GENETIC DIVERSITY AND RESISTANCE TO CASSAVA BROWN STREAK DISEASE IN CENTRAL UGANDA

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DECLARATION

I, OGWAL SAM hereby declare that this is my original work and has never been submitted to any University or institution of higher learning for award.

Signed.....

Date.....25/11/2019.....

APPROVAL

This is to certify that this work was conducted under our supervision as University supervisors and is now ready for submission for examination.

Signed.....

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Date....26/11/19.....

Signed.....

Dr. Jolyn Runyararo Rukarwa

Regional University Forum for capacity building in Agriculture (RUFORUM).

Date.....

DEDICATION

This work is dedicated to my parents the late Charles Dickens Obong and Mrs. Concy Obong, brothers (Amwata Benson, Atine Godfrey, Okullo Obong Geoffrey, Obong Morton), sisters (Apio Loyce, Achola Carolin, Ejang Susan, Alele Shara and Arao Kay Ruth), wife Jolly Ogwal and children Auma Lucky Ogwal, Apio Charity Ogwal, Obong John, Amwata Benjamin, Okullo Phliphs, Ayo Roy Musa and Akite Juliet.

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ACRONYMS

CBSD	Cassava brown streak disease
CBSV	Cassava brown streak virus
COX	Cytochrome oxidase
CP	Coat protein
CTAB	Cetyltrimethyl ammonium bromide
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
dsRNA	Double strand ribonucleic acid
FAO	Food and agriculture organization
IC-PCR	Immuno Capture-Polymerase Chain Reaction
MAbs	Monoclonal antibodies
IITA	International institute of Tropical Agriculture
NaCRRI	National crop resources research institute
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
RNAi	Ribonucleic acid interfering
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
siRNA	Small interfering ribonucleic acid
ssRNA	Single stranded ribonucleic acid
TAE	Tris acetate
UBOS	Uganda Bureau of Statistics
UCBSV	Uganda cassava brown streak
VSRs	Virus suppressors of RNA silencing

ABSTRACT

Cassava (*Manihot esculenta* Crantz), a common food security crop in many sub-Saharan African countries is being threatened by cassava brown streak disease (CBSD) which causes up to 100% yield loss in susceptible varieties .Cassava brown streak disease is caused by two distinct *Ipomoviruses* namely Cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV Cassava brown streak disease causes symptoms characterised by chlorotic patches on leaves, dark brown "streaks" on stems, cracked constricted corky, yellow-brown necrotic root tubers of cassava. However, variability of symptoms exists within cassava genotypes and CBSV strains depending on the environmental conditions. Currently in central Uganda, there is limited information on CBSV genetic and symptom diversity, as well as sources of resistance to CBSV. Yet development of CBSD control strategies requires knowledge of CBSV genetic diversity, virus-host interaction and availability of resistant cassava germplasm to CBSD. The objective of this study was therefore to 1) assess genetic diversity and distribution of cassava brown streak disease causal viruses in central Uganda. 2) unravel the symptom diversity of CBSD causal viruses on selected cassava genotypes. 3) identify potential sources of resistance to cassava brown streak disease in central Uganda.

Symptomatic leaf samples collected from the four districts in central Uganda were used for total RNA extraction using CTAB protocol. The purified samples were subjected to PCR amplification of specific DNA sequence by *in vitro* DNA synthesis done as prescribed by Mullis and Faloona (1988). PCR products were separated using electrophoresis and the sequencing of PCR products was done in Netherland at Macrogen laboratory and the sequenced products were edited using Edit Seq in DNA Star, aligned using clustal logarithm MEGA 6 software and coat protein nucleotide sequence data analysed using AMOVA. The results revealed that there was variation in nucleotide sequence and the coat protein among and within CBSV strains with isolates clustering into two groups. Additionally, CBSV was the dominant strain, followed by UCBSV and last mixed infection among 18 out of 20

symptomatic leaves sampled. Symptoms diversity and sources of resistance were identified by inoculating disease free cassava grown in screen house with CBSV strains singly and in combinations using chip bud method of grafting. Symptom diversity was identified based on the dominant symptoms from symptoms appearance at 1 MAI to 3 MAI. For resistance, incidence and severity was assessed. Later severity scores were used to compute area under disease progress curve (AUDPC). Findings showed that there was significant difference ($P<0.001$) in reaction of cassava genotypes to CBSV viral strains. In addition, significant variety x viral strains interactions ($P<0.001$) was observed. The highest CBSD incidence and severity was recorded from Kwatampola and the lowest in NASE14. Overall, NASE 14 and NAROCASS were resistant while Aladu, NASE12, Njule, Bamunanika and Bukalasa were moderately susceptible.

Symptom diversity and severity was scored visually from symptom appearance and continued at monthly intervals for three months using a scale of 1 – 5. Findings showed Variation in symptoms of CBSD existed on leaves and stems of cassava genotypes inoculated with CBSV, UCBSV and CBSV+UCBSV strains. Infact, the symptoms varied from chlorotic spots on leaf tips to mild chlorosis, mottling of secondary to primary veins and mild stem streaks. It was concluded that CBSV and UCBSV were dominant strains of CBSV. Additionally genotype NASE 14 was most resistant to CBSD. Accordingly CBSD symptoms varied from chlorotic spots to mild stem streaks. This study has an implication on the management of CBSD in Uganda. However another study to assess the relationship of CBSV viral load, CBSD symptom severity and resistance of cassava genotypes needs to be conducted.

CHAPTER ONE: INTRODUCTION

1.1: Background to the study

According to FAO (2004) global cassava production projection are likely to remain around 192 million tones up to 2020. Yet, estimates from larger producing countries in Africa point production level of 103 million tones. However, in Asia, increase in production is expected to rise largely due to high domestic and export prices whereas in Latin America and the Caribbean, cassava output was forecasted to increase to 23.9 million tons in 2005.Accordingly, the global projections of supply, demand, trade for roots and tubers to the year 2020 is forecasted to contribute to a clearer vision of the roles that cassava and other root crops can play in the food systems of developing countries in the decades ahead (Dunstan and Spencer.,2017).

Table 1: Global cassava production projection for the year 2020

Country	Production (000,000ton	Base line (000,000ton	High growth (000,000ton
Africa	88.06	168.54	184.04
Asia	42.35	47.95	48.30
Latin America and the Caribbean	30.51	42.93	50.49
World total	160.92	259.42	282.83

Source: Danstan and Spencer (2017)

According to Dunstan and Spencer (2017), Asia and sub-Saharan Africa are the main centers for production and utilization for individual root and tuber crop in the whole world.

Cassava (M.*esculenta*) is thought to have been directly derived from wild sub –species (Scott *et al.*, 2000). Cassava was domesticated in the southern Amazon basin and later introduced into Africa in the 17th century (Hillocks *et al.*,2003).The common varieties of cassava in Uganda are NAROCASS,TME 204,NASE12,NASE14,Njule,BAO,Nigeria,Bukalasa (Abele et al ., 2007).

1.2: Importance of cassava

Cassava is a major staple food for about 800 million people in the tropics and sub-tropical regions of the world (Raji *et al.,* 2008). Infact estimates show that about 160 million people of the population of sub-Saharan Africa alone consume cassava as a staple food (FAO, 2009; UBOS, 2010). Cassava is grown throughout sub-Saharan Africa where it ranks second in importance to maize as human staple food (FAO, 2013), Cassava is an ideal famine reserve crop providing household income, poverty alleviation and employment generation for man (Hillocks *et al.,* 2001; Katz and Weaver, 2003; Kenyon *et al.*, 2006; UNCST, 2009; UBOS, 2010; Mbanzibwa *et al.,* 2011., Gwandu, 2014; Onyenwoke *et al.*, 2014 and FAO, 2015).

Although, described as a subsistence crop for rural households, cassava is increasingly becoming a cash crop in Africa. According to Nweke *et al.* (2002), cassava is a famine-reserve crop and to a minor extent, raw material for feed and products exported for many industrial uses in Africa, South America and Asia. However, about 88% of cassava produced is used for human food (Hillocks *et al.*, 2001). Accordingly, cassava production and consumption continues to grow in Africa, Asia and South America (Hillock *et al.*, 2003). However , 56.8 % of the global cassava farmers are grappling with the challenge of two deadly virus diseases namely, cassava mosaic disease and cassava brown streak disease respectively (Nweke *et al.*, 2013). Cassava brown streak disease was first identified in 1936 in Tanzania although it has now spread to the other coastal areas of east Africa from Kenya to Mozambique (Ntawuruhunga *et al.*, 2007) to the high altitude areas such as Rwanda,

Burundi, Democratic Republic of Congo and Uganda (Mulimbi *et al.*, 2012; Tomlinson *et al.*, 2013). Globally, cassava is currently used for two main purposes namely, human food and industrial materials. Primary industrial products from cassava are cassava flour, crude ethanol, native starch, and animal feed/cassava chips/pellets while the secondary products from cassava include modified cassava starch, glucose syrup, extra neutral alcohol, bakery, confectionery, meat and textile processing, coating of pharmaceutical products, manufacture of glues/adhesives and oil drilling starch (UNCST ,2009).

According to UNCST (2009) about 74% of farm households grow cassava for food in Uganda. The roots of sweet cassava varieties are eaten raw, roasted, fried or boiled. Fresh cassava roots may be sliced, grated, fermented or pounded, then dried and further processed into dried chips and balls. The bitter type of cassava (cynogenic) can only be used after fermentation. The dried chips are milled into flour which can be used alone or as a composite with millet, sorghum and maize flour to make a pasty product commonly called *ugali*, *atap* or *kalo* in Uganda (FAO,1990).

1.3: Cassava production

According to FAO (2015), world cassava production was 289 million tons in 2015 but was forecasted to increase by 12.9 percent by 2025. According to Nteranya (2015), cassava is one of the world's most important food crops, with annual global production at approximately 276 million metric tons (MT) in 2013. The top cassava producing countries globally in 2017 were as indicated in the Table 2.

Table 2: Top world cassava producing countries in 2017

Rank	Area	Production cassava(tons)
1.	Nigeria	47,406,770
2	Thailand	30,227,542
3	Indonesia	23,936,920
4	Brazil	21,484,218
5	Angola	16,411,674
6	Ghana	15,989,940
7	Democratic Republic of the Congo	14,611,911
8	Viet Nam	9,757,681
9	Cambodia	7,572,344
10	India	7,236,600
11	Malawi	4,813,699
12	United Republic of Tanzania	4,755,160
13	Cameroon	4,596,383
14	China, mainland	4,585,000
15	Mozambique	4,303,000
16	Benin	3,910,036
17	Sierra Leone	3,810,418
18	Madagascar	3,114,578
19	Uganda	2,979,000
20	Rwanda	2,948,121

Source: FAOSTAT (2019)

Cassava production and consumption continues to grow in Africa, Asia and South America (Hillock *et al.*, 2002). For instance, 163 million tons of cassavas are estimated to be produced in Africa annually (Amisse, 2013). Cassava is cultivated in around 40 African countries, stretching from Madagascar in the southeast to Senegal and Cape Verde in the northwest (IFAD and FAO, 2000).However, major cassava producers in Africa are Nigeria, Madagascar, Ghana, Angola, Mozambique, Malawi, Uganda, D.R. Congo and Benin(FAO, 2014., Nteranya , 2015) as shown in table 3 below. In Uganda, the leading cassava growing districts include Apac, Tororo, Masaka and Hoima (UBOS, 2015). According to Fermont *et al.,* (2009), higher yields per unit area are reportedly obtained in central and eastern Uganda under good management practices.

Table 3: Major cassava producers in Africa in the year 2013

Country	**Cassava production in metric tones**
Nigeria	54,831,600
Madagascar	30,878,100
Ghana	16,534,000
Angola	10,106,000
Mozambique	5,114,750
Malawi	4,910,810
Uganda	2,812,000
Democratic Republic of Congo	1,660,890
Benin	1,370,092

Source: FAO (2014).

1.4: Constraints to cassava production

Cassava is the most important food crop in Africa which is strongly affected by genetic erosion, infestation by pests and diseases. According to Abate *et al.* (2000), cassava is plagued by a host of production constraints causing up to 100% yield loss. Diseases and pests constitute the most important biotic constraint to cassava production in East Africa (Mbanzibwa *et al.,* 2011). The most widespread and devastating diseases of cassava are CMD and CBSD. The other diseases of cassava include stem rot, anthracnose, bacterial blight and the brown leaf spot (Hahn and Keyser, 1985; Thomas *et al.*, 1986; Guthrie, 1987; Silvestre, 1989; Alaux and Fauquet, 1990; Hong *et al.*, 1993; Swanson and Harrison, 1994; Legg and Thresh, 2003).

However, in the recent years, the main biotic constraint to cassava production in Uganda is CBSD (Winter *et al.,* 2010; Rwegasira *et al.*, 2011). CBSD alone is reported to cause over 80% losses under farmers conditions (Kumakech *et al.,* 2013; Nuwamanya *et al.*, 2015). A biotic factor includes inadequate rains and soil infertility (FAO, 2015). Cassava production is also affected by socio economic factors such as agronomic problems, land degradation, shortage of planting materials, access to markets, limited processing options and inefficient/ ineffective extension delivery systems (Yuguda *et al.*, 2013). Yield of cassava harvested under optimal experimental conditions (over 80 tons/ha) and the average yield harvested by African farmers of around 8-12 tons/hectare indicates numerous limiting factors in cassava subsistence agriculture FAO (2013).

1.5: Economic importance of CBSD

In East and central Africa productivity is significantly constrained by two viral diseases, cassava mosaic disease (CMD) and cassava brown streak disease (CBSD), which together are estimated to cause annual losses worth US$1 billion (IITA, 2014a) and adversely affect food security in the entire region (Patil *et al.*, 2015). CBSD causes significant losses in both yield

and quality of cassava storage roots (Patil *et al.,* 2015) and poses a serious threat to food and income security because crop yields can be reduced drastically to as high as 70% (Maruthi *et al.,* 2014) and quality of edible storage roots by up to 100 % (Hillocks *et al.*, 2001). It is estimated that African farmers collectively lose revenue ofup to $100 million annually due to the devastating disease (Gwandu, 2014). According to (Refs), in Uganda, the overall incidence of CBSD per district is 20% compared to the time when the CBSD epidemic was first observed in 2004 (Alicai *et al.*, 2007).CBSD is currently widespread in the Eastern, Central and Southern Africa region in countries such as Burundi, DRC, Kenya, Malawi, Mozambique, Rwanda, and Zambia (Alicai *et al.*, 2007; Rwegasira, 2009; Bigirimana *et al.*, 2011).In fact, CBSD is now occurring in areas that were believed to be unsuitable for the disease such as high altitude areas away from the Indian coastal belt in Kenya, Tanzania and Mozambique (Alicai *et al.,* 2007; Jeremiah, 2008; Bigirimana *et al.*, 2011; Mulimbi *et al.*, 2012 and Tomlinson *et al.*, 2013).

1.6: Statement of the Problem

Whereas cassava is important for food security and poverty alleviation in Uganda and other parts of the world (FAO, 2009; UBOS, 2010; Burns *et al.*, 2010; Mbanzibwa, 2011; FAO, 2015), the crop has been severely attacked by *Ipomoviruses* CBSV responsible for inciting cassava brown streak disease (CBSD). Cassava brown streak disease is very devastating because it makes storage roots unfit for human consumption and can lead to total yield loss (Legg *et al.*, 2011; Maruthi *et al.,* 2014; Patil *et al.,* 2015). Mbanzibwa *et al.* (2011) report on the distribution of the two strains indicates that UCBSV is predominant in Uganda while there is even distribution between UCBSV and CBSV in other regions around Lake Victoria and lowland of Tanzania. Also, the study by Kathurima *et al.,* (2016) indicated that UCBSV is the most predominant strain in Kenya and its coastal region hitherto thought to be infested by CBSV strain only). Although, breeding for resistance is one of the strategies

recommended for controlling CBSD (Munga, 2008), it requires clear understanding of the interactions between CBSV and cassava genotypes However, information on the genetic diversity of the causal organisms and sources of resistance including symptom diversity of the different strains of virus in Uganda is scanty and limited. . Yet, it is vital to identify isolates which can be used to screen for resistance and hence the development of materials with durable resistance to CBSD. It is therefore against this background that this study was undertaken to unravel the genetic diversity of CBSD causal viruses, symptom diversity of CBSD causal viral strains and resistance to CBSD in central Uganda.

1.7: Justification of the study

Although, CBSD was first reported in 1936, it has received much less attention than CMD. The etiology and epidemiology of CBSD are not fully elucidated, yet the alarming rate of spread is worrying to the livelihoods, food security and loss of diversity of cassava varieties (Hillocks and Jennings, 2003). In response to the challenge, it was highly recommended to unravel the genetic diversity of the causal virus, screen local and introduced cassava germplasm against CBSD so that resistant genotypes or genes for resistance to CBSD can be identified and used to introgress genes for resistance into the preferred cultivars (NARO, 2006The knowledge on the diversity of the pathogen strains is vital in determining its evolution and virulence while information on different cassava genotypes resistance genes will be useful in breeding cassava plants that are resistant. Thus, renewed measures to identify, characterize, and preserve CBSD resistance in cassava germplasm are required for sustainable disease management strategies (Ravi *et al.*, 2016).

1.8.0: General objective

The overall objective of this study was to unravel the genetic diversity of CBSV and resistance to cassava brown streak disease in Uganda.

1.8.1: Specific objectives

(i) To assess genetic diversity and distribution of cassava brown streak disease causal viruses in central Uganda.

(ii) To unravel the symptom diversity of CBSD causal viruses on selected cassava genotypes.

(iii)To identify sources of resistance to cassava brown streak disease in central Uganda.

1.8.2: Hypotheses

(i) The diversity of CBSD viral strains exist in central Uganda.

(ii) Cassava brown streak disease manifests as a syndrome in different cassava genotypes depending on the viral strain.

(iii)Sources of resistance to CBSD exist among cassava genotypes in central Uganda.

1.9: Significance of the study

The findings from this study will help in improving cassava production through:-

(i) Identification of the prevalent CBSV isolates, distribution of CBSV strains in central Uganda to help plant pathologists monitor the trend in CBSV existence evolution and specificity of host infection.

(ii) To develop understanding of the CBSD incidence, symptom diversity and severity of CBSD with different CBSV strains which are useful in screening resistant cassava genotypes by breeders and set policy guidelines for selection of clean planting materials by farmers.

(iii) Identification of cassava genotype that is resistant to CBSD for plant breeders to develop more resistant varieties through breeding.

CHAPTER TWO: LITERATURE REVIEW

2.1: History of Cassava brown streak disease

CBSD is a devastating disease that causes loss of cassava root (tuber) production and quality. It can render susceptible varieties unusable if cassava roots are left in the ground for over nine months. CBSD is a threat to the major cassava growing countries such as Burundi, Democratic Republic of Congo, Madagascar and it is likely to move to West African belt which produces over half of the cassava on African continent through sharing of infected planting materials (Mahungu *et al.*, 2003; Alicai *et al.*, 2007).CBSD was first reported to occur in Tanzania in 1936 at the foothills of Usambara Mountains (Story, 1936). Historically CBSD was known to be endemic to the coastal lowlands of Eastern Africa lying below 500 meters above sea level. It was not until 2007 and 2009 when the disease was reported at higher altitude (1200 meters above sea level) in Uganda and Tanzania, respectively (Rwegasira, 2009). CBSD is now occurring in areas that were believed to be unsuitable for the disease such as high altitude areas away from the Indian coastal belt in Kenya, Tanzania and Mozambique (Alicai *et al.*, 2007; Jeremiah, 2008; Bigirimana *et al.*, 2011; Mulimbi *et al.*, 2012 and Tomlinson *et al.*, 2013).CBSD is currently widespread in the Eastern, Central and Southern Africa region in countries such as Burundi, DRC, Kenya, Malawi, Mozambique, Rwanda, and Zambia (Alicai *et al.*, 2007; Rwegasira, 2009; Bigirimani *et al.*, 2011). In Uganda CBSD was first reported at Bukalasa government research station in 1945 when infected material was taken from Tanzania in 1936, but the disease was eradicated by destroying all plants showing symptoms and use of six month quarantine (Nichols, 1950);Jameson, 1964; Alicai *et al.*, 2007). However, it re –occurred in November 2004, where symptoms were observed on cassava at Mukono in central Uganda (Alicai *et al.*, 2007; Rwegasira *et al.*, 2011; Mbanzibwa, 2011)

2.1.1: Distribution of Cassava brown streak disease in East Africa

Cassava brown streak disease (CBSD) is caused by Cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV) (Legg *et-al.,* 2011). Mbanzibwa *et al.* (2011) reported that UCBSV is predominant in Uganda while there is even distribution between UCBSV and CBSV in other regions of East Africa, around Lake Victoria and lowland of Tanzania. Also, the study by Kathurima *et al.* (2016) indicated that UCBSV is the most predominant strain in Kenya and its coastal region hitherto thought to be infested by CBSV strain only. In fact, the recent outbreak of CBSD in mid-altitude areas of the great lakes region of east and central Africa (Alicai *et al.*, 2007; Mbanzibwa *et al.*, 2011) have demonstrated that there is no intrinsic barrier to the spread of the CBSD-causing viruses at elevations above 1000 meters above sea level. However, the widespread nature of UCBSV compared to CBSV which is a more virulent isolate remains unclear (Mulenga *et al.*, 2018).

2.1.2: Genetic diversity of cassava brown streak disease causal virus

CBSD is caused by Cassava brown streak virus(CBSV) and Uganda cassava brown streak virus(UCBSV)strains (Calvert and Thresh, 2002; Mbanzibwa *et al.*,2009; Monger *et al.*,2010; Winter *et al.*,2010; Rwegasira *et al.,* 2011., Bua *et al.*, 2013; Nuwamanya *et al.*, 2015). Phylogenetic analysis of 12 complete genome sequences available in the GenBank indicates that CBSD is caused by two distinct virus strains with several populations within each strain (Monger et al., 2001; Winter. 2010). CBSV's share 69.0 – 70.3% nucleotide, and 73.6 – 74.4% amino acid sequence identity, respectively (Mbanzibwa, 2011). Nucleotide identity between CBSV and UCBSV is ~70%, whereas it ranges from ~87 – 99% and ~79 – 95% among UCBSV and CBSV isolates, respectively (Mbanzibwa, 2011). Considerable variation between the genomes of CBSV and UCBSV, together with the presence of several isolates within each virus strain indicates that the viruses are evolving fairly rapidly. Recent reports from Malawi (Mbewe *et al.*, 2014) and Mayotte Island (Roux-Cuveller *et al.*, 2014)

showed that UCBSV is more prevalent compared to CBSV and revealed the presence of intermediate isolates in both geographical locations, which cluster between previously described isolates of UCBSV and CBSV.

2.1.3: Detection of CBSD causal virus

A study by Mohammed *et al.* (2012) in east Africa reveal the existence of two species of CBSV (CBSV and UCBSV) that were distinguished using virus-specific primers which amplified CBSV(344 bp) and UCBSV (440 bp), respectively. The deduced amino acid sequences for the complete coat protein (CP) of CBSV and UCBSV isolates consisted of 378 and 367 bases, respectively. The parsimony analysis grouped the CP amino acid sequences into two major clusters (CBSV and UCBSV) where members within each group were conserved with an average amino acid similarity of 95.8% for CBSV group, 96.6% similarity for UCBSV group and the similarity between the two groups to each other by only 80.3% (Ndunguru *et al.,* 2015)

2.1.4: Diversity of CBSD causal virus

A considerable variation in partial coat protein of CBSV isolates has been found in Rwanda among samples collected in east Africa. For instance, Monger *et al.* (2001), reported variation within CBSV isolates collected from Tanzania and Mozambique which has been further substantiated by a phylogenetic analysis of partial coat protein gene sequences of isolates from Tanzania (Mbanzibwa *et al.*, 2009). In fact, later studies have demonstrated that CBSD is caused by isolates of at least two phylogenetically distinct species of single stranded RNA (ssRNA) filamentous virus particles belonging to the Potyviridae family and genus Ipomovirus. Indeed, the comparison of coat protein (CP) and full length sequences of the isolates lead to the description of the two genetically distinct populations of CBSV causing CBSD (Winter *et al.*, 2010; Mbanzibwa *et al.*, 2011).

Additionally, Ndunguru *et al.*(2015) reported that the complete genomes of the various

CBSV and UCBSV isolates range from 8,900 to 10,818 nt in length, sequence differences exists between CBSV and UCBSV hence the existence of wide genetic diversity among the isolates of CBSV (79.3–95.5% at nt level) than UCBSV (86.3–99.3%) in cassava growing regions of Tanzania. Accordingly, CBSV isolates are more genetically diverse than UCBSV (Mbanzibwa *et al.*, 2009; Ndunguru *et al.*, 2015; Alicai *et al.*, 2016). However, Legg *et al.* (2015) reported that UCBSV is more widespread than CBSV in eight countries against four districts with CBSV. Interestingly, Alicai *et al.* (2016) observed that CBSV has a faster rate of evolution compared to UCBSV. According to Adams *et al.* (2005), members of *Potyviridae* share similar genomic organization, with levels of amino acid identity in polyproteins ranging from 42 to 56% among different species of the same genus and from 25 to 33% among viruses from different genera.

Also, the high level of genetic diversity of CBSV isolates has been produced by variations in symptoms in indicator plants (*Nicotiana benthamiana*). Research done by Monger *et al.*, (2001) on the diversity of short coat protein (CP) sequence comparisons revealed only 8% differences in nucleotides and 6% differences in deduced amino acids, respectively. Furthermore, comparisons of isolates from Mozambique reveal similar level of sequence divergence (Amisse *et al.*, 2019). This was found to be consistent with the fact that the two strains of CBSV that are responsible for the CBSD are CBSV and UCBSV, respectively (Bua *et al.*, 2013; Nuwamanya *et al.*, 2015). However, the extent of mixed infections compared to single infections across different cassava genotypes is unknown (Maruthi *et al.*, 2005).

2.2: Symptoms of CBSD

CBSD manifests on cassava leaves as yellow /chlorotic spots or mottling, stem streaks and brownish leaf stalk scars and corky necrotic root tubers (Hillocks and Thresh, 2000). However, some cassava plant varieties may not show symptoms on leaves or tubers; other varieties may only express symptoms on leaves and not on roots; while others do not show symptoms on leaves but on roots only (Omongo *et al.*, 2000; Mohammed *et al.*, 2012). According to Mbanzibwa (2011); Nuwamanya *et al.* (2015), the CBSD symptoms are variable depending on different factors such as age of plant, cultivar, geographical location and environmental conditions.

On the leaves, the symptoms of the CBSV include; chlorosis on mature (bottom) leaves than younger ones. Compared to cassava mosaic virus, the leaves infected with CBSV do not become distorted in shape; chlorosis is often also associated with secondary veins; at the advanced stages of the CBSV, irregular yellow blotchy chlorosis that is most pronounced in the periphery (margins or edge) of lower leaves is usually evident (Alicai *et al.*, 2007).

On the stem, the symptoms are; dark brown "streaks" and "spots" on stems, with dead spots on leaf scars; most common on upper, green portions of the stem; streaks may appear as scratch-like wounds on stems; and the diseased plant may show shoot tip death, which may progress into cassava stem die-back (Alicai *et al.*, 2007).

On the root tubers the symptoms include; cracks and discoloration in the storage roots; root constriction and malformation; root rot becomes evident; harvested roots have corky, yellow-brown necrotic spots; dry, hard rot which is irregular, yellow blotchy chlorosis; necrosis is most pronounced in the edges of the root when the root/tuber is cut across with a knife (Hillocks *et al.*, 2001, 2002).

2.2.1: Symptom diversity of CBSD causal viral strains on cassava genotypes.

Cassava affected by CBSD in the field shows diverse symptoms. However, yellowing of the small veins is commonly observed on older leaves, but is less obvious on younger leaves and absent on the top most leaves. Brown lesions can be observed on stems, and brownish necrotic regions seen within the edible storage roots. In fact, some cassava genotypes display very severe symptoms including complete necrosis of storage roots and stem dieback, while others show mild symptoms. In some cases, symptoms are restricted to a few branches, with the rest of the infected plant remaining symptomless (Hillocks *et al.,* 2003). According to Nuwamanya *et al.* (2015), the CBSD symptoms are variable depending on a number of factors including age of plant, cultivar, geographical location and environmental conditions. For example, earlier, Mohammed *et al.* (2012) observed that some cassava plant varieties may not show symptoms on leaves or tubers; other varieties may only express symptoms on leaves and not on roots; while others do not show symptoms on leaves but on roots only.

Key symptoms of CBSD appear on leaves with varying patterns of chlorosis on margins, veins resulting into blotches; symptoms on stems are; dark brown "streaks" and "spots" , with dead spots on leaf scars; on upper, green portions of the stem; and the diseased plant may show shoot tip death, which may progress into cassava stem die-back (Alicai *et al.*, 2007). Root tubers the symptoms include; cracks and discoloration in the storage roots; root constriction, root rot, root tubers have corky, yellow-brown necrotic spots; dry, hard rot which is irregular, necrosis is most pronounced at the edges of the root when the tuber is cut across with a knife (Hillocks *et al.*, 2002). Distinct symptoms are produced on cassava and indicator hosts by CBSV. CBSV strain causes more severe root necrosis and feathery chlorosis along vein margins, which develops into chlorotic blotches, whereas UCBSV causes circular chlorotic blotches between veins in cassava (Nichols, 1950; Winter *et al.*,

2010; Mohammed *et al.*, 2012).According to Monger *et al.*, (2001), cassava cultivars exhibit different symptoms when affected with CBSD. However, no clear research on the reaction of cassava cultivars to the two strains of CBSV in east Africa (Tadeo *et al.,* 2014) and information relating CBSD phenotype to CBSV, UCBSV and mixed infection is scanty (Ogwok,2015).

2.3: Management of cassava brown streak disease

Earlier research by Mahungu *et al.* (1994) demonstrated that host plant resistance is the most efficient and sustainable approach towards the management of CBSD in East Africa. Indeed, breeding for resistance to CBSD is a major breeding objective of the Uganda National cassava breeding program with focus on sourcing for resistance genes in east Africa with the long term objective of developing cultivars with multiple resistances to CBSD, CMD and whiteflies (Mahungu *et al.*, 1994; NARO, 2006). Tolerant cassava varieties exist in some countries though some of them are not consistent in showing tolerance to CBSD (Zengh *et al.*, 2010). For instance, in Tanzania and Mozambique, six local and two improved cultivars with some levels of tolerance to CBSD were identified through evaluation and screening of cassava cultivars (Hillocks, 2002). Recently, Yadav *et al.* (2011) demonstrated that UCBSV can be effectively controlled using RNA interference (RNAi) technology in cassava. Also, a cross between wild *Manihot* species (*M.glaziovii*, M.*dichotoma* and *M.melanobasis*) and cassava (*M.esculenta*) cultivars is proposed as potential source of CBSD resistance genes where hybrids with resistance to CBSD showing only occasional, mild leaf symptoms (Hillocks and Jennings, 2003). Kaweesi (2014) reported Kigoma red, Namikonga (46106/27), Kiroba, Muzungu and NDL90/034 cultivars with high tolerance to CBSD but only Namikonga (*M. esculenta*×*M. glaziovii)* and NDL90/034 were recommended for release and currently, Namikonga is being used as breeding stocks for resistance to CBSD in different countries in east and southern Africa. According to Ntawuruhunga *et al.*, (2007), NASE 3 is

tolerant and MH96/2961, TME 204, TME 14, 00061(AKENA) /roots are not affected by CBSD. However, research report indicates that cassava landraces may possess useful traits for breeding for resistance to CBSV but it is not clear if these cassava genotypes possess resistance or tolerance traits therefore is need to screen tolerant genotypes to establish their adaptability and reaction to CBSD in Uganda (Wendy *et al.*, 2010).

2.3.1: Resistance to CBSD

Tolerant cassava varieties exist in some countries but some do not show consistence in tolerance to CBSD (Zengh *et al.*, 2010). Different varieties have been showing different levels of resistance to CBSD (Monger *et al.*, 2001). CBSD tolerant varieties like Nacinyaya, Kirooba, Kigoma red and Namikonga have been identified among local varieties in Tanzania and Mozambique (Hillocks and Thresh, 2000).

Resistance to CBSV can be through prevention of virus inoculation by vector, mature plants, immunity, virus movement in the host cells virus multiplication and acquisition by vector and availability of vector (Lecoq *et al.*, 2004).

2.3.2: Incidence and severity of CBSD

CBSD incidences have been reported to increase with decrease in altitude (Hillocks *et al.*, 2002). CBSD symptoms can be expressed at altitudes greater than 1000 metres above sea level when infected cuttings are planted(Jameson ,1964).CBSD have not been prevalent in Uganda, although CBSD symptoms were observed on plants in central Uganda in 1994 when it re- occurred (Thresh *et al.*,1994).

2.3.3: Plant response to CBSV infections

Plants offer resistance to viruses using various mechanisms once a virus has infected a plant such as inhibition of replication, inhibition of cell-to-cell movement, inhibition of systemic infection as well as defense response mechanisms (Palukaitis and Carr, 2008). The first line of defense for plants to viral infection is physical and chemical barriers. The second line of

defense is the plant recognition of virus virulence factors which involve development of basal immunity or RNA silencing (Pallas and Garcia, 2011). Despite of these defense mechanisms, viruses may overcome them by producing silencing suppressors as a counter defensive strategy many plant viruses have evolved viral suppressors of RNA silencing (VSRs) to counteract anti VSRs by viruses. This provides strong evidence for the antiviral nature of RNA silencing where activation of plant VSRs leads to the recovery of plants from viral infections (Pallas and Garcia,2011).

2.4: Literature summary

Cassava Brown streak disease poses a serious threat to food and income security because crop yields can be reduced drastically by as high as 70% (Maruthi *et al.,* 2014). CBSD is caused by CBSV and UCBSV strains (Bua *et al.*, 2013; Nuwamanya *et al.*, 2015).However, the extent of mixed infections compared to single infections across different cassava genotypes is unknown (Maruthi *et al.*, 2005). Hillocks & Thresh (2000) pointed the need for knowledge of serological diversity and resistant cassava genotypes to CBSD as important factors in the assessment of its intensity and severity. According to Winter *et al.* (2010), all improved cassava varieties bred or selected for resistance to CMD are susceptible to CBSD and this reverses most of the gains made in breeding cassava in the eastern and southern Africa region. Cassava landraces may possess useful traits for breeding for resistance to CBSV; it is unclear if these materials possess resistance or tolerance traits. Therefore there was need to screen tolerant genotypes to establish their adaptability and reaction to CBSD in Uganda as recommended by Wendy *et al.*, (2010). Accordingly, the effect by viral strains as well as symptom diversity is dependent in environment conditions and genotype of cassava. However, this information is scanty in Uganda, a reason this study was undertaken.

CHAPTER THREE:

GENETIC DIVERSITY OF CASSAVABROWN STREAK VIRUS IN UGANDA

3.1: Introduction

Cassava brown streak disease is reported to be caused by CBSV and CBSUV. These viral strains can be distinguished using virus-specific primers like CBSVF2 & CBSVR7 and CBSVF2 and UCBSVR8 (Mohammed *et al.,* 2012). However, there is increase in the number of CBSV and UCBSV sequences available hence a need to have primers that would detect all isolates of CBSV and UCBSV is required, particularly for field surveys (Abarshi *et al.,* 2017). For example, some amplified products may contain approximately 1600 nucleotide sequences upon sequencing. The variation in the nucleotide sequences of CBSV strains make the similarity between CBSD viral strains to be different. For example, a similarity of 95.8% for CBSV group, and a 96.6% similarity for CBSUV group in Mozambique was recorded (Mohammed *et al.,* 2012). A report by Abashi *et al.* (2017) also indicated that based on the available sequences (~1600 bases), the CBSV group shared 93.7% nucleotide identities, UCBSV 93.1%, and there was ~70% identity between the two groups. Also complete virus genome and CP encoding sequences of the six isolates of UCBSV and CBSV showed 90.7-99.5 and 93.7-99.5% identities at the nucleotide and amino acid levels. (Mbanzibwa *et al.,* 2009b). Currently, all these information is scanty in Uganda hence this study was conducted.

3.2: Materials and methods

3.2.1: Sample collection and laboratory analysis

Samples were obtained from the central districts of Uganda and laboratory extraction of viral RNA done in NaCRRI in Uganda from 2017 to 2018.

3.2.2: Sample collection

Samples of symptomatic cassava leaves were collected from four districts of Mukono, Kayunga, Luwero and Masaka all found in central Uganda. The districts were selected

because central Uganda is considered hot spot areas for CBSD infection.

In each district, five samples of symptomatic cassava leaves, with at least two leaves from symptomatic plant in each field of cassava crop affected by CBSD were picked. Fields sampled were identified with the help of the sub county agricultural staff who served as guide. The sampled sub-county was based on the intensity of production and prevalence of the disease. The selection of farmers depended on the type of cassava grown, field size and the number of years in growing cassava. Samples were picked by moving diagonally in the field. Sampled fields were estimated to be six (6) kilometers apart to attain representation of factors such as cassava genotypes sharing of planting materials, mobility of CBSV vectors, farming practices, natural factors such as soil and weather of the place. In general, one field per village, 5 villages for every two sub counties per district were sampled. A total of 20 symptomatic leaf samples were collected, placed in newsprint papers and taken to the laboratory for isolation of possible causal pathogens.

3.2.3: Laboratory analysis of samples

Laboratory analysis of leaf samples was carried out at the National Crop Resources Research Institute (NaCRRI), Namulonge. NaCRRI is located in Wakiso district, 27 km North of Kampala in central Uganda at an altitude of 1200 meters above sea level.

3.2.4: RNA extraction and purification

Extraction of total RNA was done using the modified CTAB method as described by Lodhi *et al.* (1994) and Xu *et al.* (2010). Approximately 50g of dry leaves of cassava were used because of its high yield of about 5-30µg total RNA compared to fresh leaf sample grounded in a mortar with 1 ml of 2% CTAB extraction buffer (2.0 M NaCl, 2.0% PVP, 25 mM EDTA, 100 mMTris-HCl pH 8.0 and 5 mM TCEP) (Sharama and Purohit,2012).

The homogenized paste of the samples was transferred to Eppendorf tubes and incubated at

65 °C for 15 minutes. Seven hundred fifty micro liters (750 µl) of a 24:1 mix of chloroform and isoamyl alcohol were added to each sample, mixed and then centrifuged at 12,000 rpm for 10 min at 4 ^{0}c. The upper phase was transferred to new tubes and 300 µl of ice-cold isopropanol added. The extracts were incubated for at least 10 minutes at -20 ^{0}c, then centrifuged for 10 minutes at 13,000 rpm and the supernatant was removed.

The pellet was purified by addition of 700µl ethanol followed by incubation at -20 ^{0}c for at least 10 minutes and centrifuged at 4 ^{0}c for 5 minutes at 13,000 rpm. The ethanol was decanted from the tubes and the pellets were left to dry for approximately 30 minutes at room temperature. Dry pellets were re-suspended in 100 µl of sterile distilled water (SDW). The quantity and purity of RNA was checked by measuring the absorbance of the sample volumes of 0.5–2µl in the wave length range of 190–840nm nanodrop (Thermo Scientific). Pure CBSV RNA obtained was used as template for reverse transcriptase polymerase chain reaction (RT-PCR) to detect CBSVs (Forslund, 2014) (Plate 1).

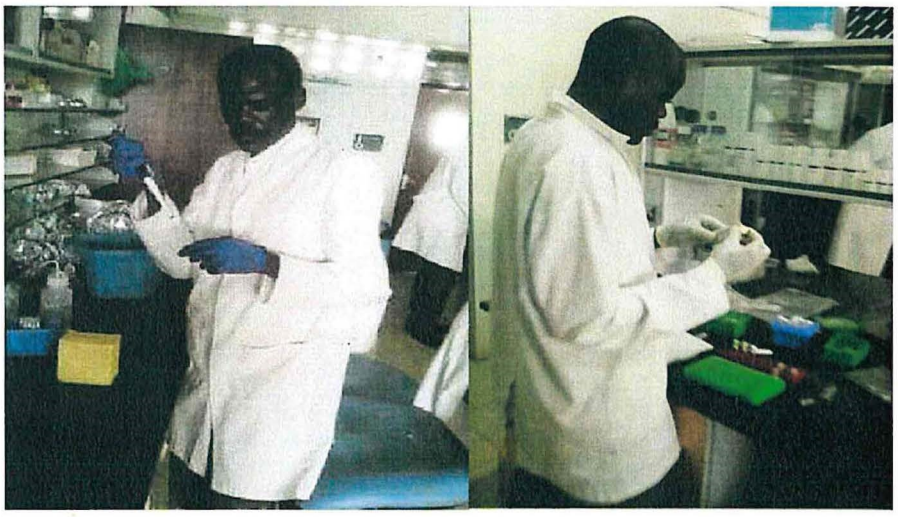


Plate 1: Extraction and purification of viral RNA at NaCRRI laboratory, 2017

3.2.5: cDNA synthesis from total RNA

The cDNA was prepared by adding 1 µl of 10xdsDNASE buffer, 1 µl of dsDNASE, 1pg -5µg

of total RNA and topped with 10 µl of nuclease free water in PCR tubes. The content was mixed gently and centrifuged, then incubated for 2 minutes at 37 ^{o}C in water bath followed by chilling on ice, centrifuging and placing on ice. Later 4 µl of 5 x reaction mixes, 2 µl of maxima enzyme mix and 4 µl of NFW were added, mixed gently and centrifuged.

It was then incubated for 10 minutes at 25 ^{o}C followed by 15 minutes at 50 ^{o}C in preheated thermomixer and the temperature increased to 65 ^{o}C for large amounts of secondary structures. This reaction can was then terminated by heating at 85 ^{o}C for 5 minutes. This first strand of cDNA was used for qPCR and balance stored at -20 ^{o}C and -70 ^{o}C for short and long periods of storage, respectively.

3.2.6: PCR amplification

PCR protocol was used to exponentially amplify specific DNA sequence by *in vitro* DNA synthesis (Mullis and Faloona, 1988). Successful RNA extract was used as template for RT-PCR to detect CBSV using master mixes made with primer 10 and 11 ($^{5'}$ATCAGAATAGTGTGACTGCTGG$^{3'}$ and $^{5'}$CCACATTATTATCGTCACCAGG$^{3'}$).

Amplification of CBSVs was done using one set of primer CBSV F/R targeting to amplify CBSV (344bp) and UCBSV (440bp) (Mbanzibwa *et al.*, 2011). The total volume master mix for the RT-PCR was 10 µl concentrations of 1 x buffer, 10 mM DTT, 60 µM dNTPs, 0.2 mM primer, 0.04 U/µl Taq DNA polymerase, 0.6U/µl MMLV-reverse transcriptase and 1 µl of RNA template was used. Amplification was done in a gene-Amp PCR system 9700 thermocycler (Perkin Elmer, Wellesey, Mass, USA).

The RT-PCR programme was set to 30 min at 42 ^{o}C, a denaturation step of 1 min at 94 ^{o}C, an annealing step of 15 seconds at 52 ^{o}C, elongation 3 min at 72 ^{o}C and then looping with 30 cycles of 94 ^{o}C for 30 seconds, 52 ^{o}C for 30 seconds, 72 ^{o}C 40 seconds and a final step at 72 ^{o}C

for 10 minutes. Two positive controls used were from infected cassava plants with CBSV and UCBSV. Two negative controls consisting of RNA from a non-infected cassava plant confirmed by laboratory test and sterile distilled water (SDW) were added instead of template. Both primer pairs CBSV 10 (^{5}ATCAGAATAGTGTGACTGCTGG^{3}) and CBSV11 (^{5}CCACATTATTATCGTCACCAGG^{3}) were used (Table 2).

Table 4: PCR primers used to detect cassava brown streak viruses (CBSV and UCBSV) in leaf samples from selected districts in central Uganda, 2017

Primer	Sequences (5'-3')	Specificity	Product size (bp)
CBSD (F) (CBSV1) Sense	ATCAGAATAGTGTGACTGCTGG	CBSV and UCBSV	344(CBSV) and 440(UCBSV)
CBSD(R) (CBSV1) Antisense	CCACATTATTATCGTCACCAGG	CBSV and UCBSV	344(CBSV)and440 (UCBSV)

*Cassava brown streak viruses (CBSVs) specific primers used for the study (Mbanzibwa *et al.*, 2011a).

3.2.7: Electrophoresis and visualization of PCR products

A 1.2% agarose gel was prepared by mixing 1.2 g of agarose with 100ml 1x TBE (Trisbase Boric Acid EDTA) buffer (Refs). The solution was then heated in a microwave for proper mixing. The gel was left to cool to approximately 60 ^{oC} and stained with 0.01 µl/ml ethidium

bromide. The gel was then casted in two trays and combs inserted. The gel was then allowed to set before loading PCR products. Each well was loaded with 5µl of the samples mixed with 1 µl of the loading dye (orange G) (Plate 2). PCR products were electrophoretically separated in a 1.2 % agarose gel in 0.5X Tris Acetate EDTA (TAE) buffer, at 80 volts for 1 hour. Five (5) µl of one Step ladder (50 bp) or 1 kb plus DNA ladder was used to determine the band size of the products. Amplicons were visualized under Ultra Violet (UV) light and recorded using an image analyser (SYNGENE UK: GENIUS) the base pairs 344 (bp) for CBSV and 440(bp) for UCBSV was recorded.

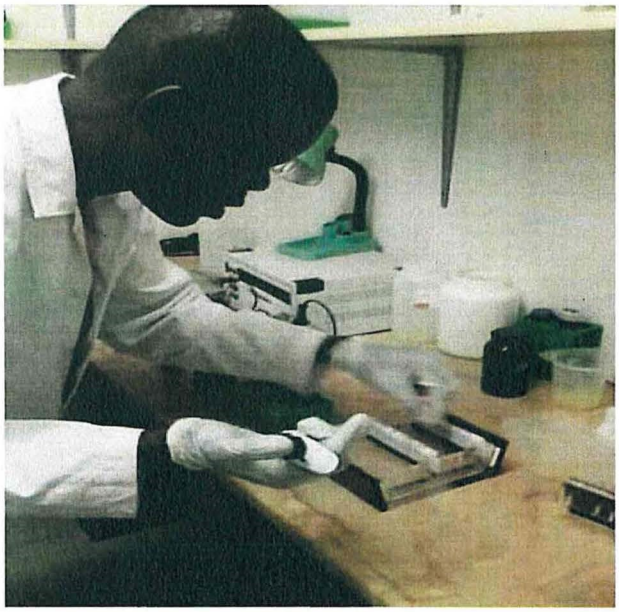


Plate 2: Loading PCR products for electrophoresis at NaCRRI laboratory, 2017

3.2.8: Sequencing of PCR products

Purified PCR products for sequencing were packed in PCR tubes up to 40µl and sent to Korea for sequencing using FEDEX courier services. Each sequence was edited manually to produce a consensus sequence for each individual isolate using the Edit Seq Programme in

DNA Star and returned in AB1 FASTA files formats and PDF chromatogram file. The sequences were aligned with reference sequences of CBSV and amino acid CP available in Gene Bank using the Clustal .W algorithm available in the MEGA 6 software programme (Thompson *et al.*, 1994).

3.2.9: Analysis of molecular variance

The genetic diversity data were subjected to two ways analysis of molecular variance (AMOVA).The means was separated using the least significant difference (L.S.D) at 5%.

3.2.10: Phylogenetic analysis of CBSV coat protein gene for sequenced samples

This was done on 18 isolates that tested positive for CBSV out of the 20 samples collected from central Uganda using the Maximum composite Likelihood model inferred by Neighbour joining tree search available in MEGA6 software program (Tamura *et al.*, 2013). Parsimony analysis was performed with PAUP using the heuristic option for 1000 replication at a 70% confident limit (Swofford, 1998). A pairwise comparison of the partial CP nucleotide sequences, expressed as percent nucleotide similarity and divergence between CBSV and UCBSV isolates identified in the 4 districts of central Uganda was calculated by ClustalW (weighted) algorithm as described by Thompson *et al.* (1994).

The evolutionary history of CBSV isolates was inferred using the Neighbour-Joining method (Saitou *et al.*, 1987). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004) for the number of base substitutions per site. All positions containing gaps and missing data were eliminated.

3.3: Results

3.3.1: Total RNA extraction and absorbance ratio

The lowest and highest nucleic acid concentrations recorded from the samples ranged from 571 to 1924.7, respectively. While the lowest and highest absorbance ratio at 260/280 was 1.74 to 1.91, for isolate G12 from Mukono and isolate G12, G14,G1 from Masaka respectively (Table 5).

Table 5: Total RNA extracts and CBSV strains diversity for 20 symptomatic CBSD leaf samples from central Uganda, 2017

District	Genotype	N/A conc.	Absorbance		Absorbanc
			260	280	
Mukono	N 14	392.9	9.82	5.58	1.76
Mukono	N 14	571	14.27	8.21	1.74
Mukono	N 12	857.7	21.44	11.46	1.87
Mukono	BAO	1741	43.52	23.44	1.86
Mukono	N 13	1875.5	46.88	24.96	1.88
Kayunga	Bukalasa	1299.8	32.49	17.44	1.86
Kayunga	NAROCASS	961.7	24.04	13.09	1.84
Kayunga	TME 204	1058.7	26.43	14.09	1.88
Kayunga	N 13	1924.7	26.46	14.73	1.8
Kayunga	N 12	1416.8	48.11	25.59	1.88
Masaka	N 13	1416.8	35.41	18.80	1.88
Masaka	N 12	1039.7	25.99	13.64	1.91
Masaka	Bukalasa	1718.7	42.96	23.15	1.86
Masaka	N 14	1242.6	31.06	16.27	1.91
Masaka	Bukalasa	1457.2	36.42	19.10	1.91
Luwero	TME 204	1252.0	31.29	16.97	1.84
Luwero	TME 204	1090.6	27.26	14.61	1.87
Luwero	Bamunanika	1572.3	39.30	21.15	1.86
Luwero	Bukalasa	1023.9	25.59	13.91	1.84
Luwero	Aladu	864.8	21.62	11.91	1.81
Mean total		1238.9	31.26	15.81	1.50

3.3.2: PCR amplification of CBSV isolates from central Uganda

Eighteen samples subjected to PCR tested positive and two samples tested negative of CBSV. Out of the 18 positive samples, eight, seven and three samples were CBSV, UCBSV and CBSV and UCBSV strains, respectively (Table 6).

Table 6: Distribution CBSV strains amplified using CBSV 10 and CBSV11 from symptomatic CBSD leaf samples from four (4) districts in central Uganda, 2017

District	Genotype	**Band size(bp)**	PCR result
Mukono	N 14	344	CBSV
Mukono	N 14	344	CBSV
Mukono	N 12	344	CBSV
Mukono	BAO	344	CBSV
Mukono	N 13	344	CBSV
Kayunga	Bukalasa	440	UCBSV
Kayunga	NAROCASS	344 and	CBSV+UCBSV
Kayunga	TME 204	344 and	CBSV+UCBSV
Kayunga	N 13	344 and	CBSV+UCBSV
Kayunga	N 12	440	UCBSV
Masaka	N 13	440	UCBSV
Masaka	N 12	440	UCBSV
Masaka	Bukalasa	440	UCBSV
Masaka	N 14	440	UCBSV
Masaka	Bukalasa	440	UCBSV
Luwero	TME 204	344	CBSV
Luwero	TME 204	344	CBSV
Luwero	Bamunanika	-	Negative
Luwero	Bukalalasa	344	CBSV
Luwero	Aladu	-	Negative

3.3.3: Electrophoresis of DNA of CBSV isolates from central Uganda

Two band sizes (344 and 440 bp) were amplified from the CBSD symptomatic samples (Figure 1).

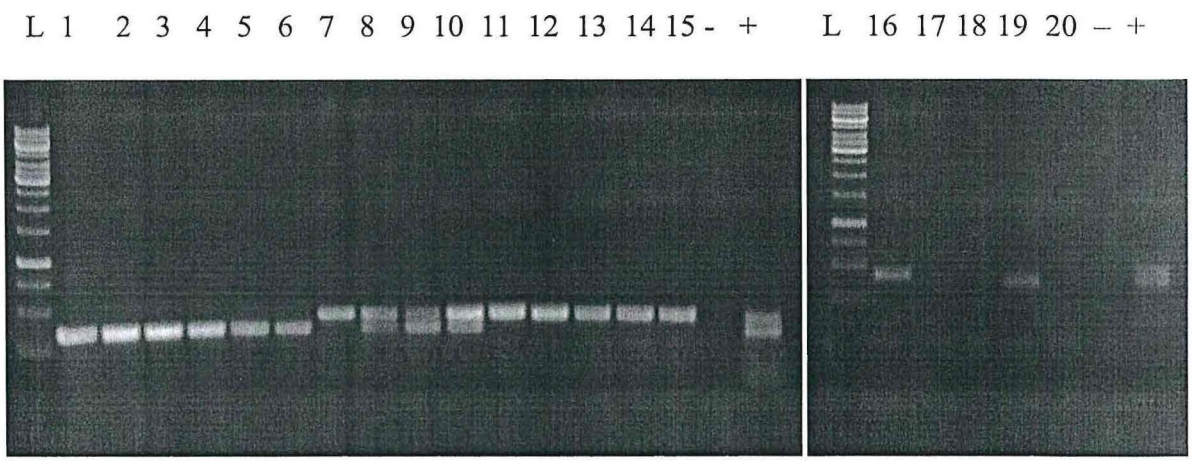


Figure 1: Amplified PCR products of the coat protein of CBSV at 344 (bp) and UCBSV at 440(bp)

KEY

L=DNA Ikb plus ladder, 1=CBSV, 2= CBSV, 3= CBSV, 4= CBSV, 5= CBSV,6 = CBSV, 7= UCBSV, 8=CBSV+UCBSV, 9= CBSV+UCBSV, 10= CBSV+ UCBSV, 11= UCBSV, 12= UCBSV, 13= UCBSV, 14 = UCBSV, 15=UCBSV, -ve=SDW, +ve=CBSV+UCBSV

(All control), L=DNA Ikb plus ladder, 16=UCBSV, 17=CBSV, 18 = -ve, 19=CBSV, 20 = -ve

-ve= SDW, +ve= CBSV+UCBSV

3.3.4: Genetic distribution of CBSV in central Uganda

The distribution of the CBSV isolates is presented in Table 7. Forty four percent of the isolates from Mukono and Luwero were CBSV strain. In contrast, 38.9% of the isolates from Kayunga and Masaka were UCBSV strain whereas 16.7% of the the isolates from Kayunga were mixed infection of CBSV and UCBSV, respectively .

Table 7: Distribution of CBSV strains from four districts from central Uganda, 2017

District	Viral species (%) in samples per district			
	CBSV	UCBSV	CBSV+UCBSV	100
Kayunga	-	40	60	100
Masaka	-	100	-	100
Luwero	100	-	-	100
Total viral species	44.4	38.9	16.7	100

3.3.5: Genetic identity of CBSV DNA coat proteins genes from isolates in central Uganda

3.3.5.1: Blasting the sequences against the NCBI databases

The blast result showing the genetic base sequence query coverage and identity percentage is presented in Table8.. The highest and lowest query percent were 96% and 44%, respectively. However, the percentage identities between the isolates ranged from 89 to 99% within the population CBSV isolates (Table 8).

Table 8: Blast result of PCR products from 18 cassava leaf samples, from central Uganda in 2017

S/NO	Source	% query	E –value	%	GI-	Identification
G1	Muk_CBSDF	73	3e-311	95	LT560306.1	CBSV
G2	Muk _CBSDF	76	3e-151	98	KR911743.1	CBSV
G3	Muk _CBSDF	69	3e-152	96	LT560299.1	CBSV
G4	Muk _CBSDF	68	3e-137	96	KR911743.1	CBSV
G5	Muk _CBSDF	70	4e-175	97	KR911743.1	CBSV
G6	Kay _CBSDF	67	2e-138	97	LT560330.1	CBSV
G7	Kay _CBSDF	86	0.0	97	KJ606226.1	UCBSV
G8	Kay _CBSDF	96	7e163	93	EU916831.1	CBSV
G9	Kay _CBSDF	45	3e88	89	KR911739.1	CBSV
G10	Kay_CBSDF	78	3e157	93	HM453039.1	UCBSV
G11	Mas_CBSDF	79	0.0	97	LT560266.1	UCBSV
G12	Mas_CBSDF	63	0.0	98	KJ606226.1	UCBSV
G13	Mas_CBSDF	72	3e-177	98	LT560266.1	UCBSV
G14	Mas_CBSDF	74	0.0	99	LT560266.1	UCBSV
G15	Mas_CBSDF	85	7e-178	96	KJ606226.1	UCBSV
G16	Luw_CBSDF	86	0.0	98	KJ606226.1	UCBSV
G17	Luw_CBSDF	44	5e-146	98	KR911743.1	CBSV
G19	Luw_CBSDF	68	2e-148	99	HM453034.1	CBSV

3.3.5.2: Analysis of molecular variance (AMOVA)

There was no significant difference (P>0.05) in diversity of CBSV strains among and within population probably the virus could have evolved from the same ancestry. The molecular analysis of variance revealed that up to 96% of the diversity was distributed within the population leaving only 4% among the population (Table 9).

Table 9: Analysis of molecular variance (AMOVA) for 18 CBSD causal viruses from central Uganda, 2017

Source	d.f.	SS	MS	Est.Var	%variance	p-value	F_{ST}
Among Population	1	0.639	0.639	0.015	4%	_	_
Within	34	12.722	0.374	0.374	96%	0.188	0.38
Total	35	13.336	1.013	0.389	100%		

Key

d. f. – degree of freedom, SS – sum of squares, MS – mean squares, p – level of significance, FST – fixation index for genetic differentiation between populations. Probability, P (rand >= data), for FST is based on standard permutation across the full data set. FST = AP/ (AP+WP) = AP/TOT AP = Est. Var. among Pops, WP = Est. Var. within Pops.

3.3.5.3: Blast sequence output

Multiple parameters on the nucleotide sequences reveals two main clusters which are sub divided into sub groups comprising of CBSV species clustered in phylo group I UCBSV species clustered in phylo group II. The phylogenetic tree grouped the isolates into two distinct clusters with one cluster comprising of isolates G1, G2, G3, G4, G5, G6, G9, G17 and G19 while the second cluster consisted of G7, G8, G10, G11, G12, G13, G14, G15 and G16. However, isolates G4 (ab 1461) and G13 (ab 1495) did not fall in either category (Figure 2).

The optimal tree with the sum of branch length = 1.01383652 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches (Felsentein *et al.*, 1985).

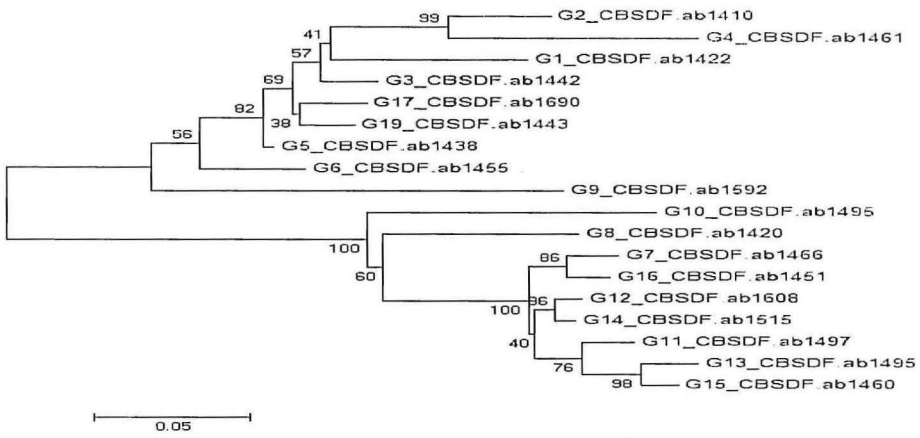


Figure 2: Phylogenetic relationships among CBSV isolates from central Uganda using cDNA polyproteins genes sequence alignment inferred by Neighbour joining tree search

3.3.5.4: Pair wise population matrix

The pairwise population matrix (Figure 3) reveals that variation occurs positively within isolates with increase of genetic distance of the isolate.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. G1_CBSDF.ab1422																		
2. G2_CBSDF.ab1410	43																	
3. G3_CBSDF.ab1442	27	31																
4. G4_CBSDF.ab1461	51	35	40															
5. G5_CBSDF.ab1438	30	26	15	35														
6. G6_CBSDF.ab1455	41	43	28	54	19													
7. G7_CBSDF.ab1466	94	98	86	108	78	78												
8. G8_CBSDF.ab1420	93	96	83	106	76	79	43											
9. G9_CBSDF.ab1592	63	65	51	75	47	59	96	90										
10. G10_CBSDF.ab1495	91	98	86	103	78	79	52	49	99									
11. G11_CBSDF.ab1497	94	99	89	109	81	81	20	46	96	53								
12. G12_CBSDF.ab1608	93	96	84	105	76	79	15	39	90	48	13							
13. G13_CBSDF.ab1495	98	102	87	109	81	79	21	46	100	58	18	25						
14. G14_CBSDF.ab1515	94	96	84	105	76	79	15	38	92	47	12	5	23					
15. G15_CBSDF.ab1460	95	98	83	105	78	78	23	47	96	56	15	23	10	21				
16. G16_CBSDF.ab1451	96	96	84	107	74	77	10	40	94	51	23	13	28	13	23			
17. G17_CBSDF.ab1690	35	35	17	45	13	28	85	83	50	84	89	84	88	84	85	83		
18. G19_CBSDF.ab1443	33	28	14	42	10	27	84	84	49	85	86	83	87	83	84	80	13	

Figure 3: Pairwise population matrix of Tamura-Nei genetic distance between 18 CBSD isolates using Number of difference model

3.4: Discussion

The result of this study has shown that the concentration of Total RNA of CBSV was variable from 392.9 - 1875.5ng but acceptable lower range of 100 ng total RNA for a 20 µl reaction can as well produce good result. Glasel (1995) research supports this finding on validity of

nucleic purity where he examined the ratio of absorption at 260 and 280 nm with UV spectrophotometry, and found high-quality RNA at absorbance ratio of 260/A280 in the range of 1.9–2.1.

The result of this study has shown the existence of three different strains of CBSV including CBSV, UCBSV, CBSV and UCBSV, respectively in central Uganda. However, CBSV was the most widespread followed by UCBSV, CBSV and UCBSV, respectively. In fact, CBSV was more prevalent in Mukono and Luwero, respectively. In contrast, UCBSV was more widespread in Masaka though 40% was detected in Kayunga. The mixed infection was detected only in Kayunga.. This finding therefore corroborates the earlier findings by Mbanzibwa *et al.* (2009) and (Mohammed *et al.,* 2012) confirmed the existence of the two strains of CBSD causal virus in east Africa. This could have been due to the sharing of infected planting material among the farmers and the buildup of that pathogen in the vectors within the locality of central Uganda. Additionally, this result corroborates with the findings of Maruthi *et al.* (1986); Amisse (2013) who highlighted the existence of the same viral species within the same locality resulting from the sharing of infected planting materials among the farmers. In fact, there is need to determine whether the same trend exists in other parts of the country other than central Uganda. However, two (02) isolates tested negative despite clear symptoms with severity score of 3 and this could have resulted from laboratory procedure errors or contaminations that led to degradation of viral RNA.

The result of this study also revealed genetic diversity among 18 CBSV isolates as seen in the amino acid CP sequence leading to two genetically distinct populations of CBSV strains. The genetic diversity of CBSV isolates was high within the population and low among the population. Members within each group were conserved with an average amino acid similarity of 89% - 99% for CBSV group, and a 93%-99% similarity for UCBSV group. This

variability could have been due to origin of isolates from different lines of ancestry. This result is similar to the previous findings by Monger *et al.* (2001) and Mohammed *et al.* (2011) work on parsimony analysis of CP amino acid sequences identified two major clusters (CBSV and UCBSV) amongst the field-collected samples with CBSV average amino acid similarity of 95.8% and 96.6% for UCBSV. Also earlier research findings on partial coat protein sequences by (Mbanzibwa *et al.*, (2009) and Amisse *et al.*, (2019) also identified differences in partial CP that lead to the description of two genetically distinct populations of CBSV strains. However, there is need to sample symptomatic leaves from wide geographical area other than central Uganda alone for this study. The relationship in the nucleotide sequence variation indicates the closeness of the isolates from sampled districts implying very close similarity among the isolates. This is in line with the findings of Ndunguru *et al.* (2015) on existence of sequence differences between CBSV and UCBSV and genetic diversity is wider among the isolates of CBSV (79.3–95.5%) than UCBSV (86.3–99.3%).

The variation among the population of 4 % (0.015) estimated variance could be as a result of samples all picked from central region of Uganda hence existence of CBSV from the same origin. While variation between the populations of 96 % (0.374) estimated variance could be due to genetic mutation within the population. The high value of FST fixation index (0.38, *p=0.203*) indicates a strong variability within the population. The AMOVA result with no significant ($p>0.05$) genetic differences within the populations at LSD 5% could be the product of difference in ancestry of members of the two phylla groups.

BLAST analysis of the nucleotide sequences obtained in the study, showed that nine (9) isolates comprising of CBSV strain clustered in phylogroup I with 89 - 99% variation. Similarly, nine (9) isolates comprising of UCBSV strain clustered in phylogroup II with 93-99% variation.

The phylogenetic analyses revealed that there were two distinct strains of CBSV suggesting that clusters originate from common ancestry but may be separated by mutational change in the genome. According to Ndunguru *et al.* (2015), two distinct clades or strains of CBCV have been found associated with CBSD infection in cassava genotypes. The loose clustering of lines among and within groups in the phylogenetic tree confirmed the relationships within the strains and the existence of two main groups with members that are closely related. The result of the study showed no new distinct CBSV isolates in central Uganda when compared with the gene accessions of isolates at NCBI data base gene bank. The pairwise comparisons values showed a high correlation between and within sub- populations. The parsimony analysis grouped the CP amino acid sequences into two major clusters: CBSV and UCBSV.

CHAPTER FOUR:

SYMPTOM DIVERSITY AND IDENTIFICATION OF SOURCES OF RESISTANCE TO CASSAVA BROWN STREAK DISEASE IN CENTRAL UGANDA

4.1: Introduction

Different cassava genotypes are reported to exhibit different symptoms when infected with CBSD (Monger *et al.*, 2001). CBSD symptoms can be observed on leaves, stems and roots of cassava (Ntawuruhunga and Legg, 2007). However Omongo *et al.* (2000) highlighted the variation of CBSD symptoms on leaves, stem and roots of host plants. Earlier research result highlighted that cassava genotypes that are tolerant to CBSD are not consistent in showing the tolerance to CBSD (Zengh *et al.*, 2010).

Currently, breeding for resistance to CBSD is focused on sourcing for genes resistant CBSD and whiteflies (Mahungu *et al.,* 1994; NARO, 2006), breeding for resistance requires clear understanding of the interactions of CBSV with cassava genotypes and host plant resistance are the most efficient and sustainable approach for the management of CBSD (Mahungu *et al.,* 1994 Hillocks & Jennings, 2003; Munga, 2008). Research findings by Ntawuruhunga *et al.* (2007) in Tanzania and Mozambique highlighted that NASE 3 to be tolerant and MH96/2961, TME 204, TME 14, 00061(AKENA) cultivars with some levels of tolerance to CBSD. However, in Uganda, there are no known sources of resistance in cassava germplasm to CBSD (Ogwok, 2015). The underlying question of this study was whether sources of resistance against CBSD can be found among commonly grown cassava germplasm picked from selected areas.

4.2: Materials and methods

4.2.1: Experimental site

The experiment was conducted in the screen house at the Department of Agriculture; Kyambogo University from August 2017 to April 2018. Kyambogo University is located

8km east of Kampala City Centre in central Uganda. The coordinates of Kyambogo University are: 0°20'54.0"N 32°37'49.0"E (Latitude: 0.348334; Longitude: 32.630275), 1,240 meters above sea level (en.wikipedia.org/wiki/ Kyambogo).

4.2.2: Cassava genotypes used for the study

Healthy test genotypes used were (NAROCASS, NASE 12, NASE14, and TME204) released in Uganda and landraces (Bamunanika, Aladu, Kwatampola, Njule, and Bukalasa) were used to screen for resistance. These materials were chosen because they were the most preferred and commonly grown cassava genotypes in central Uganda.

4.2.3: Sample collection

Stem cuttings of 4 disease-free improved cassava genotypes (NASE 12, NASE 14, TME 204 and NAROCASS) and the local landraces (Aladu, Bamunanika, Bukalasa, Njule and Kwatampola) collected from different parts of the country including NaCRRI (Wakiso), Masindi, Luwero, Nakasongola, Apac, Gulu and Kitgum

4.2.4: Virus indexing of samples

Selected cassava plants were virus-indexed by observation of CBSD symptoms on leaves and stems as described by Gondwe *et al.* (2003) and Wagaba *et al.* (2016). This was done to avoid picking plants with latent infection in central Uganda which is the hot spot for CBSD. Stem cuttings of about 30cm obtained from the mature portion of the stem with prominent nodes were planted in the screen house for one month and monitored for CBSD symptom development. Cutting with shoots free from CBSD symptoms were then planted and used in CBSV strain inoculation experiments.

4.2.5: Media sterilization and potting

Forest soil was sterilized by heating in an open pan for 30minutes at approximately . 40°C The sterilized forest soil was allowed to cool down over night, mixed with river sand

and rock ballast in a ratio of 4:1:1 and filled into plastic buckets of 20kgs capacity to the brim (Teshome *et al.,* 2016; Jeremiah *et al.,* 2017).

4.2.6: Experimental design

The experiment was laid out in complete randomized design (CRD) with three replications.

4.2.7: Planting of cuttings

Cuttings (30 cm in length) from the virus-indexed improved and landrace cassava genotypes were planted vertically in plastic buckets filled with amended sterilized soil up to the brim. One cutting was planted in amended sterilized soil per bucket. The buckets were spaced 45cm from each other giving a total of nine (9) plants per treatment unit and thirty six (36) plants per replicate. In total, there were one hundred and eight (108) plants per trial and a total of 216 plants for both trials. Watering was done twice a week plants were maintained at 28 ± 5°C, 50–60% relative humidity in the screen house at Kyambogo University. Improved genotypes and landraces were subjected to four treatments (CBSV, UCBSV, CBSV+UCBSV and control (non-infected leaf graft).

4.2.8: Inoculation of the cassava plants

Symptomatic cassava leaves petiole and bud that tested positive for CBSV, UCBSV, mixed infections or healthy plants were used to graft inoculate cassava plants from each cassava genotype in screen house. Chip bud grafting was done using leaves from symptomatic plants for easy transfer of virus where graft union was performed according to Kester *et al.* (2002). Axillary buds between 3 and 6 mm in width were obtained from non-lignified stems of 3-6 months old symptomatic plants and used as the source of inocula. Buds with the petiole and leaf attached were excised to a depth of about 2 mm, sufficient to expose the cambium layer from 4- 12 nodes below the apical point from plants infected by virus and control (healthy) plants by making a triangular cut with a double-edged razor blade. Axillary buds of equivalent size were excised from the rootstock test plants 6-8 nodes above soil level at 6-8

weeks after their establishment in soil. The inocula buds were inserted into and secured to the test plants by wrapping it to the rootstock stem using parafilm (Pechiney Plastic Packaging Company, Chicago, IL). The petiole, leaf blade and the bud from the scion and grafted plants were kept in the screen house under growth conditions of 28 ± 5°C, 50–60% relative humidity.

One week after bud graft insertion the parafilm wrapping was removed and success or failure of graft union assessed. Successful graft was ascertained by the scion bud retaining its green color and had fused to the rootstock with visible callus tissue formed at the graft union edges.

4.2.9: Data collection

Data were collected on CBSD incidence and severity using the disease symptoms on cassava leaves and stems at one month interval.

4.2.9.1: Incidence

Disease incidence on cassava leaves and stems was done by counting the number of plants with symptoms and expressing it as percent of the total number of plants in the pots inoculated with CBSV strains.

$$\text{Percentage disease incidence (\%DI)} = \frac{\text{Number of disease plants}}{\text{Total number of plants assessed}} \times 100\%.$$

Data collection for CBSD incidence commenced on symptoms appearance and continued on a monthly interval as described by Wagaba (2013) (Plate 3).

4.2.9.2: Symptom diversity and Severity of CBSD on leaves and stem

Symptom diversity and severity was scored visually from symptom appearance and continued at monthly intervals until three months for both trials (first trial September-December 2017) and (Second trial February to April 2018). Severity of CBSD was scored on

plant to plant basis using the scale of 1 – 5 where 1 = no symptoms, 2 = mild foliar feathery chlorosis, no stem lesion, 3 = pronounced foliar feathery chlorosis, mild stem lesions and no die back, 4 = severe feathery chlorosis, severe stem lesion and no die back and 5 = severe chlorosis, defoliation, severe stem lesions and die back (Gondwe *et al.*, 2003; Wagaba *et al.*, 2016). Symptom severity data of CBSD was used to calculated area under disease progress curve (AUDPC). The symptoms observed (Plate 3) were correlated to the CBSD isolates used for grafting, to determine the level of resistance of cassava genotypes to viral strains (Mohammed *et al.*, 2012).

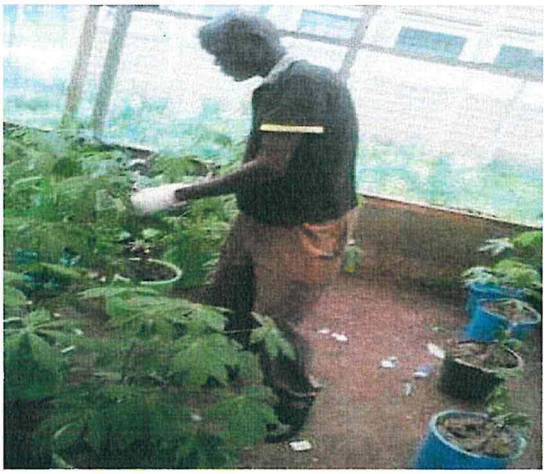


Plate 3: Scoring of CBSD incidence and symptoms severity in Screen house at Kyambogo University, 2018

4.2.10: Data analysis

Data on CBSD incidence and severity was analyzed using two-way ANOVA of Genstat (13th edition) computer package. Means were separated using least significant difference test (LSD) test at 5% probability level.

4.3: Results

4.3.1: Symptom diversity of cassava brown streak disease

Variation in symptoms of CBSD existed on leaves and stems of cassava genotypes inoculated

with CBSV, UCBSV and CBSV+UCBSV strains from 1-3 months after inoculation (MAI). CBSD symptoms observed varied among cassava genotypes and over MAI; as chlorotic spots on leaf tips to mild chlorosis/mottling of secondary to primary and later mild stem streaks on resistant genotypes. Susceptible genotypes had chlorotic spots on leaves that progressed to chlorosis of veins, leaf lamina and later necrotic spots on stems across all CBSV strains. However symptom diversity of CBSD in cassava genotypes varied with the CBSV strains.

Asymptomatic **CBSV**

UCBSBV **CBSV+UCBSV**

Plate 4: Aladu genotype showing asymptomatic leaves and CBSD symptoms with different CBSV strains

Asymptomatic **CBSV**

UCBSV **CBSV+UCBS**

Plate 5: Bamunanika genotype showing asymptomatic leaves and CBSD symptoms with different CBSV strains

Asymptomatic **CBSV**

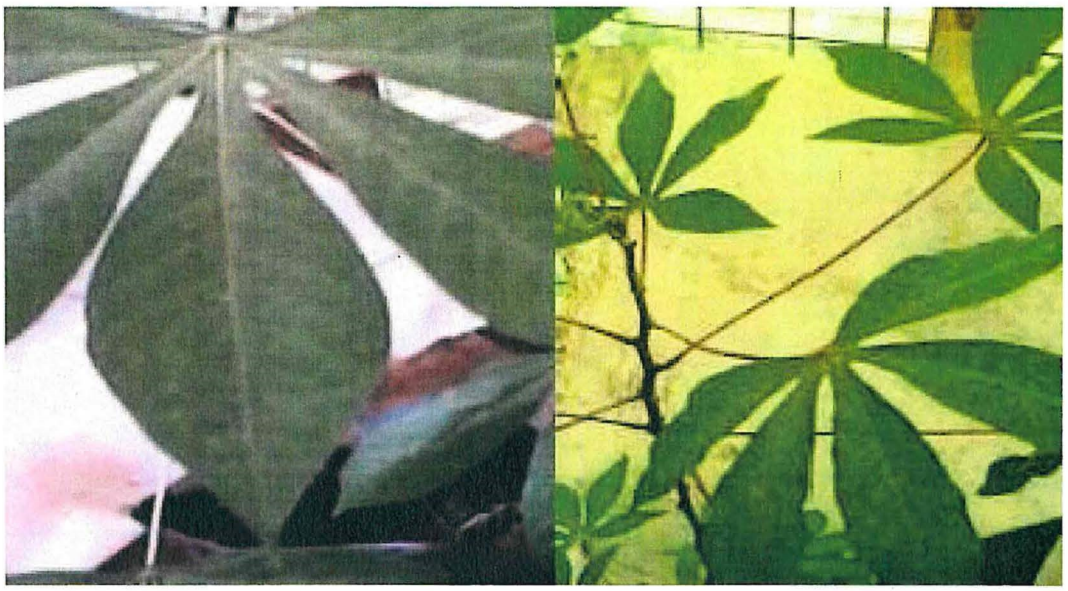
UCBSV **CBSV+UCBSV**

Plate 6: Bukalasa genotype showing asymptomatic leaves and CBSD symptoms with different CBSV strains

Asymptomatic **CBSV**

UCBSV **CBSV+UCBSV**

Plate 7: Njule genotype showing asymptomatic leaves and CBSD symptoms with different viral strains



Asymptomatic **CBSV**

UCBSV **CBSV+UCBSV**

Plate 8: NAROCASS genotype showing asymptomatic leaves and CBSD symptoms with different viral strains

Asymptomatic **CBSV**

UCBSV **CBSV+UCBSV**

Plate 9: NASE 12 genotype showing asymptomatic leaves and CBSD symptoms with different viral strains

Asymptomatic **CBSV**

UCBSV **CBSV+UCBSV**

Plate 10: NASE 14 genotype showing asymptomatic leaves and CBSD symptoms with different viral strains

Asymptomatic **CBSV**

UCBSV **CBSV+UCBSV**

Plate 11: TME 204 genotype showing asymptomatic leaves and CBSD symptoms with different viral strains

4.3.2: Cassava brown streak disease symptom incidence

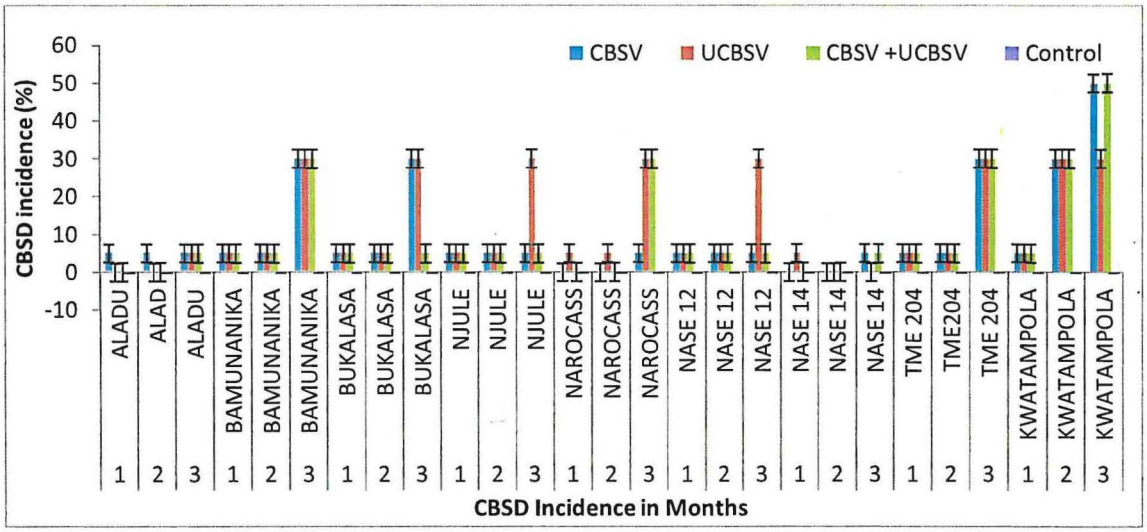


Figure .4a.

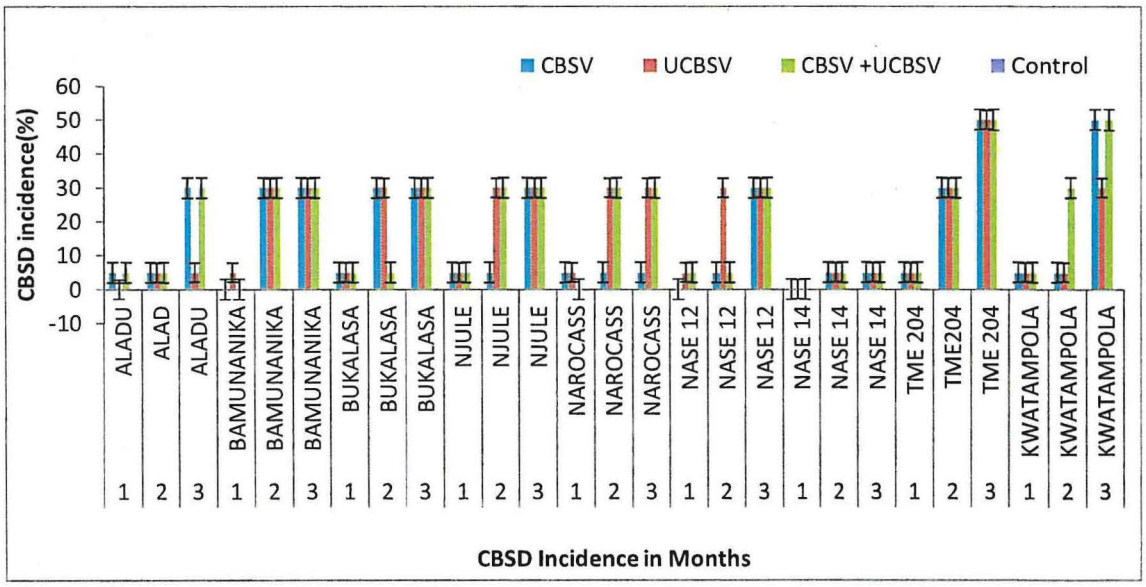


Figure .4.b.

Figure.4: Cassava brown streak disease incidence percentage in 9 cassava genotypes inoculated with CBSV, UCBSV and mixed isolate

The highest and lowest CBSD incidence of 5% and 30% were recorded across CBSV strains in cassava genotypes. From the assessments done on the test plants, there was 100% incidence as all genotypes treated with all the three viral strains showed signs of cassava brown streak disease. Lowest and the highest incidence percentage of 5 and 50% respectively was recorded cassava genotypes inoculated with different CBSV strains (Figure 3a and 3b).

4.3.3: Cassava brown streak disease progress in cassava genotypes

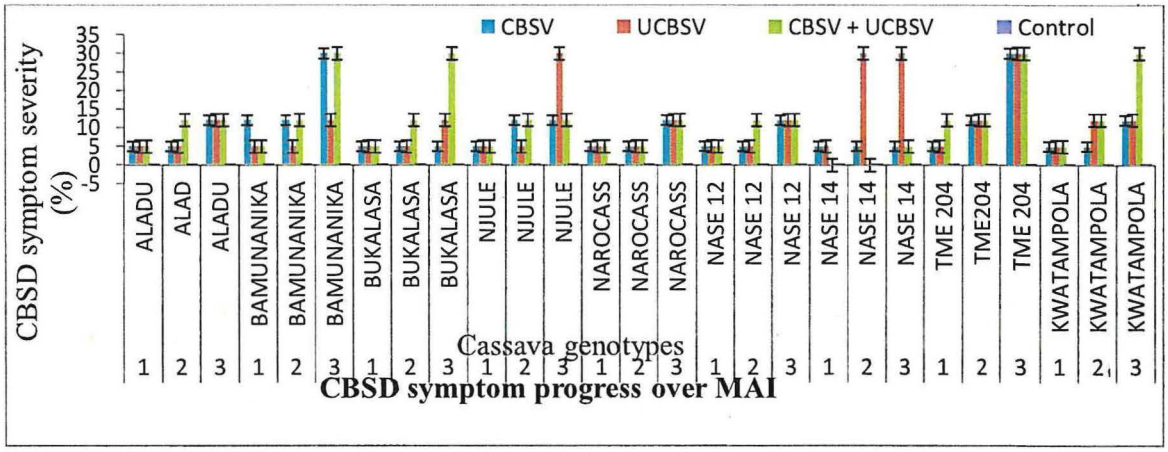


Figure .5a.

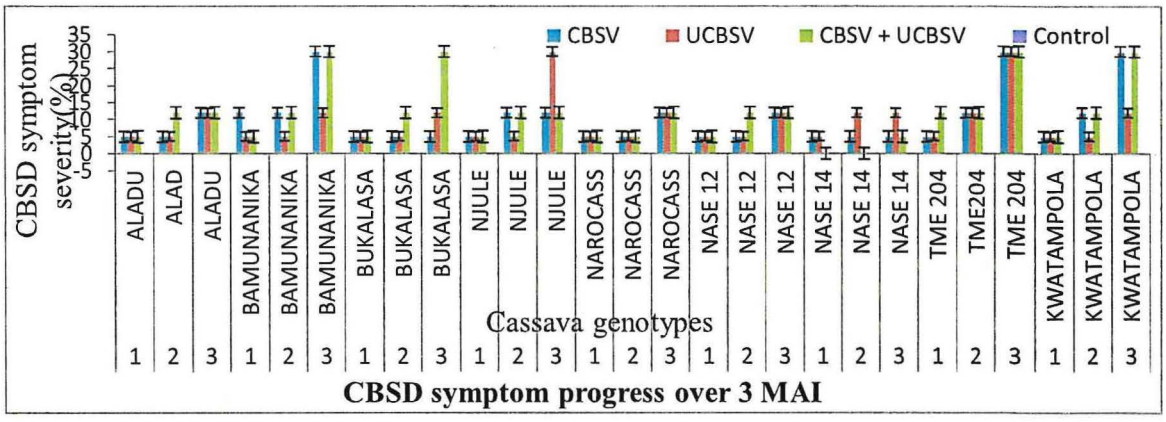


Figure. 5b.

Figure 5: Cassava brown streak disease progress in percentage among 9 cassava genotypes inoculated with CBSV, UCBSV and mixed isolate

CBSD symptom severity among cassava genotypes varied from 5 to 30% across all viral strains. CBSD symptoms increase with the increase in time after inoculation. CBSD progresses rapidly on Bamunanika, Bukalasa, Njule,TME 204 and Kwatampola

4.3.4: Cassava brown streak disease symptom severity

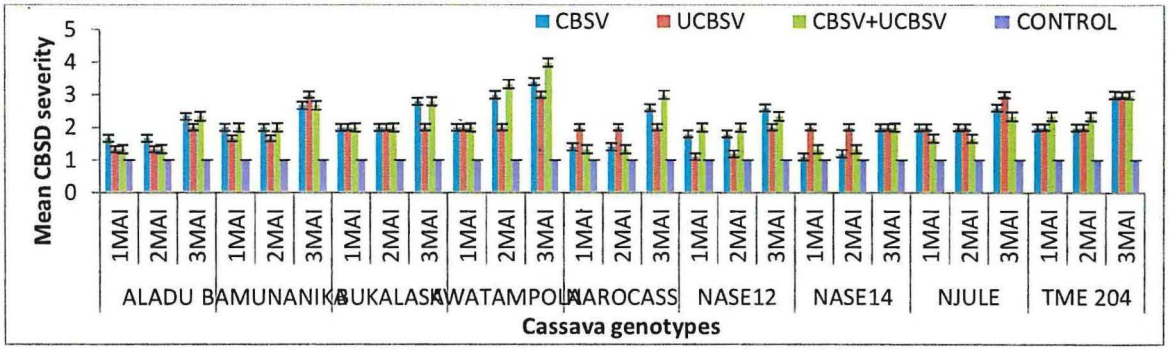


Figure .6a.

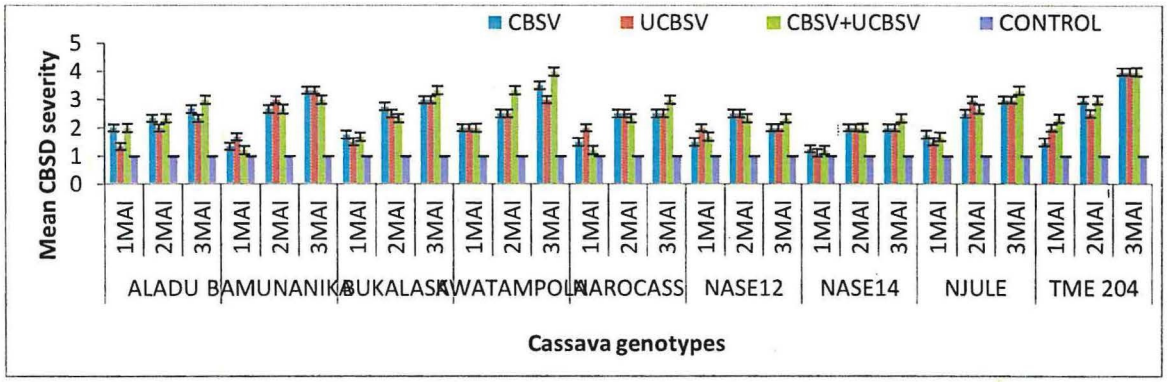


Figure .6b.

Figure 6: Cassava brown streak disease symptom severity in 9 cassava genotypes inoculated with CBSV, UCBSV and mixed isolate

Table 10: Summary of analysis of variance (ANOVA) for cassava genotypes inoculated with CBSV isolates at Kyambogo University, 2017/2018

		Trial one 2017			Trial two2018		
Source of variation	Degrees	ofl MAI	2 MAI	3 MAI	1 MAI	2 MAI	3 MAI
Variety	8	0.63^{***}	1.78^{***}	1.35^{NS}	0.52***	0.55***	1.99***
Viral strains	3	3.84^{***}	5.14^{***}	18.31^{***}	2.58***	15.8***	29.5***
Variety*Treatment	24	0.19^{*}	0.36***	0.29***	0.24***	0.18***	0.32***
Residual	72		0.1389	0.1981	0.1516	0.2396	0.1713

MAI = Month after inoculation, *** = Significant at <0.001 ** = Significant at 0.01 and * =0.05, NS Not Significant

There were significant differences (P<0.001) in reaction of cassava genotypes to CBSV viral strains in both trials, respectively. In addition, significant variety x viral strains interactions (P<0.001) was observed during the first two months after inoculation but not the third month after inoculation for trial one. However, significant variety x viral strains interactions (P<0.001) was observed throughout for trial two (Table 10).

4.3.5: Cassava brown streak disease means symptom severity across cassava genotypes

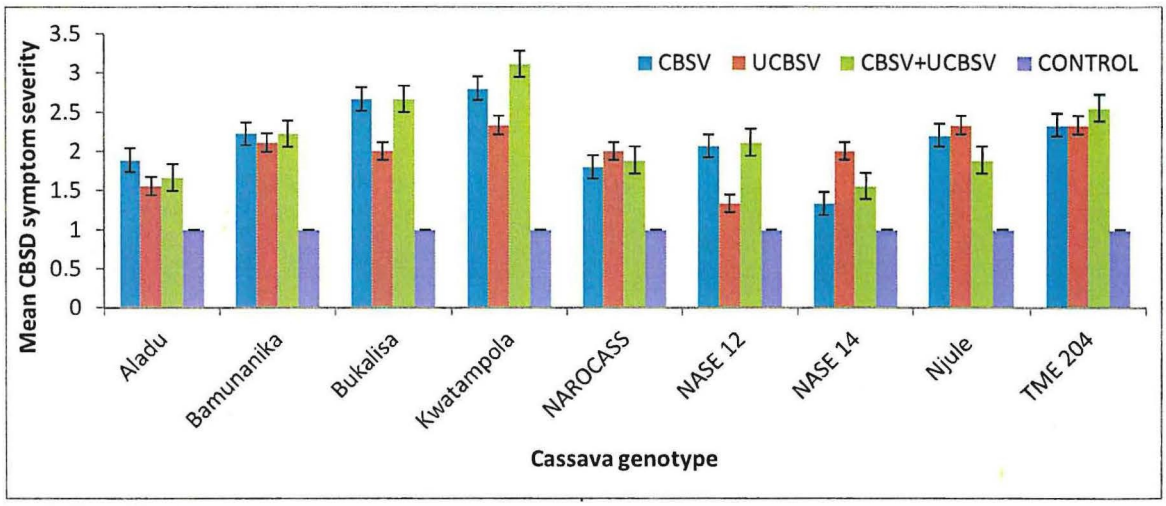


Figure .7a.

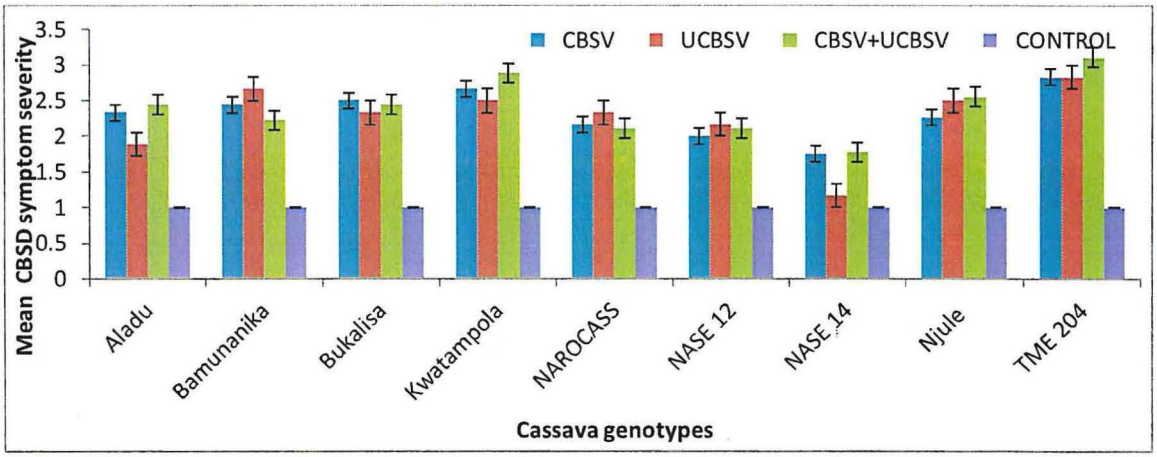


Figure .7b.

Figure 7: Cassava brown streak disease mean symptom severity in 9 cassava genotypes inoculated with CBSV, UCBSV and mixed isolate

CBSD mean symptom severity varies across cassav

strains used for inoculation. The highest and lowest mean symptom severity from CBSV strain of 2.8 and 1.3 was recorded on Kwatampola, TME 204 and NASE 14, respectively. Similarly, the highest and lowest mean symptom severity of 3.1 and 1.5 from the mixed

infection was recorded on TME 204 and Kwatampola and NASE 14, respectively (Figures 6a and 6b). In contrast, highest and lowest mean symptom severity from UCBSV strain of 2.8 and 1.2 was recorded on TME 204 and NASE 14, respectively. Overall, mixed infection of CBSV had the highest mean severity of 3.1 followed by CBSV and UCBSV, respectively. In fact, CBSD symptom severity increases with time after inoculation.

All cassava genotypes succumbed to CBSD infection during both trials one and two, respectively.

4.4: Discussion

The results of this study have shown that all the cassava genotypes used in the study succumb to CBSD infection although there was variation in severity amongst the genotypes and viral strains. According to Agrios (2005), the variation in severity could be attributed to the virulence of the viral isolates and genetic make-up of the cassava genotypes. However, higher symptom severity was recorded from mixed viral infection followed by CBSV and UCBSV, respectively. The higher symptom severity registered from mixed viral infection could be attributed to the synergistic effects of the two viral strains. A similar scenario was reported for SPVD which is due to co-infection of SPCSV and SPFMV (Karyeija *et al.*, 2000). Also, the increase in disease over time is expected because of factors such as pathogenicity, favorable environmental conditions and susceptibility of the hosts among others (Agrios, 2005). In fact, Mohammed *et al.* (2012) and Nuwamanya *et al.* (2015) reported that the virulence of CBSV strains increases across cassava genotypes with differing resistance levels and possibly cassava genotypes infected with different viral strains which affect symptom development. However, Hillocks and Jennings (2003), suggested that some inherent characteristics of the varieties control resistance or susceptibility to CBSD. Campbell and Madden (1990) noted that symptom severity increases when an infectious disease remains infectious and the host tissues can support pathogen establishment and development.

Therefore, susceptibility to CBSD infection is dependent on viral strains and genes controlling resistance in the genotype (Monger *et al.*, 2001; NARO, 2006; Alicai *et al.*, 2007). Earlier, Hillocks (2002) reported five categories of resistance in cassava including 1= completely resistant, 2= mild resistance, 3 = moderately susceptible, 4 = susceptible, and 5= highly susceptible. However, in this study, NASE 14, NASE 12, NAROCASS and Aladu showed mild resistance to CBSD at varying levels suggesting a possibility of potential source of resistance that can be explored for future resistance work. Resistance of cassava genotypes was also probably due to plant defense mechanisms to CBSV strains such as inhibition of replication, cell- to-cell movement and systemic infection but this need to be clarified in another study. Earlier Palukaitis and Carr, (2008) reported that some genotypes resist CBSD due to the development of barriers and basal immunity or RNA silencing to virus virulence factors. In another study, Pallas and Garcia (2011) highlighted that CBSD symptoms can easily be suppressed by virus silencing suppressors (VSRs) or recovery where plants with mild symptoms recover from viral infections due to lack or inactivation of virus silencing suppressors.

CHAPTER FIVE:

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1: General discussion

The objectives of this were to 1) assess the genetic diversity of cassava brown streak disease causal viruses, 2) assess the symptoms diversity, incidence and severity of CBSD; 3) identify potential sources of resistance to cassava brown streak disease in central Uganda.

The result of this study points out clearly the existence and diversity of CBSV in central Uganda. Two CBSV strains were found to exist in central Uganda. Accordingly, this study has confirmed that cassava brown streak disease was largely a result of single infection attributed to either CBSV or UCBSV and mixed infection by both CBSV and UCBSV. Infact, CBSV was more widespread in Mukono and Luwero districts than UCBSV and CBSV +UCSBV, respectively. However, UCBSV was more widespread in Kayunga and Masaka districts. Interestingly, the mixed infection of CBSD was only detected in samples from Kayunga only. This could either mean that Kayunga 1) provides common front where both virus strains are concentrated and therefore can coexists or 2) provides habitats where the vectors for the two virus strains are able to transmit CBSD with better efficiency. However, it was beyond the scope of this study to establish whether there was synergism or antagonism between the two viral strains on CBSD infection. According to Ogwok et al. (2016), the genetic diversity of CBSV in east Africa was found to be widespread.

Additionally, CBSV isolates were found to cluster in two main groups namely cluster one (phylla I) and cluster two (phylla II), respectively. However, more diversity was detected among the UCBSV (93-99%) than CBSV (89-99%) an indication that UCBSV is more distantly related that CBSV. Similar result was reported in Tanzania by Ndunguru et al. (2015).

Although the parsimony results revealed two clusters from the amino acid CP sequence of the two strains, the average amino acid similarity of 95.8% and 96.6% for CBSV and UCBSV were not significantly different.

Nei genetic distance variation occurs positively within and with increase in genetic distance of CBSV isolates. Significant differences in genetic differentiation with distance exist within sub population as represented in the AMOVA (Table 9). This finding is substantiated by the work of Mbewe et al (2017) that members of Potyviridae share similar genomic organization, with levels of amino acid identity in their polyproteins.

This study used nine (09) different cassava genotypes from local and improved sources. CBSD incidence and symptom severity showed that all the cassava genotypes used in the experiment were susceptible to CBSD. The incidence of CBSD was 100% in all cassava genotypes but severity varied across genotypes over time. Low symptom severity was recorded in the 1MAI of the experiment across all cassava genotypes. Variability in severity of CBSD exists within cassava genotypes and within viral strains.

CBSD symptom severity increases from 1MAI, 2MAI and 3MAI across all viral strains. Mixed CBSV strains infection registered highest score followed by CBSV and lastly UCBSV. CBSV pathogenicity increases with increase in days after inoculation. Mixed strain CBSV+UCBSV was more pathogenic followed by CBSV while UCBSV was the least in pathogenic. Among the nine cassava genotypes NASE14 and NAROCASS were resistant while Aladu, NASE12, Njule, Bamunanika and Bukalisa were moderately susceptible. The most susceptible genoptyes to CBSV were Kwatampola and TME 204. According to Ogwok et al. (2016), CBSV strains interact differently in different host plants due to the genotype-specific resistance to CBSD viruses and resistance by tolerant cassava genotype could be by restriction in virus replication and movement within the plant system supports. Additionally,

specificity of the different CBSV strains could have resulted in the variability in the symptom severity observed in the different cassava genotypes evaluated (Monger et al., 2001; Hillocks et al., 2002; Alicai et al., 2007). CBSD symptoms were recorded on all plant parts in highly susceptible genotypes, while for more tolerant varieties symptom was common on the leaves only. Higher CBSD symptom severity score could possibly be due to the susceptibility hence spread of the symptoms within host plants.

This finding implies that NASE 14 and NAROCASS could be improved further by developing their genetic potential to resist CBSV. Additionally, resistant genotype can be used in the development of integrated disease management strategies, which addresses components of the disease cycle. However, there is still considerable scope for further research into several aspects of the resistance of cassava genotypes and the genetics of the pathogen, especially in understanding the pathogenicity of viral strains, host specificity of CBSV pathogens so as to develop sustainable and effective integrated disease management practices.

5.2: Conclusions

Two strains of CBSV were found to exist in central Uganda suggesting the genetic diversity among the CBSD causal viruses. Accordingly, CBSD infection on cassava genotypes could be caused singly by either CBSV or UCBSV and a combination of both viral strains. However, the distribution of CBSV was not uniform in central Uganda. CP and amino acid sequence variation exist among and within CBSV population.

There was resistance to viral infection varies across cassava genotypes and viral strains. NASE 14 and NAROCASS were resistant while Aladu, NASE12, Njule, Bamunanika and Bukalasa were moderately susceptible. The most susceptible genotypes to CBSV were Kwatampola and TME 204. Variability of CBSD symptoms exist across cassava genotypes

and between the viral strains infecting cassava genotypes. This responds to the hypothesis that symptom variation of CBSD exits among cassava genotypes and across CBSV strains

5.3: Recommendations

This research recommended the followings;

(i) Sampling wide area of the country to ascertain the distribution of CBSV strains in all geographical locations of Uganda.

(ii) NASE 14, NAROCASS and Aladu should be bred and incorporated with more resistant gene for the management and control of CBSD.

(iii)Also there is need to conduct a study to relationship of CBSV viral load, CBSD symptom severity and resistance of cassava genotypes.

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APPENDICES

Appendix 1: Origin of the samples used in the study

District	GPS coordinates			Genotype	Severity score	Sample identity
Mukono	0.68243	32.6273	1,366m	N 14	2	1
Mukono	0.74583	32.78086	1,366m	N 14	2	2
Mukono	0.670458	32.840285	1,079m	N 12	3	3
Mukono	0.670458	32.840285	1,079m	BAO	2	4
Mukono	0.670458	32.84028	1,079m	N 13	2	5
Kayunga	0.680058	32.925553	1,092m	Bukalasa	2	6
Kayunga	0.680058	32.925553	1,092m	NAROCASS	2	7
Kayunga	0.680058	32.925553	1,092m	TME 204	3	8
Kayunga	0.680058	32.925553	1,092m	N 13	2	9
Kayunga	0.680058	32.925553	1,092m	N 12	3	10
Masaka	-0.362146	31.742332	1,290m	N 13	2	11
Masaka	-0.47000	31.741135	1,283m	N 12	3	12
Masaka	-0.359258	31.744705	1,334m	Bukalasa	3	13
Masaka	-0.359258	31.744705	1,334m	N 14	2	14
Masaka	-0.451172	31.746892	1,282m	Bukalisa	3	15
Luwero	0.707807	32.693448	1,085m	TME 204	4	16
Luwero	0.707807	32.693448	1,085m	TME 204	4	17
Luwero	0.848242	32.697788	1,086m	Bamunanika	3	18
Luwero	0.924963	32.696032	1,099m	Bukalisa	2	19
Luwero	0.925733	32.701860	1,096m	Aladu	3	20

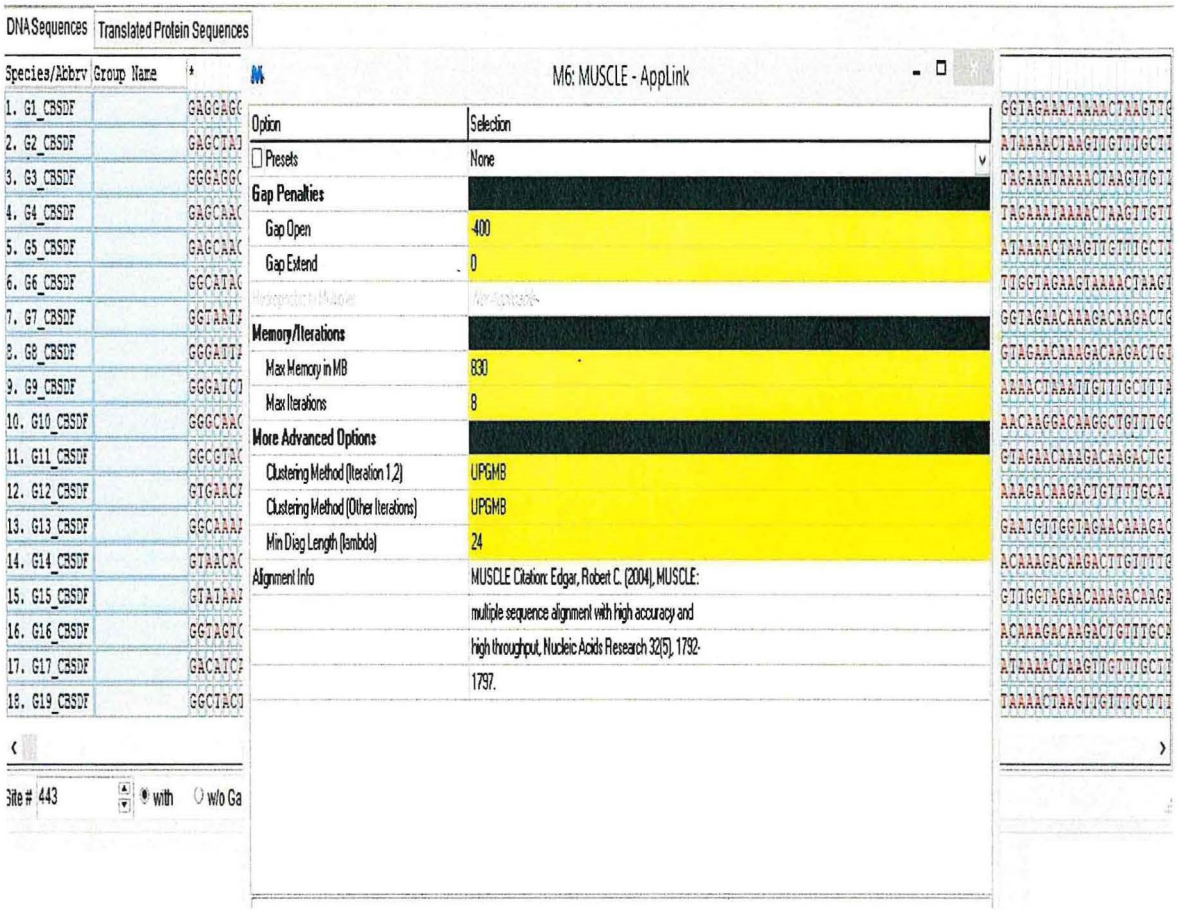
Appendix 2: Base sequence for PCR products

S/n	Code	Bases	Base sequence
G1	A01	422	GAGGAGGATGGGGAAAAGCTGGGTAGATATGACCGCCCCGCCGAAGCAATTTGTCACAAAGCCCTTTGATAT TAAACATCAAATACTGGCTGCTAATGTTGGTAGAAATAAAACTAAGTTGTTTGCTTTACCTGCTCCGGG TGATGATAATAAGGTGGATAAAGAAAGGCACACCACGCGTGATGTTAGTGCAACCAGGCACAGCTATG CGGGTGCCGCTATCGAATAAATTAGTTTGAAAGCCAAGTTTGGTGGAGTTTTAGGTAGCTTGTTATGTTT TGGTTGTGAAGCCAAGACATATTCAGGAGCTCTTTCTCCATATCCATTTTGTTTTCAGGTGGAGGGGGA GTTTTGGAAAGCCAATTATATTTTGGTTGGGAAACCGAATGTGTATTGTGGACTCCCCCCCCCACAAAA CA
G2	C01	410	GAGCTATTAGTGTTAGGTATTGACTTCCTAGCCGAGCACAATTTGTCACAAAGCCAACTTGATATTAAACATC AAATACTGGCTGCTAATGTTGGTAGAAATAAAACTAAGTTGTTTGCTTTAGCTGCTCCTGGTGATGATA ATAATGTGGATAAAGAAAGGCACACCACGCGTGATGTTAGTGCAACCAGGCACAGCTACGCGGGTGCC GCTATCGAATAAATTAGTTTGAAAGTCAAGTTTGGTGGAGTTTTAGGTAGCTTGTTATGTTTTGGTTATG AAGCCAAGACATATTCAGGAGCCCTCTCCCCATATCCTGCGCCGCGCCCCCATTGGGGGTTTGAAGCAT TTTTTGGTTAAACCGATTATTGAGCTCCCTCAAAAAAATTTGACAATCATATTAAGATATG
G3	E01	442	GGGAGGCTTGATGGTGGGTAGGTATTGACTTCCTAGCCGACGCACAATTGTCACAAAGCCAACTTGATATTAA ACATCAAATACTGGCTGCTAATGTTGGTAGAAATAAAACTAAGTTGTTTGCTTTAGCTGCTCCTGGTGA TGATAATAATGTGGATAAAGAAAGGCACACCACGCGTGATGTTAGTGCAACCAGGCACAGCTATGCGG GTGCCGCTATCGAATAAATTATTTTGAAAGCCAAGTTTGGTGGAGTTTTAGGTAGCTTGTTATGTTTTGG TTGTGAAGCCTAGACATATTCAGGAGCCCTTTCTCCATATCCACAATCGTTTTCAAGGGTGGAGGAGTT TTGGATAGCCAATTATATTTTGGTTAGATAAGACAGAATGTAGGTTGTAGCCTCTTCCTTCAAAACAAC AAAAAAACAACCCCACTCCGCGGC
G4	G01	461	GAGCAACCTAGTTGTTGGTATGACTTCCTTAGCCTGAAGCACAATTCGTCCACAAAGCCAACTTGATATTAAA CATCAAATACTGGCTGCCTAATGTTGGTAGAAATAAAACTAAGTTGTTTAGCTTTAGCTGCTCCTGGTG ATGATAATAATGTGGATAAAGAAAGGCACACCACGCGTGATGTTAGTGCAACCAGACACAGCTACGCG GGTGCCGCTATCGAATAAATTAGTTTGAAAGTCAAGTTTGGTGGAGTTTTAGGTAGCTTGTTATGTTTTG GTTATGAAGCCAAGACATATTCAGGAGCCTCCTCCCCATATCCAGCCCCCACAAACGCGGAATAATTTA ATATCCACTAATATATTTTTTGTTAACCAACCAGAATGTTGGGAGTGCCTCCCCCTATAAAAAAACATA AATTGGTTCCCAATCCCCCCCCTAAAATATATCAGTCTTCCCC
G5	I01	438	GAGCAACCTGTTGTTAGGTATGACTTCCTAGCCGAGCACAATTGTCCACAAAGCCAACTTGATATTAAACATC AAATACTGGCTGCTAATGTTGGTAGAAATAAAACTAAGTTGTTTGCTTTAGCTGCTCCTGGTGATGATA ATAATGTGGATAAAGAAAGGCACACCACGCGTGATGTTAGTGCAACCAGACACAGCTACGCGGGTGCC GCTATCGAATAAATTAATTTGAAAGTCAAGTTTGGTGGAGTTTTAGGTAGCTTGTTATGTTTTGGTTATG AAGCCAAGACATATTCAGGAGCCCTCTTCCCATATCCAGAATTTCGTTTTCAAGTTTGGTGGAGTTTTGG ATAGCCAATTATATTTTGGTTAGATAAGCCAGAATGTATTTTGGAGCCCTTTTTCCATATCCAGGAAAA ACAAACCCCCCCCCCAAAA
G6	K01	455	GGCATAGTAGTTAGTCTCCATTATTGACTTAGTAGCTCGTATGCATCAATTGTCACAAAGCCAACTTGATATC AAAGCATCAAATATCTGGCTGCTAATGTTGGTAGAAGTAAAACTAAGTTGTTTGCTTTAGCTGCTCCTG GTGATGATAATAATGTGGATAAAGAAAGGCACACTACGCGGGATGTTAGTGCAACTAGGCATAGCTAT GCTGGTGCTGCTATTGAATAAATTAATTTGAAAGTCAAGTTTGGTGGAGTTTTAGGTAGCTTGTTATATT TTGGTTGTGAAGCCAAGATATATTCAGGAGCCCTCTTCCCATATCCAAATTTCGTTTTCAAGTTTGGTGG AGTTTTGGATAGCCAATTATATTTTGGTTAGATAAGCCAGAATGTATTTTGGAGCCCTTTTTCCATATCC ACGAGGGGCCGCCCGCCCCAAAAAAAAAAAATTTA
G7	M01	466	GGTAATAGCTTAACTAGGATCAGATTTTCTGAGCCGAGTGTCTCACATTAGTCATCAGAGTCAACTTGATATC AAGCATCAGATTCTGGCAGCGAATGTTGGTAGAACAAAGACAAGACTGTTTGCATTAGCAGCTCCTGG AGATGATAATAATGTAGATAGAGAAAGGCACACGACACATGATGTCAGTGCAAACAGGCACAGCTACA GTGGTGCCGCAATTGAATAAATGAAATATTAGGTTTTCTGATTGGGTTTGAATGAGATTATAGCTTAGT TGGAAAGCTAAGTTATATTTCAAATTTAAATTCAAATACTTAAATATTGTATTTCGTTTTCAAGTTTGGT GGAGTTTTGGATAGCCAATTATATTTTGGTTAGATAAGCCAGAATGTATTTTGGAGCCCTCTCCCCAAA ATCCAGGGAAAAGATTTTTTTGGAAAGGAGAGGGAAGGTGGGCGGGGGG
G8	O01	420	GGGATTAGATTCAATTGGTTAAGTATTGACTTCCTAGCCGAAGCTCAGTGTCACAGAGTCTACTTGATATCAA GCATCAGATTCCGGCTGCTAATGTTGGTAGAACAAAGACAAGACTGTTTGCTTTAGCTGCTCCTGGGGA CGATAACAATGTAGATAAAGAAAGGCACACCACACACGATGTCAGTGCAAACAGGCACAGTTATAGTG GTGCCGCAATTGAATAAAAAAAATATTAATGTTTCTATTTGAGTATGAATGAAATTATAGCTTGGTTGG AAAGCTAAGTTGTATTTCAAATTTGAATTCCATTACTTAAATATAGTATTTTGTTTCTAAGTTTGGTGGA GTTTTGGATTGCCAATTATATTTTGGTTAGATAAGCCAAAATGTATTTTGGAGCCCTTTCTCCATATCCA
G9	A03	592	GGGATCTGTGCTAGGTATTGACTTCTAGCCGAGCACAATTGTCACAAAGCCAACTTGATATTAAAGCATCAGA TATCTGGCTGCTAATGTTGGTAGAACTAAAACTAAATTGTTTGCTTTAGCTGCTCCTGGTGATGATAATA ATGTGGATAAAGAAAGGCACACCACGCGTGATGTTAGTGCAACCAGGCCAAGCTTCGCGGTCCCCGCT TTCGAAAAATTTAATTTGAAAGCTTCGTTTGGAGTATTTTTAAGTTTCTTGTTAGGTTGTGGTTATAAGT CCTATATCTAATCTGAATTCCTTCCTTATATACAGTATTTTGTTTCTAAGTTTGGTGGAGTTTTGGATTGC CAATTATATTTTGGTTAGATAAGCCAAAATGTATTTTGGAGCCCTTCCCCATATACAAAGGGTGCGGGC CCGCGAACGAGGGGTGGCAACTCGCCACGGTGCTATAGGTCACACCGAGGAAAGAGACAGAGCAGGG GGTCGCAAGGAAGAAACCATCCAGGCAGATAATGAGGAGTATGCAGGATTGCGGGAGGGAGTCCCGA AGCACCCTACAGAAGAACAAGGCAATACGTGGTACCC
G10	C03	495	GGGCAACATGAGGCATGGTATGACCTTTTCCTTGCCGAAGCCCATTATCACAAAGTCAACTTGATATTAAGCA

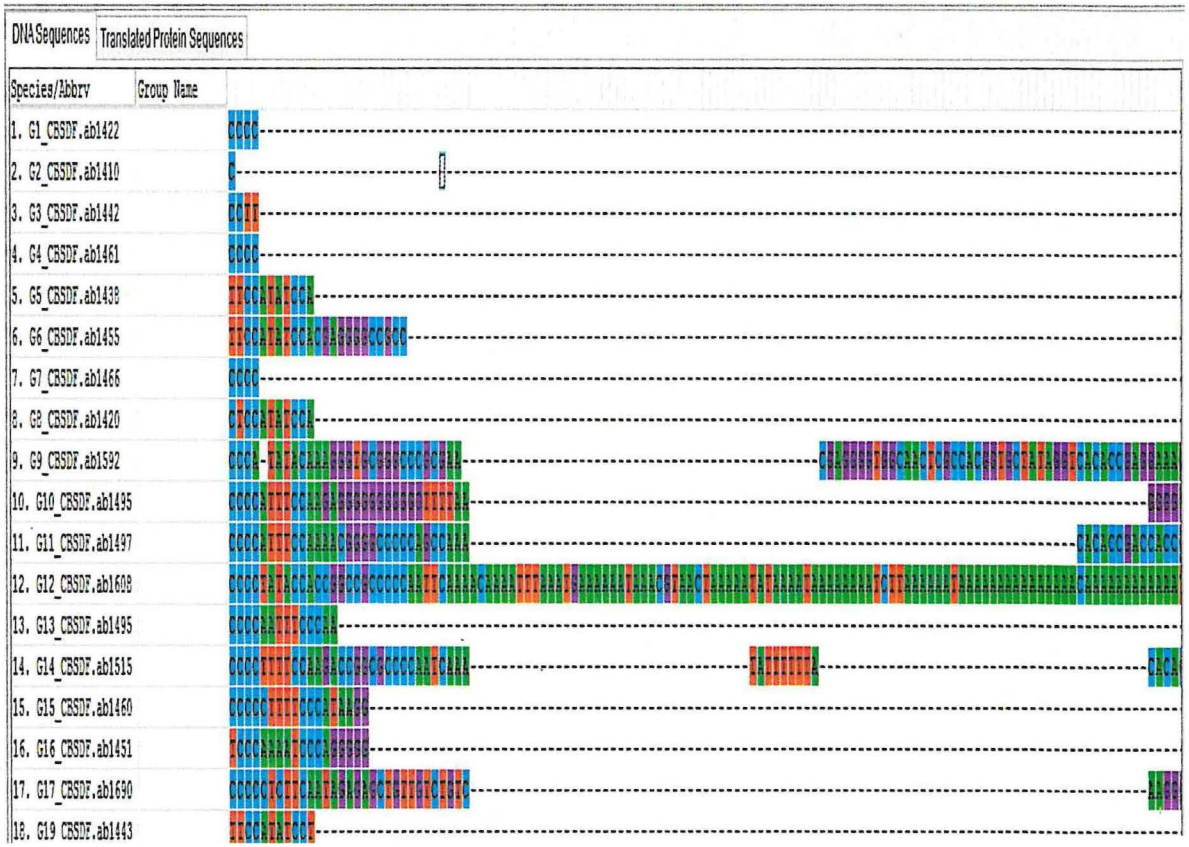
			TCAAATTCTGGCAGCTAATGTTGGTAGAACAAGGACAAGGCTGTTTGCTTTAGGCTGCTCCTGGTGATG ACAACAATGTAGATAAAGAAAGGCACACGACACACGATGTCAGTGCAAATAGGCACAGTTACAGTGGT GCCGCAATTGAATAAATAAAATGTTAATATTTCTAATTAAGTATGATGAGACTATAGCTTAGTTGGAAA GCTGAGTTATTTTCAAATTCGAATTTCATACTTTCAAATATCATATTTTATTTTCAAGTTTGGTGGAGTTT TGGATAGCCAATTATATTTTGGTTGGGTAAGCCAAAATGTATTTTGGAGCCCTCCCCCCATTTCCAAGA GGGGGGGGGGGTTTTAAGGGGGGGGGGGGGGGGAGTTTTATTTGTATAAATATTATCCGCCATTATTTA AGTT
G11	E03	497	GGCGTACAGAGCCTAGGTTCAGATCCTCTTGCCGAGGCCTTCAATTGTCATCCAGAGTCCAACTTGATATCAA GCATCAGATTCCTGGCAGCGAATGTTGGTAGAACAAAGACAAGACTGTTTGCATTAGCAGCTCCTGGA GATGATAATAATGTAGATAGAGAAAGGCACACGACACACGATGTCAGTGCAAACAGGCACAGCTACA GTGGTGCCGCAATTGAATAAATGAAATATTAGGTTTTCTAATTGGATTTGAATGAGATTATAGCTTAGT TGGAAAGCTAAGTTATATTTCAAATTTAAATTCAAATACTTAAATATTGTATTTCGTTTTCAAGTTTGGT GGAGTTTTGGATAGCCAATTATATTTTGGTTAGATAAGCCAGAATGTATTTTGGAGCTATCCCCCCATTT CCAAAACGGGGCCCCCAGCCAAACACACCGACCACCGGGGGGAGAAGGACATACTAGGATGAACAGT TGATTTGAAATAT
G12	G03	608	GTGAACAGAGATACGAATAGATTTCTTGCCGAGGCTCAATTGTCATCAGAAGTCAACTTGATATCAAGCATCA GATTCTGGCAGCGAATGTTGGTAGAACAAAGACAAGACTGTTTTGCATTAGCAGCTCCTGGAGATGATA ATAATGTAGATAGAGAAAGGCACACGACACACGATGTCAGTGCAAACAGGCACAGCTACAGTGGTGCC GCAATTGAATAAATGAAATATTAGGTTTTCTAATTGGATTTGAATAAGATTATAGCTTAGTTGGAAAGC TAAGTTATATTTCAAATTTAAATTCAAATACTTAAATATTGTATTTCGTTTTCAAGTTTGGTGGAGTTTTG GATAGCCAATTATATTTTGGTTAGATAAGCCAAAATGTATTTTGGAGCCCTCCCCCCTATACCACCGGC CGCCCCCAATTCAAAACAAAATTTAAATGAAAAAATAAACGTAACTAAAAATATAAAATAAAAAAAAT CTTAAAAAATAAAAAAAAAAAAAAACAAAAAAAAAAAATTAAAAAAAAAAAAAAAAAAAAAAAAA AAAAGAAAAAAAAAAAAGAAAAAACAAAAGAAAGACAAAAAAAAAGAAAGGGATG
G13	I03	495	GGCAAAATGCCTCACTTAGCATTCCGATACTCGTGAGCCGATGTCTCCACTTACGTCCATCCAGAGTCCAACT TGATATCAAGCATCAGATTCTGGCAGCGAATGTTGGTAGAACAAAGACAAGACTGTTTGCATTAGCAG CTCCTGGAGATGATAATAATGTAGATAGAGAAAGGCACACGACACACGATGTCAGTGCAAACAGGCAC AGCTACAGTGGTGCCGCAATTGAATAAATGAAATATTAGGTTTTCTAGTTGGGTTTGAATGAGATTATA GCTTAGTTGGAAAGCTAAGTTATATTTCAAATTTGAATTCAAATACTTAAATATTGTATTTCGTTTTCAA GTTTGGTGGAGTTTTGGATAGCCAATTATATTTTGGTTAGATAAGCCAGAATGTATTTTGGAGCCTATTC CCCAATTTCCCAAAAAGCCTTAAAAAAAAAATCCAAAACCAAACCCAAGGGGGCGAGCACGGAGGGAA AGCCACGAAG
G14	K03	515	GTAACACGTCGGCACGTTCAGATTTTCTTGCCGAGGCTCAATTGTCATCAGAAGTCAACTTGATATCAAGCAT CAGATTCTGGCAGCGAATGTTGGTAGAACAAAGACAAGACTTGTTTTGCATTAGCAGCTTCCTGGAGAT GATAATAATGTAGATAGAGAAAGGCACACGACACACGATGTCAGTGCAAACAGGCACAGCTACAGTG GTGCCGCAATTGAATAAATGAAATATTAGGTTTTCTAATTGGGTTTGAATGAGATTATAGCTTAGTTGG AAAGCTAAGTTATATTTCAAATTTAAATTCAAATACTTAAATATTGTATTTCGTTTTCAAGTTTGGTGGA GTTTTGGATAGCCAATTATATTTTGGTTAGATAAGCCAGAATGTATTTTGGAGCCATCCCCCCTTTTCCA AGACCGGCGCCCCAATCAAATATTTTTTACACACCCGGAGGGGAGAGAGAAAAACAGAAAAAATGGA AACAGTAAAAATTAAACGAAAGAAGGGTGC
G15	M03	460	GTATAAAGATCCTTGGTTCAGATTCTCTTGCCGAGGCCTTCCAATTGTTCCATCCAGAAGTCCAACTTGATATT CAAGCATCAGATTTCTGGCAGCGAATGTTGGTAGAACAAAGACAAGACTGTTTGCATTAGCAGCTCCTG GAGATGATAATAATGTAGATAGAGAAAGGCACACGACACACGATGTCAGTGCAAACAGGCACAGCTA CAGTGGTGCCGCAATTGAATAAATGAAATATTAGGTTTTCTGATTGGGTTTGAATGAGATTATAGCTTC GTTGGAAAGCTAAGTTATATTTCAAATTCAAATTCAAATACTTAAATATTGTATTTCGTTTTCAAGTTTG GTGGAGTTTTGGATAGCCAATTATATTTTGGTTAGATAAGCCAGAATGTATTTTGGAGCCTCTTCCCCCT TTTCCCATAAGGGGGAAAACAAACAAAAAGGGAAAGGGGAA
G16	O03	451	GGTAGTCTGACTAGCATCAGATTTTCTGAGCCGATGTCTCAATTGTCATCAGAGTCAACTTGATATCAAGCAT CAGATTCTGGCAGCGAATGTTGGTAGAACAAAGACAAGACTGTTTGCATTAGCAGCTCCTGGAGATGA TAATAATGTAGATAGAGAAAGGCACACGACACACGATGTCAGTGCAAACAGGCACAGCTACAGTGGTG CCGCAATTGAATAAATGAAATATTAGGTTTTCTGATTGGGTTTGAATGAGATTATAGCTTCGTTGGAAA GCTAAGTTATATTTCAAATTCAAATTCAAATACTTAAATATTGTATTTCGTTTTCAAGTTTGGTGGAGTT TTGGATAGCCAATTATATTTTGGTTAGATAAGCCAGAATGTATTTTGGAGCCCTCTTCCCAAAATCCCAG GGGGGGAATAAAAGAAAAAAAAAGAAGGAAAAA
G17	A05	690	GACATCATGCTGCTAGATCTTGACTTCCTAGCCGAAGCACAATTGTCACAAAGCCAACTTGATATTAAACATC AAATACTGGCTGCTAATGTTGGTAGAAATAAAACTAAGTTGTTTGCTTTAGCTGCTCCTGGTGATGATA ATAATGTGGATAAAGAAAGGCACACCACGCGTGATGTTAGTGCAACTAGGCACAGCTACGCGGGTGCC GCTATCGAATAAATTAGTTTGAAAGTCAAGTTTGGTGGAGTTTTAGGTAGCTTGTTATGTTTTGGTTATG AAGCCTAGACATATTCAGGAGCCCTTCTTCCATATCCACAATTTCGTTTTTGCCGTTTGGTGGAGTTTTG GATAGCCAATTACATCTTGGTTAGATAAGCCAGCTTTTTTTTCTTCAACCCTTTCTCCCAATAAAAAATT CTCAAGTCCGATCTTGCCCATCGGAGCCTTTGGGGAAATACCCATGTGTTTGGAGGCTTACACATCGGG GCGGAGCTGGGATCGATGGGCGCCAATTTTTCTTGCTGGTCCCCCTCTTCAATAGAGAGCTGTTGTCTGT CAAGGGGGGCGTCAAGAGGCCACAACGAGAGAAAATCTCCCGCCTTTTCGATATTTAGAATGCCCCTG AAGTCGCCATGAAAATGCTCGAGTAACGATCCGTTTACACGAGTATCGGCGAAGGTGAAAAAC
G19	C05	443	GGCTACTAGTGCTAGGTATTGACTTCCTGGCCGAAGCACAATTGTCACAAAGCCAACTTGATATTAAACATCA AATACTGGCTGCTAATGTTGGTAGAAATAAAACTAAGTTGTTTGCTTTAGCTGCTCCTGGTGATGATAA TAATGTGGATAAAGAAAGGCACACCACGCGTGATGTTAGTGCAACCAGGCACAGCTACGCGGGTGCCG CTATCGAATAAATTAGTTTGAAAGCCAAGTTTGGTGGAGTTTTAGGTAGCTTGTTATGTTTTGGTTATGA AGCCTAGACATATTCAGGAGCCCTTCTCCCATATCCAAAATTTCGTTTTCAAGTTTGGTGGAGTTTTGGA

			TAGCCAATTATATTTTGGTTAGACAAGCCAGAATTTTTTTTTAGCTTTTTTTCCATATCCTAAATTTATAA

Appendix 3: Multiple sequence alignment of Cassava brown streak virus isolates from central Uganda 2018



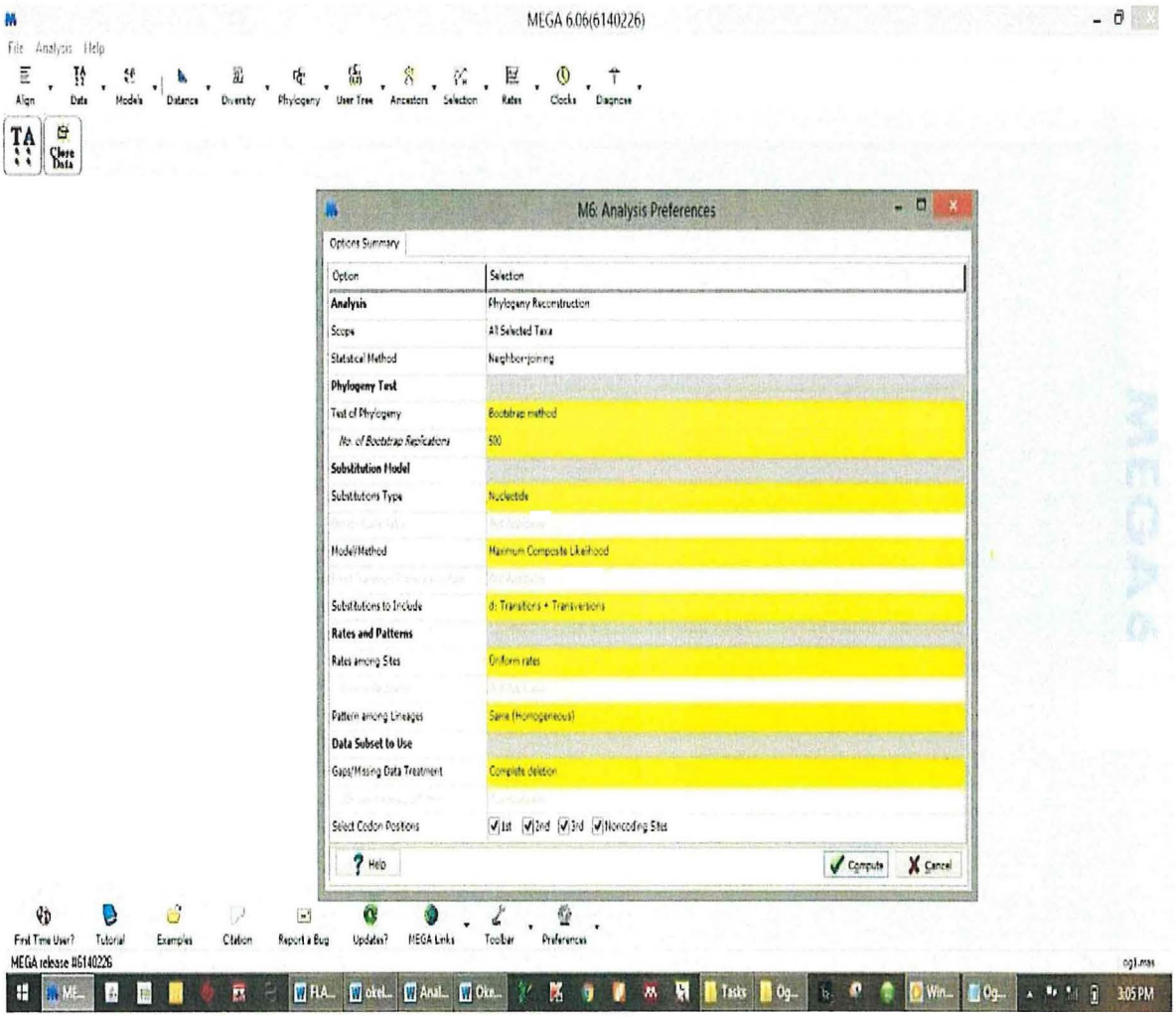
Appendix 4: Aligned sequences



Appendix 5: Phylogenetic analysis parameter

(TEST: Bootstrap, MODEL: Maximum composite Likelihood model, NO. OF BOOTSTRAP REPLICATIONS/BOOTSPRAP PARAMETER: 500 and STATISTICAL METHOD:

Neighbour joining).



Appendix 6: CBSD incidence percentage across cassava genotypes with CBSV strains over time trial one, 2017

MAI	GENOTYPE	CBSV	UCBSV	CBSV+UCBSV	CONTROL
1	ALADU	5	5	5	0
2	ALADU	5	5	12	0
3	ALADU	12	12	12	0
1	BAMUNANIKA	12	5	5	0
2	BAMUNANIKA	12	5	12	0
3	BAMUNANIKA	30	12	30	0
1	BUKALASA	5	5	5	0
2	BUKALASA	5	5	12	0
3	BUKALASA	5	12	30	0
1	NJULE	5	5	5	0
2	NJULE	12	5	12	0
3	NJULE	12	30	12	0
1	NAROCASS	5	5	5	0
2	NAROCASS	5	5	5	0
3	NAROCASS	12	12	12	0
1	NASE 12	5	5	5	0
2	NASE 12	5	5	12	0
3	NASE 12	12	12	12	0
1	NASE 14	5	5	0	0
2	NASE 14	5	30	0	0
3	NASE 14	5	30	5	0
1	TME 204	5	5	12	0
2	TME204	12	12	12	0
3	TME 204	30	30	30	0
1	KWATAMPOLA	5	5	5	0
2	KWATAMPOLA	5	12	12	0
3	KWATAMPOLA	12	12	30	0

Appendix 7: CBSD incidence percentage across cassava genotypes with CBSV strains over time trial two, 2018

MAI	GENOTYPES	CBSV	UCBSV	CBSV+UCBSV	CONTROL
1	ALADU	5	5	5	0
2	ALADU	5	5	12	0
3	ALADU	12	12	12	0
1	BAMUNANIKA	12	5	5	0
2	BAMUNANIKA	12	5	12	0
3	BAMUNANIKA	30	12	30	0
1	BUKALASA	5	5	5	0
2	BUKALASA	5	5	12	0
3	BUKALASA	5	12	30	0
1	NJULE	5	5	5	0
2	NJULE	12	5	12	0
3	NJULE	12	30	12	0
1	NAROCASS	5	5	5	0
2	NAROCASS	5	5	5	0
3	NAROCASS	12	12	12	0
1	NASE 12	5	5	5	0
2	NASE 12	5	5	12	0
3	NASE 12	12	12	12	0
1	NASE 14	5	5	0	0
2	NASE 14	5	12	0	0
3	NASE 14	5	12	5	0
1	TME 204	5	5	12	0
2	TME204	12	12	12	0
3	TME 204	30	30	30	0
1	KWATAMPOLA	5	5	5	0
2	KWATAMPOLA	12	5	12	0
3	KWATAMPOLA	30	12	30	0

Appendix 8: CBSD symptom severity percentage across cassava genotypes with CBSV strains over time trial one, 2017

MAI	GENOTYPES	CBSV	UCBS	CBSV+UCBS	Control
1	ALADU	5	0	0	0
2	ALAD	5	0	0	0
3	ALADU	5	5	5	0
1	BAMUNANIKA	5	5	5	0
2	BAMUNANIKA	5	5	5	0
3	BAMUNANIKA	30	30	30	0
1	BUKALASA	5	5	5	0
2	BUKALASA	5	5	5	0
3	BUKALASA	30	30	5	0
1	NJULE	5	5	5	0
2	NJULE	5	5	5	0
3	NJULE	5	30	5	0
1	NAROCASS	0	5	0	0
2	NAROCASS	0	5	0	0
3	NAROCASS	5	30	30	0
1	NASE 12	5	5	5	0
2	NASE 12	5	5	5	0
3	NASE 12	5	30	5	0
1	NASE 14	0	5	0	0
2	NASE 14	0	0	0	0
3	NASE 14	5	0	5	0
1	TME 204	5	5	5	0
2	TME204	5	5	5	0
3	TME 204	30	30	30	0
1	KWATAMPOLA	5	5	5	0
2	KWATAMPOLA	30	30	30	0
3	KWATAMPOLA	50	30	50	0

Appendix 9: CBSD symptom severity percentage across cassava genotypes with CBSV strains over time trial two, 2018

MAI	GENOTYPES	CBSV	UCBSV	CBSV+UCBSV	CONTROL
1	ALADU	5	0	5	0
2	ALAD	5	5	5	0
3	ALADU	30	5	30	0
1	BAMUNANIKA	0	5	0	0
2	BAMUNANIKA	30	30	30	0
3	BAMUNANIKA	30	30	30	0
1	BUKALASA	5	5	5	0
2	BUKALASA	30	30	5	0
3	BUKALASA	30	30	30	0
1	NJULE	5	5	5	0
2	NJULE	5	30	30	0
3	NJULE	30	30	30	0
1	NAROCASS	5	5	0	0
2	NAROCASS	5	30	30	0
3	NAROCASS	5	30	30	0
1	NASE 12	0	5	5	0
2	NASE 12	5	30	5	0
3	NASE 12	30	30	30	0
1	NASE 14	0	0	0	0
2	NASE 14	5	5	5	0
3	NASE 14	5	5	5	0
1	TME 204	5	5	5	0
2	TME204	30	30	30	0
3	TME 204	50	50	50	0
1	KWATAMPOLA	5	5	5	0
2	KWATAMPOLA	5	5	30	0
3	KWATAMPOLA	50	30	50	0

Appendix 10: Means of CBSD symptom severity across cassava genotypes under screen house September –December 2017 after 1 MAI interval trial one

Viral spp	CBSV	UCBSV	CBSV+ UCBSV	CBSV	UCBSV	CBSV+ UCBSV	CBVS	UCBSV	CBSV + UCBSV
Time (wks)	1MAI			2MAI			3MAI		
Genotype									
Aladu	1.667	1.333	1.333	1.667	1.333	1.333	2.333	2.0	2.333
Bamunanika	2.0	1.667	1.2	2.0	1.667	2.0	2.667	3.0	2.667
Bukalisa	2.0	2.0	2.0	2.0	2.0	2.0	2.8	2.0	2.333
Kwatampola	2.0	2.0	2.0	3.0	2.0	3.333	3.4	3.0	4.0
NAROCAS S	1.4	2.0	1.2	1.4	2.0	1.333	2.6	2.0	3.0
NASE12	1.8	1.1	2.0	1.8	1.2	2.0	2.6	2.0	2.333
NASE 14	1.2	1.1	1.2	1.2	2.0	1.333	2.0	2.0	2.0
Njule	2.0	2.0	1.667	2.0	2.0	1.667	2.6	3.0	2.333
TME	2.0	2.0	2.333	2.0	2.0	2.333	3.0	3.0	3.0
LSD (5%)	0.4024			0.4699			0.5612		

Appendix 11: Means of CBSD symptom severity across cassava genotypes under screen house in January –April 2018 after 1MAI interval trial one

Viral spp	CBSV	UCBSV	CBSV+ UCBVS	CBSV	UCBSV	CBSV+ UCBVS	CBSV	UCBSV	CBSV+ UCBVS
Time (wks)	1MAI			2MAI			3MAI		
Genotype									
Aladu	2.0	1.333	2.0	2.333	2.0	2.333	2.667	2.333	3.0
Bamunanika	1.333	1.667	1.2	2.667	3.0	2.667	3.333	3.333	3.0
Bukalisa	1.75	1.5	1.667	2.75	2.5	2.333	3.0	3.0	3.333
Kwatampola	2.0	2.0	2.0	2.5	2.5	3.333	3.5	3.0	4.0
NAROCAS	1.5	2.0	1.2	2.5	2.5	2.333	2.5	2.5	3.0
NASE 12	1.5	2.0	1.667	2.5	2.5	2.333	3.0	2.5	3.667
NASE14	1.25	1.1	1.2	2.0	2.0	2.0	2.0	2.0	2.333
Njule	1.75	1.5	1.667	2.5	3.0	2.667	3.0	3.0	3.333
TME	1.5	2.0	2.333	3.0	2.5	2.5	4.0	4.0	4.0
LSD (5%)	**0.5489**			**0.6900**			**0.5834**		

Appendix 12: Mean CBSD symptom severity within and across cassava genotypes under screen house September –December 2017 after 3MAI trial one

Genotype (n=3)	**Mean severity (scale 1-5)**			
	CBSV	**UCBSV**	**CBSV+UCBSV**	**CONTROL**
Aladu	1.889	1.555	1.666	1
Bamunanika	2.222	2.111	2.222	1
Bukalisa	2.666	2	2.666	1
Kwatampola	2.8	2.333	3.111	1
NAROCASS	1.8	2.0	1.888	1
NASE 12	2.066	1.333	2.111	1
NASE 14	1.333	2.0	1.555	1
Njule	2.2	2.333	1.889	1
TME 204	2.333	2.333	2.555	1
Grand mean	**2.145**	**1.999**	**2.184**	**1**

Appendix 13: Mean CBSD symptom severity within and across cassava genotypes under screen house January – April 2018 after 3MAI for trial two.

Genotype (n=3)	Mean severity (scale 1-5)			
	CBSV	UCBSV	CBSV+UCBSV	CONTROL
Aladu	2.333	1.888	2.444	1
Bamunanika	2.444	2.666	2.222	1
Bukalisa	2.5	2.333	2.444	1
Kwatampola	2.666	2.5	2.888	1
NAROCAS	2.166	2.333	2.111	1
NASE 12	2.0	2.166	2.111	1
NASE 14	1.75	1.166	1.777	1
Njule	2.266	2.5	2.555	1
TME 204	2.833	2.833	3.111	1
Grand mean	**2.427**	**2.265**	**2.407**	**1**