozambique, Republic of South Africa and eSwatini with the Transvaal region of South Africa as the centre of speciation for *Vigna unguiculata*. However, cowpea could have been brought under cultivation in any part of the continent (Padulosi and Ng, 1997).

The centre of maximum diversity of cultivated cowpea is reportedly found in West Africa (Padulosi and Ng, 1997; Madamba *et al.*, 2006). Some reports suggest the origin of cowpea to be in West Africa (Harlan, 1971; Rawal, 1975; Pienaar and van Wyk, 1992). Lush and Evans (1981) however, proposed the origin of cowpea to be West and Central Africa. Presence of high diversity and unique isozyme (Pasquet, 1996) and allozyme (Pasquet, 2000) polymorphisms among the northeast African accessions from Cv.gr. Biflora compared to West African accessions suggests that northeast Africa is a possible center of domestication for cowpea. Analysis of AFLP data by Coulibaly *et al.* (2002) suggests that cowpea originated from the north eastern Africa due to great diversity in the wild taxon, however, precise localization of domestication was not possible as a result of extensive gene flow between wild and domesticated forms. The wide distribution of ssp. *unguiculata* var. *spontanea*, the progenitor of wild cowpea across Africa also makes it difficult to define the center of origin for cultivated cowpea (Pasquet, 1999). Based on RAPD marker analysis, Ba *et al.* (2004) reported higher diversity within species *Unguiculata* var. *spontanea* from the East African region than from Southern and Western Africa suggesting that wild populations that gave rise to domesticated cowpea must be located in the northern part of Africa as earlier proposed by Pasquet (1999) and Coulibaly *et al.* (2002). The studies based on AFLPs (Coulibaly *et al.*,

2002) and RADPs (Ba *et al.*, 2004) showed that domestication of cowpea in eastern or southern Africa is highly unlikely. The actual point of origin of cowpea can only be concluded after collection and studying of wild accessions from northeastern Africa especially from Sudan, Eritrea, and Ethiopia (Pasquet, 1999; Coulibaly *et al.*, 2002).

Cowpea was probably introduced into Europe around 300 BC and to India 200 BC (Madamba *et al.*, 2006). As a result of human selection in China, India and South-East Asia, cowpea underwent further diversification to produce two cultivar-groups, *Sesquipedalis* with long pods used as a vegetable, and *Biflora* grown for the pods, dry seeds and for fodder. It is estimated that cowpea was introduced to tropical America in the 17th century by the Spanish traders and is widely grown in the United States, the Caribbean region and Brazil (Madamba *et al.*, 2006).

1.2 Taxonomy of cultivated cowpea

Cowpea, *Vigna unguiculata* (L.) Walp, is divided into ten perennial subspecies and one annual subspecies, ssp. *Unguiculata* (Pasquet, 1993a; 1993b; 1997). The ssp. *Unguiculata* is further split into var. *unguiculata* (cultivated cowpea) and var. *spontanea* (Schweinf.) Pasquet (annual wild cowpea). Cultivated cowpea is subdivided into five cultivar-groups (cv.gr) based on seed and pod characters (Westphal, 1974; Mithen and Kibblewhite, 1993) as well as ovule number and photo sensitivity (Pasquet, 1996; 1998). The cv.gr include *Unguiculata*, *Biflora*, *Melanophthalmus*, *Sesquipedalis* and *textilis*.

cv.gr. *Unguiculata* is characterized by high ovule numbers (>16), late flowering even under inductive conditions, lower epicotyl length, higher peduncle length, thick and shiny testa, and pod length of less than 30cm. cv.gr. *Unguiculata* occurs both in Africa and Asia but in Africa, it is more characteristic of southern and eastern Africa although it has been introduced to west Africa as well (Pasquet, 1998). Cv.gr. *Biflora* also known as catjang/catiang has ovule numbers less than 17, flowers quickly under inductive conditions, has thick testa and is very close to wild cowpea in terms of primary leaf width, flower length, calyx-lobe length, pod length, pod width, seed width and pod dehiscence (Pasquet, 1998). Cv. *Biflora* occurs both in Africa and Asia (Pasquet, 1998) and is mainly used as a forage legume (Coulibaly *et al.*, 2002). Cv.gr. *Melanophthalmus* is found mainly in West Africa and has white flower and

white seed, thin and wrinkled seed testa, lower ovule number (<17), flowers quickly under inductive conditions, unfleshy short pods ($<30\text{cm}$) and seeds are not spaced within the pods. Blackeyed cowpeas fall in this group. Cv.gr. *Sesquipedalis* also known as “yard long” or “asparagus bean” occurs mainly in Asia and is characterized with long fleshy pods ($>30\text{ cm}$) that are wrinkled when ripe, spaced seeds within the pods, seeds are kidney shaped and has higher ovule number (>17). This group is mainly grown as a vegetable or snap bean. Cv.gr. *Textilis* is mainly cultivated for its fibres and has long floral peduncles ranging from 40cm to one-meter length. Based on allozyme diversity (Pasquet, 2000), cv.gr. *Melanophthalmus* and cv.gr. *Sesquipedalis* are less diverse than their putative primitive progenitors, *Biflora* and *unguiculata*.

1.3 Importance of cowpea

Cowpea is a multifunctional legume (Timko and Singh, 2008) with every part of this crop serving a useful purpose. The crop is used for human food, hay, pasture, silage, soil cover, green manure and as a concentrate for livestock (Westphal, 1974; Pienaar and van Wyk, 1992; Tarawali *et al.*, 1997; Hall *et al.*, 2003). The young leaves and shoots are consumed as spinach (vegetable) and thus provide one of the most widely used pot herbs in Africa (Westphal, 1974; Timko and Singh, 2008). In the arid and semi-arid regions, young leaves and shoots may be preserved by sun drying or boiling and then sun-drying to be used during the dry season (Westphal, 1974; Pienaar and van Wyk, 1992; Madamba *et al.*, 2006; Timko and Singh, 2008). When the apical meristems are cut back regularly starting at an early stage, the crop continues to produce new leaves thus ensuring availability of the pot herb for a longer period.

The grain or seed which is the most important part of cowpea for human consumption is rich in proteins (23-32%), minerals, and vitamins especially folic acid (B vitamin) that prevents defects in the brain and the spinal cord of unborn children (Hall *et al.*, 2003). Cowpea protein nutritionally complements low protein cereal or tuber crop (starch foods) which are common in many African diets. The seeds are often harvested, dried for storage and consumption at a later time either after boiling/roasting whole or dehulled or after being milled into a flour product for use in several recipes. The young seeds are also edible and in many parts of the world especially in the United States, consumption of fresh seeds and green pods is preferred to the cooked dried seeds (Hall *et al.*, 2003; Madamba *et al.*, 2006). Cowpea is used as a

fodder especially in West Africa, Asia, and Australia where it is used for grazing or cut and mixed with dry cereals for animal feed (Tarawali *et al.*, 1997; Madamba *et al.*, 2006). The haulms of cowpea have higher crude protein, digestibility and mineral composition and therefore compare very well with commonly used forage legumes (Tarawali *et al.*, 1997).

In the southern United States, cowpeas are mainly produced for use as canned and frozen products (Ehlers *et al.*, 2002; Hall *et al.*, 2003). It is also grown as a green manure and as a cover crop (Ehlers *et al.*, 2002; Hall *et al.*, 2003) in the United States. Cowpea improves soil fertility through fixation of atmospheric nitrogen and often does not require additional fertilizers to achieve high grain yields (Hall *et al.*, 2003). In Nigeria, varieties from cv.gr. Textilis are grown for fibres extracted from long peduncles for making fishing gear and good quality paper (Pienaar and van Wyk, 1992; Madamba *et al.*, 2006). Cowpea is also reported to have medicinal values; for instance, seeds and leaves are used to treat inflammations and skin infections, leaves treat tooth ailments. The roots are used as an antidote for snake bites, treating epilepsy, chest pain and constipation (Madamba *et al.*, 2006). Culturally, seeds of wild or weedy cowpea are used as an aphrodisiac (for arousing sexual desire) and for puberty as well as initiation rites for adolescent males in several West African communities (Rawal, 1975). In Uganda, cowpea is used in important cultural occasions such as child naming and funerals (Mundua, 2010).

1.4 Cowpea production status

Dry grain production is the only commodity of cowpea formally estimated on a worldwide basis. The United Nations Food and Agricultural Organization (FAO) estimated that nearly 7,407, 924 tons of dry cowpea grain were produced in 2017 on about 12,577,845 million hectares of land worldwide (<http://faostat3.fao.org/>). Africa was responsible for over 95% (7,107,334 tons) of the global production. In the same year, Nigeria was the largest producer of cowpea, producing 3,409,992 tons of dry grain followed by Niger, Burkina Faso, Tanzania, Cameroon and Myanmar (<http://faostat3.fao.org/>). The average yield worldwide in 2017 was estimated at 589 kg ha⁻¹, the lowest value of the major tropical grain legumes. In 2017, western Africa produced the highest amount of cowpea (6,185,750 tons), followed by eastern Africa (508,187 tons), middle Africa (270,780 tons), northern Africa (136,959 tons) and southern Africa (5,658 tons) respectively (<http://faostat3.fao.org/>).

In Uganda, total production of cowpea in 2018 was estimated at 15,838 tons from an area of about 53,823 ha with average national yield estimated at less than 500 kg ha⁻¹ (UBOS, 2019). The eastern and northern regions of the country are the main producers of cowpea compared to the western and central regions (UBOS, 2010). While cowpea is grown in two seasons in Uganda, most production/cultivation occurs in the second season (UBOS, 2010). Production goals and socio-economic status of farmers are key determinants in the production of cowpea in Uganda. For instance, commercial and dual purpose farmers grow cowpea on larger pieces of land compared to subsistence farmers (Isubikalu *et al.*, 1999).

1.5 Cowpea production constraints

Average cowpea yield in Uganda is low (less than 500 kg ha⁻¹) (UBOS, 2019). Several factors such as diseases, pests, low plant population densities and poor weed management contribute to the low cowpea yields in Uganda (Adipala *et al.*, 1999). Production is mainly done by subsistence farmers on small pieces of land (0.6 ha), and often lack resources to acquire required inputs (Isubikalu *et al.*, 1999).

Several viral, bacterial and fungal diseases attack cowpea and contribute greatly to the low productivity of the crop. Bacterial blight and bacterial pustule are the major bacterial diseases while bacterial wilt and halo blight are regarded as minor disease of cowpea (Emechebe and Florini, 1997). Eleven major fungal diseases are reported to infect cowpea worldwide (Kannaiyan and Haciwa, 1993; Emechebe and Florini, 1997). In Uganda, the major fungal diseases include cercospora leaf spot, anthracnose, scab, false rust, dactuliophora leaf spot, rust and powdery mildew (Edema *et al.*, 1997; Adipala *et al.*, 1999).

In addition to bacterial and fungal diseases, the high prevalence of viral diseases (Edema *et al.*, 1997) in the main cowpea growing areas is a major limitation to cowpea production in Uganda. Yield losses of up to 87% or total cowpea crop failure due to viral infection were reported in Iran (Kaiser and Mossahebi, 1975). In addition to yield reduction, infection of cowpea by viral diseases also reduces the protein content of the grain (Taiwo and Akinjogunla, 2006; Kareem and Taiwo, 2007). Cowpea is susceptible to more than 140 viruses (Hughes and Shoyinka, 2003), of which nine were reported in Uganda. These include; *Cucumber mosaic virus* (CMV), *Cowpea mild mottle virus* (CPMMV), *Cowpea mottle virus* (CPMoV), *Cowpea chlorotic mottle virus* (CCMV), *Cowpea yellow mosaic virus* (CYMV),

Cowpea severe mosaic virus (CPSMV), *Cowpea aphid-borne mosaic virus* (CABMV) and *Southern bean mosaic virus* (SBMV) and *Cowpea mosaic virus* (CPMV) (Amayo *et al.*, 2012; Orawu *et al.*, 2015). Transmission of these viruses can be through seed, sap or vectors by several insect species such as aphids, whiteflies and beetles (Ndiaye *et al.*, 1993; Bashir *et al.*, 2002a). Transmission through seed is very destructive because young plants/seedlings are exposed early to viral inoculums thus producing greater damage (Ndiaye *et al.*, 1993). With increasing exchange or sharing of cowpea seed/germplasm globally, it is highly likely that there could be other unidentified viruses infecting cowpea in the country. Previous attempts to identify cowpea viruses in Uganda were specific in nature (Edema *et al.*, 1997; Orawu *et al.*, 2005; Amayo, 2008; Amayo *et al.*, 2012; Orawu *et al.*, 2015) and thus not helpful in identifying previously uncharacterized pathogens (Adams *et al.*, 2009; Kreuze *et al.*, 2009). Deployment of accurate and reliable diagnostic tools as well as use of varieties with broad resistance are thus critical to successful management of viral infections (Ndiaye *et al.*, 1993; Bashir *et al.*, 2002a; Naidua and Hughes, 2003). However, previously assays used to detect cowpea viruses were less reliable (Amayo, 2008; Amayo *et al.*, 2012). Additionally, earlier attempts to improve resistance were narrow in scope focusing only on *Cowpea aphid-borne mosaic virus* (CABMV) (Orawu, 2007).

1.6 Problem Statement

Diseases caused by viruses are widespread and a major constraint to cowpea production in the main growing areas of Uganda negatively affecting the livelihoods of many subsistence farmers that rely on it. Earlier studies have documented the occurrence of up to nine cowpea viruses in Uganda (Edema *et al.*, 1997; Orawu *et al.*, 2005; Amayo *et al.*, 2012; Orawu *et al.*, 2015). With increasing and ongoing exchange or movement of seed/genetic resources through research/trade, it is highly likely that other unknown viruses may have been inadvertently introduced in Uganda. The limited available knowledge on the identity of viruses in the country is a great hinderance to effective management of cowpea viruses. Also, reliable diagnostic assays that can accurately detect cowpea viruses in the country are lacking (Amayo, 2008; Amayo *et al.*, 2012). There is a therefore a likelihood that other viruses were not been detected in previous studies. Previous attempts to improve resistance to cowpea viruses in locally grown susceptible germplasm focused solely on *Cowpea aphid-borne mosaic virus* (CABMV) (Orawu, 2007). These studies screened 54 cowpea genotypes of

which five were identified as resistant to CABMV. The five identified accessions were later used to study genetic inheritance of resistance to CABMV by Orawu *et al.* (2012). However, no improved varieties with broad virus resistance were developed. Breeding for resistance focusing on a single pathogen or strain is less durable and thus can be easily broken down by other or unknown viruses or strains (Rossel and Thottappilly, 1985; Ndiaye *et al.*, 1993). Cowpea farmers in Uganda are mainly resource constrained hence virus disease management strategies that have been proposed such as chemical control of vectors, roguing, intercropping and timing of planting (Kannaiyan and Haciwa, 1993; Thresh, 2003; Ambang *et al.*, 2009) are not easily adopted by farmers.

1.7 Justification

Developing resistant varieties would reduce infections caused by viral diseases significantly improving the nutritional quality and productivity of cowpea positively impacting the livelihoods of smallholder farmers in Uganda. Resistant varieties would also be easily adopted by farmers compared to management practices. However, developing resistant varieties requires better understanding of the identity and variability of the pathogen(s). There is need therefore for proper identification of cowpea viruses in Uganda. Secondly, in order to effectively manage cowpea viral infections, there is need to develop reliable and accurate virus diagnostic assays. Additionally, developing resistant varieties to reduce the damage by viral infections requires identification of new sources of resistance coupled with high yield potential in the country. There is therefore need to screen and evaluate a wide range of cowpea genotypes in different conditions to identify promising sources of resistance.

1.8 Objectives of the study

1.8.1 Main objective

The main objective of this study was to generate knowledge required for improved management of cowpea viral diseases in Uganda.

1.8.2 Specific objectives

- 1) To identify and characterize viruses infecting cowpea in Uganda.
- 2) To develop a molecular diagnostic assay for *Cowpea aphid-borne mosaic virus* (CABMV) in Uganda.

- 3) To determine the level of virus resistance among cowpea genotypes under field conditions.

1.8.3 Hypotheses

1. New or novel viruses infect cowpea in Uganda.
2. Molecular assays can accurately detect *Cowpea aphid-borne mosaic virus* in Uganda.
3. Virus resistant and high yielding cowpea genotypes exist among Ugandan cowpea germplasm.

CHAPTER TWO

LITERATURE REVIEW

2.1 Taxonomy of viruses

Unlike cellular organisms that have a last universal common ancestor (LUCA), viruses do not have a presumed common ancestor (Glansdorff *et al.*, 2008). The origin of viruses is thus complicated with three hypotheses proposed to explain their origin: 1) they arose from mobile genetic elements that gained the ability to move between cells; 2) are remnants of cellular organisms that adopted a parasitic replication strategy; and 3) they predate or coevolved with their current cellular hosts (Wessner, 2010). The viruses can be divided into classes each of which has its own method of transmitting its genetic material from one generation to the next and its own style of expressing its genetic information (Baltimore, 1971). The Baltimore system classifies viruses into seven trees of life based on nature of the nucleic acids in the virion particle;

Class I consists of double strand DNA (dsDNA) viruses which include viruses with the largest known genomes (up to 800 kb) with a single genome component which may linear or circular. Well known viruses in this group include the head tail viruses such as herpesvirus. There are no plant viruses in this group. Class II consists of single stranded DNA (ssDNA) with two families of plant viruses, *Geminiviridae* and *Nanoviridae*. Viruses in this group have small circular genome components of up to 5.6 kb. Class III consists of double stranded RNA viruses (dsRNA); plant viruses in this group include *Endornaviridae*, *Partitiviridae* and *Reoviridae*. Class IV includes positive sense single stranded RNA viruses (ssRNA+); this group includes many viruses that cause respiratory infections for instance SARS- coronavirus, (SARS-CoV) and also the causal agents of polio, hepatitis, foot and mouth disease (FMD). This group also includes the majority of plant virus families such as *Bromoviridae*, *Closteroviridae*, *Comoviridae*, *Potyviridae*, *Tombusviridae*, *Virgaviridae*, *Secoviridae*, *Marnaviridae*, *Tymoviridae*, *Alphaflexiviridae* and *Betaflexiviridae*. Class V includes negative sense single stranded RNA (ssRNA-) and has some plant viruses in families such as *Rhabdoviridae*, *Bunyaviridae*, *Ophioviridae*. Class VI and VII include the retro-transcribing viruses which are single stranded (ssRNA) or double stranded, (dsRNA), respectively. This

group of retro-viruses includes HIV. There is a single family of plant viruses (*Caulimoviridae*) in this group that is characterized by a mono-partite open circular double stranded DNA of between 7-8.2 kb with discontinuities in both strands (Hulo *et al.*, 2011).

Within each of the above virus classes or groups, different characteristics are used (often in combination) to classify the viruses into families, genera and species. The most important characteristics are particle morphology, genome, serological and biological properties. Among these, serological properties such as ELISA are still widely used although their significance in taxonomy has reduced because they are based on the antigenic properties of the virus coat protein, which represents only about 10% of the total virus genome thus does not take into account the rest of the virus genome. In addition, serology based approaches have limited application in the detection of viroids, satellite RNAs, viruses which lack particles, viruses which occur as extremely diverse serotypes and viruses that are poor immunogens or are difficult to purify (Naidua and Hughes, 2003). Availability of nucleotide sequence data (partial/full) for conserved regions such as coat protein and/or full genome sequences makes it easy to classify viruses into various families, genera and species (Adams *et al.*, 2005a).

2.2 Viruses infecting cowpea and their transmission

Cowpea is infected by more than 140 viral diseases worldwide (Hughes and Shoyinka, 2003), of which 11 naturally occur in Sub-Saharan Africa (Thottappilly and Rossel, 1992; Hughes and Shoyinka, 2003). Most cowpea viruses are seed-borne and hence have been transmitted to many parts of the world through germplasm exchanges (Hampton *et al.*, 1997). Secondary spread in the field is carried out by insect vectors especially aphids and beetles (Hampton *et al.*, 1997). Some of the most damaging seed-borne naturally occurring viruses in sub-Saharan Africa are; *Bean common mosaic virus* strain *blackeye cowpea* mosaic (BCMV-BICM), *Cowpea aphid-borne mosaic potyvirus* (CABMV), *Cucumber mosaic cucumovirus* (CMV), *Cowpea mosaic comovirus* (CPMV), *Cowpea yellow mosaic comovirus* (CYMV), *Cowpea severe mosaic comovirus* (CPSMV), *Southern bean mosaic sobemovirus* (SBMV), *Cowpea mottle carmovirus* (CPMoV), *Cowpea mild mottle carlavirus* (CPMMV). Important non seed-borne cowpea viruses include *Cowpea golden mosaic* (CpGMV) *begomovirus* and *Cowpea chlorotic mottle* (CCMV) *bromovirus*. These viruses (seed-borne or non-seed-borne) cause

diseases either alone or in combination with other viruses (Thottappilly and Rossel, 1992; Hampton *et al.*, 1997).

2.2.1 Cowpea aphid-borne mosaic virus (CABMV)

CABMV causes a serious mosaic on cowpea and occurs in several countries wherever cowpea is grown (Bashir *et al.*, 2002a). The virus has rod-shaped filamentous particles of about 750 nm in length with a wide host range that includes both legume and non-legume species (Lovisolo and Conti, 1966; Bock, 1973a; Bock and Conti, 1974; Kaiser and Mossahebi, 1975; Ndiaye *et al.*, 1993). CABMV is seed-borne in cowpea although its seed transmission is very erratic depending on the virus strain and cowpea variety. For instance, Lovisolo and Conti (1966) reported a seed transmission rate of 0.3-1.59%, Bock (1973a) reported 0-3%, Kaiser and Mossahebi (1975) recorded 1.1-39.8%, Kannaiyan and Haciwa (1993) reported up to 10% transmission rates. Ndiaye *et al.* (1993) reported 0-30% seed transmission rates in five Senegalese cowpea genotypes while Bashir (1992) reported seed transmission rates of 0.0% to 55%. However, there appears to be no correlation between yield reduction and the percentage of seed transmission (Kaiser and Mossahebi, 1975).

The virus is also transmitted non-persistently (stylet-borne) by several aphid species such as *Aphis craccivora*, *A. fabae*, *A. gossypii*, *A. medicaginis*, *Macrosiphum euphorbiae* and *Myzus persicae* (Lovisolo and Conti, 1966; Bock, 1973a). Infected plants show a range of symptoms for instance, dark green vein banding, interveinal chlorosis, leaf distortion, blistering and stunting (Bock and Conti, 1974; Bock, 1973a; Kaiser and Mossahebi, 1975). Four strains of the virus have been reported; the *European (type) strain* (Lovisolo and Conti, 1966) causes a severe distorting mosaic in cowpea (Bock and Conti, 1974); the *African (neo-type) strain* (Bock, 1973a) induces irregular, angular, dark-green, broken vein-banding on a light green mosaic (Bock, 1973a; Bock and Conti, 1974). This strain expresses symptoms similar to the European strain; the *African mild strain* (Bock, 1973a) induces very mild mottle symptoms (Bock, 1973a; Bock and Conti, 1974); the *African vein banding strain* (Bock, 1973a) which induces broad dark-green vein-banding symptoms on a greenish-yellow background (Bock, 1973a; Bock and Conti, 1974).

Yield losses due to natural field infection of cowpea by CABMV are significant. For instance Kaiser and Mossahebi (1975) reported yield reduction by 13% to 87% while Kannaiyan and

Haciwa (1993) reported 48% to 60% yield loss. Additionally when CABMV interacts with CMV, cowpea stunt disease (Pio-Ribeiro and Kuhn, 1980) is caused. This is a very severe disease that causes significant yield losses (up to 87.3%) (Pio-Ribeiro *et al.*, 1978).

Mlotshwa *et al.* (2002) reported the first complete genome sequence of CABMV from Africa (CABMV-Z). CABMV-Z sequence (Figure 1) has a single large open reading frame (ORF) of 9,159 nucleotides which encodes for a large polyprotein of 3,053 amino acids. The 3' terminus of the CABMV-Z consists of 1,221 nucleotides with ORF of 990 nucleotides and a non-coding region of 231 nucleotides followed by a poly (A) tail (Sithole-Niang *et al.*, 1996).

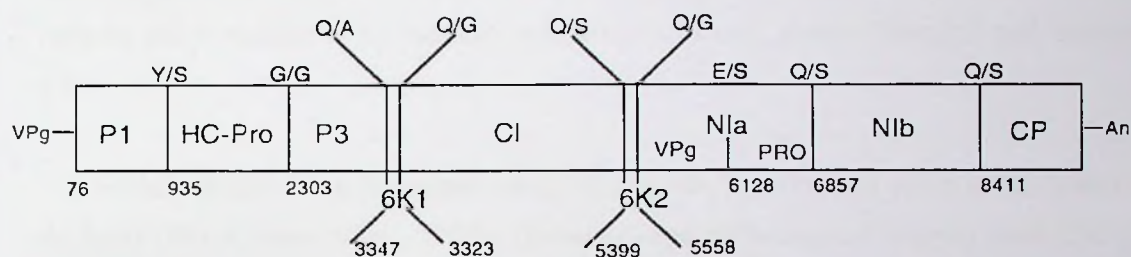


Figure 1: Genomic map of *Cowpea aphid-borne mosaic potyvirus*. The open bar represents the ORF and UTRs are indicated by a single line. Vertical lines indicate putative cleavage sites within the polyprotein. Amino acids on either side of each cleavage site are shown above the site. Ten mature proteins are predicted from the polyprotein. Numbers below the map indicate the position of the first coding nucleotide of each gene. An: poly (A) tail; VPg: genome linked protein. **Source:** Mlotshwa *et al.* (2002).

2.2.2 *Bean common mosaic virus- blackeye cowpea mosaic strain (BCMV-BICM)*

BCMV-BICM has similar distribution like CABMV and the two viruses were previously thought to be serologically related or the same (Purcifull and Gonsalves, 1985; Dijkstra *et al.*, 1987). However, a close examination of their serological properties revealed that they belong to different serogroups (Taiwo and Gonsalves, 1982; Huguenot *et al.*, 1993) and have distinct differential cowpea hosts (Taiwo *et al.*, 1982). Comparison of the sizes of the capsid (capsid Mr) and HPLC capsid peptide profiles (Huguenot *et al.*, 1994) further showed that BCMV-BICM and CABMV are distinct potyviruses. BCMV-BICM causes a mosaic disease on cowpea (*V. unguiculata* ssp. *unguiculata*, asparagus bean (*V. unguiculata* ssp. *Sesquipedalis*) and *Crotalaria spectabilis* (Purcifull and Gonsalves, 1985). Systemic symptoms due to BCMV-BICM include severe mottling, distortion, yellowing, mosaic and vein necrosis

(Thottappilly and Rossel, 1992). The virus has a wide host range that includes both legumes and non-legumes (Purcifull and Gonsalves, 1985; Dijkstra *et al.*, 1987).

The virus is readily transmitted through seed and by aphids especially *A. craccivora* and *Myzus persicae* (Purcifull and Gonsalves, 1985), however transmission through seed is variable depending on the virus strain/isolate and the variety (Bashir, 1992). Zettler and Evans (1972) reported 0.0% to 30.9% seed transmission rates while Bashir (1992) reported up to 48.5% seed transmission rates. BCMV-BICM has flexuous filamentous particles with a length of 720-760 nm with a mode at 743 nm that contain single stranded RNA (Taiwo *et al.*, 1982). It induces the formation of cylindrical inclusions in its host plants (Purcifull and Gonsalves, 1985).

While BCMV-BICM is an important virus on cowpea, its effect on yield is minimal (2.5% yield loss) (Pio-Ribeiro *et al.*, 1978). However, dual infection of cowpea with CMV and BCMV-BICM results into cowpea stunt, a disease associated with up to 87.5% yield loss (Pio-Ribeiro and Kuhn, 1980; Pio-Ribeiro *et al.*, 1978). In addition to stunting, the doubly infected plants have small, mottled, blistered and malformed leaves.

The genome is monopartite with linear ssRNA (+). The 3' terminus has a poly (A) tract while the 5' terminus has a genome-linked protein (VPg) as shown in Figure 2. The complete genome of BCMV-BICM is 9,612 nucleotides long, excluding a 3'-terminal poly(A) tail. The putative start codon located at nucleotide positions 170-172 initiates one large open reading frame that is terminated with a UAA codon at position 9,368-9,370. The predicted polyprotein has 3,066 amino acids and an M(r) of 340.3 kDa. The positions of putative cleavage sites are analogous to consensus sequences in other potyviruses (Fang *et al.*, 1995).

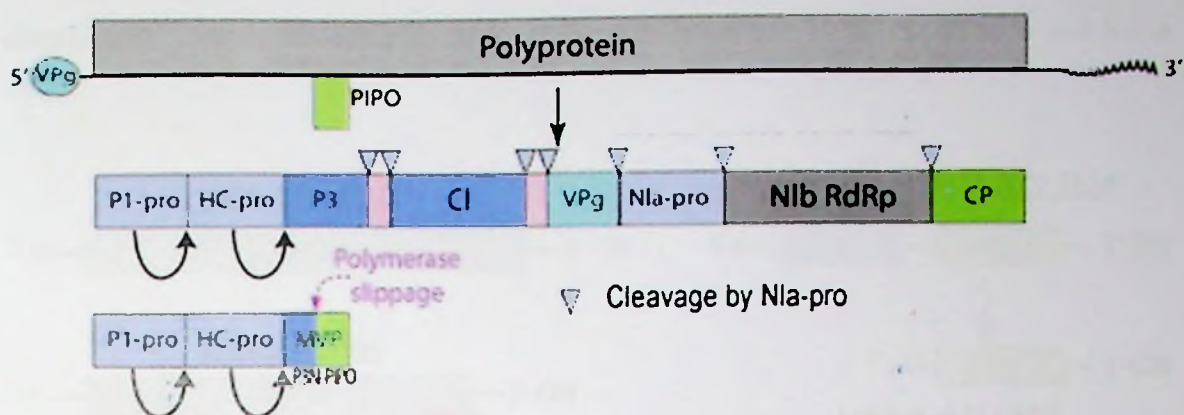


Figure 2: Genome structure of potyviruses. Source: Hulo *et al.* (2011).

2.2.3 Cucumber mosaic virus (CMV)

CMV belonging to the genus *Cucumovirus* has a very wide host range (host range breadth) and a worldwide distribution (Francki *et al.*, 1979). The wide host range of CMV is largely attributed to its segmented genome (multipartite) which offers increased genome stability, high replication fidelity, increased replication as well as better regulation of gene expression and genome exchange through reassortment between particles (Ojosnegros *et al.*, 2011; Sicard *et al.*, 2016; Moury *et al.*, 2017). CMV has isometric particles (Fischer and Lockhart, 1976a; Francki *et al.*, 1979) with approximately 28 nm diameter containing single stranded RNA with a tripartite/multipartite genome composed of RNA1, RNA2 and RNA3 (Figure 3). Each genomic segment has a 3'tRNA-like structure and a 5'cap. RNA1 and RNA2 encode proteins 1a and 2a, respectively and are both involved in genome replication and internal transcription of sgRNA4 from the minus-strand copy of RNA3. RNA3 and sgRNA4 are translated into movement and capsid proteins, respectively. ORF2b encodes a viral suppressor of RNA silencing (VSR) and is expressed probably as a sub genomic RNA (sgRNA4A).

CMV is readily transmitted through sap, seed as well as several aphid species (Fischer and Lockhart, 1976b). Symptoms caused by CMV in cowpea are often mild and include; mild mottle, mosaic, leaf distortion with characteristic ringspots. However, in a few varieties, severe leaf symptoms with reddish vein necrosis can develop (Thottappilly and Rossel, 1992). Yield loss due to CMV alone is about 14% (Pio-Ribeiro *et al.*, 1978). Mixed infections involving CMV with either BCMV-BICM or CABMV lead to a cowpea stunt, a severe

disease with yield losses of up to 87.5% (Pio-Ribeiro *et al.*, 1978; Pio-Ribeiro and Kuhn, 1980).

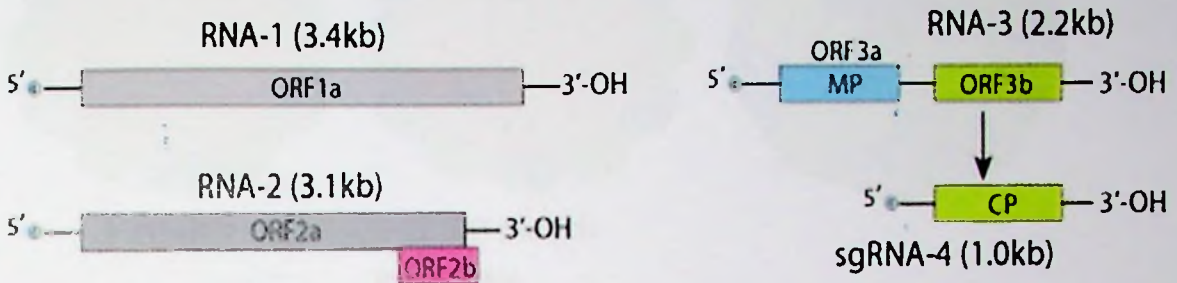


Figure 3: Genome organization of *Cucumber mosaic virus*. Source: Hulo *et al.* (2011).

2.2.4 Cowpea mosaic virus (CPMV)

CPMV comovirus initially referred to as *Cowpea yellow mosaic virus* (CYMV) by (Chant, 1959) occurs in several countries in West and East Africa (Chant, 1959; Thottappilly, 1992; Thottappilly and Rossel, 1992). Susceptible varieties display a range of systemic symptoms from inconspicuous light green to a distinct yellow mosaic. Infected plants are also characterized by slow growth, stunting, shortened internodes, vein clearing, and leaf distortion (Chant, 1959; Thottappilly and Rossel, 1992). The virus is transmitted through sap, seed (0-10%) and by several beetle species. Chant (1959) and Whitney and Gilmer (1974) reported galericid beetles (*Ootheca mutabilis*) as the most effective vector in transmission of CPMV. Reduction in cowpea yield due to CPMV infection of up to 95% has been reported (van Kammen *et al.*, 2001).

CPMV is a single stranded RNA virus with isometric particles of approximately 28 nm in diameter. Infected plants contain two kinds of nucleoprotein components (Figure 4); middle (M) and Bottom (B) that are similar in size but differing in RNA content. Additionally, empty shells/top component (T) without RNA are produced in infected plants (van Kammen *et al.*, 2001). The M and B components contain a single RNA molecule of 3,481 (RNA2) and 5,889 (RNA1) nucleotides, respectively.

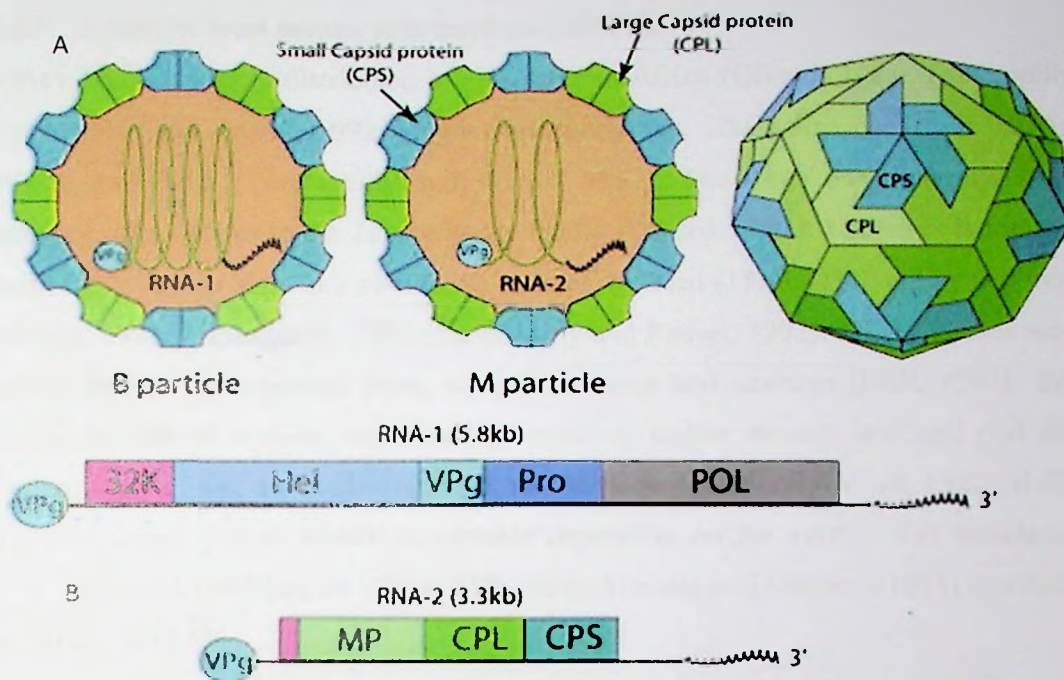


Figure 4: Particle morphology (A) and genome organization (B) of comoviruses. Source: Hulo *et al.* (2011).

2.2.5 Cowpea severe mosaic virus (CPSMV)

CPSMV was mainly restricted to countries in the western hemisphere such as USA, Trinidad, Puerto Rico, El Salvador, Costa Rica, Venezuela, Surinam, Brazil and Peru (de Jager, 1979). However, due to movement of infected seed lots through germplasm exchanges, it is assumed to be distributed in many parts of the world (Hampton *et al.*, 1997). It is readily transmissible mechanically and by beetles especially Chrysomelid beetles as well as through seed with up to 10% transmission reported (de Jager, 1979). CPSMV has isometric particles, approximately 25 nm in diameter with two types of nucleoproteins (middle and bottom components) which are morphologically and serologically identical but containing different RNA molecules (de Jager, 1979). The genome organization of CPSMV is similar to that of CPMV (Figure 4). The virus is known to infect only legumes. Symptoms on inoculated leaves include necrotic or chlorotic lesions while systemic symptoms include mottling, mosaic, severe blistering and distortion of leaves. Early infection of cowpea at the seedling stage by CPSMV in susceptible cultivars leads to significant yield loss ranging from 57% to 88% with the greatest losses occurring in the dry season (Booker *et al.*, 2005).

2.2.5 *Southern bean mosaic sobemovirus (SBMV)*

SBMV virus is widely distributed in Sub-Saharan Africa (Givord, 1981; Thottappilly, 1992; Thottappilly and Rossel, 1992; Hughes and Shoyinka, 2003) also occurs in the Americas, Europe and India (Thottappilly and Rossel, 1992). The virus has a narrow host range, infecting only species in the *Leguminosae* family (Givord, 1981). The virus is transmitted by beetles (*Chrysomelidae*) in a circulative manner, by seed (15 to 44%) and by sap inoculation (Givord, 1981; Thottappilly, 1992; Thottappilly and Rossel, 1992). SBMV causes mosaic and mottle diseases on common bean, cowpea, urdbean and soybean (Hull, 2004). Symptoms caused by SBMV include vein clearing, mottling, coarse mosaic, leaf and pod distortion, stunting, leaf chlorosis, puckering, and leaf blistering (Cupertino *et al*, 1982; Hull, 2004). Yield reduction due to SBMV is variable depending on the variety. For instance, Givord (1981) reported yield loss of 11% to 59% while Morales and Castano (1985) reported a yield reduction of 47.5%.

SBMV has non enveloped isometric particles (28 nm to 30 nm in diameter) with a monopartite, linear, ssRNA (+) genome of about 4 kb. The particles have a $T = 3$ structure with 180 protein subunits. The 5' terminus has a genome-linked protein (VPg) with a non-polyadenylated 3' terminus (Figure 5). The virion RNA is infectious and serves as both the genome and viral messenger RNA. ORF1 and ORF2 are translated via leaky scanning. ORF2 is a polyprotein which is presumably cleaved into a serine-protease, a VPg and a RdRp protein. The viral serine protease is responsible for all the cleavages. ORF3 is translated via a -1 ribosomal frame shifting. Replication produces a sub-genomic RNA which encodes for the capsid protein (ORF4) (Hulo *et al.*, 2011).

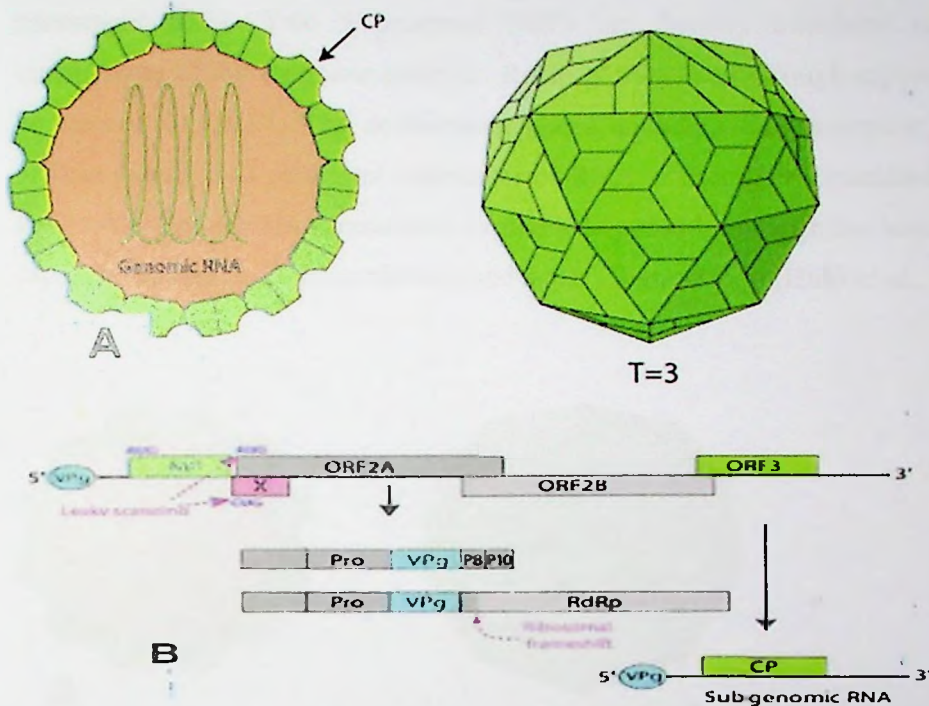


Figure 5: Particle morphology (A) and genome organization (B) of *Southern bean mosaic virus*. **Source:** Hulo *et al.* (2011).

2.2.6 Cowpea mottle virus (CPMoV)

Cowpea mottle virus is a single stranded RNA virus with isometric particles of approximately 30 nm (Shoyinka *et al.*, 1978; Bozarth and Shoyinka, 1979). The virus was initially reported only in Nigeria (Shoyinka *et al.*, 1978) but was later reported in other African countries (Thottappilly and Rossel, 1992). It has a wide host range infecting many members of the *Leguminosae* and *Solanaceae* families (Shoyinka *et al.*, 1978; Bozarth and Shoyinka, 1979). CPMoV is readily transmitted by beetles (*Ootheca mutabilis*), by seed (0-10%) and by sap inoculation (Shoyinka *et al.*, 1978; Thottappilly and Rossel, 1992). Susceptible hosts show a combination of symptoms such as severe chlorosis, uniform mosaic or mosaic-mottle, local lesions with or without systemic spread, vein necrosis (Shoyinka *et al.*, 1978). Thouvenel *et al.* (1990) reported a 65% reduction in yield due to natural infection of cowpea. However, (Kareem and Taiwo, 2007) reported total yield loss especially when cowpea is infected with CPMoV at early stages/seedling either alone or in combination with other viruses.

CPMoV has a linear, ssRNA (+) genome of 4-5.4 kb, which lacks a cap structure and a poly(A)tail (Figure 6). The virion RNA is infectious and serves as both the genome and viral messenger RNA. Two 5'-proximal ORFs are directly translated to produce the viral constituents of the replicase complex. RdRp is translated through suppression of termination at the end of ORF1. The downstream genes encoding the movement proteins and capsid protein (which acts as a viral suppressor of RNA silencing) are translated from sub-genomic RNAs. Cap-independent translation is mediated possibly through the interaction between a 3'-cap-independent translation element and a 5'UTR stem-loop (Hulo *et al.*, 2011).

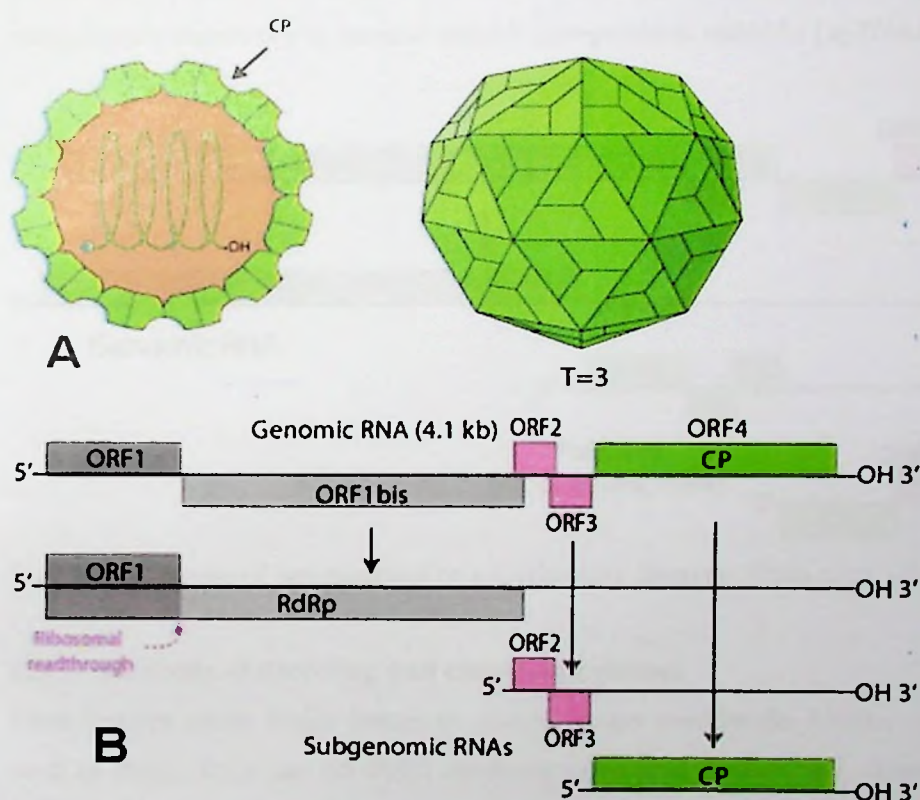


Figure 6: Particle morphology (A) and genome organization (B) of *Cowpea mottle virus*.
Source: Hulo *et al.* (2011).

2.2.7 Cowpea mild mottle carlavirus (CPMMV)

CPMMV was first reported on cowpea in eastern Ghana (Brunt and Kenten, 1973) but later spread to several parts of the world (Thottappilly and Rossel, 1992). The virus infects several species in the *Papilionaceae* and *Solanaceae* families (Brunt and Kenten, 1973; 1974; Naidu *et al.*, 1998). Naturally infected cowpea may be symptomless or have leaves with either mild

systemic chlorotic mottle or conspicuous chlorosis (Brunt and Kenten, 1973). CPMMV is readily transmitted by sap, seed (0-90%) and by whitefly (*Bemisia tabaci*) in a non-persistent manner (Naidu *et al.*, 1998). The virus has flexous filamentous particles of approximately 650 nm that are typical of carlaviruses (Brunt and Kenten, 1973; Thottappilly and Rossel, 1992). Yield reduction due to CPMMV ranges from 10-15% in single infections but in mixed infections, it can exacerbate the deleterious effects of other viruses (Brito *et al.*, 2012). The genome arrangement is similar to those of other carlaviruses (Figure 7). The virion RNA is infectious and serves as both the genome and viral messenger RNA. RNA-dependent RNA polymerase (RdRp) is translated directly from the genomic RNA. The other ORFs are transcribed presumably as mono-cistronic sub-genomic mRNAs (sgRNAs).

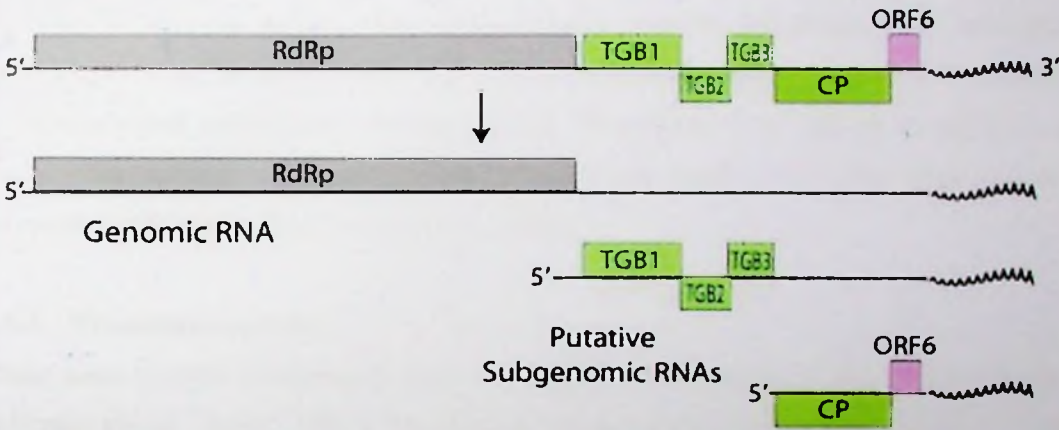


Figure 7: Genome of organization of a Carlavirus. Source: Hulo *et al.* (2011).

2.3 Methods of detecting and classifying viruses

Plant viruses cause major losses to several crops worldwide. Unlike other plant pathogens such as fungi, there are no direct methods to control viruses and, consequently, the current measures rely on indirect tactics to manage the viral diseases. Hence, methods for detection and identification of viruses both in plants and vectors play a critical role in virus disease management. Diagnostic techniques for viruses fall into two broad categories: biological properties related to the interaction of the virus with its host and/or vector for instance symptoms and intrinsic properties of the virus itself (coat protein and nucleic acid). Detection methods based on coat protein include enzyme-linked immunosorbent assays (ELISA) and immunoblotting while viral nucleic acid-based techniques include dot-blot hybridization

assays and polymerase chain reaction (PCR). Nucleic acid-based are more sensitive than other methods.

2.3.1 Symptomatology

Symptoms on infected plants can be used to characterize or identify viruses since such plants display abnormal phenotypes as a result of virus infection. Visual inspection is relatively easy when symptoms clearly are characteristic of a specific disease. However, asymptomatic or covert virus infections are ubiquitous and such viruses may accumulate to relatively low titers in the host organism or become latent such that virus production ceases altogether. These viruses would not readily be detected by use of symptoms (Liu *et al.*, 2011). Symptom expression is influenced by other factors such as virus strain, host plant cultivar/variety, time of infection and the environment (unfavourable weather conditions, soil mineral/nutrient imbalances, infection by non-viral pathogens, damage caused by insect/mite/nematode pests, air pollution and pesticides) (Matthews, 1980; Mbanzibwa *et al.*, 2011). In addition, different viruses can produce similar symptoms or different strains of a virus may cause distinct symptoms in the same host (Aliyu *et al.*, 2012).

2.3.2 Transmission tests

These tests include mechanical, graft and vector transmission of the viruses to susceptible indicator plants (Jones, 1993). Mechanical transmission by sap inoculation to herbaceous indicator plants can be used for viruses that are mechanically transmissible and the characteristic symptoms produced by these plants allow virus identification. Non mechanically transmissible viruses can be diagnosed by vector transmission or grafting onto suitable indicator hosts. However, these tests are time and resource consuming, and are often beset with the same difficulties in discerning viruses based on symptoms expressed in the field (Naidua and Hughes, 2003).

2.3.3 Electron microscopy (EM)

Early classification of viruses depended heavily on the morphology of the viral capsid as revealed by EM. One of the primary advantages for the use of EM is that organism or virus specific reagents are not required for virus identification. Although identification of a virus beyond the family or genus level may not be possible (Adams *et al.*, 2009; Goldsmith and Miller, 2009), EM provides leads for more detailed characterization of the virus for instance

filamentous and rod-shaped viruses such as potyviruses, potexviruses and tobamoviruses can more readily be differentiated in negatively stained leaf-dip preparations than isometric viruses and other viruses (Naidua and Hughes, 2003; Liu *et al.*, 2011). However, viruses that occur in low concentrations in plant sap are not easily seen unless the virus in the test material is concentrated before visualization. EM is also labour intensive and expensive thus it cannot often be used for the rapid processing of multiple samples (Naidua and Hughes, 2003).

2.3.4 Serological methods

Serological or immunological assays have been used successfully for the detection of many plant viruses. These tests are broadly subdivided into liquid and solid phase tests. In the former, both antigen and antibody react in solution to form a visible precipitate. In the latter, assays are conducted on a solid surface such as on a micro-titre plate or nitrocellulose membrane and the antigen-antibody reaction is visualized by means of a suitable detection system such as an enzyme-labelled antibody. The commonly used serological methods in virus detection are; Immunosorbent electron microscopy (ISEM) and Enzyme-linked immunosorbent assay (ELISA).

2.3.4.1 Immunosorbent electron microscopy (ISEM)

ISEM shares similarity with solid phase immunoassays and combines the specificity of serological assays with the visualization capabilities of the EM. Virus particles are selectively “trapped” on antibody-coated grids with little contaminating host-plant material. Hence, the technique is more sensitive for detecting viruses than the leaf dip method. In addition to diagnosis, ISEM can also be used to estimate the degree of serological relationship between viruses. Although ISEM is a sensitive technique, it has the same drawbacks as EM (Naidua and Hughes, 2003).

2.3.4.2 Enzyme-linked immunosorbent assay (ELISA)

ELISA is used in a wide range of situations especially to test a large number of samples in a relatively short period of time for detection of viruses in plant material, insect vectors, seeds, and vegetative propagules since its introduction by Clark and Adams (1977). While ELISA techniques are favoured because of their specificity and speed in detection of morphologically different viruses in purified preparations and unclarified extracts of infected plants (Clark and Adams, 1977), these techniques suffer from several drawbacks especially when trying to

identify 'unknown' agents e.g. either a pathogen infecting a new host or a previously uncharacterized pathogen. Such specific assays require a prediction of the infective agent based on reliable knowledge of which pathogens infect which hosts (Adams *et al.*, 2009; Liu *et al.*, 2011). Reliable information is frequently incomplete or even totally absent especially for many of the more obscure or novel hosts which complicates virus identification. Also ELISA techniques involve use of specific reagents such as antibodies covering a single strain or small group of pathogens which leads to incapability of detecting variants such as new strains that can arise unexpectedly (Adams *et al.*, 2009).

2.3.5 Molecular methods

Molecular techniques that involve direct detection of viral genomes in the specimen have increasingly been used in plant virology. As opposed to immunological methods that are based on the antigenic properties of the virus coat protein which represents only about 10% of the total virus genome (Gould and Symons, 1983), molecular methods can be targeted at any region of the genome to develop a diagnostic test. Molecular methods are also applicable in detection of viroids, satellite RNAs as well as viruses which lack particles and thus cannot be detected by serological methods (Naidua and Hughes, 2003). Molecular techniques can be divided into two categories: those that do not involve amplification i.e. hybridization with nucleic acid probes, and those that involve amplification such as polymerase chain reaction.

2.3.5.1 Nucleic acid hybridization assays

Nucleic acid hybridization assays are based on the homology between two strands of nucleic acid (DNA: DNA, DNA: RNA or RNA: RNA). In these assays, a single-stranded complementary nucleic acid (either DNA or RNA) which has been "labelled" with a reporter molecule is used as a probe to form a hybrid with the target nucleic acid. The double-stranded probe-target hybrid molecules are then detected by several methods such as autoradiography for probes labelled directly using radioactive isotopes (Wetmur and Fresco, 1991). Since these assays are specific and require prior knowledge of the virus expected in a sample, only a specific /single strain or small group of pathogens can be detected which leads to incapability of detecting new strains (Adams *et al.*, 2009).

2.3.5.2 Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) provides a simple, ingenious method to exponentially amplify specific DNA sequences by *in vitro* DNA synthesis (Henson and French, 1993). Because of its speed, specificity, sensitivity and versatility PCR has been widely applied in many areas of research in biology. Since PCR has the power to amplify the target nucleic acid present at an extremely low level and form a complex mixture of heterologous sequences, it has become an attractive technique for the diagnosis of plant virus diseases.

PCR consists of three steps: (i) denaturation at high temperature (usually 94–95°C) to separate the two complementary strands of the double-stranded DNA, (ii) annealing of two oligonucleotide primers to their complementary sequences in the opposite strands of the target DNA (annealing temperature depends on the nucleotide composition and length of the primer, usually anywhere between 35 and 65°C), and (iii) extension of each primer through the target region (usually at 72 °C) using a thermostable DNA polymerase (e.g. *Taq polymerase*). Each DNA strand made in one cycle will serve as a template for synthesis of a new DNA strand in the next cycle. This results in an exponential increase in PCR product as a function of cycle number. The three step cycles are repeated many times (between 30 and 40 cycles) in an automated thermal cycler until sufficient product is produced (Henson and French, 1993; Naidua and Hughes, 2003). This procedure is applicable directly to DNA plant viruses (caulimo, gemini and badnaviruses); however, for diagnosis of plant viruses with RNA genomes, the RNA target has to be “converted” to a complementary DNA (cDNA) copy by reverse-transcription before PCR is done. On completion of the reaction, the amplified DNA can be analysed by agarose gel electrophoresis. While PCR is sensitive compared to serological methods, it detects specific target viruses, requires prior knowledge of the target virus and therefore cannot be used to identify variants or unknown viral agents (Adams *et al.*, 2009; Liu *et al.*, 2011; Adams *et al.*, 2012).

2.3.6 Next-generation sequencing (NGS)

Next-generation sequencing is a non-Sanger-based and high-throughput methodology (Schuster, 2008) which allows for generation of millions of sequences or reads in a single run (Hall, 2007). NGS methods have high-throughput strategies for *in vitro* amplification that are very cheap and also eliminate the inherent biases of *in vivo* methods used in Sanger sequencing (Hall, 2007; Liu, 2009). Recently several methods have been reported based on

next-generation sequencing technology (Adams *et al.*, 2009; Kreuze *et al.*, 2009; DeBoever *et al.*, 2013; Kamala *et al.*, 2015) that provide a rapid and efficient way of identifying viruses. These methods enable the viral genome to be sequenced within a background of host nucleic acid whilst bioinformatics approaches enable the identification of pathogen sequences by similarity comparison to known viruses or virus-like sequence motifs. Because of the depth of sampling associated with next-generation platforms, novel and rare pathogen variants can easily be detected (Liu, 2009).

Briefly, the steps involved in preparing RNA/DNA for sequencing involve; (i) fragmenting and/or sizing the target sequences to a desired length, (ii) converting target to double-stranded DNA (cDNA), (iii) attaching oligonucleotide adapters to the ends of target fragments, (iv) quantifying the final library product for sequencing. Fragmentation can be achieved through physical, enzymatic or chemical methods. DNA fragmentation is typically done by physical methods (i.e., acoustic shearing and sonication) or enzymatic methods (i.e., non-specific endonuclease cocktails and transposase tagmentation reactions (Head *et al.*, 2014), (v) amplification of target fragments through multiple PCRs to enhance fluorescent or chemical signals, (vi) amplified DNA templates are then attached to a solid support in a reaction chamber or flow cell, (vii) extension/elongation of fragments to the desired length is then carried out by repetitive application and washing of sequencing reagents (Liu, 2009; Egan *et al.*, 2012).

4 Management of cowpea viral diseases

Cowpea viral diseases spread under field conditions through increase in vector populations, the presence of susceptible host plants and the presence of initial infection foci through seed-borne infection, therefore appropriate control could be achieved by controlling these three vectors (Bashir *et al.*, 2002a). Integrated control measures i.e. involving combination of all possible measures that operate in different ways such that they complement each other and can be applied together in farmers' fields as one overall control package (Makkouk and Umari, 2009) are however more effective. The different control measures available can be classified as host resistance, cultural and chemical methods.

2.4.1 Cultural measures

Cultural control methods are the most diverse and include both phytosanitary and agronomic measures for instance; early sowing and intercropping of cowpea with cereals reduces incidence of CABMV (Kannaiyan and Hagiwa, 1993). Early planting promotes rapid crop canopy development which decreases insect vector landing rates and shades out infected source plants while intercropping with non-host plants decreases overall vector numbers and the proportion that are viruliferous (Jones, 2004). Since most of the viruses affecting cowpea are seed-borne, use of healthy seed stocks is an important method of preventing virus spread to new areas. Production of healthy seed stocks can be ensured through field inspection and rouging to remove sources of virus infection from within crops. It is only effective when symptoms are conspicuous and easily recognized and when the symptomatic plants are removed early before vectors have visited them and also where numbers of vectors are low (Thresh, 2003; Makkouk and Kumari, 2009; Makkouk *et al.*, 2014). However, since there is evidence that some cowpea viral diseases may also occur occasionally to be seed transmitted in symptomless plants (Aboul-Ata *et al.*, 1982; Bashir and Hampton, 1996), it is important to implement a rapid indexing procedure for the detection of such viruses in seed lots. Also using early maturing cultivars is an important measure for virus control in grain legume crops (Jones, 2001; 2004) since sowing of short duration cultivars can be timed so that they approach maturity and express mature plant resistance to virus infection during the period of greatest insect vector activity.

2.4.2 Chemical control

While pesticides have been used to prevent virus spread by decreasing vector populations or by impeding transmission, their use is inappropriate (Thresh, 2003) because the relationships between viruses and their vectors are complex and a decrease in vector populations does not necessarily achieve a commensurate decrease in virus spread. For instance, use of insecticides to control vectors of non-persistent viruses have limited effectiveness (Thresh, 2003). These are acquired and transmitted most effectively in brief probes and often by vectors that visit but do not colonize the crop being sprayed. Moreover, there are problems due to the difficulty and cost of treating crops throughout the entire vulnerable period of growth. There may also be damage to the natural enemies of vectors and risks to the environment and to human health. Consequently, pesticides play only a limited role in plant virus control even in developed

countries where they are mainly used against the vectors of persistent viruses of commercial crops (Thresh, 2003; Makkouk and Kumari, 2009).

2.4.3 Host resistance

The use of disease-resistant genotypes has obvious advantages because it provides a convenient means of control that can be used alone or in combination with other measures. Moreover, there are no harmful side effects and no additional costs once resistant varieties are made available that are comparable or superior in performance to the susceptible ones being grown (Thresh, 2003). Successful use of host resistance in controlling viral diseases depends on the availability of sources of resistance and the incorporation of resistance into susceptible germplasm (Ndiaye *et al.*, 1993; Arshad *et al.*, 1998). Sources of resistance to virus infection have been reported among cowpea germplasm from several countries such as in Iran (Kaiser and Mossahebi, 1975), Zambia (Kannaiyan and Haciwa, 1993), Senegal (Ndiaye *et al.*, 1993), Pakistan (Bashir, *et al* 2002b) and Uganda (Orawu, 2007).

2.4.4 Integrated approaches

Yield losses and the resulting financial damage due to viral infection can be limited by controlling epidemics using measures that minimize virus infection sources or suppress virus spread. For each combination of virus, cultivated plant and production system, there is an 'economic threshold' above which the financial damage is sufficient to justify using such measures. However, individual measures used alone may bring only small benefits and they may become ineffective especially over the long term. When diverse control measures that act in different ways are combined and used together, their effects are complementary resulting in far more effective overall control (Jones, 2004). Such experiences have led to the development of integrated management concepts for virus diseases that combine available host resistance, cultural and chemical control measures. Selecting the ideal mix of measures for each pathosystem and production situation requires detailed knowledge of the epidemiology of the causal virus and the mode of action of each individual control measure so that diverse responses can be devised to meet the unique features of each of the different scenarios considered (Thresh, 2003; Jones, 2004). Supporting evidence from several field trials show that a combination of measures is effective against viruses. For instance, combination of insecticide application with less susceptible genotypes significantly reduced development of cowpea viral diseases (Ambang *et al.*, 2009). A combination of high to

moderate plant density together with moderately resistant cowpea genotypes greatly reduced the effect of cowpea viruses (Aliyu and Balogun, 2011). Early planting, cereal intercrops with varied spatial arrangements reduced viral disease damage in cowpea (Kannaiyan and Hacıwa, 1993; Aliyu and Balogun, 2012).

CHAPTER THREE

MOLECULAR CHARACTERIZATION OF VIRUSES INFECTING COWPEA IN UGANDA USING NEXT-GENERATION SEQUENCING

3.1 Introduction

Characterization and accurate identification of viruses is a prerequisite for effective management of viral diseases (Hamilton *et al.*, 1981; Thottappilly *et al.*, 1995). Worldwide, about 140 viruses have been reported on cowpea (Hughes and Shoyinka, 2003) of which 11 viruses were reported in Africa (Thottappilly, 1992; Thottappilly and Rossel, 1992; Hughes and Shoyinka, 2003). In Uganda, nine viruses were reported (Amayo *et al.*, 2012). Previous studies (Edema *et al.*, 1997; Orawu *et al.*, 2005; 2015; Byarugaba, 2008; Amayo, 2008; Amayo *et al.*, 2012) that attempted to identify and characterize cowpea viruses in Uganda used species specific assays/methods applicable to only a few potential viruses. Species specific methods are often incapable of detecting new species, strains or variants as well as pathogens that have changed hosts or geographical range (Adams *et al.*, 2009; Adams *et al.*, 2012). Specific assays rely on accurate prediction of the infective agent based on a reliable *a priori* knowledge of pathogens infecting a particular host. However, such information is often unavailable or incomplete making it difficult to identify previously unknown or uncharacterized agents (Adams *et al.*, 2009; Kreuze *et al.*, 2009). It is therefore likely that several viral species and/or their strains have not been identified in Uganda. Methods based on next-generation sequencing (NGS) technology offer a powerful and generic frontline approach for rapid identification of viruses (Cox-Foster *et al.*, 2007; Adams *et al.*, 2009; Kreuze *et al.*, 2009; DeBoever *et al.*, 2013). The main objective of this study was to identify and characterize putative cowpea viruses in Uganda using NGS.

3.2 Materials and methods

3.2.1 Sample collection and preservation

Leaf samples were collected from cowpea plants showing characteristic virus symptoms such as mosaic, chlorosis, distortion, mottling, leaf curling and necrosis were collected from fields in twelve districts of Uganda namely; Ngora, Kumi, Pallisa, Namutumba, Serere, Dokolo, Lira, Apac, Soroti, Oyam, Wakiso and Amuria. In Wakiso district, 20 samples were collected

from a single experimental field at Makerere University Agricultural Research Institute (MUARIK). In the remaining districts, five plants were randomly selected from four different/distant fields (a minimum of 5 kilometres apart). A total of 240 samples was collected from all the districts. Type and severity of symptoms and associated with each sample per field were also recorded. The samples were preserved immediately by wrapping them in paper towels and then inserted in ziploc bags containing about 50g of silica gel. The ziploc bags containing samples were placed in a plastic container and tightly covered. Silica gel was changed once or twice to ensure complete drying of samples. Drying was achieved within two to three days at room temperature (25-28°C). The dried leaf samples together with silica gel were transported to Biosciences eastern and central Africa (BecA), Nairobi, Kenya for total RNA extraction and sequencing.

3.2.2 RNA extraction

Total RNA was extracted from the dried leaf samples (240) using TRI Reagent (Sigma-Aldrich, St. Louis, USA) with some modifications to the manufacturer's protocol. The following modifications were made; after homogenizing the samples in TRI reagent, samples were spinned at 12,000 x g for 10 minutes at 4°C to remove insoluble material. A supernatant containing RNA was then transferred to a fresh sterile micro-centrifuge tube for phase separation using 0.2ml chloroform. RNA was precipitated using 0.25ml of chilled isopropanol and 0.25ml of a mixture of 1.2M NaCl and 0.8M sodium citrate solution. The precipitate was spinned at 12,000 × g for 10 minutes at 4°C to form a pellet. Precipitated RNA (pellet) was washed with 1ml of 75% ethanol by gentle vortex and then spinning at 8,000 × g for 5 minutes at 4°C. Later the RNA pellet was air dried for 10 minutes before resuspension in 30µl of nuclease free water (AMRESCO, Ohio, USA). To facilitate dissolution, samples were incubated at 60°C for 10 minutes with repeated pipetting, chilled on ice and then stored at -80°C.

The quality and concentration of extracted RNA was determined using NanoDrop spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA). Samples with a 260/280 ratio of ≥ 2.0 were considered as having good RNA. RNA quality was further confirmed by visualizing samples using TAE/Formamide agarose gel electrophoresis as described by Masek *et al.* (2005) and only those showing no genomic DNA contamination or RNA degradation

were selected. Samples showing intact 18S and 28S subunits on agarose gels were selected and diluted to 20ng/μl.

3. 2. 3 Preparation of cDNA libraries and sequencing

Four cDNA libraries for sequencing were prepared from the diluted RNA samples using 1μg of pooled RNA. The first cDNA library comprised of 10 samples from Wakiso (MUARIK), the second cDNA library comprised of two samples each from Ngora, Kumi, Pallisa, Namutumba, Serere and Soroti districts (a total of 12 samples), the third cDNA library comprised of 12 samples only from Amuria district. The fourth cDNA library comprised of 10 samples from Dokolo (3 samples), Lira (2 samples), Apac (2 samples) and Oyam (3 samples) districts. cDNA libraries were prepared following Truseq Stranded mRNA sample preparation kit (Cat. No. RS-122-2101; Illumina, San Diego, CA, USA). The final size and concentration of the prepared library was checked using 2100 Bio-analyser Desktop System (Agilent, Santa Clara, CA, USA) and quantified using Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA). Paired-end sequencing (2 x 300 bp) was performed using a MiSeq Desktop Sequencer (Illumina sequencing platform) at BecA-International Livestock Research Institute (BecA-ILRI), Nairobi, Kenya.

3. 2. 4 De novo assembly and sequence analysis

Raw sequences were analysed using CLC Genomics workbench version 7.0.4 (CLC bio, Qiagen, CA, USA) as follows; sequences were subjected to quality control (QC) and filtering to identify regions with low quality reads (Del Fabbro *et al.*, 2013). Based on the QC report, trimming was done using quality parameter settings score limit set to 0.01, maximum number of ambiguities set to 2 and removing any reads with less than 50 nucleotides (nt). Read trimming removes low quality portions and preserves the longest high-quality parts of the NGS read thus increasing reliability and quality of fragment analysis (Del Fabbro *et al.*, 2013). The remaining paired sequences were assembled *de novo* in both directions into contiguous sequences (contigs) while the unpaired or orphan reads were discarded. Assembled contigs were subjected to BLASTN and BLASTX homology searches against a plant viral database of 57,417 sequences downloaded from National Centre for Biotechnology Information (NCBI). BLASTN and BLASTX searches were carried out using NCBI-blast-

2.2.29+ (Altschul *et al.*, 1997). BLASTN and BLASTX searches were carried using default settings (-word size 28 -reward 1-penalty -2 -gap open 0 -gap extend 2.5).

3. 2. 5 Phylogenetic analysis

Contiguous sequences that gave significant virus hits on BLASTN analysis were aligned with sequences of other virus isolates downloaded from the DDBJ/ENA/GenBank. Alignments were done using ClustalW tool in MEGA Version 7.0.25 (Kumar *et al.*, 2016). Phylogenetic analysis was carried out using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). The Tamura-Nei model of nucleotide substitution accounts unequal base frequencies in a dataset. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbour-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The trees were drawn to scale with branch lengths measured in the number of substitutions per site. To assess the robustness of the lineages in the trees, alignment data was bootstrapped (re-samplings) 1,000 times.

3. 2. 6 Pair wise comparison of Ugandan cowpea virus isolates with other isolates

Pair wise nt and aa comparisons were done between the virus contigs assembled in this study with other sequences obtained from the DDBJ/ENA/GenBank database. Pair-wise comparisons were made both at nucleotide and protein levels using EMBOSS Stretcher (<http://www.ebi.ac.uk/Tools/psa/>) and BioEdit version 7.0.4.1 (Hall, 1999).

3.3 Results

3. 3. 1 Sequence analysis and de novo assembly

The four libraries gave a total of 18,318,168 pair-end reads with read length ranging from 35-301 nucleotides (nt). After trimming of low-quality reads based on the QC report (section 3.2.3), a total of 13,781,621 reads remained with an average length of 178 nt. *De novo* assembly of trimmed paired reads resulted into a total of 75,862 contiguous sequences (contigs) from the four libraries. Analysis of contigs using BLASTN and BLASTX against a database of 57,417 viral sequences gave similar results. Thus, results that follow are based only BLASTN analysis. Eight contigs out of 75,862 contigs ranging from 227 bp to 9,904 bp

gave significant hits on BLASTN search against the database (Figure 8-15). The identified viruses were *Cowpea aphid-borne mosaic virus* (CABMV), *Peanut mottle virus* (PeMoV), *Sugarcane mosaic virus* (SCMV) and *Maize chlorotic mottle virus* (MCMV). Contigs corresponding to the identified viruses were deposited in the DDBJ/ENA/GenBank under accession numbers KT970623, KT970624, KT970618, KT970619, KT970621, KT970622, KT970625 and KT726938. The longest contig was for CABMV (KT726938) and on further analysis was confirmed as a complete genome sequence of CABMV. The results presented in the subsequent sections (genome annotation, pair wise nt/aa comparison and phylogenetic analysis therefore solely focus on the complete genome of CABMV assembled in this study.

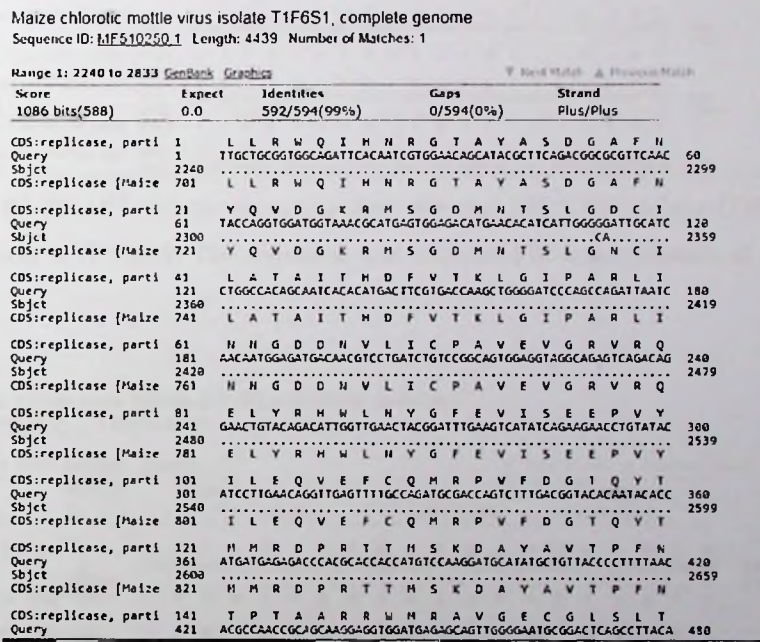


Figure 8: NCBI BLAST of the sequence data for the NMF1 isolate (594 bp) under accession number KT970618. The sequence was identified as a replicase gene of MCMV in the database.

Peanut mottle virus isolate Habin, complete genome
Sequence ID: [KF977830.1](#) Length: 9709 Number of Matches: 1

Range 1: 7401 to 9421		GenBank	Graphics			Next Match	Previous Match
Score	Expect	Identities		Gaps	Strand		
3616 bits(1958)	0.0	2000/2021(99%)		0/2021(0%)	Plus/Plus		
CDS:polyprotein, par	1	M K A A V G A L Y K G K K K E Y F E S L					
Query	1	ATGAAGGCTGCGGTTGGAGCCTTATACAAAGGGAAGAAAGAACTATTGAGAGTTTG					60
Sbjct	7401						7460
CDS:polyprotein [Pea	2427	M K A A V G A L Y K G K K K E Y F E S L					
CDS:polyprotein, par	21	S E P E K H H I V Q A S C E R L F Y G E					
Query	61	TCAGAACAGAAACATCACCATTGTACAAGCGATTGTGAAAGGCTGTCTATGGAGAG					120
Sbjct	7461						7520
CDS:polyprotein [Pea	2447	S E P E K H H I V Q A S C E R L F Y G E					
CDS:polyprotein, par	41	M G V W N G S L K A E L R P K E K V A L					
Query	121	ATGGGAGTGTGAATGGATCCCTCAAGCTGAATTGAGACAAAGGAAAGTTGCACAT					180
Sbjct	7521						7580
CDS:polyprotein [Pea	2467	M G V W N G S L K A E L R P K E K V A L					
CDS:polyprotein, par	61	N K T R T F T A A P I D T L L G G K C C					
Query	181	AATAAGACAAGGACATTCACAGCCGCTCTATTGATACACTGCTGGAGGGAAGTGTGT					240
Sbjct	7581						7640
CDS:polyprotein [Pea	2487	N K T R T F T A A P I D T L L G G K C C					
CDS:polyprotein, par	81	V D D F N N R F Y S L N I E G P W T V G					
Query	241	GTGGATGATTTTAATAATAGGTTTATAGTTTAAATATAGAGGCCCTTGGACAGTGGT					300
Sbjct	7641						7700
CDS:polyprotein [Pea	2507	V D D F N N R F Y S L N I E G P W T V G					
CDS:polyprotein, par	101	H T K F Y G G W D K L M R K L P D G W R					
Query	301	ATGACTAAGTTTATGGTGGATGGGACAGTTAATGCGCAAACTACTGTGATGGATGGGC					360
Sbjct	7701						7760
CDS:polyprotein [Pea	2527	H T K F Y G G W D K L M R K L P D G W R					
CDS:polyprotein, par	121	Y C H A D G S Q F D S S L T P F L L N A					
Query	361	TACTGTGATGCTGATGGTTTCAATTTGACAGTTCCTTAACACCATCTTGTAAATGCG					420
Sbjct	7761						7820
CDS:polyprotein [Pea	2547	Y C H A D G S Q F D S S L T P F L L N A					
CDS:polyprotein, par	141	V L A V R L H F H E D W W V G E Q H L R					
Query	421	GTTTTAGCGGTGCGACTAATGTTTCATGGAGATTGGTGGGTAGGTGAGCAATGCTGCGC					480

Figure 9: NCBI BLAST of the sequence data for the SELKB2 isolate (2,021 bp) under accession number KT970619. The sequence was identified as a polyprotein of PeMoV in the database.

Maize chlorotic mottle virus isolate B3_S3, complete genome
Sequence ID: [JF510251.1](#) Length: 4429 Number of Matches: 1

Range 1: 3857 to 4083		GenBank	Graphics			Next Match	Previous Match
Score	Expect	Identities		Gaps	Strand		
420 bits(227)	1e-113	227/227(100%)		0/227(0%)	Plus/Plus		
CDS:coat protein, pa	1	M P Q N R T V F R C I Q A G Q F A A L G					
Query	1	ATGCCACAAAACCGCACTGTGTTCAAGTGTATACAGGCTGGTCAGTTTGCTGCGTTGGGC					60
Sbjct	3857						3916
CDS:coat protein [Ma	161	M P Q N R T V F R C I Q A G Q F A A L G					
CDS:coat protein, pa	21	S A A D K Q M Y S P G R F I V A I P K A					
Query	61	AGCGCGGCTGACAAGCAAATGTATCTCCCGGACGATTGTTGTTGGCTATCCCCAAAGCT					120
Sbjct	3917						3976
CDS:coat protein [Ma	181	S A A D K Q M Y S P G R F I V A I P K A					
CDS:coat protein, pa	41	S A T Q A V G Q I K I S Y S V S Y R G A					
Query	121	AGTGCAACACAAGCGGTAGGCCAAATCAAATCTCGTACTCCGTGCTACCCGTTGGAGCA					180
Sbjct	3977						4036
CDS:coat protein [Ma	201	S A T Q A V G Q I K I S Y S V S Y R G A					
CDS:coat protein, pa	61	A I L Q P A L V P G P G L A N					
Query	181	GCAATCCTACAACTGCCCTGGTTCCAGGCCAGGGCTGGCAAATCA					227
Sbjct	4037						4083
CDS:coat protein [Ma	221	A I L Q P A L V P G P G L A N H					

Figure 10: NCBI BLAST of the sequence data for the NMF3 isolate (227 bp) under accession number KT970621. The sequence was identified as a coat protein of MCMV in the database.

Maize chlorotic mottle virus isolate B3_S3, complete genome
Sequence ID: MF510251.1 Length: 4429 Number of Matches: 1

Range 1: 3857 to 4086					GenBank	Graphics	▼ Next Match ▲ Previous Match																
Score	Expect	Identities		Gaps	Strand																		
425 bits(230)	2e-115	230/230(100%)		0/230(0%)	Plus/Plus																		
CDS:coat protein, pa	1	M	P	Q	H	R	T	V	F	R	C	I	Q	A	G	Q	F	A	A	L	G		
Query	1	ATGCCACAAAACCGCACTGTGTTCAGGTGTATACAGGCTGGTCAGTTTGCTGCGTTGGGC																					60
Sbjct	3857																						3916
CDS:coat protein [Ma	161	M	P	Q	H	R	T	V	F	R	C	I	Q	A	G	Q	F	A	A	L	G		
CDS:coat protein, pa	21	S	A	A	D	K	Q	H	Y	S	P	G	R	F	I	V	A	I	P	K	A		
Query	61	AGCGCGGCTGACAAGCAAAATGTATTCTCCGGACGATTTCATTGTGGCTATCCCCAAAGCT																					120
Sbjct	3917																						3976
CDS:coat protein [Ma	181	S	A	A	D	K	Q	H	Y	S	P	G	R	F	I	V	A	I	P	K	A		
CDS:coat protein, pa	41	S	A	T	Q	A	V	G	Q	I	K	I	S	Y	S	V	S	Y	R	G	A		
Query	121	AGTGCAACACAAGCGGTAGGCCAAATCAAAATCTCGTACTCCGTGCTCTACCGTGGAGCA																					180
Sbjct	3977																						4036
CDS:coat protein [Ma	201	S	A	T	Q	A	V	G	Q	I	K	I	S	Y	S	V	S	Y	R	G	A		
CDS:coat protein, pa	61	A	I	L	Q	P	A	L	V	P	G	P	G	L	A	N	H						
Query	181	GCAATCCTACAACCTGCCCTGGTTCCAGGCCAGGGCTGGCAATCATTTG																					230
Sbjct	4037																						4086
CDS:coat protein [Ma	221	A	I	L	Q	P	A	L	V	P	G	P	G	L	A	N	H						

Figure 11: NCBI BLAST of the sequence data for the NMF6 isolate (230 bp) under accession number KT970622. The sequence was identified as a coat protein of MCMV in the database.

Sugarcane mosaic virus isolate b nonfunctional polyprotein gene, partial sequence
Sequence ID: KP835284.1 Length: 1678 Number of Matches: 2

Range 1: 623 to 1394		GenBank	Graphics	▼ Next Match		▲ Previous Match	
Score	Expect	Identities	Gaps	Strand			
1066 bits(577)	0.0	715/784(91%)	13/784(1%)	Plus/Plus			
CDS:polyprotein, par	1	H V I L A F N Y A M L S S G I R E E E I					
Query	1	ATGGTTATACATAGCTTTCACATATGCTATGCTATCGAGTGGTATTAGAGAAAGAGGATT					60
Sbjct	623	A.....					682
CDS:polyprotein, par	21	D N C C R H F A N G D D L L L A V H P D					
Query	61	GATAACTGCTGTAGGATGTTTGCAAATGGTGGTACCTCTTCTAGCAGTGCACCCGAT					120
Sbjct	683	C.....A..T..T..					742
CDS:polyprotein, par	41	F E Y I L N G F Q D H F G N L G L N F E					
Query	121	TTTGAATACATTTTAAACGGATTTCAAGATCAC1TTGGGAATCTCGGATTAATTTTGAG					180
Sbjct	743	C.....C.....A.....G.....					802
CDS:polyprotein, par	61	F T S R T R D K S E L W F H S T R G I K					
Query	181	TTTACATCACGAACACGAGATAAGTCCGAGCTGTGGTTTCATGTCCACAAAGAGGAATCAA					240
Sbjct	803	A.....A.....T.....T.....					862
CDS:polyprotein, par	81	C E G I Y I P K L E K E R I V A I L E W					
Query	241	TGTGAAGGAATCTACATACCTAACTCGAGAAAGAAAGATAGTCGCAATACCTTGAATGG					300
Sbjct	863						922
CDS:polyprotein, par	101	D R S H L P E H R L E A I C A A M V E A					
Query	301	GATCGATCAAACTACCTGAGCATAGATTGGGAAGCATTTGTGCAGCTATGTGTCGAAGCA					360
Sbjct	923	Y.....					982
CDS:polyprotein, par	121	W G Y S D L V H E I R K F Y A W L L E H					
Query	361	TGGGGTTACTCAGATCTGTGCTAGAAATCCGGAATTTTATGCGTGGCTTCTAGAAATG					420
Sbjct	983	Y..Y..T.....Y.....					1042
CDS:polyprotein, par	141	Q P F A N L A K E G K A P Y I A E T A L					
Query	421	CAACCTTTCGCAAAATTAGCAAAAAGAGGCAAGGCGCATACATAGCAGAAACAGCGCTC					480
Sbjct	1043	C.....A..T.....					1102
CDS:polyprotein, par	161	R N L Y L G T G I K E E E I E K Y F K Q					
Query	481	CGTAACCTTTTATCTTGGACGGGCATTAAAGAGAGAAATGAGAAATATTTTAAACAA					540
Sbjct	1103						1162

Figure 12: NCBI BLAST of the sequence data for the LVIC1 isolate (833 bp) under accession number KT970623. The sequence was identified as a polyprotein of SCMV in the database.

Sugarcane mosaic virus isolate R3 polyprotein gene, complete cds
Sequence ID: [KF44390.1](#) Length: 9552 Number of Matches: 1

Range 1: 8715 to 9373		GenBank	Graphics	▼ Next Match & Previous Match	
Score	Expect	Identities	Gaps	Strand	
1195 bits(647)	0.0	655/659(99%)	0/659(0%)	Plus/Plus	
CDS:coat protein, pa	1	M S K K M R L P K A K G K D V L H L D F			
Query	1	ATGTCAGAGAAAATGCGCTTACCGAAAGCAAAAGGAAAAGATGCTTTCATCTGGACTTC			60
Sbjct	8715				8774
CDS:polyprotein [Sug	2860	M S K K M R L P K A K G K D V L H L D F			
CDS:coat protein, pa	21	L L T Y K P Q Q Q D I S N T R A T K E E			
Query	61	TTGCTCACATACAGCCCAOCAGCAAGACATACAAACACAAAGCAACTAAGGAGAG			120
Sbjct	8775				8834
CDS:polyprotein [Sug	2880	L L T Y K P Q Q Q D I S N T R A T K E E			
CDS:coat protein, pa	41	F D R W Y D A I K K E Y E I D D I O H I			
Query	121	TTTGATAGATGGTACGATGCCATAAGAAAGGAATACGAGATTGATGACACACAAATGACA			180
Sbjct	8835				8894
CDS:polyprotein [Sug	2900	F D R W Y D A I K K E Y E I D D I O H I			
CDS:coat protein, pa	61	V V H S G L H V W C I E N G C S P N I N			
Query	181	GTTGTCATGAGCGGTCTATGGTGTGGTGCATTGGAGAGGTTGCTCACCAACATAAAC			240
Sbjct	8895				8954
CDS:polyprotein [Sug	2920	V V H S G L H V W C I E N G C S P N I N			
CDS:coat protein, pa	81	G N W T M H M D G D E Q R V F P L K P V I			
Query	241	GGAAATGGCAATGATGGATGGAGTGAACAAAGGATCTTTCACCTCAACCAAGTCATT			300
Sbjct	8955				9014
CDS:polyprotein [Sug	2940	G N W T M H M D G D E Q R V F P L K P V I			
CDS:coat protein, pa	101	E N A S P T F R Q I H H H F S D A A E A			
Query	301	GAAACGGATCTCCAACTTTCGACAAATATGATCATTTTAGTGTGACAGTGAAGACA			360
Sbjct	9015				9074
CDS:polyprotein [Sug	2960	E N A S P T F R Q I H H H F S D A A E A			
CDS:coat protein, pa	121	Y I E Y R N S T E R Y M P R Y G L Q R N			
Query	361	TACATAGAGTACAGAACTCTACTGAGCGATACATGCCAAGATACGGACTTCACGCGAAT			420
Sbjct	9075				9134
CDS:polyprotein [Sug	2980	Y I E Y R N S T E R Y M P R Y G L Q R N			
CDS:coat protein, pa	141	L T D Y S L A R Y A F D F Y E M T S R T			
Query	421	CTCACCACATATGCTTAGCAGGATGATTTTGATTTCTATGAAATGACITCAGCGACA			480

Figure 13: NCBI BLAST of the sequence data for the LVIC2 isolate (659 bp) under accession number KT970624. The sequence was identified as coat protein of SCMV in the database.

Sugarcane mosaic virus isolate b nonfunctional polyprotein gene, partial sequence
Sequence ID: [KP835284.1](#) Length: 1678 Number of Matches: 2

Range 1: 623 to 1394		GenBank	Graphics	▼ Next Match & Previous Match	
Score	Expect	Identities	Gaps	Strand	
1066 bits(577)	0.0	715/784(91%)	13/784(1%)	Plus/Plus	
CDS:polyprotein, par	1	M V I L A F N Y A M L S S G I R E E E I			
Query	1	ATGGTTTACTAGCTTTCAACTATGCTATGCTATCGAGTGGTATTAGAGAAGAAGAGATT			60
Sbjct	623				682
CDS:polyprotein, par	21	D N C C R M F A N G D D L L L A V H P D			
Query	61	GATAACTGCTGTAGGATGTTGCAATGGTGTGACCTGCTTCTAGCAGTGCACCCCGAT			120
Sbjct	683				742
CDS:polyprotein, par	41	F E Y I L N G F Q D M F G H L G L N F E			
Query	121	TTTGAATACATTTTAAACGGATTCAAGATCATTCTGGGAATCTCGGATTAAATTTTGAG			180
Sbjct	743				802
CDS:polyprotein, par	61	F T S R T R D K S E L W F M S T R G I K			
Query	181	TTTACATCACGAACACGAGATAAGTCCGAGCTGTGGTTCATGTCCACAAAGAGGAATCAAA			240
Sbjct	803				862
CDS:polyprotein, par	81	C E G I Y I P K L E K E R I V A I L E N			
Query	241	TGTGAAGGAATCTACATACCTAACTCGAGAAAGAAAGATAGTCGCAATAC TTGAATGG			300
Sbjct	863				922
CDS:polyprotein, par	101	D R S N L P E H R L E A I C A A M V E A			
Query	301	GATCGATCAAATCTACCTGAGCATAGATTGGAAGCCATTGTGCACTATGGTCGAGCA			360
Sbjct	923				982
CDS:polyprotein, par	121	W G Y S D L V H E I R K F Y A W L L E H			
Query	361	TGGGGTACTCAGATCTTGTCCATGAAATCCGGAATTTTATGCGTGGCTTCTAGAAATG			420
Sbjct	983				1042
CDS:polyprotein, par	141	Q P F A N L A K E G K A P Y I A E T A L			
Query	421	CAACCTTTTCGAAATTTAGCAAAAGAAAGGCGCCATACATAGCAGAAACAGCGCTC			480
Sbjct	1043				1102
CDS:polyprotein, par	161	R N L Y L G T G I K E E E I E K Y F K Q			
Query	481	CGTAACCTTTATCTTGGAAACGGGCATTAAAGAAAGAAATTTGAGAAATATTTTAAACAA			540
Sbjct	1103				1162

Figure 14: NCBI BLAST of the sequence data for the LVIC3 isolate (833 bp) under accession number KT970625. The sequence was identified as polyprotein of SCMV in the database.

Range 1: 1195 to 9893		Genbank		Genbank		W. R. Smith		W. R. Smith													
Score	Expect	Identities		Gaps		Strand		Plus/Minus													
6839 bits(3703)	0.0	7069/8734(81%)		72/8734(0%)																	
CD5:polyprotein [Cow]	360	H	Q	S	I	I	K	R	G	D	S	G	L	V	P	P	Q	E	I	1246	
Query	1189	CATCAGATGATTATCAAAAGAGGATAGATGGTCTTGATGTATCCCCACAGG-AA-ATA																		1252	
Subject	1195	A.G.G.T.....G.A.....G.....G.....A.....G.A.....																			
CD5:polyprotein [Cow]	352	H	Q	N	V	F	K	K	G	D	S	G	L	V	P	T	G	K	I		
CD5:polyprotein [Cow]	379	K	A	L	P	E	Q	E	K	E	I	F	I	I	R	G	R	Q	D	G	V
Query	1247	AAGCATCTCCGAGACAGGAAAGAGATTTTATATATAGAGGAGGACAGATGGATGT																		1306	
Subject	1253	T.T.C.....T.G.....T.H.G.....G.T.G.....G.....C.....T.T.G.....A.C.T.																		1312	
CD5:polyprotein [Cow]	371	K	A	L	P	A	H	E	K	D	V	F	V	F	R	G	R	I	D	G	I
CD5:polyprotein [Cow]	399	L	I	N	A	L	D	W	H	S	E	J	A	S	V	Q	H	M	S	H	Q
Query	1307	TAATTAACGCTCTTCAGCTGGCATAGTGAATATGGCAGG-GTCCACACATATTACACACAG																		1365	
Subject	1313	.G..A.....T.....T.G.....C.....C.A.T.....A.....G.....T.....T..A																		1371	
CD5:polyprotein [Cow]	351	L	I	N	A	L	D	W	H	S	E	J	A	S	V	Q	H	M	S	H	Q
CD5:polyprotein [Cow]	419	L	E	I	Q	F	F	Q	G	W	K	K	V	F	D	K	L	V	P	M	T
Query	1366	TTAGAAATTCATTTCTCCAGCTGGAAAGAGGTGTTGCACAAATAGTCCGCCACAGC																		1425	
Subject	1372	C.C.....G.....T.....T.G.....I.....G.....A.....C.....G.....A.....G.....C																		1431	
CD5:polyprotein [Cow]	411	P	E	V	Q	F	F	Q	G	W	K	K	V	F	D	K	L	V	P	R	T
CD5:polyprotein [Cow]	429	E	D	H	E	C	T	V	D	Y	S	N	E	Q	G	C	G	E	L	A	S
Query	1436	GAGGATCATGAAGTCACCGCTTGTATACAGCAATGACGCTCGCGGGAGTTGGCAGGCTCA																		1485	
Subject	1432	C.A.....C.....T.....G.....C.....C.....C.....T.....G.....C.....A.....S																		1491	
CD5:polyprotein [Cow]	431	Q	D	H	E	C	T	V	D	Y	S	N	E	Q	G	C	G	E	L	A	S
CD5:polyprotein [Cow]	459	L	C	Q	T	L	P	V	P	K	K	L	S	C	R	C	R	F	H	I	
Query	1486	TTGTCTCAAACTTTGTATCATGTAAGAAGAACTTTTCATGTAAGAAATAGAGTTCACATC																		1545	
Subject	1492	.A.....S.....G.....G.....G.....C.....C.....C.....C.....G.....I																		1551	
CD5:polyprotein [Cow]	451	L	S	Q	I	L	P	V	P	K	K	L	S	C	R	C	R	F	H	I	
CD5:polyprotein [Cow]	479	K	D	L	S	W	E	E	Y	K	E	F	I	A	V	H	F	G	C	C	A
Query	1546	AAAGATTTTGAGCTGGGAAGAAATACAAAGATTCATTCAGATTCACATTGGATGCTGTGCA																		1605	
Subject	1552	G.....T.G.....A.C.....T.....T.....																		1611	
CD5:polyprotein [Cow]	471	K	D	L	S	W	E	E	Y	K	E	F	I	A	V	H	F	G	C	C	A
CD5:polyprotein [Cow]	499	E	T	L	K	D	Q	G	S	I	G	F	K	N	V	Q	T	L	V	E	R
Query	1606	GAGACATCTCAAGATCAGCAAGATATAGGTTTCAAGATNTTCAGACATCTTCAGACATCTTCAGAAAGG																		1665	

3.3.2 Annotation of CABMV isolate (Serere1) genome sequence

37

inclusion protein), 5,824-5,982 (6K2, 6 Kilo Dalton Protein 2), 5,983-6,552 (VPg, Viral genome Linked Protein), 6,553-7,281 (NIa, Nuclear Inclusion Protein a), 7,282-8,832 (Nib, Nuclear Inclusion Protein b), 8,833-9,660 (CP, Coat protein) constituted the mature proteins (Figure 16). The expected conserved motifs FRNK in HC-Pro, GDD in Nib, and DAG in CP were found. Aphid transmission associated DAG motif was also observed in the CI region of the genome. All the predicted cleavage sites (amino acids before and after the cleavage site) were as expected for accurate cleavage (Adams *et al.*, 2005b).

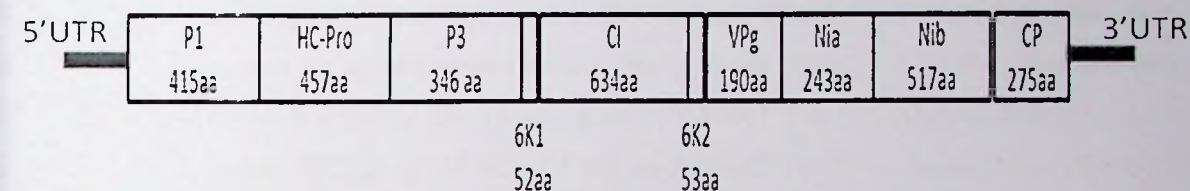


Figure 16: Schematic representation of the genome of Serere1 isolate. The length of nucleotide and amino acid sequences of mature proteins are indicated. The proteolytic cleavage sites for the 10 proteins identified in this isolate were as follows; P1/HC-Pro(VQHYYS), HC-Pro/P3(YKVG/G), P3/6K1(VETQ/A), 6K1/CI(VRMQ/S), CI/6K2(VCLQ/S), 6K2/VPg (VITQ/G), VPg/NIa(VGVE/S), NIa/Nib(VATQ/S), Nib/CP (VMLQ/S).

3. 3. 3 Comparison of proteins in Serere1 with other CABMV isolates

The 5' UTR nt identity between Serere1 and other isolates ranged from 36.9-52.6% with the KM655833 and AF348210 having the lowest identity (Table 1). However, CABMV Serere1 had a high identity (75.7-78.0%) with all isolates in the 3'UTR. The P1 protein identities varied from 43.7-69% and 41.7-64.3% for nt and aa levels, respectively. The lowest % nt identity level was with KM655833 while at the aa level, it was with the Zimbabwean isolate AF348210) and KM655833. Therefore, for CABMV Serere1, 5' UTR and P3 were the most variable regions compared to other regions. At the whole genome sequence level, Serere1 nt identity with other CABMV isolates ranged from 67.1-77.8% (Table 1). With the exception of KM655833, the genome sequence identity between Serere1 and the Zimbabwean isolate was low (75.8%). CP nt identity ranged from 68.4-83.1% while the aa identity ranged from 76.8-77.0%. The lowest aa identity was with HQ280243 while at the nt level, it was with KM655833.

The 5' UTR and the 3' UTR for Serere1 were 111nt and 244nt compared to 75 nt and 228 nt for AF348210, respectively (Table 1). The size of the P1 protein for Serere1 was 415aa much larger than that of AF348210 (286 aa). The P3 protein for AF348210.1 was larger than of Serere1 by 3nt (1,044 nt) compared to 1,041nt for Serere1 while the sizes of CI, VPg, NIa, Nib and 6K2 proteins for Serere1 and AF348210 differed by either one or two aa. However, for the 6K1 and CP, AF348210 and Serere1 had the same size (52 aa and 275 aa), respectively.

Serere1 had all the expected cleavage sites (Adams *et al.*, 2005b) however, for some sites there were differences in aa sequences around them when compared to the Zimbabwean isolate (AF348210). For instance, the aa sequence at P3/6K1 was VETQ/A in Serere1 while in AF348210.1, it was GRHQ/A. At 6K1/CI site in AF348210, the sequence was VRVQ/G and VRMQ/S in Serere1. For 6K2/VPg site in AF348210, aa sequence was VTTQ/G and VITQ/G in Serere1. At the NIa/Nib site, the aa sequence in AF348210 was GCTQ/S compared to VATQ/S in Serere1 and at the Nib/CP site in AF348210.1, aa sequence was VVLQ/S compared to VMLQ/S in Serere1 (Figure 16 and Figure 17).

Table 1: Sequence identity (%) comparison of mature proteins of CABMV obtained in this study with five genome sequences

Genomic region of Serere1		nt identity (%) with complete genome sequences					Amino acid identity (%) with complete genome sequences				
Nucleotide position	Region and size	AF3482	HQ8802	KM655	KM597	HQ88024	AF3482	HQ8802	KM655	KM597	HQ8802
		10	43	833	165	2	10	43	833	165	42
1-111	5'UTR	36.9	52.6	36.9	50.7	50.7	-	-	-	-	-
112-1,356	P1 (415aa)	45.4	69.0	43.7	60.5	59.1	41.7	64.3	41.7	45.0	45.0
1,357-2,724	HC-Pro (457aa)	82.4	79.9	70.4	81.1	81.8	92.8	90.8	91.9	90.8	91.7
2,725-3,765	P3 (346aa)	79.4	79.3	79.7	79.7	79.7	82.5	83.3	83.3	83.9	83.9
3,766-3,921	6K1 (32aa)	78.8	80.1	75.0	79.5	79.5	92.3	94.2	92.3	92.3	92.3
3,922-5,823	CI (634aa)	80.5	80.8	71.7	80.8	80.8	91.3	92.1	91.6	91.8	91.8
5,824-5,982	6K2 (53aa)	78.6	79.2	79.2	79.2	79.2	88.7	86.8	86.8	86.8	86.8
5,983-6,552	VPg (190aa)	77.9	76.8	70.7	77.0	77.0	88.0	84.8	84.8	84.8	84.8
6,553-7,281	NIa (243aa)	79.3	80.2	69.8	80.9	80.9	91.0	90.6	90.6	90.6	90.6
7,282-8,832	NIb (317aa)	79.5	78.7	71.4	79.3	79.3	88.6	90.0	89.6	89.8	89.8
8,833-9,660	CP (275aa)	82.3	77.7	68.4	83.1	83.1	85.5	76.8	85.5	87.0	87.0
9,661-9,904	3'UTR	82.6	83.1	82.6	82.6	82.6	-	-	-	-	-
1-9,904	Genome	75.8	77.8	67.1	77.4	77.5	-	-	-	-	-
112-9,657	Polyprotein	75.8	78.0	67.1	77.7	77.8	82.9	85.1	82.9	83.3	83.4

Full names for the 10 proteins are mentioned in text. Information in parentheses refers to the number of amino acids of each protein.

AF348210 = CABMV-Z (Zimbabwe), HQ880243 = MG-Avr (Brazil), KM655833 = RR4 (India), KM597165 = RR3 (India), HQ880242 = BRI (Brazil)

In Serere1, two motifs (ESKKL in P1 and LVYV in CI) that are conserved among potyviruses were missing. The DAG motif which is known to occur only in the CP region also occurred in the CI protein. Thus, DAG motif was found at two positions 1787-1789 and 2921-2923. Other expected potyvirus motifs were present in the Serere1 however, when compared to AF348210.1 (CABMV-Z), there were differences in their positions. For instance, the GDSC motif found in P1 was found at 367-370 position in Serere1 compared to 238-241 in CABMV-Z. For HC-Pro, the conserved sequence GYCY for putative proteinase active site in CABMV Serere1 was found at 756-759 while in CABMV-Z, it was at 626-629. Also, the KLSC, FRNK, PTK, and CCC motifs in Serere1 were located at 469-472, 595-598, 726-728, 706-708 respectively, while in CABMV-Z, they were located at 339-342, 465-468, 594-596 and 576-578, respectively.

The DPY-(X)7-SP-(X)2-L motif in P3 was found at position 902-916 in Serere1 compared to 772-786 in CABMV-Z. In the CI protein, GAVGSGKST (1,227-1,235), VLLLEPTRPL (1,247-1,256), KVSAT (1,343-1,347), LVYV (1,394-1,397), VATNIIENGVTL (1,443-1,454) and GERIQRLGRVGR (1,487-1,498) were identified in CABMV-Z while in Serere1, these motifs except LVYV occurred at positions; GAVGSGKST (1,356-1,364), VLLLEPTRPL (1,376-1,385), KVSAT (1,472-1,476), VATNIIENGVTL (1,574-1,585) and GERIQRLGRVGR (1,618-1,629). The overlapping sequence pretty interesting potyvirus open reading frame (PIPO) was at 2,755-2,978 and 3,178-3,401 in CABMV-Z and Serere1, respectively. In Nib, the SG-(X)3-T-(X)3-NT-(X)30-GDD and CHADGS motifs were at positions 2,571-2,614 and 2506-2511, respectively in CABMV-Z while in Serere1, these conserved sequences were at 2,704-2,747 and 2,639-2,644, respectively. In the CP, the DAG motif was found at positions 2,787-2,789 and 2,921-2,923 in CABMV-Z and Serere1, respectively.

3. 3. 4 Phylogeny of CABMV Serere1 with other isolates

Phylogenetic analysis of complete nucleotide genome, polyprotein and coat protein (CP) sequences placed Serere1 in a cluster different from other CABMV isolates (Figures 18A, 18B and 19). These figures also show a great amount of genetic change both in the whole genome as well as the coding regions of the Ugandan isolate compared to other isolates. Among the five isolates analysed, Serere1 isolate was more distinct from RR4 isolate from

India. Analysis of CP sequences showed that the ancestry of CP sequences for the Ugandan isolate lies within that of the Zimbabwean isolate (Figure 19).

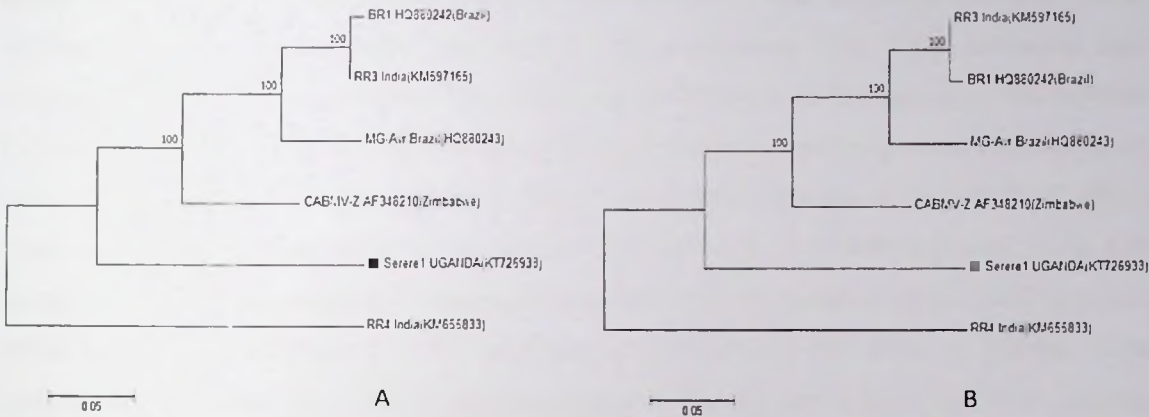


Figure 18: Maximum Likelihood method tree for complete genomes (A) and coding sequences (B) of CABMV isolates.

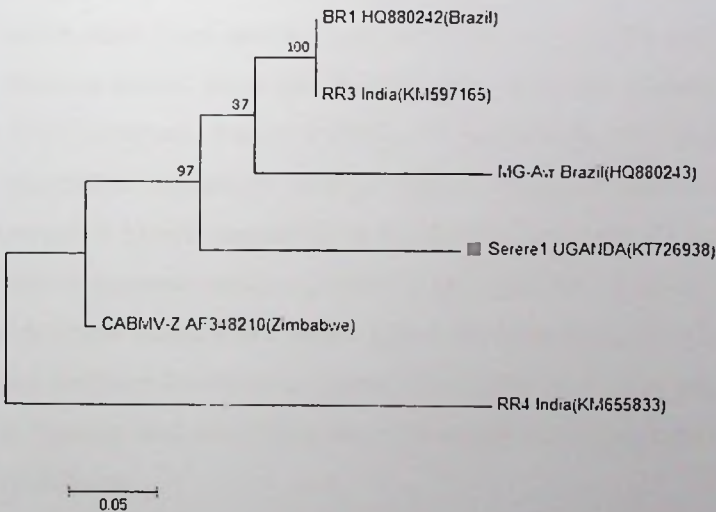


Figure 19: Maximum Likelihood tree for coat protein sequences of CABMV isolates.

3.4 Discussion

The identity of viruses infecting cowpea in Uganda was determined using next-generation sequencing (NGS). Three potyviruses; *Cowpea aphid-borne mosaic virus* (CABMV), *Peanut mottle virus* (PeMoV), *Sugarcane mosaic virus* (SCMV) and a Machlomovirus, *Maize chlorotic mottle virus* (MCMV) were detected by sequencing total RNA extracted from symptomatic cowpea leaf samples. The occurrence of CABMV in Uganda was first reported by Anon (1981) and is widely distributed in the major cowpea growing areas (Edema *et al.*, 1997; Orawu *et al.*, 2005; Amayo, 2008, Amayo *et al.*, 2012; Orawu *et al.*, 2015). CABMV causes significant cowpea yield losses for instance Kaiser *et al.* (1968) reported up to 80% reduction in seed yield in Iran, Kaiser and Mossahebi (1975) reported up to 13-87% in Iran while Kannaiyan and Haciwa (1993) observed a yield loss of 48- 60% in Zambia. Total cowpea crop loss due to CABMV was also reported Raheja and Leleji (1974) in Nigeria. CABMV is the most economically important virus worldwide (Bashir *et al.*, 2002a).

Although cowpea is a known natural host for PeMoV (Demski *et al.*, 1983) and the virus widely occurs in East Africa (Bock, 1973b), this is the first time that it was found on cowpea in Uganda. In East Africa, PeMoV is mainly widespread on groundnut and soybean but also infects other plant species such as *Phaseolus vulgaris* and *Cassia occidentalis* (Bock, 1973b). Previous studies show that PeMoV expresses mild disease reaction on cowpea (Demski *et al.*, 1983) however, the possibility of occurrence of naturally several strains and potential synergistic interaction with unrelated viruses makes it an important cowpea virus. Since cowpea is grown frequently in fields close or adjacent to other legumes susceptible to PeMoV such as soybean and groundnut, it can serve as a source of inoculum (Demski *et al.*, 1983). Additional isolates of PeMoV across the country need to be studied to establish their diversity and virulence levels on cowpea. The distribution/incidence and severity of PeMoV on cowpea in Uganda and identification of cowpea genotypes with resistance to the virus need to be carried out.

Results from this study also showed that cowpea is a potential natural host for SCMV and MCMV. The two viruses either singly or in mixed infections are known to infect only cereals (Gordon *et al.*, 1984; Teakle *et al.*, 1989) and have never been detected on legumes. MCMV and a potyvirus such as SCMV causes maize lethal necrosis (MLN) disease in maize

(Uyemoto, *et al.*, 1981). The occurrence of MCMV and MLN on maize in East Africa were initially reported by Wangai *et al.* (2012) and later by Adams *et al.* (2012) but SCMV was reported earlier in Kenya (Louie, 1980). Identification of MCMV and SCMV on cowpea has epidemiological implications on the spread of MLN since cowpeas are often intercropped or grown in close proximity to maize fields.

The identification of SCMV, MCMV and PeMoV highlights the importance of NGS in enabling rapid identification of new and unusual plant viruses and facilitating the rapid development of high throughput diagnostic assays as previously proposed by Adams *et al.* (2012). Conventional assays require reagents designed to exclusively detect their viral target and any variation in the virus genome may cause failure of the assays. However, NGS is non-biased method that requires no a priori knowledge of the target and hence is able to detect existing strains, new variants and even completely new viruses (Adams *et al.*, 2009). However, the NGS approach used could not detect several viruses that were previously reported on cowpea in Uganda using ELISA. These included; *Cucumber mosaic virus* (CMV), *Cowpea mild mottle virus* (CPMMV), *Cowpea mottle virus* (CPMoV), *Cowpea chlorotic mottle virus* (CCMV), *Cowpea yellow mosaic virus* (CYMV), *Cowpea severe mosaic virus* (CPSMV) and *Southern bean mosaic virus* (SBMV) (Edema *et al.*, 1997; Orawu *et al.*, 2005; Amayo, 2008; Amayo *et al.*, 2012; Orawu *et al.*, 2015). ELISA often gives false positives due to cross-reactivity between antisera and plant proteins or between antisera and other different viruses (Dietzgen *et al.*, 2001; Gutiérrez *et al.*, 2003; Naidua and Hughes, 2003; Tairo, 2006; Akinjogunla *et al.*, 2008; Kassif, 2009). Also, the samples analysed by NGS may have not been infected by the viruses that were previously reported. There is need to sequence additional isolates/samples to verify this finding. Future studies are needed to establish the implications of single or mixed infections of cowpea with MCMV and SCMV on virus spread and subsequent effects on cowpea productivity.

In this study, the first complete genome sequence of an isolate (Serere1) of CABMV from Uganda was obtained. The isolate had all the 10 mature proteins characteristic of potyviruses (Adams *et al.*, 2005a; 2005b). Comparison of Serere1 with CABMV-Z from Zimbabwe (another isolate with a complete genome from Africa) revealed that 5'UTR and P1 were the most variable regions in Serere1 while in CABMV-Z, P1 and P3 were the most variable regions. High variability in potyvirus genomes is often associated with P1 and P3 proteins

(Adams *et al.*, 2005a). In the Serere1 genome, two motifs, ESKKL in P1 and LVYV in CI that are conserved in potyviruses were missing. P1 protein acts as a protease in the autoproteolytic activity of P1 at the C-terminus (Verchot and Carrington, 1995) and also in RNA silencing (Mbanzibwa *et al.*, 2009) while CI plays several functions such as *in vitro* RNA dependent ATP binding, RNA helicase activities, virus replication and viral cell-cell movement (Deng *et al.*, 2015). The absence of conserved motifs in Serere1 may influence the virulence of the virus. It was observed that in Serere1 genome, a DAG motif was present in the CI protein in addition to the one present in the CP region. The DAG motif plays an important role in aphid transmission of the virus (Mlotshwa *et al.*, 2002). The additional DAG motif may influence aphid transmission of the virus although the CI is not known to play any role in virus transmission by aphids.

Pairwise nucleotide comparison of entire genome and polyprotein sequences revealed that the Serere1 isolate was very distinct from RR4 isolate (KM655833) from India with percentage nucleotide identity of 67.1%. However, the Ugandan isolate was closely related to two Brazilian isolates MG-Avr (GenBank accession number HQ880243) and BR1 (HQ880242) (Barros *et al.*, 2011) and an Indian isolate, RR3 (KM597165) (Mishra *et al.*, 2015), with 77.8%, 77.4%, and 77.5% nt identity, respectively (Table 1). Phylogenetic analysis however clustered the Ugandan isolate separately from other isolates (Figures 18 and 19). This could be attributed to affinity of isolates to their geographic origin or host ecological conditions (Abubakar *et al.*, 2003; Trovao *et al.*, 2015). While sequence data is important in clarifying the taxonomy, diversity, evolutionary relationships among viruses (Adams *et al.*, 2005a; Ndunguru *et al.*, 2015), multiple sequences from diverse geographic areas/regions are needed in order to generate reliable results.

CHAPTER FOUR

DEVELOPMENT OF A MOLECULAR DIAGNOSTIC ASSAY FOR COWPEA APHID-BORNE MOSAIC VIRUS

4.1 Introduction

Cowpea (*Vigna unguiculata* L. Walp) is an important legume crop in Uganda, third after common beans and groundnuts widely grown in eastern and northern parts of the country (UBOS, 2010). The crop improves soil fertility through fixation of nitrogen and also curbs erosion due to its rapid ground cover (Singh *et al.*, 2002; Tarawali *et al.*, 2002; Casky *et al.*, 2002). Despite its importance both as a food and cash crop, its productivity is low with yield in Uganda less than 500 kg ha⁻¹ (UBOS, 2019). Low productivity is attributed to several constraints such as insect pests, diseases, parasitic weeds, low soil fertility, drought and lack of access to inputs (Ajeigbe and Singh, 2006).

Cowpea aphid-borne mosaic virus (CABMV) is an economically important seed-borne virus with a worldwide distribution (Bashir *et al.*, 2002a). The virus may have been widely distributed and established in several areas of the world as a result of cowpea seed trade and through movement of infected seed lots/germplasm for variety trials (Salem *et al.*, 2010). The virus is capable of causing up to 100% yield losses under single or mixed infections (Raheja and Leleji, 1974; Kaiser and Mossahebi, 1975; Pio-Ribeiro *et al.*, 1978; Pio-Ribeiro and Kuhn, 1980; Kannaiyan and Haciwa, 1993). CABMV, first reported in Uganda in 1981 (Anon, 1981) is widespread in the main cowpea growing areas of the country (Edema *et al.*, 1997; Orawu *et al.*, 2005; Amayo *et al.*, 2012; Orawu *et al.*, 2015). The presence of CABMV in Uganda was recently confirmed using next-generation sequencing (Mbeyagala *et al.*, 2016; 2018a) further highlighting its importance in the country. In order to mitigate the damage and yield loss associated with CABMV and other pathogens, efficient and accurate diagnosis is critical. The most common diagnostic method often used by the national cowpea improvement programmes mainly focuses on monitoring of virus-like symptoms (Salem *et al.*, 2010). However, different cowpea viruses often show similar symptoms or different strains of the same virus cause distinct symptoms on the same host (Aliyu and Balogun, 2011; Mbanzibwa *et al.*, 2011). Conventional methods such as ELISA are often too specific to a particular virus species or strain while alternative methods such as mechanical/vector

transmission, host range tests and electron microscopy do not usually give species level diagnosis (Adams *et al.*, 2012).

Studies have been undertaken in the past focusing on detection of cowpea viruses in Uganda using different methods (ELISA and PCR) (Orawu *et al.*, 2005; Amayo, 2008; Byarugaba, 2008; Amayo *et al.*, 2012; Orawu *et al.* 2015). These studies however, gave inconsistent/ inconclusive results. This study was conducted to develop a reliable and accurate molecular diagnostic assay that could be used for the detection of CABMV in Uganda.

4.2 Materials and methods

4.2.1 Plant samples

Forty (40) leaf samples from plants with characteristic virus symptoms were collected from two fields in Serere and Ngora districts in eastern Uganda. The leaves were dried by placing them between paper towels and inserted in ziploc bags and silica gel added. The bags were placed horizontally in a plastic container and tightly closed. Complete drying of samples was achieved in 2-3 days at room temperature with at least a single change of silica gel. The dried leaf samples were used for RNA extraction and virus detection by RT-PCR. Fresh leaf samples were also collected and kept in cool boxes containing ice for serological assay using Nitrocellulose membrane-based ELISA (NCM-ELISA).

4.2.2 Nitrocellulose membrane based ELISA (NCM-ELISA)

NCM-ELISA procedure was carried out as described by Fuentes (2010) as follows; fresh leaf sample discs of approximately 1cm diameter were ground with 1ml of extraction buffer, Tris buffered saline (TBS, pH 7.5). Macerated samples were allowed to stand for 30 min and followed by blotting 10µl of supernatant on to the nitrocellulose membrane and left to dry for 30 min. Positive and negative samples (controls) were also blotted on the NCM. The dried NCM was treated with 25ml of blocking buffer (TBS + 2 % milk + 2 % TRITON X-100) and then washed with TBS Buffer pH 7.5. The membrane was placed in 25ml of Antibody buffer (TBS, pH7.5) + 4.8 g skimmed milk) containing the 1st antibody (DSMZ, Braunschweig, GERMANY) and incubated for 3 hrs at room temperature followed by washing with T-TBS buffer. Later the membrane was placed in 25ml of Conjugate buffer (TBS, pH 7.5 + 4.8 g skimmed milk) containing the 2nd antibody (conjugated with alkaline phosphatase), also from DSMZ, Braunschweig, GERMANY followed by washing in T-TBS buffer. Both antibodies

were used at a dilution of 1:500. The membrane was dried and then placed in 25ml of substrate-chromogen solution BCIP/NBT (5-Bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium chloride; pH 9.5) for ± 30 mins until the colour (purple/blue) changed as virus signals. BCIP/NBT were obtained from Sigma, St. Louis, USA. Colour development was stopped by discarding the substrate solution and immersing the membrane in distilled water for 10 mins. Positive reactions were identified by purple coloured blots while negative samples remained blue.

4.2.3 RNA extraction and cDNA synthesis

Dried leaf samples were ground into powder using a mortar and pestle with the help of liquid nitrogen. RNA extraction was carried out using AccuZol reagent (Bioneer, Seoul, Republic of Korea) according to the manufacturer's instructions. Quality was checked using TAE/Formamide agarose gel electrophoresis as described by (Masek *et al.*, 2005) and quantified using NanoDrop spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA). The oligo(dT)₁₈ primed synthesis of complementary DNA (cDNA) on 2 μ g of total RNA was achieved using RevertAid Reverse Transcriptase (RT), a recombinant M-MuLV RT according to manufacturer's instructions (Thermo Fisher Scientific, Massachusetts, USA).

4.2.4 Primer sequences used

Primers for virus detection were designed based on available data from full genome sequences as well as 3'-untranslated regions (3'-UTR) of CABMV in DDBJ/ENA/GenBank. The sequences were aligned using multiple alignment softwares; ClustalX Version 1.83 (Thompson *et al.*, 1997) and MEGA7 (Kumar *et al.*, 2016). Following multiple alignment, five primer pairs were designed with the forward primer for the first pair starting at the GNNSGQ motif located in the centre of the nuclear inclusion protein b (Nib) (Zheng *et al.*, 2010). Subsequent forward primers were located downstream of the GNNSGQ motif. Reverse primer sets were design in the 3'UTR regions. The designed primers targeted the entire coat protein region. Table 2 shows the list of primer pairs that were used in this study. An additional primer pair (sixth) designed by Gillaspie *et al.* (2001) was also included.

Table 2: Primers used for detection of CABMV in Ugandan cowpea samples

Primer name	Primer sequence (5'-3')	Predicted size (bp)	Reference
CABFF1	GGT AAC AAY AGT GGR CAA CC	1,642	This study
CABRR1	CTG AGC ACT CMA ACC GGG		This study
CABFF2	GAG AGG YTR GTK TTC TTT GC	1,514	This study
CABRR2	GGC CTC YCY GCT AAG TTC		This study
CABFF3	TGA ATT AYG AYT TCT CAG AAA G	1,333	This study
CABRR3	CTG RAT ATA TGC GTA CTA TTT AC		This study
CABFF4	AGA GAR GAR TTA TGG TTC ATG	1,281	This study
CABRR4	CTA AAA CCA ACC ATT AGC		This study
CABFF5	TAC ATA CCA AAR CTW GAG C	1,275	This study
CABRR5	CCA CAC TRR CAT ATA TAG		This study
GF	CGCTCAAACCCATTGTAGAA	221	Gillaspie <i>et al.</i> (2001)
GR	TATTGCTTCCCTTGCTCTTTC		

4.2.5 Polymerase chain reaction (PCR) conditions

After optimization of PCR conditions, only CABFF1/CABRR1 out of the five designed primer pairs was effective thus further sections on methodology, results and discussions are limited to this primer as well as GF/GR primer set designed by Gillaspie *et al.* (2001). The PCR mix consisted of 10µl of 2X PCR Master mix (Bioneer, Seoul, Republic of Korea), 0.25µM of each primer, 1µl of cDNA and 8µl of sterile nuclease free water giving a total reaction volume of 20µl. The PCR conditions for CABFF1/CABRR1 were; 94°C for 2 min followed by 35 cycles of 94°C (30 sec), 58°C (90 sec), 72°C (90 sec) for denaturation, annealing and extension, respectively with a final extension at 72°C for 5min. The PCR conditions used for GF/GR were as described by Gillaspie *et al.* (2001).

PCR products were analysed by electrophoresis in 1% agarose gel in 1×TAE buffer and stained with ethidium bromide. Gel electrophoresis was carried out at 100V for 60 min. After electrophoresis, images of the PCR products were captured/visualized using an inbuilt camera in an E-Gel® Imager System (Thermo Fisher Scientific, Massachusetts, USA) under UV light.

4.2.6 Sanger sequencing and sequence analysis

The PCR products were purified from the gel using Wizard SV Gel and PCR Clean-Up System (Promega, Madison, USA) following manufacturer's instructions and later directly sequenced. Sequencing was carried out at Macrogen Inc (Macrogen Europe Laboratory, Amsterdam, The Netherlands). Sequencing results were compared to published DNA sequences using a Basic Local Alignment Search Tool (BLAST) as described by Johnson *et al.* (2008). Related sequences identified through BLASTN analysis were selected and used in phylogenetic analysis. A total of 21 sequences including an out group (*Barley yellow dwarf virus-BYDV*) were included in the analysis. Multiple sequence alignment and computation of nucleotide sequence identities were done using the BioEdit software version 7.0.5 (Hall, 1999).

Phylogenetic relationships were inferred using the Maximum Likelihood method, based on the Tamura-Nei model (Tamura and Nei, 1993) implemented in MEGA7.0 (Kumar *et al.*, 2016). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbour-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. The trees were drawn to scale with branch lengths measured in the number of substitutions per site. Statistical significance of branching for phylogenetic trees was assessed using bootstrap analyses of 1,000 different alignments from the original dataset, and reported as percentage.

4.3 Results

CABMV was detected in all the 40 leaf samples by NCM-ELISA including the positive and negative controls. NCM-ELISA therefore was not able to distinguish between positive and negative samples since all samples gave positive signals. Among the six primer pairs that were designed, only one primer (CABFFI/CABRRI) was able to give CABMV amplification when tested on the positive controls. The primer pair was therefore used to differentiate all collected samples into negative and positives. CABFFI/CABRRI primer pair yielded the expected PCR product of approximately 1,642 bp (Figure 20). However, the GF/GR primer designed by Gillaspie *et al.* (2001) produced a PCR product of about 400 bp instead of the expected 221 bp (Figure 21).

BLAST analysis (BLASTN) of nucleotide sequences from Ugandan CABMV isolates amplified with CABFF1/CABRR1 primer showed similarity to coat protein (CP) sequences of other CABMV isolates in the DDBJ/ENA/GenBank (Figure 22). However, BLAST analysis of sequence data from GF/GR primer fragments gave no virus hits (there were no significant alignments). Nucleotide sequences for three Ugandan CABMV isolates (CABMV-H1, CABMV-H2 and CABMV-H5) were deposited in the DDBJ/ENA/GenBank under accession numbers MH151199, MH151200 and MH151201, respectively. Sequences of these isolates contained a single open reading frame (ORF) of 278 aa encoding the entire coat protein (CP) followed by a non-coding region of 147 nt, 150 nt and 144 nt, excluding the poly A tail for CABMV-H1, CABMV-H2 and CABMV-H5, respectively.

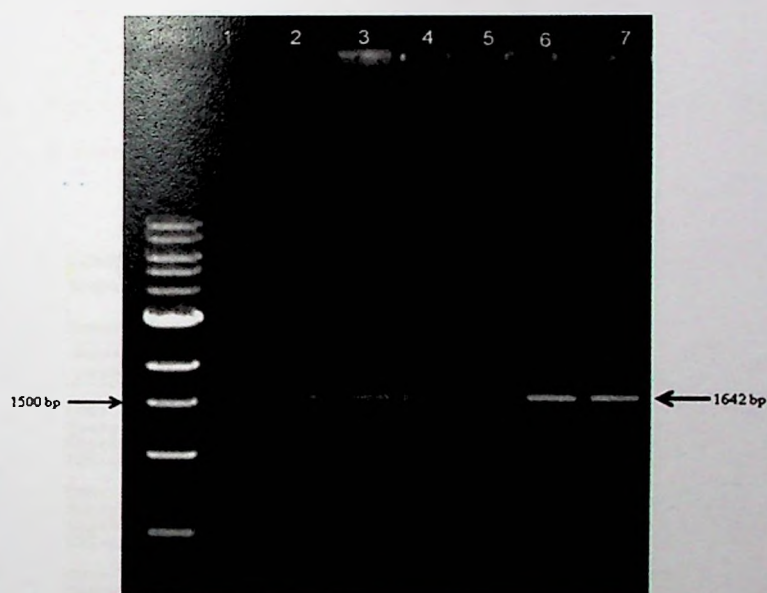


Figure 20: RT-PCR product for CABMV resulting from CABFF1/CABRR1 primer pair: Lane 1: Negative control, lanes 2-6: Samples, lane 7: Positive control.

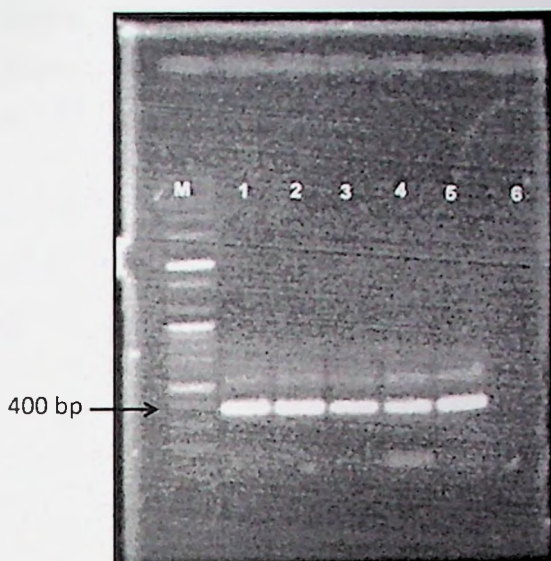


Figure 21: RT-PCR product for CABMV for GF/GR primer pair: M is 100 bp ladder, lane 1-5: Samples, lane 6: Negative control.

Cowpea aphid-borne mosaic virus isolate BF-E1 coat protein gene, partial cds

Sequence ID: [MF277031.1](#) Length: 828 Number of Matches: 1

Range 1: 1 to 828 GenBank Graphics					Next Match Previous Match	
Score	Expect	Identities	Gaps	Strand		
1419 bits(768)	0.0	808/828(98%)	0/828(0%)	Plus/Minus		
CDS: Putative 1	275	* Q G H R M G L L S H M N Q N V D T A T				
Query	147	TTACTGCCCATGCCTCATCCCAAGAAGGGAGTGTCATGTTTGGATTGACATCAGTGGCAGT			206	
Sbjct	828	C.....A.....G..			769	
CDS:coat protein, pa	275	Q G H R M G L L S H M N Q N V D T A T				
CDS: Putative 1	256	H R E T N E S T T A V N G D L G F M R T				
Query	207	GTGCCTCTCAGTGTTCTCACTAGTTGTCGCAACATTCCCATCCAGGCCAAACATCCTGGT			266	
Sbjct	768	G.....			709	
CDS:coat protein, pa	256	H R E T N E S T T A V N G D L G F M R T				
CDS: Putative 1	236	N V N S L A A A K M Q A I A E R A R D S				
Query	267	GTTAACGTTTCGACAGGGCTGCTGCTTTCATCTGTGCTATTGCTTCTCGTGCCTATCAGA			326	
Sbjct	708				649	
CDS:coat protein, pa	236	N V N S L A A A K M Q A I A E R A R D S				
CDS: Putative 1	216	T K S T V E Y F D F A Y R A L S K D R L				
Query	327	AGTTT TAGAGGTCACCTTCGTAGAAATCAAAAGCATACCTTGCTAAGCTCTTATCCCTTAA			386	
Sbjct	648	G.....			589	
CDS:coat protein, pa	216	T K S T V E Y F D F A Y R A L S K D R L				
CDS: Putative 1	196	N R L L G Y R P M Y F G E S N R H E I Y				
Query	387	ATTCTGAGTAGTCCATACCTGGGCATGTAGAACCCTCGGAATTCTCATCTCTATATA			446	
Sbjct	588	A.....			529	
CDS:coat protein, pa	196	N R L L G Y R P M Y F G E S N R H E I Y				
CDS: Putative 1	176	A E A A D S F H H M I Q R L T P K A N E				
Query	447	CGCTTCAGCTGCGTCAGAGAAGTGGTGCAATATTTGCTAAGTGTGGGTTTTGCATTTTC			506	
Sbjct	528				469	
CDS:coat protein, pa	176	A E A A D S F H H M I Q R L T P K A N E				

Figure 22: NCBI BLAST of the sequence data for the RT-PCR fragment from CABFF1/CABRR1 primer.

The Ugandan CABMV isolates exhibited 62-75% nucleotide identity with CABMV isolates from Brazil, 72-75% identity with an isolate from Nigeria and 68-75% identity with isolates from Burkina Faso (Table 3). The molecular diversity among Ugandan CABMV isolates was very limited, ranging from 89-94% with the lowest identity observed between CABMV-H1 and CABMV-H5. Overall the Ugandan isolates showed higher identity with African isolates (from Burkina Faso and Nigeria) than with the Brazilian isolates.

Phylogenetic analysis showed little divergence/genetic diversity among CP sequences for the Ugandan isolates as shown by the very short branch lengths. It is therefore possible that the three isolates belong to the same strain of CABMV. The Ugandan isolates were closely related to other CABMV isolates from Burkina Faso but very different from Brazilian isolates (Figure 23). Figure 23 also showed that virus isolates exhibited great phylogenetic affinity to their geographic origin with isolates from each country clustering together compared to other isolates.

Table 3: Pairwise sequence comparison matrix for complete coat protein nucleotide sequences of three Ugandan isolates and other isolates of CABMV^a

Virus Isolate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
^b CABMV-BF-E4																					
^b CABMV-BF-E1	99.1																				
^b CABMV-BF-E5	95.2	95.2																			
^b CABMV-BF-E10	95.2	95.2	100																		
^b CABMV-BF-E6	95.4	95.4	99.8	99.8																	
^c CABMV-M3	64.7	64.6	64.6	64.6	64.5																
^c CABMV-M2	64.8	64.7	64.9	64.9	64.9	98.6															
^c CABMV-SP	63.5	63.4	63.3	63.3	63.2	63.2	95.1	94.6													
^c CABMV-Lns10	64.0	63.7	63.3	63.3	63.2	63.2	84.8	84.9	83.4												
^c CABMV-Lns9	64.0	64.0	63.6	63.6	63.5	63.5	84.7	84.9	83.1	99.3											
^c CABMV-Lns4	63.9	63.8	63.2	63.2	63.2	63.1	84.7	84.9	83.3	99.2	98.9										
^c CABMV-Lns3	63.9	63.8	63.4	63.4	63.3	63.3	84.9	85.0	83.4	99.6	99.3	99.0									
^c CABMV-Lns2	64.0	63.9	63.5	63.5	63.4	63.4	84.9	85.1	83.4	99.6	99.7	99.0	99.6								
^c CABMV-DF-Brs	63.8	63.7	63.1	63.1	63.0	63.0	91.9	91.8	90.4	85.8	85.9	86.0	85.9	85.9							
^c CABMV-F-144	64.9	64.8	65.0	65.0	64.9	64.9	98.1	99.4	94.6	85.0	84.9	85.0	85.1	85.2	92.0						
^c CABMV-F-101	63.4	63.3	63.6	63.6	63.5	63.5	96.2	97.4	96.5	83.4	83.3	83.0	83.5	83.6	90.1	97.4					
^d CABMV-A132	56.1	55.9	55.3	55.3	55.2	77.3	77.6	76.0	77.3	77.1	77.0	77.3	77.3	77.3	77.8	77.6	76.0				
^c CABMV-H1	74.5	74.4	72.4	72.4	72.5	69.0	69.1	68.0	74.7	74.5	75.0	74.7	74.7	74.6	68.2	69.2	68.0	72.1			
^c CABMV-H2	71.6	71.5	69.7	69.7	69.7	66.7	66.8	65.7	72.1	71.9	72.0	72.1	72.1	72.1	65.8	66.9	66.0	74.8	94.3		
^c CABMV-H5	69.6	69.5	67.7	67.7	67.8	63.2	63.3	62.5	68.6	68.3	68.0	68.6	68.5	62.6	63.3	62.0	73.4	89.3	93.4		
BYDV-Montana	30.1	30.0	29.8	29.8	29.8	30.0	30.1	29.5	29.6	29.7	30.0	29.6	29.6	29.3	30.1	29.0	25.5	27.7	26.8	25.3	

^aPair wise comparisons made with BioEdit version 7.0.4.1, derived using ClustalW multiple alignments with the following parameters:

BLOSUM matrix, Gap open=10, Gap extension=0.1. ^bBurkina Faso isolates, ^cBrazilian isolates, ^dNigerian isolate, ^eUgandan isolates

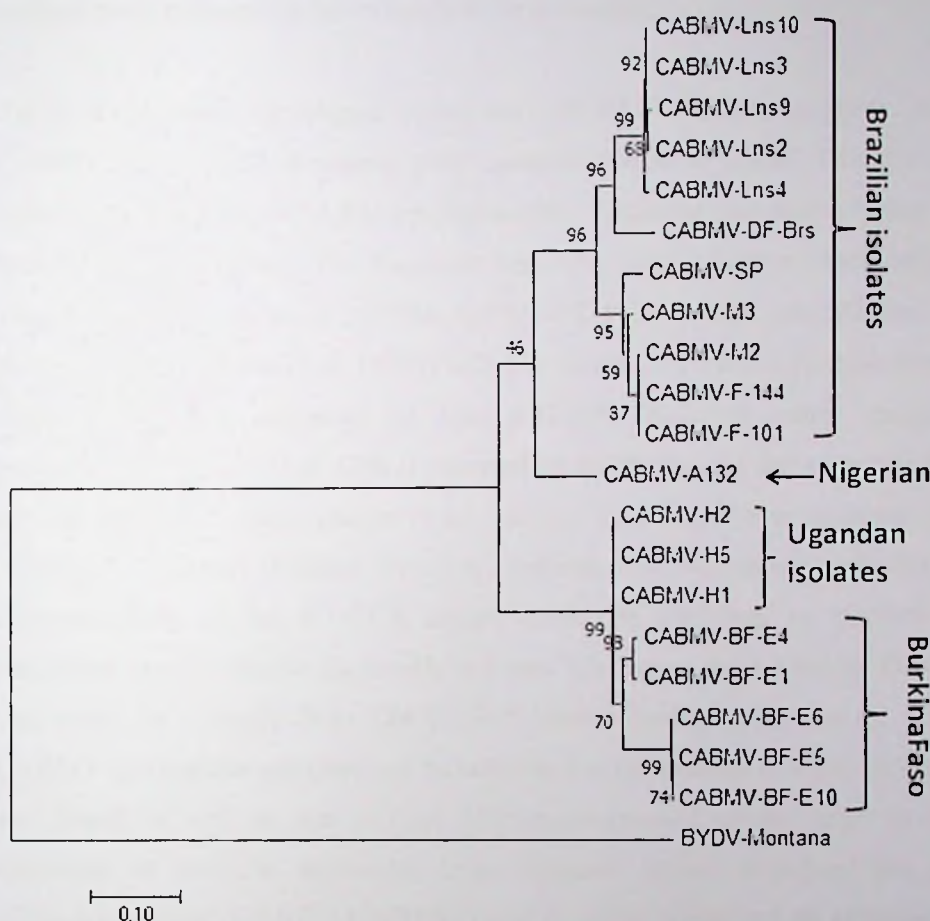


Figure 23: Phylogenetic analysis of complete CP encoding nucleotide sequences of three CABMV isolates from Uganda and other countries. The tree was rooted on the sequence of *Barley yellow dwarf virus*.

4.4 Discussion

NCM-ELISA test was unable to distinguish between positive and negative samples since all samples gave positive signals. ELISA tests are often associated with giving false signals (false positives) and this could be due to cross reaction of antibodies with plant proteins or other viruses (Dietzgen *et al.*, 2001; Gutierrez *et al.*, 2003; Kashif, 2009). Cross reaction makes it difficult to correctly distinguish/separate infected from non-infected samples rendering accurate virus detection impossible. Cross adsorption of membranes with extracts (plant sap) from healthy plants was reported as efficient in preventing reaction of antibodies with plant

proteins in sweet potato (Gutierrez *et al.*, 2003). The potential of cross adsorption with cowpea plant extracts therefore needs to be evaluated.

An RT-PCR assay developed based on CABFFI/CABRR1 primers was able to detect CABMV in the infected cowpea plant samples that were tested. This primer set yielded the expected amplification of 1,642 bp (Figure 20). While reverse transcriptase PCR (RT-PCR) is a reliable method/assay for diagnosis and allows for further characterisation of cowpea viruses (Akinjogunla *et al.*, 2008), not all RT-PCR assays are efficient. An earlier assay developed by Gillaspie *et al.* (2001) failed to detect CABMV in positive samples in this study (Figure 21) as was observed by Amayo (2008). However, other studies using the same protocol by Gillaspie *et al.* (2001) reported its ability to give the expected fragment size (221 bp) (Byarugaba, 2008; Amayo *et al.*, 2012). However, these authors (Byarugaba, 2008; Amayo *et al.*, 2012) findings were not confirmed by sequencing and analysis of amplicons. Non-specificity of the RT-PCR assays could be attributed to nucleotide differences in sequences used to design diagnostic primers. The assay developed by Gillaspie *et al.* (2001) was based on a single Brazilian CABMV strain isolate from peanut. Its failure to detect CABMV in Ugandan samples can be attributed to differences in virus strains between Uganda and Brazil as well as due to host differences (peanut vs cowpea). In the present study, alignment of multiple sequences from different strains improved the specificity of the diagnostic primer (CABFFI/CABRR1) and therefore improved its efficiency for detection of CABMV in Uganda.

Molecular analysis of the CP fragments amplified using the CABFFI/CABRR1 clearly identified them as CABMV belonging to the family *Potyviridae* (Figure 22). However, sequence analysis of fragments from the assay developed by Gillaspie *et al.* (2001) did not give any hits related to CABMV and therefore unable to detect the virus among Ugandan samples. The criteria set by the International Committee for Taxonomy of Viruses (Adams *et al.*, 2011) and other taxonomic studies (Adams *et al.*, 2005) suggested a benchmark for species demarcation in the family *Potyviridae* as a nucleotide sequence identity of less than 76% either in the CP or over the whole genome. Using this criterion, the Ugandan virus isolates from cowpea belong to the same strain of CABMV (Table 3).

Pairwise sequence comparison and phylogenetic analysis showed that the Ugandan isolates belong to the same strain of CABMV given the highest identity percentage (89-94%) following the criteria described by Adams *et al.* (2005a). Phylogenetic affinity of isolates to their geographic origin (Figure 23) could be due to restricted geographical range (isolation by distance) as well as adaptation of isolates to the ecological conditions of the host as previously reported for *Rice yellow mottle virus* (RYMV) (Abubakar *et al.*, 2003; Trovao *et al.*, 2015). The new RT-PCR assay can be deployed in breeding programs to aid selection of virus resistant progenies, CABMV epidemiological studies and plant quarantine.

CHAPTER FIVE

LEVELS OF FIELD RESISTANCE TO NATURAL VIRUS INFESTATION AMONG COWPEA GENOTYPES

5.1 Introduction

Cowpea viruses are widespread in the main cowpea growing areas of Uganda (Orawu *et al.*, 2005; Orawu, 2007; Byarugaba, 2008; Amayo, 2008; Amayo *et al.*, 2012; Orawu *et al.*, 2015). Infections by viruses are associated with severe effects on growth, nutritional quality and yield (Neya *et al.*, 2015). Given the economic potential of these viruses on the livelihoods of many smallholder farmers, breeding efforts were initiated in early 2000 at the National Semi Arid Resources Research Institute (NaSARRI) to manage virus infections. These efforts involved screening of 54 cowpea lines from Uganda, Kenya, Nigeria and South Africa for yield potential and resistance to CABMV (Orawu, 2007) at a single location. Five promising genotypes were identified and later used to study genetics of resistance to CABMV using North Carolina II mating design (Orawu *et al.*, 2012). While five promising lines were identified, there is need to evaluate additional collections of germplasm and at different locations in the country to ensure selection of stable parents both in terms of yield and disease resistance (Oloka *et al.*, 2008; Goenaga *et al.*, 2011; Maphosa *et al.*, 2013).

Most of the viruses reported on cowpea in Uganda are spread through infected seed and several insect vectors in a non-persistent manner under field conditions and therefore use of insecticides is not an effective method of control (Lecoq and Katis, 2014). This is because insecticides generally do not act quickly to prevent the vector's short sucking time during which they transmit non-persistent viruses. Host resistance therefore remains the most effective and practical approach in reducing crop losses due to these diseases (Umaharan *et al.*, 1997). This study was conducted to assess the level of resistance among cowpea germplasm, identify sources of resistance with good yield potential for use in breeding programmes.

5.2 Materials and Methods

5.2.1 Description of study sites

Field trials were established at three locations; National Semi-Arid Resources Research Institute (NaSARRI) in Serere district (1°39'N and 33°27'E; 1038 m above sea level), Tororo District Agricultural Training Information Centre (Tororo-DATIC) in Tororo district (0°39'54"N and 34°11'41"E; 1,198m above sea level) and Budaka District Agricultural Training Information Centre (Budaka-DATIC) in Budaka district (1°06'03"N and 34°00'16"E; 1,160m above sea level). The trials were conducted in two seasons of 2012 herein referred as 2012A and 2012B. These locations are known to grow cowpeas extensively and have a high prevalence of cowpea viral diseases (Orawu *et al.*, 2005).

5.2.2 Plant materials

In this study, 105 cowpea genotypes comprising of; landraces (82), a commercial variety (1) and introductions (22) were used (Table 4). The landraces were collected from the northern and eastern regions (NE), western and central regions (WC) of Uganda. The introductions were obtained from the International Institute of Tropical Agriculture (IITA). The health status of seed for all genotypes used in the study was not determined. The commercial variety (SECOW2W) was used as a check and is an improved variety released by National Semi-Arid Resources Research Institute (NaSARRI), Serere.

5.2.3 Layout of experiment

A 10 x 11 alpha-lattice design with three replicates was used in the study. Each replication had 10 blocks with each block having 11 plots. The design was generated using the Gendex DOE Toolkit with alpha program available at <http://www.designcomputing.net/gendex/alpha/>. Each genotype was planted in two rows of four meters length. Row to row spacing was 60cm while plant to plant spacing (within rows) was 30cm. Spacing between plots, blocks was one meter while spacing between replications was two meters. Experimental plots were kept free of weeds using a hand hoe. No fertilizer or supplementary irrigation was applied during the growing periods. Post-flowering pests such as flower thrips (*Megalurothrips sjostedti* Trybom), pod borers (*Maruca vitrata* Fabricius) and pod sucking bugs were controlled by 3-4 sprays using Profenofos 40% + Cypermethrin 4% starting from the budding stage. There was no inoculation of cowpea genotypes with virus during the two seasons of evaluation.

Table 4: List and source of cowpea genotypes used in the study

Code	Genotype	Source	Code	Genotype	Source	Code	Genotype	Source
G1	EBELAT	NE	G36	NE23	NE	G71	WC18	WC
G2	IT00K-835-45	IITA	G37	NE30	NE	G72	WC2	WC
G3	IT03K-124	IITA	G38	NE31	NE	G73	WC21	WC
G4	IT04K-219-2	IITA	G39	NE32	NE	G74	WC26	WC
G5	IT04K-221-1	IITA	G40	NE36	NE	G75	WC27	WC
G6	IT04K-227-4	IITA	G41	NE37	NE	G76	WC29	WC
G7	IT06K-121	IITA	G42	NE39	NE	G77	WC30	WC
G8	IT06K-123-1	IITA	G43	NE4	NE	G78	WC32	WC
G9	IT06K-124	IITA	G44	NE40	NE	G79	WC33	WC
G10	IT06K-147-1	IITA	G45	NE41	NE	G80	WC35A	WC
G11	IT06K-154-1	IITA	G46	NE42	NE	G81	WC35B	WC
G12	IT06K-281-1	IITA	G47	NE43	NE	G82	WC35C	WC
G13	IT06K-91-11-1	IITA	G48	NE44	NE	G83	WC36	WC
G14	IT07K-187-24	IITA	G49	NE45	NE	G84	WC39	WC
G15	IT07K-188-49	IITA	G50	NE46	NE	G85	WC4	WC
G16	IT07K-211-1-8	IITA	G51	NE48	NE	G86	WC41	WC
G17	IT07K-243-1-5	IITA	G52	NE49	NE	G87	WC42	WC
G18	IT07K-292-10	IITA	G53	NE5	NE	G88	WC44	WC
G19	IT07K-299-4	IITA	G54	NE50	NE	G89	WC48	WC
G20	IT07K-300-12	IITA	G55	NE51	NE	G90	WC5	WC
G21	IT89KD-288	IITA	G56	NE53	NE	G91	WC51	WC
G22	IT97K-499-35	IITA	G57	NE55	NE	G92	WC52	WC
G23	IT98K-503-1	IITA	G58	NE6	NE	G93	WC53	WC
G24	MU09B	MAK	G59	NE67	NE	G94	WC55	WC
G25	MU15	MAK	G60	NE70	NE	G95	WC6	WC
G26	MU17	MAK	G61	NE71	NE	G96	WC62	WC
G27	MU19	MAK	G62	SECOW2W	E	G97	WC63	WC
G28	MU20	MAK	G63	WC1	WC	G98	WC64	WC
G29	MU20B	MAK	G64	WC10	WC	G99	WC65	WC
G30	MU9	MAK	G65	WC11	WC	G100	WC66	WC
G31	NE13	NE	G66	WC12	WC	G101	WC67	WC
G32	NE15	NE	G67	WC13	WC	G102	WC67A	WC
G33	NE17	NE	G68	WC15	WC	G103	WC68	WC
G34	NE18	NE	G69	WC16	WC	G104	WC69	WC
G35	NE19	NE	G70	WC17	WC	G105	WC7	WC

NE = Northern and Eastern, Uganda; WC = Western and Central, Uganda; MAK = Makerere University, Uganda; IITA = International Institute of Tropical Agriculture, Nigeria.

5.2.4 Data collection

At each trial site, virus disease data (incidence and severity) were collected starting from three weeks after planting (WAP) at an interval of 14 days until the appearance of the first ripe

Pods. To record viral disease incidence, all plants per plot were assessed while severity was recorded on ten randomly selected plants in each plot. Disease severity assessment was carried out using a 1-5 scale as described by Gumedzoe *et al.* (1997) where; 1 = no symptoms on all leaves, 2 = slight symptoms (1 to 25% of the leaves infected), 3 = moderate symptoms (26 to 50 % leaves infected), 4 = prominent symptoms with stunting (51 to 75% of leaves infected), 5 = highly severe symptoms with stunting (> 75% of leaves infected). Data on agronomic traits such as days to first flowering, days to 50% flowering (mid-bloom) and days to physiological maturity were collected as the number of days between planting date and date of appearance of first flower, date at 50% flowering and date at physiological maturity, respectively. At maturity, pods from all plants in each plot were harvested, sun-dried, and threshed, and weighed to obtain plot seed yield. Plot seed yield was measured in grams and yield per hectare determined by extrapolation.

5.2.5 Data analysis

Data on symptom severity was used to compute area under disease progress curve (AUDPC) as described by Campbell and Madden (1990):

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left(\frac{Y_i + Y_{i+1}}{2} \right) (t_{i+1} - t_i)$$

Where; n = number of readings, Y_i = symptom severity at date i , t_i = number of days after the first observation on assessment date i . Data was subjected to analysis of variance (ANOVA) using the restricted maximum likelihood model (REML) in R Software Version 3.1.0 (R Core Team, 2014). Mean AUDPC and incidence per genotype were computed per location, season and per genotype. Correlation analysis among traits was carried out using Pearson's correlation coefficient (r). AUDPC, incidence and yield data were also visualized using genotype and genotype by environment (GGE) biplots as suggested by Yan *et al.* (2000); Yan (2005). GGE biplot analysis was carried out using "GGEBiplotGUI" package (Frutos *et al.*, 2014). GGE biplot analysis was based on an environment centred model/equation (Yan, 2005) expressed as;

$$P_{ij} = Y_{ij} - \mu - \beta_j = \alpha_i + \phi_{ij}$$

Where;

Y_{ij} is the value of genotype i in environment j , P_{ij} is the genotype by environment two-way table, μ is the grand mean, α_i is the genotype (row) main effect, β_j is the environment (column) main effect, ϕ_{ij} is the specific genotype by environment interaction. Genotypes with high AUDPC and/or incidence values were considered susceptible while those with low values were considered resistant or tolerant.

5.3 Results

5.3.1 Viral symptom expression among genotypes

Several symptoms were observed on cowpea genotypes at all the locations during the two seasons of evaluation. The observed symptoms included; vein banding, leaf curling, leaf distortion, mottling, mosaics, necrotic spots and purpling (Figure 24). In some instances, infected plants exhibited more than one form of symptom expression.



Figure 24: Virus symptoms on infected cowpea leaves: (A) Vein banding and leaf curling, (B) vein banding and leaf distortion, (C) leaf mottling, (D) mild mosaic, (E) intense mosaic, (F) severe mosaic, (G) Mosaic and necrotic spots, (H) Necrotic spots and (I) purpling.

5.3.2 Variation among cowpea genotypes for AUDPC, incidence and other traits

Results of analysis of variance for area under disease progress curve (AUDPC), incidence, days to first flower (first flower), days to 50% flowering (mid-bloom), days to maturity (maturity) and yield are presented in Table 5. Genotypes, locations and seasons significantly ($P<0.001$) influenced area under disease progress curve, virus incidence, yield and maturity traits. Interactions between genotypes and locations, genotype and season, location and season, genotype by site by season had significant effects ($P<0.01$) on AUDPC, incidence, first flower, mid-bloom and maturity. However, genotype by site by season interactions did not have a significant influence on incidence ($P>0.05$). The greatest variation in disease progress was due to the effect of season (63.6%) followed by genotypic effects at 12.5%. For virus incidence, season effects contributed to the greatest variation of virus incidence at 28.5% followed by genotypic effects at 24.5% (Table 5). The highly significant effects of interactions indicate inconsistent response/performance of cowpea genotypes in the three locations.

For the case of days to 75% maturity, season, genotype, genotype by site, site, site by season interactions had significant contribution to explained variation of 9.15, 9.29, 9.33, 17.17 and 31.52%, respectively at $P<0.001$. Number of days to 50% flowering over the two seasons ranged from 45 days to 73 days in Budaka, 39 days to 63 days in Serere and 42 days to 67 days in Tororo. The average number of days to 50% flowering in 2012A was 57, 44 and 53 days for Budaka, Serere and Tororo, respectively. In 2012B, the average number of days to 50% flowering was 52, 54 and 52 days in Budaka, Serere and Tororo, respectively. Number of days to maturity across the two seasons ranged from 69 days to 101 days in Budaka, 71 days to 88 days in Serere and 67 days to 99 days in Tororo. Average number of days to maturity in 2012A was 87 days in Budaka, 81 days in Serere and 85 days in Tororo while in 2012B, the average number of days to maturity was 74, 81 and 89 days in Budaka, Serere and Tororo, respectively.

Table 5: Mean squares for area under disease progress curve, incidence, yield and other agronomic traits

Source of variation	Df	AUDPC	Incidence	Yield	First flower	Mid-bloom	Maturity
Blocks	9	1214.99***	551.13***	451420.92***	52.46***	61.487***	40.15***
Replicates	2	251.72 ^{ns}	352.75 ^{ns}	410516.43*	30.85*	74.96***	10.71 ^{ns}
Genotypes	104	1488.17***	2557.32***	733000.37***	83.10***	91.84***	78.32***
Sites	2	18452.16***	40743.99***	53031087.44***	3637.20***	4846.16***	7530.83***
Seasons	1	788616.43***	309086.11***	15413989.41***	1590.32***	482.30***	8025.73***
Genotype x site	208	217.25***	201.67*	224969.52***	14.36***	20.69***	39.36***
Genotype x season	104	350.18***	413.83***	278436.75***	12.05***	15.35***	31.66***
Site x season	2	7525.95***	46850.17***	106948584.78***	6463.67***	8492.84***	13821.15***
Genotype x site x season	208	134.161**	183.24 ^{ns}	286842.12***	10.23***	11.14***	20.23***
Residuals	1228	101.36	168.01	131916.87	6.88	6.77	10.62

***, **, * = significant at $P < 0.001$, 0.01 and 0.05, respectively; and ns = non-significant at 0.05
Df = degrees of freedom

5.3.2 Correlation matrix for AUDPC, incidence and grain yield

The results of correlation analysis for AUDPC, incidence and grain yield are presented in Table 6. AUDPC had a significant ($P<0.05$) positive correlation with incidence ($r = 0.75$). This suggests that an increase in incidence is associated with increase in severity. AUDPC and incidence were negatively correlated with yield ($r = -0.08$ and $r = -0.03$), respectively although the correlation between incidence and yield was not significant ($P<0.05$). Correlation analysis results therefore show that an increase in AUDPC and incidence is associated with reduction in grain yield.

Table 6: Correlation of AUDPC, incidence and yield

Traits	AUDPC	Incidence	Yield
AUDPC	1		
Incidence	0.75	1	
Yield	-0.08	-0.03 ^{ns}	1

^{ns} means non-significant at $P<0.05$

5.3.3 Response to virus infection and yield potential

Average values for AUDPC, incidence and yield for the cowpea genotypes evaluated in Budaka, Serere and Tororo are presented in Table 7 and Appendix 1. For the two seasons, AUDPC was highest in Budaka and lowest in Tororo trial site. In 2012A, lower AUDPC was recorded than in the second season (2012B). Overall, mean AUDPC was lower in 2012A than in 2012B with Budaka recording the highest values followed by Tororo and Serere in 2012A. In 2012B, Budaka had the highest viral severity level followed by Serere and Tororo trial sites, respectively. Based on genotype source, introduced cowpea genotypes had higher AUDPC than local genotypes. Among the landraces, genotypes that were collected from the western and central regions of the country had lower viral infection levels. Average AUDPC across the three locations over the two seasons ranged from 39.4 for WC48 to 94.9 for NE46. Genotypes WC48, SECOW2W, NE15, WC18, WC33, WC53, WC35A, MU19, WC39, WC35C and NE32 had lower AUDPC (38.25, 41.79, 42.68, 42.74, 42.91, 43.83, 44.26, 44.28, 44.50, 44.53 and 44.57, respectively) while NE46, IT06K-91-11-1, IT07K-188-49, IT06K-121, MU20, IT07K-292-10, IT06K-123-1, IT07K-187-24 and IT06K-123-1 had the highest AUDPC values of 94.72, 85.49, 76.23, 75.59, 73.86, 69.98, 69.09, 68.34 and 67.44,

respectively in the three trial sites (Appendix 1). The results showed that none of the genotypes evaluated was immune to viral infection.

Virus incidences among cowpea genotypes were equally highest in Budaka and lowest in Tororo over the two seasons (Table 7). Genotypes with the lowest average incidence across the three locations were; WC48, WC35A, WC18, WC35C, WC33, WC39, MU19, WC26, SECOW2W, NE32 with incidence values 22.21%, 23.39%, 23.52%, 24.25%, 25.54%, 25.64%, 25.80%, 26.95%, 27.27% and 27.62%, respectively (Appendix 1). Genotypes with the highest average incidence across the three locations were NE46, IT06K-91-11-1, IT06K-121, MU20, IT07K-300-12, IT06K-147-1, IT07K-188-49, IT07K-187-24, IT97K-499-35, IT06K-123-1 with 71.86%, 71.14%, 69.82%, 69.32%, 65.35%, 65.02%, 64.19%, 64.11%, 62.79% and 62.12%, respectively.

Trials conducted in 2012A had lower disease incidence than those conducted in 2012B. On the basis of source of germplasm, introduced genotypes had higher virus incidence than the local genotypes. Genotypes from the western and central regions (WC) of Uganda had lower virus incidence levels among the local genotypes.

Yield over the two seasons ranged from 25 kg/ha to 3,000 kg/ha in Budaka, 8.3 kg/ha to 2,650 kg/ha in Serere and 25 kg/ha to 2,708 kg/ha in Tororo. In Budaka, higher average yields were recorded in 2012A (1,529 kg/ha) compared to 523 kg/ha obtained in 2012B. In Serere, there were higher yields in 2012B (899 kg/ha) compared to 187 kg/ha in 2012A while in Tororo, higher yields were obtained in 2012A (566 kg/ha) compared to 345 kg/ha in 2012B (Table 7). Across locations, genotypes such as WC51, NE48, NE37, MU17, NE51 and WC32, were consistently high yielding across the two seasons with 1,388.97 kg ha⁻¹, 1,191.43 kg ha⁻¹, 1,156.48 kg ha⁻¹, 1,119.67 kg ha⁻¹, 1,106.37 kg ha⁻¹ and 1,082.87 kg ha⁻¹, respectively while the lowest yielding genotypes were MU20B, IT07K-299-4, WC5, IT97K-499-35 and WC11 with 357.6 kg ha⁻¹, 363.8 kg ha⁻¹, 365.97 kg ha⁻¹, 370.34 kg ha⁻¹ and 385.87 kg ha⁻¹, respectively. Genotypes NE23, NE40 and NE15 yielded highly in 2012A while IT04K-227-4 and IT06K-91-11-1 were high yielders in 2012B. A summary of average yield values for each genotype in each location is presented in Appendix 1.

Table 7: Mean values of AUDPC, incidence and yield across three locations

Location	Season	AUDPC	Incidence (%)	Yield (kg/ha)
Budaka	12A	34.75	27.96	1,529.31
	12B	79.48	62.57	522.69
Serere	12A	31.11	25.42	187.28
	12B	78.22	64.18	899.38
Tororo	12A	31.56	28.51	565.96
	12B	64.98	34.88	344.83
LSD		12.82	15.13	61.97
CV (%)		18.83	31.84	54.18

5.3.4 Biplot analysis of disease indexes and yield data

Biplot analysis was used to explain the responsiveness of the evaluated cowpea genotypes to virus infection. The biplots were also used to identify the best and worst yielding genotypes at the three locations and group the locations into putative mega-environment(s) in relation to virus infection and yield. The results of biplot analysis are presented in Figures 25-30.

5.3.4.1 Biplot analysis based on AUDPC

Genotype and genotype by environment biplot analysis of AUDPC data explained 93.06% of the variation in the data (Figure 25). The first principal component (PC1/Axis1) accounted for 81.15%; while the second principal component (PC2/Axis2) accounted for 11.19%. The “which-won-where” feature of the GGEbiplotGUI package generated a biplot (Figure 25) with seven sectors with the most responsive (most susceptible) genotypes for each sector located at the respective vertex. The vertex genotypes were the most responsive since they had the longest distance from the biplot origin. From Figure 25, the three locations (Serere, Tororo and Budaka) formed a single mega-environment with regard to AUDPC. In this mega-environment, the most responsive (most susceptible) genotypes were G50 (NE46), G13 (IT06K-91-11-1), G15 (IT07K-188-49), G14 (IT07K-187-24), G11 (IT06K-154-1), G6 (IT04K-227-4), G19 (IT07K-299-4 and G22 (IT97K-499-35) while the most resistant were G89 (WC48), G47 (NE43), G79 (WC33) and G71 (WC18).

Ranking of genotypes based on AUDPC data using a concentric circle biplot (Blanche *et al.*, 2007) placed the most susceptible genotypes (with high AUDPC values) at or close to the centre of concentric circles (Figure 26) along the average tester axis (ATA). Accordingly, the most susceptible genotypes were G50 (NE46), G13 (IT06K-91-11-1), G15 (IT07K-188-49), G7 (IT06K-121), G28 (MU20), G10 (IT06K-147-1), G8 (IT06K-123-1), G18 (IT07K-292-10), G14 (IT07K-187-24), G20 (IT07K-300-12), G21 (IT89KD-288), G11 (IT06K-154-1), G6 (IT04K-227-4), G3 (IT03K-124) and G9 (IT06K-124) while the least susceptible genotypes included G89 (WC48), G47 (NE43), G32 (NE15), G80 (WC35A), G84 (WC39), G79 (WC33), G82 (WC35C) and G71 (WC18).

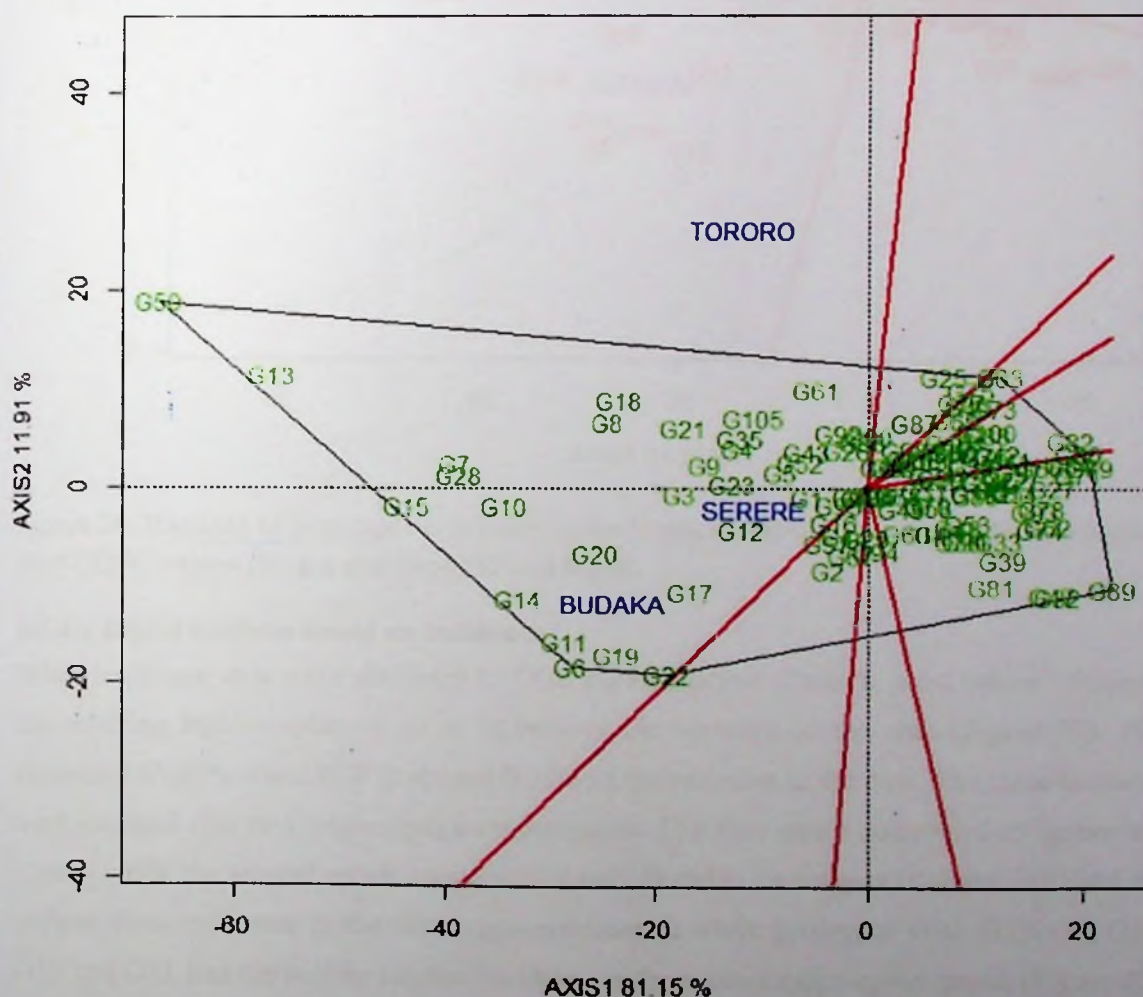


Figure 25: Mega-environment and most responsive genotypes based on AUDPC among cowpea genotypes across three locations in Uganda using a genotype focused biplot.

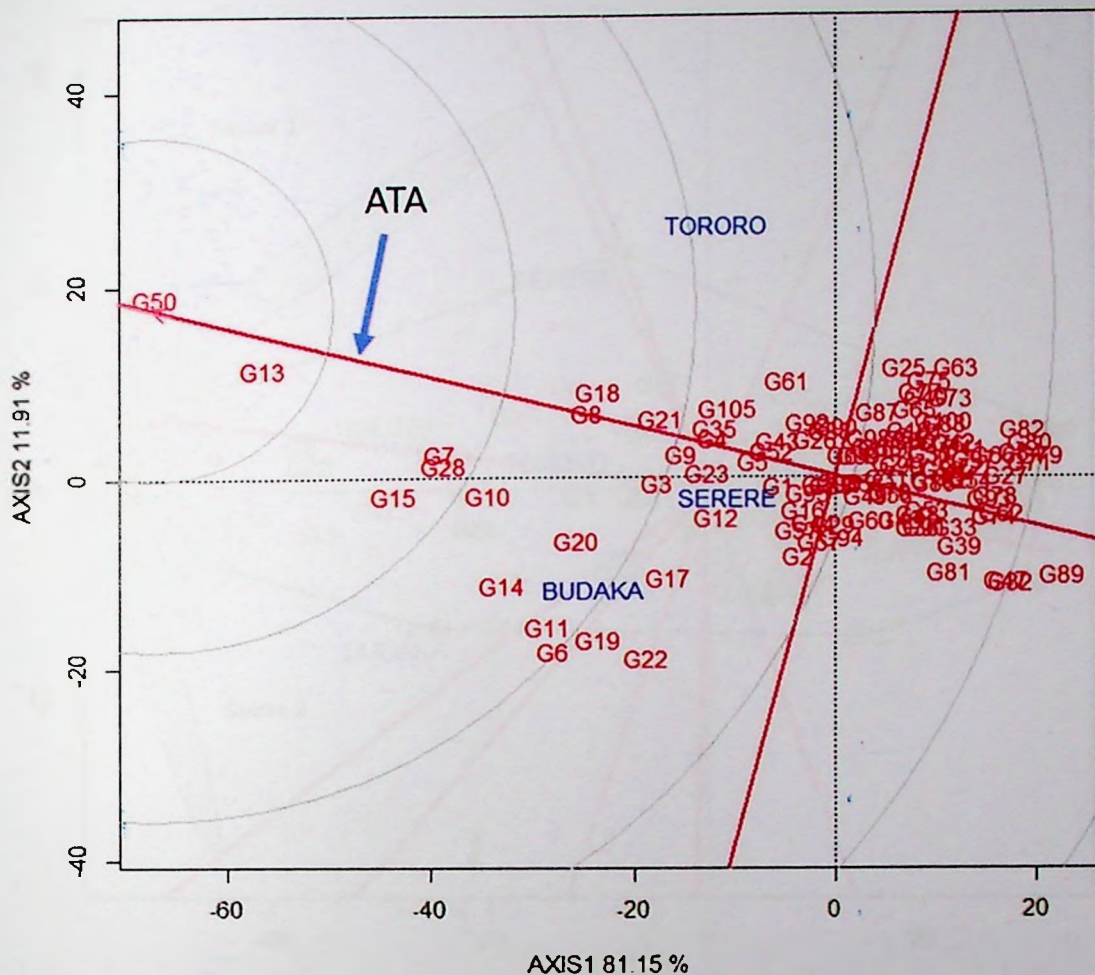


Figure 26: Ranking of genotypes in relation to the “most susceptible” to viral infection based on AUDPC values using a genotype focused biplot.

5.3.4.2 Biplot analysis based on incidence

When incidence data were subjected to GGE biplot analysis (“which. won. where” feature), the resulting biplot explained up to 93.96% of the variation in the data (Figure 27). PC1 explained 86.58% while PC2 explained 7.38% of the variation in the data. The three locations were grouped into two sectors/mega-environments. The first sector comprised of Serere and Tororo while the second sector comprised of only Budaka. Genotypes G20 and G30 had the highest virus incidence in the first mega-environment while genotypes G50, G13, G7, G10, G18 and G11 had the highest disease incidence in the second mega-environment (Figure 27). On the other hand, genotypes G80, G71, G79, G82, G72, G84, G27 and G89 had the lowest virus incidence levels.

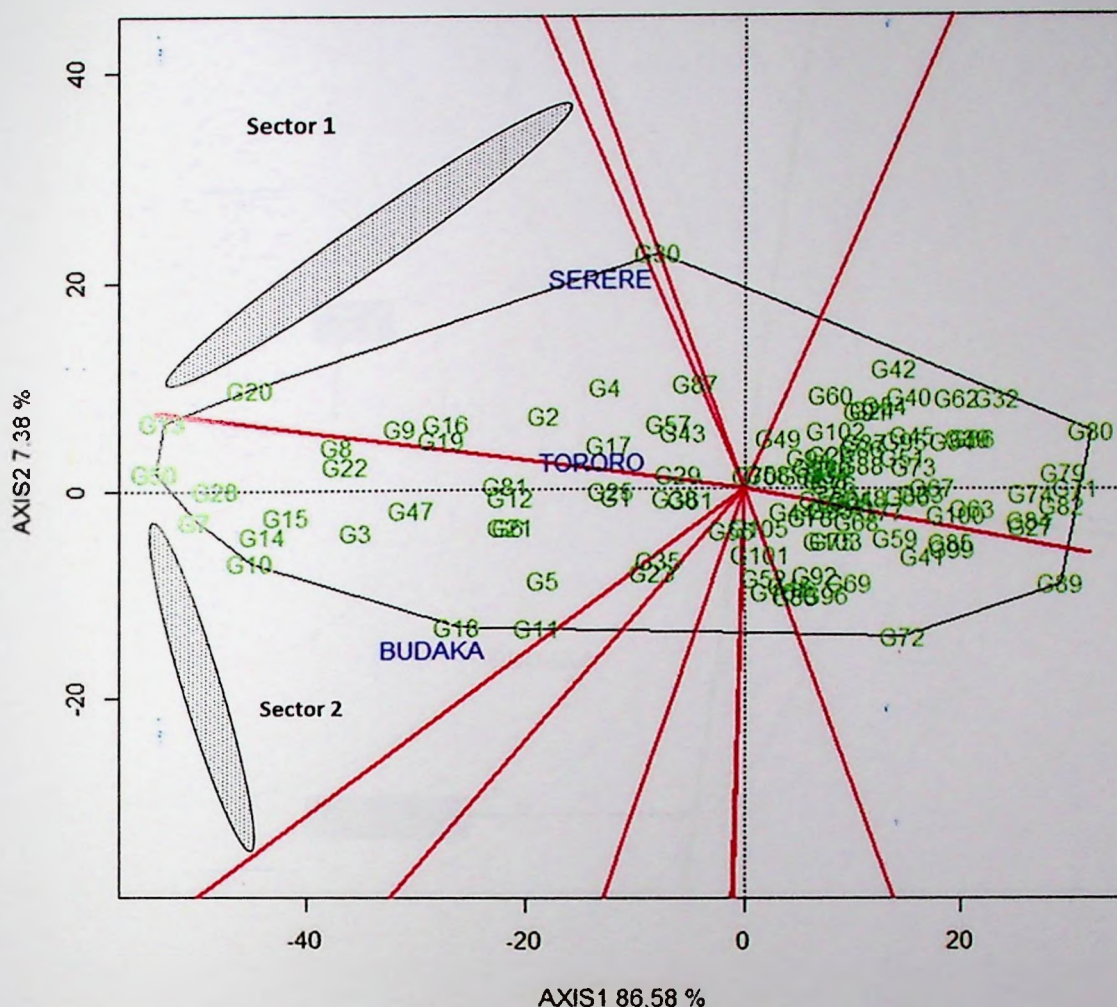


Figure 27: Mega-environments and most responsive genotypes for virus incidence in cowpea genotypes across locations in Uganda using a genotype focused biplot.

Genotype ranking on basis of virus incidence using a concentric circle biplot placed genotypes with high virus incidence closer to the centre of concentric circles (Figure 28) along the ATA while those with low incidence levels were placed further away from the centre. Genotype G13 (IT06K-91-11-1) had the highest virus incidence (at the centre of the smallest concentric circle) followed by G50 (NE46), G20 (IT07K-300-12), G28 (G28 (MU20) and G7 (IT06K-121). On the other hand, G89 (WC48), G82 (WC35C), G71 (WC18), G79 (WC33) and G74 (WC26) further to the right on the ATA and below the stability axis had the lowest incidence.

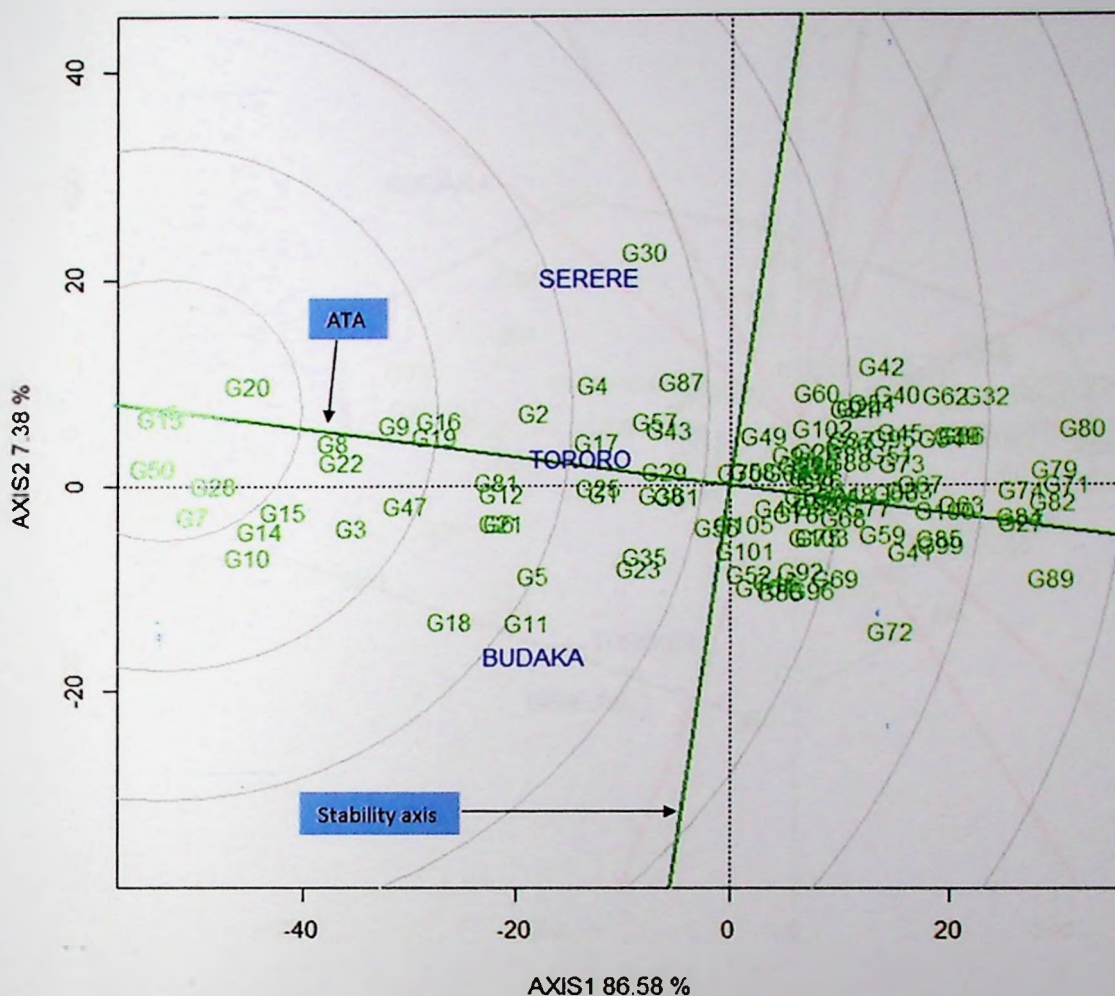


Figure 28: Ranking of cowpea genotypes based on virus incidence using a genotype focused concentric circle biplot.

5.3.4.3 Biplot analysis based on yield

Analysis of yield data using GGE biplot revealed that the two axis1 (PC1) and axis2 (PC2) explained 85.57% of the total variation (67.84% for PC1 and 17.73% for PC2) (Figure 29). The three locations (Budaka, Serere and Tororo) formed a single mega-environment with genotypes; G91 (WC51), G41 (NE37), G51 (NE48) and G54 (NE50) genotypes as winning genotypes. The average yield for G91 (WC51), G41 (NE37), G51 (NE48) and G54 (NE50) were; 1,384.7, 1,156.5, 1,191.4, and 976.2 kg ha⁻¹, respectively.

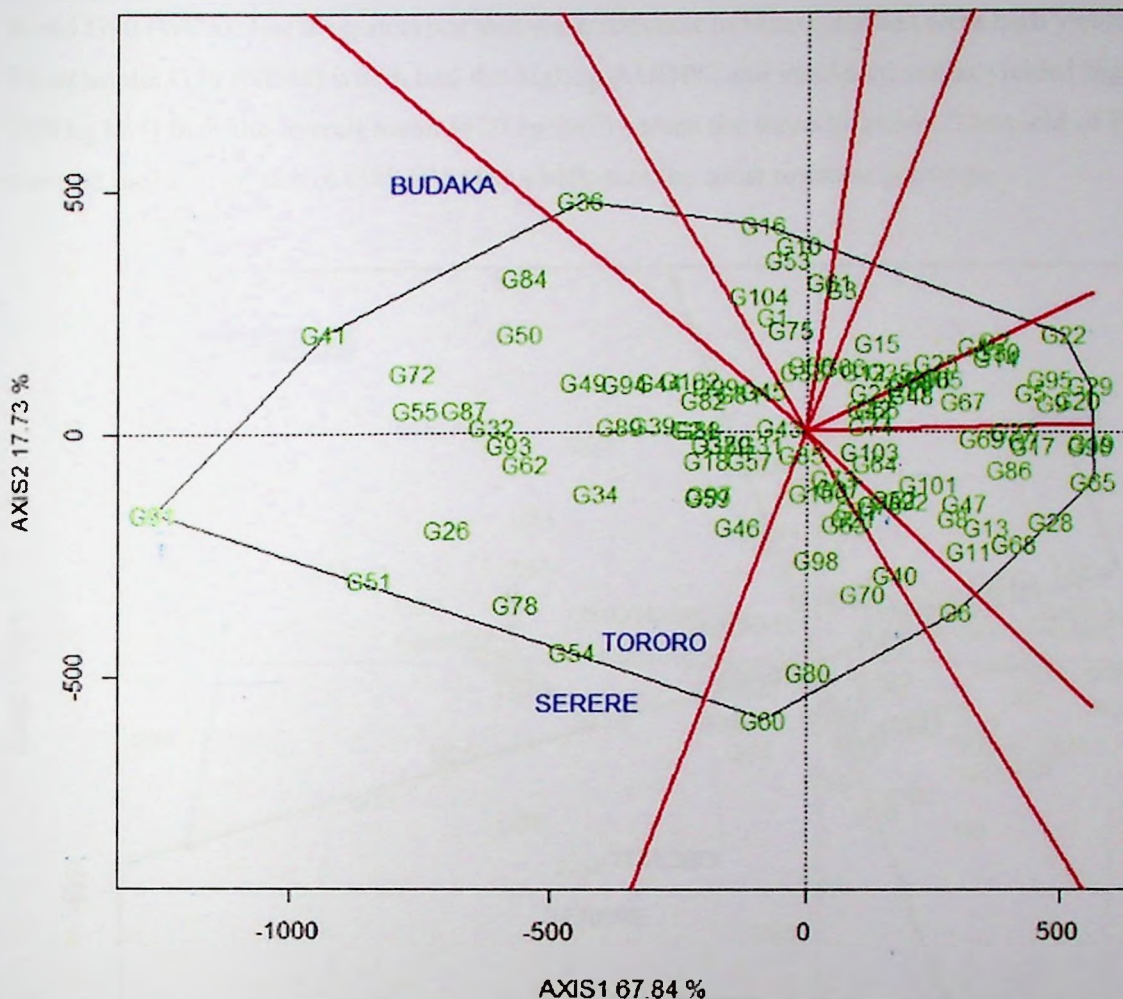


Figure 29: Biplot view of mega-environments and winning genotypes for grain yield using a genotype focused biplot.

Using a concentric circle biplot, G91 (WC51), G51 (NE48), G26 (MU17), G78 (WC32), G55 (NE51), G41 (NE37), G72 (WC2), G87 (WC42), G32 (NE15), G93 (WC53), G62 (SECOW2W) and G54 (NE50) were identified as the most desirable genotypes combining both high yield and stability across the three locations given their closeness to the “ideal genotype” (Figure 30). An “ideal” genotype (centre of the innermost concentric ring/circle) is a point on the average tester axis (ATA) (“absolutely stable”) in the positive direction and has a vector length equal to the longest vectors of the genotypes on the positive side of ATA (“highest mean performance”). The poorest genotypes in terms of yield were G22 (IT97K-499-35), G29 (MU20B), G20 (IT07K-300-12), G95 (WC6), G65 (WC11), G19 (IT07K-299-

4) and G90 (WC5). Not all genotypes that were resistant to virus infection were high yielding. For example, G50 (NE46) which had the highest AUDPC and incidence values yielded higher (929 kg ha⁻¹) than the overall mean (670 kg ha⁻¹) across the three locations. The yield of G50 was also higher than that of G89 (WC48) which was the most resistant genotype.

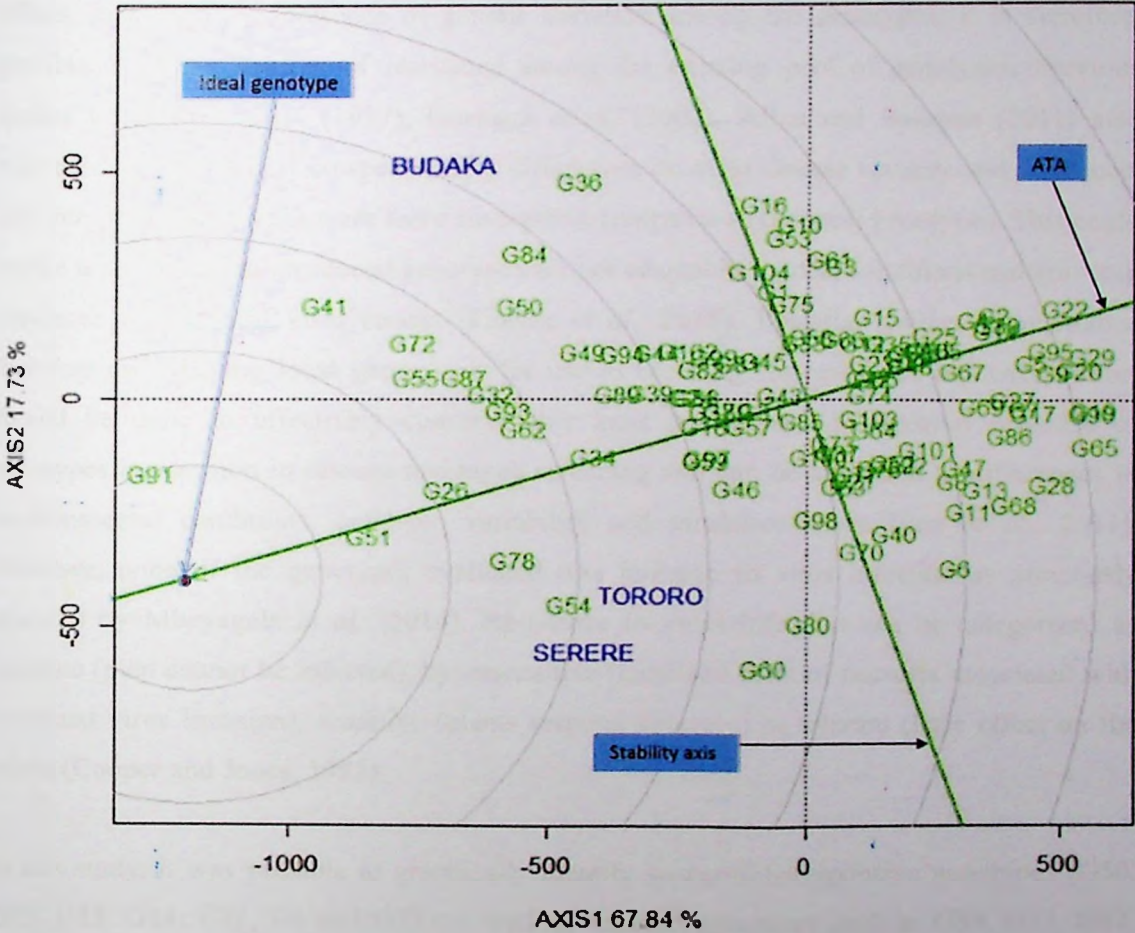


Figure 30: Ranking of cowpea genotypes relative to the ideal genotype for grain yield using a genotype focused concentric circle biplot.

5.4 Discussion

Seasonal differences had the greatest effect on AUDPC and incidence with lower virus incidence and AUDPC in the first (wetter) season compared to the second (drier) season. This suggests that virus infection and spread among cowpea genotypes was favoured by low

moisture conditions. Dry or low moisture conditions are associated with high virus vector populations and mobility and hence higher infestations compared to wet seasons as previously reported by Taiwo *et al.* (2006) and Kone *et al.* (2017a,b). However, earlier reports suggested that virus infection was higher under wet conditions (Edema *et al.*, 1997; Adipala *et al.*, 1999). Genotypic effects were the second contributor to the total explained variation after season effects, indicating the presence of genetic variation among the genotypes. It is therefore, possible to obtain sources of resistance among the existing pool of genotypes. Previous studies by Edema *et al.* (1997), Goenaga *et al.* (2008), Aliyu and Balogun (2011) also reported the influence of cowpea variety differences on viral disease severity and incidence. The introduced genotypes were more susceptible compared to the local genotypes. This could be due to the fact that introduced genotypes are not adaptable to local conditions and also lack resistance to the local virus strains (Fioreze *et al.*, 2018). Potential sources of resistance therefore exist among local germplasm for use in breeding for resistance and every effort should be done to effectively conserve this local germplasm. Differential response of genotypes is common in disease resistance screening and can be attributed to differences in environmental conditions, pathogen variability and virulence (Gremillion *et al.*, 2011). However, none of the genotypes evaluated was immune to virus infection as previously reported by Mbeyagala *et al.* (2014). Resistance to virus infection can be categorized as immune (plant cannot be infected), hypersensitive (localized form of necrosis associated with restricted virus invasion), sensitive (plants respond severely) or tolerant (little effect on the plant) (Cooper and Jones, 1983).

In this study, it was possible to graphically identify susceptible/responsive genotypes (G50, G13, G15, G14, G11, G6 and G22) as well as resistant genotypes such as G89, G32, G47, G79, G80, G82, G71, G84 and G62. The discriminative power of GGE biplots has been reported previously for other crops by several authors such as in soybean (Yan and Rajcan, 2002), wheat (Yan and Tinker, 2006) and cotton (Blanche *et al.*, 2007). Further screening of identified resistant genotypes under high viral load/inoculum is needed to ascertain their resistance.

Genotype, locations and location by season interactions had the greatest contribution to variation in yield and maturity indices. The observed variability in this study allows for selection of suitable genotypes for grain yield and maturity period as previously reported

(Hawkes, 1991). Based on the median values for 75% maturity, most of the cowpea genotypes in this pool are largely medium maturing. The range of yield values indicated that both low to potentially high yielding genotypes exist in this collection. These findings are in agreement with Agbahoungba *et al.* (2017) suggesting that it is possible to select genotypes with high yield from the assembled germplasm pool.

With regard to AUDPC, the three locations (Serere, Tororo and Budaka) formed a single mega-environment (Figure 25) in which the most responsive (most susceptible) genotypes were G50 (NE46), G13 (IT06K-91-11-1), G15 (IT07K-188-49), G14 (IT07K-187-24), G11 (IT06K-154-1), G6 (IT04K-227-4), G19 (IT07K-299-4 and G22 (IT97K-499-35) while the most resistant were G89 (WC48), G47 (NE43), G79 (WC33), G32 (NE15), G80 (WC35A), G82 (WC35C), G84 (WC39) and G71 (WC18). The three locations formed two mega-environments with regard to incidence one comprised of Serere and Tororo while the second one comprised of Budaka (Figure 27). Genotypes G20 and G30 had the highest virus incidence in the first mega-environment while genotypes G50, G13, G7, G10, G18 and G11 had the highest disease incidence in the second mega-environment. In terms of yield, the three locations formed a single mega-environment with G91, G41, G51, G26 and G54 as the high yielding and stable genotypes (Figure 29). Ceccarelli (2012) suggested that good genotypes should have the best combination of high yield and high stability. Partitioning of test locations into mega-environments and deploying different genotypes for different mega-environments is the best way to exploit positive genotype x environment interactions while avoiding negative ones (Yan and Tinker, 2006). However, data from further multiple seasons is required to confirm if the observed mega-environment pattern is repeatable (Yan and Tinker, 2005, 2006).

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General discussion

Viral diseases of cowpea are still a threat to the productivity and nutritional value of cowpea grain in Uganda putting the livelihoods of many smallholder farmers at risk. Different disease management approaches such as early planting and intercropping are still ineffective while reduction of virus vectors through chemical sprays is also associated with reduced efficiency since most viruses are transmitted in a non-persistent manner. Moreover, chemicals destroy natural enemies of vectors and have adverse environmental and human health risks. Additionally, chemical sprays are costly especially for the smallholder resource poor farmers who are the main producers of cowpea in the country. Use of resistant varieties therefore remains the most effective, cheap and reliable approach to manage viral diseases. To develop virus resistant varieties, breeders need a better understanding of the viruses present in the country; have access to reliable virus diagnostic assays for accurate detection; screening to identify sources of resistance genes for incorporation in susceptible backgrounds. These were therefore the main focus of the studies conducted.

The first study (Chapter three) deployed next-generation sequencing (NGS) to identify viruses infecting cowpea in Uganda. Results from this study identified four viruses, CABMV, PeMoV, SCMV and MCMV. With the exception of CABMV, this is the first report of PeMoV, SCMV and MCMV on cowpea in Uganda. Identification of SCMV and MCMV is also the first report of these two viruses infecting a legume crop. This shows that cowpea and/or other legumes are likely alternative hosts for SCMV and MCMV. Identification of only four viruses by NGS yet several viruses were reported in previous studies using methods such as ELISA could be due to absence of other viruses in the samples analysed by NGS. False positives due to cross-reaction in ELISA may have also contributed to erroneous reporting/identification of many viruses in previous studies. This study also generated the first complete genome of CABMV from a Ugandan isolate (Serere1). Serere1 clustered separately from other isolates including CABMV-Z from Zimbabwe. The genome of Serere1 lacked two conserved motifs ESKKL and LVYV in the P1 and CI regions, respectively. This may likely affect the virulence of the virus. It was also observed that Serere1 had a DAG motif in the CI

in addition to another present in the CP region yet CI is not known to play any role in virus transmission by aphids aided by DAG motifs. This finding may have an impact on insect transmission of Ugandan CABMV virus isolates. Also, variability in SerereI was greatest in the 5'UTR and P1 regions yet in potyviruses, the greatest variability is found in P1 and P3 regions.

The second study (Chapter four) developed and tested a new RT-PCR assay for diagnosis of CABMV, one of the four viruses identified in Chapter three. CABMV causes the greatest economic loss on cowpea. The new assay with CABFFI: 5'-GGT AAC AAY AGT GGR CAA CC-3'/CABRRI: 5'-CTG AGC ACT CMA ACC GGG-3 successfully detected CABMV. Analysis of Ugandan CABMV isolates amplified with CABFFI/CABRRI showed that they were closely related (89.3%-94.3% nucleotide identity). Phylogenetic analysis of Ugandan isolates with others from different countries revealed affinity of isolates to their geographic origin. Therefore, ecology of the host plays a key role in the variability of the virus. The new assay is recommended in breeding programs for rapid screening of segregating progenies at an early stage, quarantine approaches and in studying the epidemiology of CABMV.

Developing virus resistant cowpea cultivars depends on identification of good sources of resistance to serve as resistance donors. Thus, in order to identify cowpea genotypes with broad resistance, 105 genotypes were screened in study three (Chapter five) under natural conditions in Serere, Tororo and Budaka. Results from this study showed variability among the genotypes with landraces exhibiting reduced virus infection than introduced materials. Genotypes WC48, NE43, NE15, WC35A, WC39, WC33, WC35C and WC18 had low AUDPC. This suggests that these genotypes can be used as donors of resistance genes to develop resistant varieties. The three study locations formed a single mega-environment with regard to yield and AUDPC, thus a genotype that consistently performs better in one location is likely to show the same performance in any of the other locations. Several genotypes with the best combination of high yield and yield stability were identified, these included; WC51, NE48, MU20, WC32, NE51, NE37, WC2, WC42, NE15, WC53, SECOW2W and NE50. These genotypes can be promoted for cultivation by farmers.

6.2 General conclusions

Using next-generation sequencing (NGS) approach, four viruses; CABMV, PeMoV, SCMV and MCMV were identified. With the exception of CABMV, the other three viruses had never been reported to infect cowpea in Uganda. This is also the first report of the occurrence or ability of SCMV and MCMV to infect a leguminous crop like cowpea. NGS also led to the assembly of the first complete genome for a Ugandan isolate of CABMV. The Ugandan isolates of CABMV clustered separately from other African isolates. Ugandan CABMV isolates also had a high nucleotide percentage identity (89.3-94.3%). A new RT-PCR assay based on CABFF1(5'-GGT AAC AAY AGT GGR CAA CC-3')/CABRR1(5'-CTG AGC ACT CMA ACC GGG-3') was developed for detection of CABMV. Genotypes WC48, NE43, NE15, WC35A, WC39, WC33, WC35C and WC18 showed broad resistance to virus infestation under natural condition. Serere, Budaka and Tororo formed a single mega-environment with regard to yield and AUDPC. However, with regard to incidence, two mega-environments were formed, the first mega-environment comprised of Serere and Tororo while the second one comprised of Budaka. WC51, NE48, MU20, WC32, NE51, NE37, WC2, WC42, NE15, WC53, SECOW2W and NE50 were high yielding and stable.

6.3 Recommendations

In this study, four viruses (CABMV, PeMoV, SCMV, MCMV) were identified in cowpea. Further sequencing of additional virus isolates from symptomatic plants collected from the main cowpea growing areas in the country is needed for a better understanding of their diversity and geographical distribution. The optimized RT-PCR assay [CABFF1(5'-GGT AAC AAY AGT GGR CAA CC-3')/CABRR1(5'-CTG AGC ACT CMA ACC GGG-3')] for CABMV has potential downstream applications in crop protection such as plant quarantine, virus indexing for virus elimination and clean seed production as well as for screening segregating populations. Genotypes WC48, NE43, NE15, WC35A, WC39, WC33, WC35C and WC18 which showed horizontal resistance are recommended as donors in cowpea breeding programs for developing cultivars with broad virus resistance. However, there is need to confirm their resistance under high virus pressure by artificial inoculation.

REFERENCES

- Aboul-Ata, A.E., Allen, D.J., Thottappilly, G. and Rossel, H.W. 1982. Variation in rate of seed transmission of *Cowpea aphid-borne mosaic virus* in cowpea. *Tropical Grain Legume Bulletin* 25:2-7.
- Abubakar, Z., Ali, F., Pinel, A., Traore, O., N'Guessan, P., Notteghem, J., Kimmins, F. and Konate, G. 2003. Phylogeography of *Rice yellow mottle virus* in Africa. *Journal of General Virology* 84:733–743. doi: 10.1099/vir.0.18759-0.
- Adams, M.J., Zerbini, F.M, French, R., Rabenstein, F., Stenger, D.C. and Valkonen, J.P.T. 2011. Family *Potyviridae*. In: King, A.M.Q., Adams, M.J., Carstens, E.B. and Lefkowitz, E.J. (Eds.), *Virus Taxonomy*, Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, London, UK. pp. 1069-1089.
- Adams, I.P., Glover, R.H., Monger, W.A., Mumford, R., Jackeviciene, E., Navalinskiene, M., Jackeviciene, E., Navalinskiene, M., Samuitiene, M. and Boonham, N. 2009. Next-generation sequencing and metagenomic analysis: a universal diagnostic tool in plant virology. *Molecular Plant Pathology* 10(4):537-545. <https://doi.org/10.1111/j.1364-3703.2009.00545.x>.
- Adams, I.P., Miano, D.W., Kinyua, Z.M., Wangai, A., Kimani, E., Phiri, N., Reeder, R., Harju, V., Glover, R., Hany, U., Souza-Richards, R., Deb Nath, P., Nixon, T., Fox, A., Barnes, A., Smith, J., Skelton, A., Thwaites, R., Mumford, R. and Boonham, N. 2012. Use of next-generation sequencing for the identification and characterization of Maize chlorotic mottle virus and Sugarcane mosaic virus causing maize lethal necrosis in Kenya. *Plant Pathology* 62(4):741–749. <https://doi.org/10.1111/j.1365-3059.2012.02690.x>.
- Adams, M.J., Antoniw, J.F. and Fauquet, C.M. 2005a. Molecular criteria for genus and species discrimination within the family Potyviridae. *Archives of Virology* 150(3):459-479. <https://doi.org/10.1007/s00705-004-0440-6>.
- Adams, M.J., Antoniw, J.F. and Beaudoin, F. 2005b. Overview and analysis of the polyprotein cleavage sites in the family Potyviridae. *Molecular Plant Pathology* 6(4):471–487. <https://doi.org/10.1111/j.1364-3703.2005.00296.x>.

- Adipala, E., Omongo, C.A., Sabiti, A., Obuo, J.E., Edema, R., Bua, B., Atwang, A., Nsubuga, E.N. and Ogenga-latigo, M.W. 1999. Pests and diseases on cowpea in Uganda: Experiences from a diagnostic survey. *African Crop Science Journal* 7(4):465-478.
- Agbahoungba, S., Karungi, J., Odong, T.L., Badji, A., Sadik, K. and Rubaihayo, P.R. 2017. Stability and extent of resistance of cowpea lines to flower bud thrips in Uganda. *African Crop Science Journal* 25(1):1-24. <https://doi.org/10.4314/acsj.v25i1.1>.
- Ajeigbe, H.A and Singh, B.B. 2006. Integrated pest management in cowpea: Effect of time and frequency of insecticide application on productivity. *Crop Protection*, 25:920–925.
- 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* 7(13):2099–2103.
- Aliyu, T.H. and Balogun, O.S. 2011. Effects of variety and planting density on the incidence of common viral diseases of cowpea (*Vigna unguiculata*) in a southern Guinea savannah agro-ecology. *Asian Journal of Plant Pathology* 5:126–133. <https://doi.org/10.3923/ajppaj.2011.126.133>.
- Aliyu, T.H. and Balogun, O.S. 2012. Effect of spatial arrangement on the incidence of virus diseases associated with cowpea intercropped with maize. *International Journal of Virology* 8:299–306. <https://doi.org/10.3923/ijv.2012.299.306>.
- Aliyu, T.H., Balogun, O.S. and Kumar, L. 2012. Survey of the symptoms and viruses associated with cowpea (*Vigna Unguiculata* (L). Walp) in the agroecological zones of Kwara State, Nigeria. *Ethiopian Journal of Environmental Studies and Management* 5(4):613–619.
- Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D.J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25(17):3389–402.
- Amayo, R., Arinaitwe, A.B., Mukasa, S.B., Tusiime, G., Kyamanywa, S., Rubaiyaho, P.R., and Edema, R. 2012. Prevalence of viruses infecting cowpea in Uganda and their molecular detection. *African Journal of Biotechnology* 11(77):14132–14139. <https://doi.org/10.5897/AJB11.398>.
- Amayo, R. 2008. Occurrence and transmission of seed-borne cowpea viruses in Uganda. MSc. Thesis, Makerere University, Kampala, Uganda. 70 pp.

- Ambang, Z., Ndongo, B., Amayana, D., Djil , B., Ngoh, J.P. and Chewachong, G.M. 2009. Combined effect of host plant resistance and insecticide application on the development of cowpea viral diseases. *Australian Journal of Crop Science* 3(3):167–172.
- Anonymous. 1981. Viruses, virus-like diseases and mycoplasma-like diseases. In: *Pest control in tropical grain legumes*. Centre for Overseas Pest Research, London, United Kingdom. pp. 95-110.
- Arshad, M., Bashir, M., Sharif, A. and Malik, B.A. 1998. Inheritance of resistance in cowpea (*Vigna Unguiculata* (L.) Walp to Black eye cowpea potyvirus. *Pakistan Journal of Biological Sciences* 30(2):263–270.
- Ba, F.S., Pasquet, R.S. and Gepts, P. 2004. Genetic diversity in cowpea [*Vigna unguiculata* (L.) Walp.] as revealed by RAPD markers. *Genetic Resources and Crop Evolution* 51(5):539–550.
- Baltimore, D. 1971. Expression of animal virus genomes. *Bacteriological Reviews* 35(3): 235–241.
- Barros, D.R., All nas-Zerbini, P., Beserra, J.E. Jr., Antunes, T.F. and Zerbini, F.M. 2011. Comparative analysis of the genomes of two isolates of cowpea aphid-borne mosaic virus (CABMV) obtained from different hosts. *Archives of Virology* 156(6):1085–1091.
- Bashir, M., Ahmad, Z. and Ghafoor, A. 2002a. Cowpea aphid-borne mosaic potyvirus: A review. *International Journal of Pest Management* 48(2):155–168. <https://doi.org/10.1080/09670870110118722>.
- Bashir, M., Ahmad, Z. and Ghafoor, A. 2002b. Cowpea germplasm evaluation of virus resistance under greenhouse conditions. *Asian Journal of Plant Sciences* 1(5):585–587.
- Bashir, M. 1992. Serological and biological characterization of seed-borne isolates of blackeye cowpea mosaic and cowpea aphid-borne mosaic potyviruses in *Vigna unguiculata* (L.) Walp. PhD thesis, Oregon State University, Corvallis, OR, USA. 247pp.
- Bashir, M. and Hampton, R.O. 1996. Sources of genetic resistance in cowpea (*Vigna unguiculata* (L.) Walp to cowpea aphid-borne mosaic potyviruses. *European Journal of Plant Pathology* 102:411–419.

- Blanche, S.B., Myers, G.O. and Kang, M.S. 2007. GGE biplots and traditional stability measures for interpreting genotype by environment interactions. *Journal of Crop Improvement* 20(1):123–135. https://doi.org/10.1300/J411v20n01_07.
- Bock, K.R. 1973a. East African strains of Cowpea aphid-borne mosaic virus. *Annals of Applied Biology* 74(1):75–83. <https://doi.org/10.1111/j.1744-7348.1973.tb07724.x>.
- Bock, K.R. 1973b. Peanut mottle virus in East Africa. *Annals of Applied Biology* 74(2):171–179. <https://doi.org/10.1111/j.1744-7348.1973.tb07736.x>.
- Bock, K.R. and Conti, M., 1974. *Cowpea aphid-borne mosaic virus*. CMI/AAB Description of Plant Viruses No. 134, Kew, Surrey, England.
- 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.
- Bozarth, R.F. and Shoyinka, S.A. 1979. Cowpea mottle virus. CMI/AAB. Description of Plant Viruses. No.212.
- Brito, M., Fernández-Rodríguez, T., Garrido, M.J., Mejías, A., Romano, M. and Marys, E. 2012. First report of *Cowpea mild mottle carlavirus* on yardlong bean (*Vigna unguiculata* subsp. *sesquipedalis*) in Venezuela. *Viruses* 4(12):3804–11. <https://doi.org/10.3390/v4123804>.
- Brunt, A.A and Kenten, R.H. 1974. Cowpea mild mottle virus. CMI/AAB. Description of Plant Viruses. No. 140.
- Brunt, A.A. and Kenten, R.H. 1973. Cowpea mild mottle, a newly recognized virus infecting cowpeas (*Vigna unguiculata*) in Ghana. *Annals of Applied Biology* 74(1):67–74.
- Byarugaba, A.A. 2008. Occurrence and pathogenesis of cowpea viruses in Uganda. MSc. Thesis, Makerere University, Kampala, Uganda. 69 pp.
- Campbell, C.L. and Madden, L.V. 1990. Introduction to Plant Disease Epidemiology. John Wiley & Sons, New York, USA. 532pp.
- Carsky, R.J., Vanlauwe, B. and Lyasse, O. 2002. Cowpea rotation as a resource management technology for cereal-based systems in the savannas of West Africa. In: Fatokun, C.A., Tarawali, S.A., Singh, B.B., Kormawa, P.M. and Tamò, M (Eds.), *Challenges and opportunities for enhancing sustainable cowpea production*. Proceedings of the World Cowpea Conference III held at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, 4–8 September 2000. IITA, Ibadan, Nigeria. pp. 252-266.

- Ceccarelli, S. 2012. Plant breeding with farmers –A technical manual. ICARDA, Aleppo, Syria. 139pp.
- Chant, S.R. 1959. Viruses of cowpea, *Vigna unguiculata* L.(Walp.), in Nigeria. *Annals of Applied Biology* 47(3):565–572.
- Clark, F.M. and Adams, A.N. 1977. Characteristics of the microplate method of Enzyme-Linked Immunosorbent Assay for the detection of plant viruses. *Journal of General Virology* 34:475–483.
- Cooper, J.I. and Jones, A.T. 1983. Responses of Plants to Viruses: Proposals for the Use of Terms. *Phytopathology*, 73(2):127-128.
- Coulibaly, S., Pasquet, R.S., Papa, R. and Gepts, P. 2002. AFLP analysis of the phenetic organization and genetic diversity of *Vigna unguiculata* (L.) Walp reveals extensive gene flow between wild and domesticated types. *Theoretical and Applied Genetics* 104:358–366. <https://doi.org/10.1007/s001220100740>.
- Cox-Foster, D.L., Conlan, S., Holmes, E.C., Palacios, G., Evans, J.D., Moran, N.A., P.L., Briese, T., Hornig, M., Geiser, D.M., Martinson, V., vanEngelsdorp, D., Kalkstein, A.L., Drysdale, A., Hui, J., Zhai, J., Cui, L., Hutchison, S.K., Simons, J.F., Egholm, M., Pettis, J.S. and Lipkin, W. I. 2007. A metagenomic survey of microbes in honey bee colony collapse disorder. *Science* 318(5848):283–287. <https://doi.org/10.1126/science.1146498>.
- Cupertino, F.P., Lin, M.T., Kitajima, E.W. and Costa, C.L. 1982. Occurrence of southern bean mosaic virus in central Brazil. *Plant Disease* 66:742-743.
- DeBoever, C., Reid, E.G., Smith, E.N., Wang, X., Dumaop, W., Harismendy, O., Carson, D., Richman, D., Masliah, E. and Frazer, K.A. 2013. Whole transcriptome sequencing enables discovery and analysis of viruses in archived primary central nervous system lymphomas. *PloS One* 8(9):e73956–e73956. <https://doi.org/10.1371/journal.pone.0073956>.
- de Jager, C.P. 1979. Cowpea severe mosaic virus. CMI/AAB. Description of Plant Viruses. No. 209.
- Del Fabbro, C., Scalabrin, S., Morgante, M. and Giorgi, F.M. 2013. An extensive evaluation of read trimming effects on Illumina NGS data analysis. *PloS One* 8(12): e85024–e85024. <https://doi.org/10.1371/journal.pone.0085024>.

- Demski, J.W., Alexander, A.T., Stefani, M.A. and Kuhn, C.W. 1983. Natural infection, disease reactions and epidemiological implications of peanut mottle virus in cowpea. *Plant Disease* 67:267–269.
- Deng, P., Wu, Z. and Wang, A. 2015. The multifunctional protein CI of potyviruses plays interlinked and distinct roles in viral genome replication and intercellular movement. *Virology Journal* 12:141. <https://doi.org/10.1186/s12985-015-0369-2>.
- Dietzgen, R.G., Callaghan, B., Higgins, C.M., Birch, R.G., Chen, K. and Xu, Z. 2001. Differentiation of peanut seed-borne potyviruses and cucumoviruses by RT-PCR. *Plant Disease* 85(9):989–992.
- Dijkstra, J., Bos, L., Bouwmeester, 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 virus and cowpea aphid-borne mosaic virus. *European Journal of Plant Pathology* 93(3):115–133.
- Edema, R., Adipala, E. and Florini, D.A. 1997. Influence of season and cropping system on occurrence of cowpea diseases in Uganda. *Plant Disease* 81:465–468.
- Ehlers, J.D., Fery, R.L. and Hall, A.E. 2002. Cowpea breeding in the USA: new varieties and improved germplasm. In: Fatokun, C.A., Tarawali, S.A., Singh, B.B., Kormawa, P.M. and Tamò, M (Eds.), *Challenges and opportunities for enhancing sustainable cowpea production*. Proceedings of the World Cowpea Conference III held at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, 4–8 September 2000. pp. 62–77.
- Egan, A.N., Schlueter, J. and Spooner, D.M. 2012. Applications of next-generation sequencing in plant biology. *American Journal of Botany*, 99(2):175–185. <https://doi.org/10.3732/ajb.1200020>.
- Emechebe, A.M. and Florini, D.A. 1997. Shoot and pod diseases of cowpea induced by fungi and bacteria. In: Singh, B.B., Mohan Raj, D.R., Dashiell, K.E. and Jackai, L.E.N (Eds.), *Advances in cowpea research*. A co-publication of International Institute of Tropical Agriculture (IITA) and Japan International Research Centre for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria. pp.176–192.
- Fang, G.W., Allison, R.F., Zambolim, E.M., Maxwell, D.P. and Gilbertson, R.L. 1995. The complete nucleotide sequence and genome organization of bean common mosaic virus (NL-3 strain). *Virus Research* 39:13–23.

- Fioreze, A.C.C.L., Grigolo, S., Piva, C.A.G. and Sartori, L. 2018. Common bean landraces as potential sources of resistance to anthracnose. *Pesq. Agropec. Trop., Goiânia*, 48(2):126-133.
- Fischer, H.U and Lockhart, B.E. 1976a. A strain of cowpea aphid-borne mosaic virus isolated from cowpeas in Morocco. *Phytopathology* 85:43-48.
- Fischer, H.U and Lockhart, B.E. 1976b. A strain of cucumber mosaic virus isolated from cowpeas in Morocco. *Phytopathology* 85:132-138.
- Food and Agriculture Organisation Statistics (FAOSTAT), Site internet: <http://faostat3.fao.org/>
- Francki, R.I.B., Mossop, D.W. and Hatta, T. 1979. Cucumber mosaic virus. CMI/AAB. Description of Plant Viruses. No.213.
- Frutos, E., Galindo, M.P. and Leiva, V. 2014. An interactive biplot implementation in R for modeling genotype-by-environment interaction. *Stochastic Environmental Research and Risk Assessment* 28:1629-1641.
- Fuentes, S. 2010. NCM-ELISA detection of sweetpotato viruses. OP21, Version 56. International Potato Centre (CIP), Lima, Peru. pp. 5.
- Gillaspie, A.G., Pio-Ribeiro, G., Andrade, G.P. and Pappu, H.R. 2001. RT-PCR detection of seed-borne *Cowpea aphid-borne mosaic virus* in Peanut. *Plant Disease* 85:1181-1182.
- Givord, L. 1981. Southern bean mosaic virus isolated from cowpea in the ivory Coast. *Plant Disease* 65:755-756.
- Glansdorff, N., Xu, Y. and Labedan, B. 2008. The Last Universal Common Ancestor: emergence, constitution and genetic legacy of an elusive forerunner. *Biology Direct* 3:29. <https://doi.org/10.1186/1745-6150-3-29>.
- Gordon, D.T., Bradfute, O.E., Gingery, R.E., Nault, L.R. and Uyemoto, J.K. 1984. Maize Chlorotic Mottle Virus. CMI/AAB Description of plant viruses. No. 284
- Goenaga, R., Quiles, A. and Gillaspie, A.G. 2008. Assessing yield potential of cowpea genotypes grown under virus pressure. *Hortscience* 43(3):673-676.
- Goldsmith, C.S. and Miller, S.E. 2009. Modern uses of electron microscopy for detection of viruses. *Clinical Microbiology Reviews* 22(4):552-563. <https://doi.org/10.1128/CMR.00027-09>.
- Gould, A.R. and Symons, R.H. 1983. A molecular biological approach to relationships among viruses. *Annual Review of Phytopathology* 21:179-199.

- Gremillion, S., Culbreath, A., Gorbet, D., Mullinix, B., Pittman, R., Stevenson, K. and Condori, M. 2011. Field evaluations of leaf spot resistance and yield in Peanut genotypes in the United States and Bolivia. *Plant Disease* 95:263-268. <http://dx.doi.org/10.1094/PDIS-06-10-0454>.
- Gumedzoe, M.Y.D., Thottappilly, G. and Asselin, A. 1997. Occurrence of southern bean mosaic virus (SVMV) in Togo and its interaction with some cowpea cultivars. *African Crop Science Journal* 5(2):215-222.
- Gutierrez, D.L., Fuentes, S. and Salazar, L.F. 2003. Sweet potato virus disease (SPVD): distribution, incidence, and effect on sweet potato yield in Peru. *Plant Disease* 87:297-302.
- Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41:95-98.
- Hall, A.E., Cisse, N., Thiaw, S., Elawad, H.O.A., Ehlers, J.D., Ismail, A.M., Fery, R.L., Roberts, P.A., Kitch, L.W., Murdock, L.L., Boukar, O., Philips, R.D. and McWatters, K.H. 2003. Development of cowpea cultivars and germplasm by the Bean/Cowpea CRSP. *Field Crops Research* 82:103-134. [https://doi.org/10.1016/S0378-4290\(03\)00033-9](https://doi.org/10.1016/S0378-4290(03)00033-9).
- Hall, N. 2007. Advanced sequencing technologies and their wider impact in microbiology. *Journal of Experimental Biology* 210(9):1518-1525.
- Hamilton, R.I., Edwardson, J.R., Francki, R.I.B., Hei-Ti, H., Hull, R., Koenig, R. and Milne, R.G. 1981. Guidelines for the Identification and Characterization of Plant Viruses. *Journal of General Virology* 54: 223-241. DOI: 10.1099/0022-1317-54-2-223.
- Hampton, R.O., Thottappilly, G. and Rossel, H.W. 1997. Viral diseases of cowpea and their control by virus resistance-conferring genes. In: Singh, B.B., Mohan Raj, D.R., Dashiell, K.E. and Jackai, L.E.N (Eds.), *Advances in cowpea research*. A co-publication of International Institute of Tropical Agriculture (IITA) and Japan International Research Centre for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria. pp. 159-175.
- Harlan, J.R. 1971. Agricultural Origins: Centers and Noncenters. *Science* 174:468-473.
- Hawkes, J.G. 1991. The importance of genetic resources in plant breeding. *Biological Journal of the Linnean Society* 43(1):3-10.
- Head, S.R., Komori, H.K., LaMere, S.A., Whisenant, T., Van Nieuwerburgh, F., Salomon, D.R. and Ordoukhanian, P. 2014. Library construction for next-generation sequencing:

- overviews and challenges. *Biotechniques* 56(2):61.
<https://doi.org/doi:10.2144/000114133>.
- Henson, J.M. and French, R. 1993. The polymerase chain reaction and plant disease diagnosis. *Annual Review of Phytopathology* 31:81–109.
<https://doi.org/10.1146/annurev.py.31.090193.000501>.
- Hughes, J.d'A and Shoyinka, S.A. 2003. Overview of viruses of legumes other than groundnut in Africa. In: Hughes, J.d'A. and Odu, O.B (Eds.), *Plant virology in Sub Saharan Africa*. Proceedings of a conference held at International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, 4-8 June 2001. pp. 553–568.
- Huguenot, C., Furneaux, M.T. and Hamilton, R.I. 1994. Capsid protein properties of cowpea aphid-borne mosaic virus and blackeye cowpea mosaic virus confirm the existence of two major subgroups of aphid-transmitted, legume-infecting potyviruses. *Journal of General Virology* 75:3555–3560.
- Huguenot, C., Furneaux, M.T., Thottappilly, G., Rossel, H.W. and Hamilton, R.I. 1993. Evidence that cowpea aphid-borne mosaic and blackeye cowpea mosaic viruses are two different potyviruses. *Journal of General Virology* 74:335–40.
- Hull, R. 2004. Southern bean mosaic virus. CMI/AAB. Description of Plant Viruses. No.408.
- Hulo, C., de Castro, E., Masson, P., Bougueleret, L., Bairoch, A., Xenarios, I. and Le Mercier, P. 2011. ViralZone: a knowledge resource to understand virus diversity. *Nucleic Acids Research* 39(Database issue):D576–82. doi: 10.1093/nar/gkq901.
- Isubikalu, P., Erbaugh, J.M., Semana, A.R. and Adipala, E. 1999. Influence of farmer production goals on cowpea pest management in Eastern Uganda: Implications for developing IPM programmes. *African Crop Science Journal* 7(4):539–548.
- Johnson, M., Zaretskaya, I., Raytselis, Y., Merezhuik, Y., McGinnis, S. and Madden, L. T. 2008. NCBI BLAST: a better web interface. *Nucleic Acids Research* 36(2):W5–W9.
<https://doi.org/10.1093/nar/gkn201>.
- Jones, R.A. 2004. Using epidemiological information to develop effective integrated virus disease management strategies. *Virus Research* 100(1):5–30.
<https://doi.org/10.1016/j.virusres.2003.12.011>.
- Jones, R.A.C. 2001. Developing integrated disease management strategies against non-persistently aphid-borne viruses: A model program. *Integrated Pest Management Reviews* 6:15–46.

- Jones, R.A.C. 1993. Effects of cereal borders, admixture with cereals and plant density on the spread of bean yellow mosaic potyvirus into narrow-leaved lupins (*Lupinus angustifolius*). *Annals of Applied Biology* 122:501–518.
- Kaiser, W. and Mossahebi, H. 1975. Studies on Cowpea aphid-borne mosaic virus and its effect on cowpea yield in Iran. *FAO Plant Protection Bulletin* 23(2):33–40.
- Kaiser, W.J., Danesh, D., Okhovat, M. and Mossahebi, H. 1968. Diseases of pulse crop (edible legumes) in Iran. *Plant Disease Reporter* 52:687-691.
- Kamala, S., Makesh Kumar, T., Sreekumar, J. and Chakrabarti, S.K. 2015. Whole transcriptome sequencing of diseased elephant foot yam reveals complete genome sequence of Dasheen mosaic virus. *Virology Reports* 5:1–9. <https://doi.org/10.1016/j.virep.2014.11.001>.
- Kannaiyan, J. and Hachiwa, C.H. 1993. Diseases of food legume crops for the scope of their management in Zambia. *FAO Plant Protection Bulletin* 41(2):73–90.
- Kareem, K.T. and Taiwo, M.A. 2007. Interactions of viruses in cowpea: effects on growth and yield parameters. *Virology Journal* 4:15–22. <https://doi.org/10.1186/1743-422X-4-15>.
- Kashif, M. 2009. Detection and characterization of viruses of sweet potatoes (*Ipomoea batatas* L.) from Guatemala and Honduras. MSc. Thesis, University of Helsinki, Finland. 72 pp.
- Kone, N., Asare-Bediako, E., Koita, O., Kone, D. and Winter, S. 2017b. Seasonal and spatial variation in the prevalence of viral diseases and associated aphid-borne viruses in cucurbits in Cote d'Ivoire. *Annals of Agricultural Sciences* 62(2):227–234. <https://doi.org/10.1016/j.aoas.2017.12.005>.
- Kone, N., Asare-Bediako, E., Silue, S., Kone, D., Koita, O., Menzel, W. and Winter, S. 2017a. Influence of planting date on incidence and severity of viral disease on cucurbits under field condition. *Annals of Agricultural Sciences* 62(1):99–104. <https://doi.org/10.1016/j.aoas.2017.05.005>.
- Kreuze, J.F., Perez, A., Untiveros, M., Quispe, D., Fuentes, S., Barker, I. and Simon, R. 2009. Complete viral genome sequence and discovery of novel viruses by deep sequencing of small RNAs: a generic method for diagnosis, discovery and sequencing of viruses. *Virology* 388(1):1–7. <https://doi.org/10.1016/j.virol.2009.03.024>.
- Kumar, S., Stecher, G. and Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33:1870–1874.

- Lecoq, H. and Katis, N. 2014. Control of cucurbit viruses. *Advances in virus research* 90:255-296.
- Liu, G.E. 2009. Applications and case studies of the next-generation sequencing technologies in food, nutrition and agriculture. *Recent Patents on Food, Nutrition and Agriculture* 1:75-79.
- Liu, S., Vijayendran, D. and Bonning, B.C. 2011. Next generation sequencing technologies for insect virus discovery. *Viruses* 3(10):1849-69. <https://doi.org/10.3390/v3101849>.
- Louie, R. 1980. Sugarcane mosaic virus in Kenya. *Plant Disease* 64:944-947.
- Lovisolo, O. and Conti, M. 1966. Identification of an aphid-transmitted cowpea mosaic virus. *Netherlands Journal of Plant Pathology* 72(3-6):265-269.
- Lush, W.M. and Evans, L.T. 1981. The domestication and improvement of cowpeas (*Vigna unguiculata* (L.) Walp.). *Euphytica* 30(3):579-587.
- Madamba, R., Grubben, G.J.H., Asante, I.K. and Akromah, R., 2006. *Vigna unguiculata* (L.) Walp. Internet Record from PROTA4U. Brink, M. and Belay, G. (Eds.). PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands. <http://www.prota4u.org/search.asp>. Accessed 30 October 2017.
- Makkouk, K.M. and Kumari, S.G. 2009. Epidemiology and integrated management of persistently transmitted aphid-borne viruses of legume and cereal crops in West Asia and North Africa. *Virus Research* 141(2):209-218. <https://doi.org/10.1016/j.virusres.2008.12.007>.
- Makkouk, K.M., Kumari, S.G., van Leur, J.A.G. and Jones, R.A.C. 2014. Control of plant virus diseases in cool-season grain legume crops. In: *Advances in Virus Research* 90:207-253.
- Maphosa, M., Talwana, H. and Tukamuhabwa, P. 2013. Assessment of comparative virulence and resistance in soybean using field isolates of soybean rust. *Journal of Agricultural Science* 5(5):249-257. <https://doi.org/10.5539/jas.v5n5p249>.
- Masek, T., Vopalensky, V., Suchomelova, P. and Pospisek, M. 2005. Denaturing RNA electrophoresis in TAE agarose gels. *Analytical Biochemistry* 336(1):46-50. <https://doi.org/10.1016/j.ab.2004.09.010>.
- Matthews, R.E.F. 1980. Host plant responses to virus infection. In: Fraenkel-Conrat, H. and Wagner, R.R (Eds.), *Comprehensive virology, virus-host interaction, viral invasion, persistence, and diagnosis*, Volume 16. Plenum Press, New York, USA. pp. 297-359.

- Mbanzibwa, D.R., Tian, Y.P., Tugume, A.K., Mukasa, S.B., Tairo, F., Kyamanywa, S., Kullaya, A. and Valkonen, J.P.T. 2011. Simultaneous virus-specific detection of the two cassava brown streak-associated viruses by RT-PCR reveals wide distribution in East Africa, mixed infections, and infections in *Manihot glaziovii*. *Journal of Virological Methods* 171(2):394–400. <https://doi.org/10.1016/j.jviromet.2010.09.024>.
- Mbanzibwa, D.R., Tian, Y., Mukasa, S.B. and Valkonen, J.P.T. 2009. Cassava brown streak virus (Potyviridae) encodes a putative Maf/HAM1 pyrophosphatase implicated in reduction of mutations and a PI proteinase that suppresses RNA silencing but contains no HC-Pro. *Journal of Virology* 83(13):6934–6940. <https://doi.org/10.1128/JVI.00537-09>.
- Mbeyagala, E.K., Tukamuhabwa, P. and Mukasa, S.B. 2018c. Detection of *Cowpea aphid-borne mosaic virus* (CABMV) in cowpea by reverse transcription polymerase chain reaction. *African Crop Science Journal* 26(3):377-388. <http://dx.doi.org/10.4314/acsj.v26i3.4>.
- Mbeyagala, E.K., Bisikwa, J., Tukamuhabwa, P. and Mukasa, S.B. 2018b. Trait association and stability of virus resistance among cowpea genotypes in Uganda. *African Crop Science Journal* 26(2):259-275. <http://dx.doi.org/10.4314/acsj.v26i2.8>.
- Mbeyagala, E.K., Tukamuhabwa, P., Bisikwa, J., Holton, T. and Mukasa, S.B. 2018a. Next-generation sequencing reveals the first complete genome sequence of *Cowpea aphid-borne mosaic virus* from Uganda. *Genome Announcements* 6:e01491-17. <https://doi.org/10.1128/genome.A.01491-17>.
- Mbeyagala, E.K., Mukasa, S.B., Holton, T., Aritua, V., Katari, S.M., Tukamuhabwa, P. and Bisikwa, J. 2016. Application of next-generation sequencing in identification of cowpea viruses. *RUFORUM Working Document Series* 14(1):653-660. ISSN 1607-9345.
- Mbeyagala, E.K., Mukasa, S.B., Tukamuhabwa, P. and Bisikwa, J. 2014. Evaluation of cowpea genotypes for virus resistance under natural conditions in Uganda. *Journal of Agricultural Science* 6(10):176–187. <https://doi.org/10.5539/jas.v6n10p176>.
- Mishra, R., Verma, R.K. and Gaur, R.K. 2015. Analysis of genome comparison of two Indian isolates of Cowpea aphid-borne mosaic virus from India. *Virus Genes* 51(2):306-309.
- Mithen, R. and Kibblewhite, H. 1993. Taxonomy and ecology of *Vigna unguiculata* (leguminosae—papilionoideae) in South-Central Africa. *Kirkia* 14(1):100–113.

- Mlotshwa, S., Verver, J., Sithole-Niang, I., Van Kampen, T., Van Kammen, A. and Wellink, J. 2002. The genomic sequence of Cowpea aphid-borne mosaic virus and its similarities with other potyviruses. *Archives of Virology* 147(5):1043–52. <https://doi.org/10.1007/s00705-002-0800-z>.
- Morales, F.J. and Castano, M. 1985. Effect of a Colombian isolate of Bean southern mosaic virus on selected yield components of *Phaseolus vulgaris*. *Plant Disease* 69:803-804.
- Moury, B., Fabre, F., Hebrard, E. and Froissar, R. 2017. Determinants of host species range in plant viruses. *Journal of General Virology* 98:862–873. doi 10.1099/jgv.0.000742.
- Mundua. 2010. Estimation of consumer preferences for cowpea varieties in Kumi and Soroti districts, Uganda. MSc thesis. Makerere University Kampala, Uganda. 102 pp.
- Naidu, R.A., Gowda, S., Satyanarayana, T., Boyko, V., Reddy, A.S., Dawson, W.O. and Reddy, D.V. 1998. Evidence that whitefly-transmitted cowpea mild mottle virus belongs to the genus Carlavirus. *Archives of Virology* 143(4):769–80.
- Naidua, R. and Hughes, J.d'A. 2003. Methods for the detection of plant virus diseases. In: Hughes, J.d'A. and Odu, O.B (Eds.), *Plant virology in Sub Saharan Africa*. Proceedings of a conference held at International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, 4-8 June 2001. pp. 233–253.
- Ndiaye, M., Bashir, M., Keller, X.E. and Hampton, R.O. 1993. Cowpea viruses in Senegal, West Africa: Identities, distribution, seed transmission and sources of resistance. *Plant Disease* 77:999–1003.
- Ndunguru, J., Sseruwagi, P., Tairo, F., Stomeo, F., Maina, S., Djinkeng, A., Kehoe, M. and Boykin, L. M. 2015. Analyses of Twelve New Whole Genome Sequences of Cassava Brown Streak Viruses and Ugandan Cassava Brown Streak Viruses from East Africa: Diversity, Supercomputing and Evidence for Further Speciation. *PLoS ONE* 10(10): e0139321. doi:10.1371/journal.pone.0139321.
- Néya, B.J., Zida, P.E., Sérémé, D., Lund, O.S. and Traoré, O. 2015. Evaluation of yield losses caused by Cowpea aphid-borne mosaic virus (CABMV) in 21 Cowpea (*Vigna unguiculata* (L.) Walp.) varieties in Burkina Faso. *Pakistan Journal of Biological Sciences* 18 (7):304-313. <https://doi.org/10.3923/pjbs.2015.304.313>.
- Ojosnegros, S., García-Arriaza, J., Escarmís, C., Manrubia, S.C, Perales, C., Arias, A., García Mateu, M. and Domingo, E. 2011. Viral genome segmentation can result from a trade-off between genetic content and particle stability. *PLoS Genetics* 7(3): e1001344. <https://doi.org/10.1371/journal.pgen.1001344>.

- Oloka, H.K., Tukamuhabwa, P., Sengooba, T. and Shanmugasundaram, S. 2008. Reaction of exotic soybean germplasm to *Phakopsora pachyrhizi* in Uganda. *Plant Disease* 92:1493–1496.
- Orawu, M., Melis, R., Milliano, W.D., Laing, M. and Adipala, E. 2005. Occurrence and prevalence of cowpea virus diseases in Uganda. *Africana Crop Science Proceedings* 7:1279–1283.
- Orawu, M., Obuo, J. and Omadi, R. 2015. Distribution and detection of cowpea viruses infecting cowpea in Uganda. *American Journal of Plant Sciences* 6(5):574–581. <https://doi.org/10.4236/ajps.2015.65062>.
- Orawu, M., Melis, R., Laing, M. and Derera, J. 2012. Genetic inheritance of resistance to *Cowpea aphid-borne mosaic virus* in cowpea. *Euphytica* 189(2):191–201. <https://doi.org/10.1007/s10681-012-0756-3>.
- Orawu, M. 2007. Occurrence of *Cowpea aphid-borne mosaic virus* and prospects of improving resistance in local cowpea landraces in Uganda. PhD thesis, University of KwaZulu-Natal, South Africa. 171 pp.
- Padulosi, S. and Ng, N.Q. 1997. Origin, taxonomy and morphology of *Vigna unguiculata* (L.) Walp. In: Singh, B.B., Mohan Raj, D.R., Dashiell, K.E. and Jackai, L.E.N (Eds.), *Advances in cowpea research*. A co-publication of International Institute of Tropical Agriculture (IITA) and Japan International Research Centre for Agricultural Sciences (JIRCAS). IITA, Ibadan, Nigeria. pp.1-12.
- Pasquet, R.S. 1996. Cultivated cowpea (*Vigna unguiculata*): Genetic organization and domestication. In: Pickersgrill, B. and Lock, J.M (Eds.), *Advances in Legume Systematics 8: Legumes of Economic Importance*. Royal Botanic Gardens, Kew. pp. 101-108.
- Pasquet, R.S. 1998. Morphological study of cultivated cowpea *Vigna unguiculata* (L.) Walp. Importance of ovule number and definition of cv gr Melanophthalmus. *Agronomie* 18:61–70.
- Pasquet, R.S. 1999. Genetic relationships among subspecies of *Vigna unguiculata* (L.) Walp. based on allozyme variation. *Theoretical and Applied Genetics* 98:1104–1119.
- Pasquet, R.S. 2000. Allozyme diversity of cultivated cowpea *Vigna unguiculata* (L.) Walp. *Theoretical and Applied Genetics* 101:211–219.

- Pasquet, R.S. 1993a. Intraspecific classification of spontaneous forms of *Vigna unguiculata* (L.) Walp. (Fabaceae) from morphological data. *Newsletter of the National Botanical Garden of Belgium* 62:127-173. DOI: 10.2307 / 3668271.
- Pasquet, R.S. 1993b. Two new subspecies of *Vigna unguiculata* (L.) Walp. (Leguminosae: Papilionoideae). *Kew Bulletin* 48:805-806.
- Pasquet, R.S. 1997. A new subspecies of *Vigna unguiculata* (Leguminosae-Papilionoideae). *Kew Bulletin* 52:840
- Pienaar, B.J. and van Wyk, A.E. 1992. The *Vigna unguiculata* complex (Fabaceae) in southern Africa. *South African Journal of Botany* 58(6):414-429.
- Pio-Ribeiro, G. and Kuhn, C.W. 1980. Cowpea stunt, heterogenous and differential reactions of cowpea cultivars. *Phytopathology* 70:244-249.
- Pio-Ribeiro, G., Wyatt, S.D. and Kuhn, C.W. 1978. Cowpea Stunt: A disease caused by a synergistic interaction of two viruses. *Phytopathology* 68:1260-1265.
- Purcifull, D. and D. Gonsalves. 1985. Blackeye cowpea mosaic virus. CMI/AAB. Description of Plant Viruses. No. 305.
- Raheja, A.K. and Leleji, O.I. 1974. An aphid-borne virus disease of irrigated cowpeas in northern Nigeria. *Plant Disease* 58:1080-1084.
- Rawal, K.M. 1975. Natural hybridization among wild, weedy and cultivated *Vigna unguiculata* (L.) Walp. *Euphytica* 24(3):699-707.
- R Core Team, 2014. R. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: <https://www.R-project.org/>.
- Rossel, H.W. and Thottappilly, G., 1985. Virus diseases of important food crops in tropical Africa. IITA Publications series, IITA, Ibadan.
- Salem, N., Ehlers, J. and Roberts, P. 2010. Biological and molecular diagnosis of seedborne viruses in cowpea germplasm of geographically diverse sub-Saharan origins. *Plant Pathology*, 59:773-784. <https://doi.org/10.1111/j.1365-3059.2010.02285.x>.
- Schuster, S.C. 2008. Next-generation sequencing transforms today's biology. *Nature Methods* 5(1):16-18. <https://doi.org/10.1038/NMETH1156>.
- Shoyinka, S.A., Bozarth, R.F., Reese, J. and Rossel, H.W. 1978. Cowpea mottle virus: a seed-borne virus with distinctive properties infecting cowpea in Nigeria. *Phytopathology* 68(5):693-699.

- Sicard A, Michalakakis Y, Guti_errez S, Blanc S. The strange lifestyle of multipartite viruses. *PLoS Pathogen* 12(11): e1005819. doi:10.1371/journal.ppat.1005819.
- Singh, B.B., Ehlers, J.D., Sharma, B. and Freire Filho, F.R. 2002. Recent progress in cowpea breeding. In: Fatokun, C.A., Tarawali, S.A., Singh, B.B., Kormawa, P.M. and Tamò, M (Eds.), *Challenges and opportunities for enhancing sustainable cowpea production*. Proceedings of the World Cowpea Conference III held at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, 4–8 September 2000. IITA, Ibadan, Nigeria. pp.22-40.
- Sithole-Niang, I., Nyathi, T., Maxwell, D.P. and Candresse, T. 1996. Sequence of the 3'-terminal region of a Zimbabwe isolate of *Cowpea aphid-borne mosaic virus* (CABMV). *Archives of Virology* 141(5):935–943.
- Tairo, F. 2006. Molecular resolution of genetic variability of major sweet potato viruses and improved diagnosis of potyviruses co-infecting sweet potato. PhD Thesis, Swedish University of Agricultural Sciences, Sweden. 52 pp.
- Taiwo, M., Gonsalves, D., Provvidenti, R. and Thurston, H. 1982. Partial characterization and grouping of isolates of black cowpea mosaic and cowpea aphid mosaic virus. *Phytopathology* 72:590–596.
- Taiwo, M.A. and Akinjogunla, O.J. 2006. Cowpea viruses: Quantitative and qualitative effects of single and mixed viral infections. *African Journal of Biotechnology* 5(19):1749-1756.
- Taiwo, M.A., Hughes, J.d'A., and Oke, K.E. 2006. Studies on Maize streak virus and Maize mottle/chlorotic stunt virus in Lagos, Nigeria. *Plant Disease*, 90:199-202.
- Taiwo, M.A. and Gonsalves, D. 1982. Serological grouping of isolates of blackeye cowpea mosaic and cowpea aphid borne mosaic viruses. *Phytopathology* 72:583-589.
- Tamura, K. and Nei, M. 1993. Estimating the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular biology and Evolution* 10(3):512-526. 10.1093/oxfordjournals.molbev.a040023.
- Tarawali, S.A., Singh, B.B., Gupta, S.C., R. Tabo., Harris, F., Nokoe, S., Fernández-Rivera, S., Bationo, A., Manyong, V.M., Makinde, K. and Odion, E.C. 2002. Cowpea as a key factor for a new approach to integrated crop–livestock systems research in the dry savannas of West Africa. In: Fatokun, C.A., Tarawali, S.A., Singh, B.B., Kormawa, P.M. and Tamò, M (Eds.), *Challenges and opportunities for enhancing sustainable*

- cowpea production. Proceedings of the World Cowpea Conference III held at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, 4–8 September 2000. IITA, Ibadan, Nigeria. pp. 233-251.
- Tarawali, S.A., Singh, B.B., Peters, M and Blade, S.F. 1997. Cowpea haulms as fodder. In: Singh, B.B, Mohan Raj, D.R., Dashiell, K.E. and Jackai L.E.N (Eds.), *Advances in cowpea research*. A co-publication of the International Institute of Tropical Agriculture (IITA) and Japan international research centre for agricultural sciences (JIRCAS). Ibadan, Nigeria.
- Teakle, D.S., Shukla, D.D. and Ford, R. 1984. Sugarcane mosaic virus. CMI/AAB Description of plant viruses. No.342.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25:4876-4882
- Thottappilly, G. 1992. Plant virus Diseases of importance to African agriculture. *Journal of Phytopathology* 134:265-288.
- Thottappilly, G. and Rossel, H.W. 1992. Virus diseases of cowpea in tropical Africa. *Tropical Pest Management* 38(4):337-348. <https://doi.org/10.1080/09670879209371724>.
- Thottappilly, G., Hamilton, R.I., Huguenot, C., Rossel, H.W., Furneaux, M.T., Gumedzoe, M.Y., Shoyinka, S.A., Naik, D.M., Konate, G., Atcham-Aanero, T., Haciwa, H.C., Anno-Nyako, F.O., Saitodine, N., Wangai, A., Lampitey, P., Gubba, A., Mbwaga, A.M., Neya, J. and Offei, S.K. 1995. Identification of cowpea viruses and their strains in tropical africa- an international pilot project. IITA Research No. 10. Pp.12-15.
- Thouvenel, J., Tia, E. and Fishpool, L.D.C. 1990. Characterization of cowpea mottle virus on cowpea (*Vigna unguiculata*) in the Ivory Coast and the identification of a new vector. *Tropical Agriculture (Trinidad)* 67:280-282.
- Thresh, J. 2003. Control of plant virus diseases in Sub-Saharan Africa: the possibility and feasibility of an integrated approach. *African Crop Science Journal* 11(3):199–223.
- Timko, M.P. and Singh, B.B. 2008. Cowpea, a Multifunctional Legume (Chapter 10). In: Moore, P.H and Ming, R (Eds.), *Genomics of Tropical Plants*. Berlin: Springer. pp. 227-258.
- Trovao, N.S., Baele, G., Vrancken, B., Bielejec, F., Marc A. Suchard, M.A., Fargette, D. and Leme, P. 2015. Host ecology determines the dispersal patterns of a plant virus. *Virus Evolution* 1(1): vev016. doi: 10.1093/ve/vev016.

- UBOS. 2010. Uganda Census of Agriculture 2008/2009. Volume iv: Crop area and production report. pp. 178.
- UBOS. 2019. Crop Area (Ha) and Production (MT) for the AAS 2018 Agricultural year. <https://www.ubos.org>
- Umaharan, P., Ariyanayagam, R.P. and Haque, S.Q. 1997. Resistance to cowpea severe mosaic virus is determined by three dosage dependent genes in *Vigna unguiculata* L. Walp. *Euphytica* 95:49-55.
- Uyemoto, J.K., Clafflin, L.E., Wilson, D.L. and Raney, R. J. 1981. Maize chlorotic mottle and maize dwarf mosaic viruses: Effect of single and double inoculations on symptomatology and yield. *Plant Disease* 65:39-41.
- van Kammen, A., van Lent, J. and Wellink, J. 2001. *Cowpea mosaic virus*. AAB Descriptions of Plant Viruses No. 378. *Association of Applied Biologists* Wellesbourne.
- Verchot, J. and Carrington, J.C. 1995. Evidence that the potyvirus P1 proteinase functions in trans as an accessory factor for genome amplification. *Journal of Virology* 69(6): 3668-3674.
- Wangai, A.W., Redinbaugh, M.G., Kinyua, Z.M., Miano, D.W., Leley, P.K., Kasina, M., Mahuku, G., Scheets, K. and Jeffers, D. 2012. First Report of Maize chlorotic mottle virus and Maize Lethal Necrosis in Kenya. *Plant Disease* 96(10):1582-1582. <https://doi.org/10.1094/PDIS-06-12-0576-PDN>.
- Wetmur, J.G. and Fresco, J. 1991. DNA Probes: Applications of the principles of nucleic acid hybridization. *Critical Reviews in Biochemistry and Molecular Biology* 26:227-259. <https://doi.org/10.3109/10409239109114069>.
- Wessner, D. R. 2010. The Origins of Viruses. *Nature Education* 3(9):37
- Westphal, E. 1974. Pulses in Ethiopia, their taxonomy and agricultural significance. PhD Thesis, Wageningen, The Netherlands. 276 pp.
- Whitney, W.K. Gilmer, R.M. 1974. Insect vectors of cowpea mosaic virus in Nigeria. *Annals of Applied Biology* 77(1):17-21.
- Yan, W. 2005. Use of biplot analysis in crop breeding. In: Swanson, C.S. (Ed.), *Proceedings of the Eastern Wheat Workers and Southern Small Grain Workers Conference*. pp. 7-29, May 9-12, 2005, Bowling Green, KY. University of Kentucky, USA.
- Yan, W., Hunt, L.A., Sheng, Q. and Szlavnyics, Z. 2000. Cultivar evaluation and mega-environment investigation based on the GGE Biplot. *Crop Science* 40:597-605.

- Yan, W. and Rajcan, I. 2002. Biplot Analysis of Test Sites and Trait Relations of Soybean in Ontario. *Crop Science* 42:11-20.
- Yan, W. and Tinker, N.A. 2005. An Integrated biplot analysis system for displaying, interpreting, and exploring genotype $\times$ environment interaction. *Crop Science* 45(3): 1004-1016. <https://doi.org/10.2135/cropsci2004.0076>.
- Yan, W. and Tinker, N.A. 2006. Biplot analysis of multi-environment trial data: Principles and applications. *Canadian Journal of Plant Science* 86(3):623-645.
- Zettler, F.W. and Evans, I.R. 1972. Blackeye cowpea mosaic virus in Florida: host range and incidence in certified cowpea seed. Florida Agricultural Experiment Stations Journal Series No. 4610:99-101.
- Zheng, L., Rodoni, B.C., Gibbs, M.J. and Gibbs, A.J. 2010. A novel pair of universal primers for the detection of potyviruses. *Plant Pathology* 59(2):211-220. <https://doi.org/10.1111/j.1365-3059.2009.02201.x>.

Appendix 1: Mean values of AUDPC, severity, incidence (%) and yield (kg ha⁻¹) for 105 cowpea genotypes evaluated in 2012

Genotype	Budaka			Serere			Tororo					
	AUDPC	Severity	Incidence	Yield	AUDPC	Severity	Incidence	Yield	AUDPC	Severity	Incidence	Yield
EBELAT	62.81	2.41	53.74	1200.00	58.80	2.23	49.55	395.14	48.72	1.81	39.88	420.00
IT00K-835-45	62.12	2.29	55.66	816.67	63.82	2.37	61.61	288.89	40.82	1.54	38.46	200.00
IT03K-124	73.00	2.80	74.71	1122.22	62.56	2.38	61.81	380.56	54.06	1.96	45.74	234.72
IT04K-219-2	63.73	2.23	52.28	848.61	67.24	2.57	63.48	198.61	55.30	2.02	30.59	377.08
IT04K-221-1	61.90	2.31	64.75	691.67	64.40	2.38	48.33	435.42	51.63	1.71	39.49	75.00
IT04K-227-4	88.73	3.15	64.10	550.00	67.90	2.38	54.33	623.61	42.28	1.52	40.61	572.22
IT06K-121	89.25	3.17	80.85	649.24	71.07	2.64	66.31	353.24	65.62	2.42	61.66	329.55
IT06K-123-1	72.73	2.67	70.38	661.11	72.32	2.83	68.71	659.17	62.74	2.13	48.39	275.00
IT06K-124	69.70	2.54	62.71	650.00	61.57	2.28	64.21	326.67	55.80	1.91	51.82	205.56
IT06K-147-1	92.58	3.33	81.51	1243.75	56.12	2.26	62.40	368.75	60.93	2.16	54.98	211.67
IT06K-154-1	91.88	3.17	69.02	595.83	58.68	2.28	46.22	685.42	45.75	1.81	36.77	298.33
IT06K-281-1	68.78	2.39	62.80	988.89	63.00	2.34	57.24	436.11	48.18	2.01	39.32	341.67
IT06K-91-11-1	98.00	3.50	78.19	600.00	82.30	3.04	76.47	621.53	80.34	2.79	61.12	286.11
IT07K-187-24	88.73	3.23	79.48	793.75	72.68	2.54	64.22	336.67	50.40	1.90	53.75	179.86
IT07K-188-49	94.24	3.30	74.44	1000.00	74.76	2.61	62.09	439.17	63.28	2.33	58.10	235.00
IT07K-211-1-8	61.97	2.21	60.64	1325.00	57.67	2.21	63.57	353.47	45.50	1.89	47.10	242.71
IT07K-243-1-5	76.77	2.55	54.79	630.56	61.37	2.42	58.09	350.00	44.80	1.64	31.78	333.33
IT07K-292-10	81.32	2.94	68.49	1123.61	45.50	1.79	43.83	808.33	66.79	2.17	53.81	393.06
IT07K-299-4	84.84	3.10	64.98	536.67	65.68	2.54	66.06	377.08	41.72	1.73	39.90	175.00
IT07K-300-12	82.74	3.04	70.20	616.67	64.75	2.65	74.50	225.00	52.03	2.15	58.27	288.89
IT89KD-288	70.47	2.54	62.07	826.39	60.67	2.40	52.19	640.97	60.14	2.10	44.87	493.06
IT97K-499-35	80.89	2.83	72.50	712.50	66.03	2.47	68.46	213.89	37.80	1.96	44.32	147.50
IT98K-503-1	69.03	2.61	56.01	950.00	59.50	2.14	42.26	420.83	53.08	1.83	38.02	402.78
MU09B	51.10	1.90	29.93	1177.08	57.17	2.10	41.67	904.17	45.92	1.85	34.15	201.67
MU15	48.27	1.94	51.49	888.89	50.75	1.92	47.80	262.50	55.07	1.92	45.79	412.50

Genotype	Budaka			Serere			Tororo		
	AUDPC	Severity	Incidence	Yield	AUDPC	Severity	Incidence	Yield	Yield
MUI7	58.57	2.09	37.42	1461.11	55.42	2.12	42.67	772.92	1125.00
MUI9	44.39	1.74	28.84	680.56	47.72	1.76	27.98	340.28	344.44
MU20	93.10	3.31	79.58	502.78	61.85	2.36	69.85	585.42	191.67
MU20B	61.95	2.21	45.02	613.89	51.92	1.96	44.61	185.42	273.61
MU9	49.00	2.08	33.88	806.25	52.58	1.99	62.89	139.58	423.33
NE13	52.91	2.05	39.63	1058.33	56.12	2.00	42.38	757.64	309.72
NE15	46.73	1.75	24.49	1505.56	53.06	1.98	40.38	831.67	676.39
NE17	48.77	1.80	36.64	1081.94	55.44	2.04	38.66	581.67	265.97
NE18	48.53	1.72	29.74	1262.50	52.03	2.02	39.17	822.22	675.00
NE19	68.39	2.41	59.93	948.61	54.02	2.12	48.66	375.00	372.22
NE23	51.57	1.88	30.75	1641.67	57.40	2.16	41.18	497.50	330.56
NE30	48.77	1.84	36.66	884.72	52.97	2.01	44.44	647.22	430.56
NE31	57.19	2.19	49.12	1140.28	53.90	2.12	45.80	577.78	643.06
NE32	49.12	1.78	29.57	1250.00	55.58	2.12	39.83	796.67	413.89
NE36	47.02	1.80	26.78	686.11	51.57	1.93	40.90	751.39	419.44
NE37	47.80	1.92	40.39	1884.72	46.38	1.80	34.12	781.25	803.47
NE39	45.73	1.69	28.65	937.50	53.55	2.12	46.57	399.31	484.72
NE4	61.09	2.24	48.17	1048.61	58.07	2.17	54.27	726.67	272.22
NE40	61.95	2.23	45.11	1306.94	56.82	2.25	42.25	458.33	734.72
NE41	47.72	1.87	27.94	1126.39	57.12	2.12	36.73	550.83	438.89
NE42	52.38	2.20	48.62	997.22	59.08	2.17	35.68	810.83	515.28
NE43	49.35	1.84	66.21	669.44	47.53	1.71	56.80	432.64	513.89
NE44	53.55	2.04	39.44	884.72	51.22	1.93	39.69	425.00	333.33
NE45	56.12	2.03	42.44	1404.17	55.44	2.16	49.34	931.67	245.83

Appendix 1 continued

Genotype	Budaka			Serere			Tororo		
	AUDPC	Severity	Incidence	Yield	AUDPC	Severity	Incidence	Yield	Yield
NE46	101.03	3.77	82.51	1569.44	92.35	3.44	73.98	768.06	450.00
NE48	49.12	1.93	35.73	1511.11	48.83	1.87	42.17	1127.08	936.11
NE49	62.86	2.28	48.60	788.33	56.51	2.05	36.05	525.69	513.89
NE5	50.14	1.88	44.26	1243.06	56.98	2.16	43.63	393.33	236.67
NE50	42.84	1.69	35.01	1096.67	60.90	2.12	48.34	1270.83	561.11
NE51	51.10	1.94	41.83	1650.00	51.38	1.94	40.98	795.00	822.22
NE53	54.02	2.24	49.99	916.67	51.68	2.02	43.60	568.75	238.89
NE55	64.05	2.39	51.08	1070.83	56.28	2.14	58.40	456.67	783.33
NE6	56.71	2.06	44.24	1081.94	47.26	1.83	45.91	459.03	398.61
NE67	49.19	1.93	38.77	1084.72	50.87	1.86	34.13	699.31	638.89
NE70	55.18	2.00	38.36	715.28	59.23	2.25	52.14	1176.39	505.00
NE71	60.20	2.19	48.37	1150.00	51.45	2.03	45.10	372.22	245.83
SECOW2W	46.32	1.76	27.76	1401.39	48.30	1.80	43.15	1013.89	469.72
WC1	42.93	1.68	30.97	837.50	51.33	1.89	31.56	563.89	633.33
WC10	58.33	1.96	40.52	855.56	49.23	1.81	42.36	568.06	395.83
WC11	48.18	1.88	38.56	491.67	53.20	1.90	37.97	339.58	326.39
WC12	41.65	1.74	34.93	1031.94	55.30	1.99	36.00	341.67	497.22
WC13	62.88	2.27	36.31	801.39	54.83	2.00	39.41	222.22	513.89
WC15	57.40	2.19	42.37	545.83	51.33	1.94	39.47	427.08	536.11
WC16	57.05	2.15	45.58	722.22	50.40	1.83	34.27	334.72	430.56
WC17	54.72	1.99	43.81	722.22	52.64	1.93	44.93	700.00	605.56
WC18	42.58	1.64	22.80	902.78	44.94	1.61	27.91	335.83	437.50
WC2	49.00	1.91	46.41	1686.11	45.85	1.76	28.97	1011.81	431.94
WC21	45.97	1.79	34.93	906.94	48.65	1.93	40.41	579.86	504.63

Appendix I continued

Genotype	Budaka				Serere				Tororo			
	AUDPC	Severity	Incidence	Yield	AUDPC	Severity	Incidence	Yield	AUDPC	Severity	Incidence	Yield
WC26	47.37	1.85	30.38	918.06	48.02	1.77	34.40	344.17	37.45	1.44	12.52	606.25
WC27	46.55	1.81	43.30	1152.78	50.40	1.90	37.04	500.69	52.66	1.78	28.00	283.33
WC29	49.23	1.95	41.30	802.78	47.60	1.95	42.89	584.72	46.51	1.69	24.93	479.63
WC30	46.67	1.90	36.11	836.11	53.55	1.95	34.44	418.06	51.80	1.89	31.33	762.50
WC32	43.75	1.66	44.00	1266.67	54.48	2.01	40.66	759.72	38.62	1.45	27.61	1222.22
WC33	40.68	1.62	25.58	1108.33	46.32	1.74	32.89	779.86	41.13	1.49	14.43	345.83
WC35A	39.08	1.57	18.58	711.11	51.33	1.88	31.64	834.03	42.35	1.50	19.98	747.22
WC35B	53.67	2.02	58.79	1159.72	49.54	1.87	54.07	578.47	33.95	1.27	48.40	454.17
WC35C	39.90	1.59	26.48	1205.56	49.70	1.71	29.21	635.42	43.98	1.48	17.05	463.89
WC36	60.67	2.38	49.82	1183.33	51.22	1.94	35.76	603.47	47.13	1.81	27.14	595.83
WC39	41.16	1.54	29.51	1635.00	49.79	1.85	29.54	584.72	42.00	1.39	18.52	530.56
WC4	49.35	1.83	37.89	995.83	53.90	1.92	33.85	430.56	42.54	1.50	17.21	701.85
WC41	50.52	1.98	33.75	641.67	51.33	1.88	40.26	350.00	47.72	1.69	32.37	441.67
WC42	50.17	1.82	43.13	1576.39	57.82	2.18	55.73	665.00	51.33	1.68	34.55	902.78
WC44	48.88	1.89	33.92	905.56	45.78	1.74	38.10	335.00	48.42	1.71	34.24	413.89
WC48	44.23	1.67	32.43	1304.17	45.97	1.67	24.83	753.47	29.13	1.15	13.65	537.96
WC5	56.00	2.14	38.27	536.11	55.18	2.07	41.69	379.86	52.15	1.85	30.91	181.94
WC51	52.27	1.90	36.23	1926.39	53.83	1.95	48.34	1312.50	38.85	1.39	20.78	915.28
WC52	62.30	2.51	42.96	768.06	45.97	1.69	31.28	479.17	54.66	2.11	36.88	556.94
WC53	47.37	1.78	41.32	1455.56	47.02	1.81	39.22	890.28	39.36	1.59	28.76	613.89
WC55	58.79	2.22	43.46	1343.06	59.33	2.16	48.44	829.17	41.07	1.51	21.13	309.03
WC6	61.95	2.31	32.50	686.11	54.60	1.99	40.98	191.67	47.25	1.77	25.78	325.00
WC62	60.20	2.20	47.67	876.39	50.05	1.90	34.16	386.81	47.25	1.60	26.07	304.17

Appendix I continued

Genotype	Budaka				Serere				Tororo			
	AUDPC	Severity	Incidence	Yield	AUDPC	Severity	Incidence	Yield	AUDPC	Severity	Incidence	Yield
WC63	43.25	1.64	38.17	1081.94	56.56	2.04	41.15	767.50	42.70	1.57	33.75	538.89
WC64	55.77	2.05	38.13	840.28	48.77	1.77	39.79	567.36	50.17	1.85	33.29	779.17
WC65	52.08	2.01	36.12	1205.00	53.20	1.97	31.15	618.75	45.73	1.60	21.88	425.00
WC66	46.78	1.90	35.82	911.11	50.75	1.90	35.74	636.81	48.30	1.57	16.98	497.22
WC67	49.00	1.87	47.91	751.39	53.08	1.90	38.63	445.83	48.30	1.70	32.85	515.00
WC67A	46.43	1.88	35.57	1245.83	59.22	2.15	44.13	701.67	42.10	1.42	30.69	345.24
WC68	54.83	2.13	46.21	886.11	49.47	1.85	40.48	384.03	48.58	1.78	20.59	620.00
WC69	60.55	2.24	52.53	1252.78	51.33	2.05	39.11	280.56	47.25	1.62	22.78	541.67
WC7	66.62	2.60	44.72	868.06	52.73	2.02	38.64	336.81	58.92	2.14	37.51	357.64