SCREENING OF PARENTAL CASSAVA GENOTYPES AND GENERATED PARTIAL INBREDS FOR RESISTANCE TO CASSAVA BROWN STREAK DISEASE IN UGANDA

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A THESIS IS SUBMITTED TO THE DIRECTORATE OF RESEARCH AND GRADUATE TRAINING IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN CROP SCIENCE OF MAKERERE UNIVERSITY

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DECLARATION

This thesis is a presentation of my original research work. Wherever contribution of others are involved, every effort is made to indicate this clearly, with due reference to the literature and acknowledgement of collaborative research and discussion.

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DEDICATION

I dedicate this thesis to my God and Lord in whom dwells all goodness – my strength and wisdom.

To my parents (Mr and Mrs Lukyamuzi) – You spent wisely.

To my dearest, Doreen and Joe – Thanks for your love, patience and support.

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I would like to thank my God for the wisdom, strength and His abiding presence that has given me reason to move on – without you I cannot do anything.

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"Everything worthy possessing, even in this world, must be secured by effort and sometimes by most painful sacrifice" E.G White.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Damage caused by CBSD associated viruses (CBSV and UCBSV) is increasing in Africa and only limited resources are available to control the spread of the disease. Therefore, more robust and durable methods of controlling these viruses are needed. The development of such methods requires the identification of additional sources of useful virus resistance and a better understanding of resistance mechanisms. Here, I report on generation of new sources of resistance and identification and characterization of resistance sources from Uganda and Tanzania. New sources of resistance were generated through inbreeding of selected parental genotypes (S_0) with a premise that the generated partial inbreds (S_1) would perform better than their respective non-inbred progenitors in terms of resistance to CBSD. According to the study, the generated partial inbreds showed higher disease index than their respective parents though the difference was not significant (5%) except for Namikonga. However, varying number of asymptomatic genotypes for CBSD was recorded from the generated partial inbreds showing that new sources of resistance can be generated through inbreeding.

Identification and characterisation of resistance to CBSD from the already available germplasm was done by both laboratory (using real time PCR) and field screening of selected genotypes at National Crops Resources Research Institute (NaCRRI), a "hotspot" for CBSD in Uganda. Ten cassava parental genotypes (three from Uganda and seven from Tanzania) were selected for evaluation. According to the results, none of these screened genotypes exhibited resistance to infection to both UCBSV and CBSV in Uganda. However one genotype (Namikonga, a cross between *M. esculenta*×*M. glaziovii)* from Tanzania was found to have comprehensive field resistance to CBSD while NASE 1 from Uganda had tolerance to CBSD. These genotypes were characterized by ranking them based on their disease index and Wilcoxon ranking, a new method developed by this study. The screened genotypes have exhibited various mechanisms of resistance to virus which included resistance to virus accumulation in NASE 14 and reversion or 'recovery' from UCBSV infection in Kiroba. In conclusion, new sources of resistance to CBSD of resistance were generated through inbreeding and four genotypes (Namikonga, NASE 1, NASE 14 and Kiroba) among those screened have been identified to have unique attributes that can be exploited for resistance to CBSD.

CHAPTER ONE

INTRODUCTION

1.1 Cassava: Origin and socioeconomic importance

Cassava (*Manihot esculenta* (Crantz) is one of the 98 species of *Manihot* known (Rogers and Appan, 1973). It is believed to have originated from Latin America where it was first domesticated in the Amazon basin and later in 17th century introduced into Africa (Olsen, 2004). Cassava production and consumption has, and continues to grow at a high rate in Africa, Asia and South America. It is the second most important root crop in the world after potatoes (*Solanum tuberosome*)and second most important staple crop in Africa after maize (*zea mays*) (FAO, 2009). Its increasing popularity can partly be attributed to its ability to grow on marginal soils with limited resource input that characterises many smallholder farmers (Devries and Toenniesen, 2001; Nasser and Ortiz, 2007). Furthermore, cassava harvest time ranges from 8-24 months after planting (Salcedo *et al.*, 2010) which allows for piece meal harvesting.

Cassava root contains 34-45% dry matter weight containing 73.7-84.9% of dietary starch (Rickard *et al.,* 1991; Okigbo, 2000). It is a major and the cheapest source of carbohydrates to over 800 million people worldwide contributing over 500 kcal per day (FAO, 2009). In addition, Cassava leaves are consumed as vegetables in many areas because they contain high amount of vitamins (A and C), minerals (iron, zinc, calcium, potassium) and proteins (USDA, 2012). Due to low protein content (0.53% of total root weight) along with other factors like naturally occurring cyanogens and rapid post harvest deterioration, cassava commercialization and/or utilization has remained a challenge in some communities.

However through transgenic biofortification techniques, novel sinks for proteins can be generated thereby increasing the amount of proteins in root tuber. Therefore cassava consumption is expected to increase in future. On the other hand, the low level of fat and protein in cassava has made cassava starch more desirable for food processing than starch derived from cereals for its non cereal taste (Ceballos *et al.,* 2007b). More so, cassava starch has greater clarity and viscosity with high stability in acidic food products. Therefore, starch and its products are

important in the paper, pharmaceutical, wood, packaging and textile industries, in ethanol and alcohol production and in the production of explosives like matches.

While global demand for cassava starch has increased over the last 20-30 years, only Thailand has made the transition from a staple food to products and raw materials for processing industries. In the Caribbean and Latin America, the cassava starch business is expanding, with a marked trend towards production of modified and hydrolysed starches that command higher prices. In Africa, most cassava starch is processed in small sized, community – level factories using labour intensive, traditional techniques. There are signs of growing interest in using locally made starch as an import substitute. Cassava starch start-ups have recently been established in Uganda, Tanzania, Malawi and Madagascar, while Nigeria has gone a step forward by producing ethanol biofuel from cassava. This means that cassava is not only a food security crop but also a raw material for many industries and therefore contributing to economic development.

1.2 Cassava production and its constraints in Uganda

According to FAO (2009) cassava production has been increasing for the last 40 years from 93.1 million metric tonnes (MMT) to 232.9 MMT in 2008 and is projected to reach 291 MMT in 2020 (Scott *et al.,* 2000). A total of 18.4 million hectares have been devoted to cassava production worldwide. Latest reports show that the 64.2% of this area is in Africa (FAO, 2011). The highest cassava producing countries include Nigeria (3,737,090 ha), Democratic Republic of Congo (2,171,180 ha), Mozambique (975,519 ha), Ghana (889,364 ha), Tanzania (739,794 ha) and Uganda (426, 148 ha).

1.2.1 Biotic and abiotic factors affecting the production of cassava in Uganda

In Uganda production of cassava in the last 20 years has increased from 3.4 MMT in 1990 to 5.3 MMT in 2010 registering a growth rate of 35%. In comparison to other cassava producing countries like Malawi with the same production area and a production growth of 95.7% (from 144 tonnes to 3.4 MMT), cassava production growth is still very low. Similar to many cassava growing areas, the actual yield recorded on farmer's fields (4-8 t/ha) in Uganda is far below the potential yield 25 – 30 t/ha under research stations. This yield gap can be accounted for by an array of biotic, abiotic, crop management and socio-ecomonic factors.

Cassava production in major producing countries is limited by numerous factors. Among these, pests and diseases cause the highest yield losses and in turn render many areas non productive. Diseases like cassava mosaic disease (CMD), cassava brown streak disease (CBSD), cassava bacterial blight (CBB) and insect pests like green mites (*Mononychellus tanojoa)* and whitefly (*Bemisia tabaci*) have had a devastating effect on cassava production in Uganda and other cassava growing countries (Hillock and Thresh 2000). Most problematic have been viral diseases, and specifically CMD and CBSD. This is because the rate of evolution among viral populations (causative agents of these diseases) is high. For instance, CMD has been reported to be caused by six viruses of genus begomovirus (Fregene *et al.,* 2004), while CBSD is caused by two viruses (UCBSV and CBSV). These overlapping pest and disease problems have been the major cause of loss and abandonment of a significant number of cassava varieties.

In response to these challenges, several interventions have been initiated to combat these disease and pests, but to varying levels of success. In most cases, cassava research efforts especially in Africa have focused on the breeding and development of integrated management strategies for the major pests and diseases. However little attention has been given to the abiotic and socioeconomic constraints such as unfavourable climatic conditions, poor soil fertility, poor quality planting materials, poor post-harvest handling technologies, poor market infrastructure and organization. Studies are needed to understand the relative importance of each of these factors to yield gap. In this thesis, efforts are tailored towards addressing the major viral challenge to cassava production in Uganda, CBSD.

1.2.2 Cassava brown streak disease in Uganda

Considering the production trend of cassava over the last 50 years, there has been a steady growth in the volume produced and consumed worldwide. According to FAOSTAT (2010), cassava production in Uganda increased from 2.2 MMt in 1995 to 5.5 MMt in 2005. However there was a sharp decline in production in the years that followed (from 5.5 MMt to 4.9 MMt in 2006 and 4.46 MMt in 2007) (Figure 1). This decline can partly be attributed to the CBSD problem in Uganda (NARO 2006; Alicai *et al.,* 2007), which causes over 70% yield losses in susceptible cultivars (Hillocks *et al.,* 2001). The disease is spread through planting of infected cuttings, mechanically and/or by vectors (Legg and Hillocks, 2003)

The disease was first reported in the country in the 1940s but was then eradicated by the Government of Uganda (Jameson, 1964). However in 2005, the disease was observed in four major cassava producing districts in Uganda which included Mukono, Luwero, Wakiso and Kaberamaido (Alicai *et al*., 2007). Later that year, the disease was reported in twelve other districts indicating a high rate of spread.

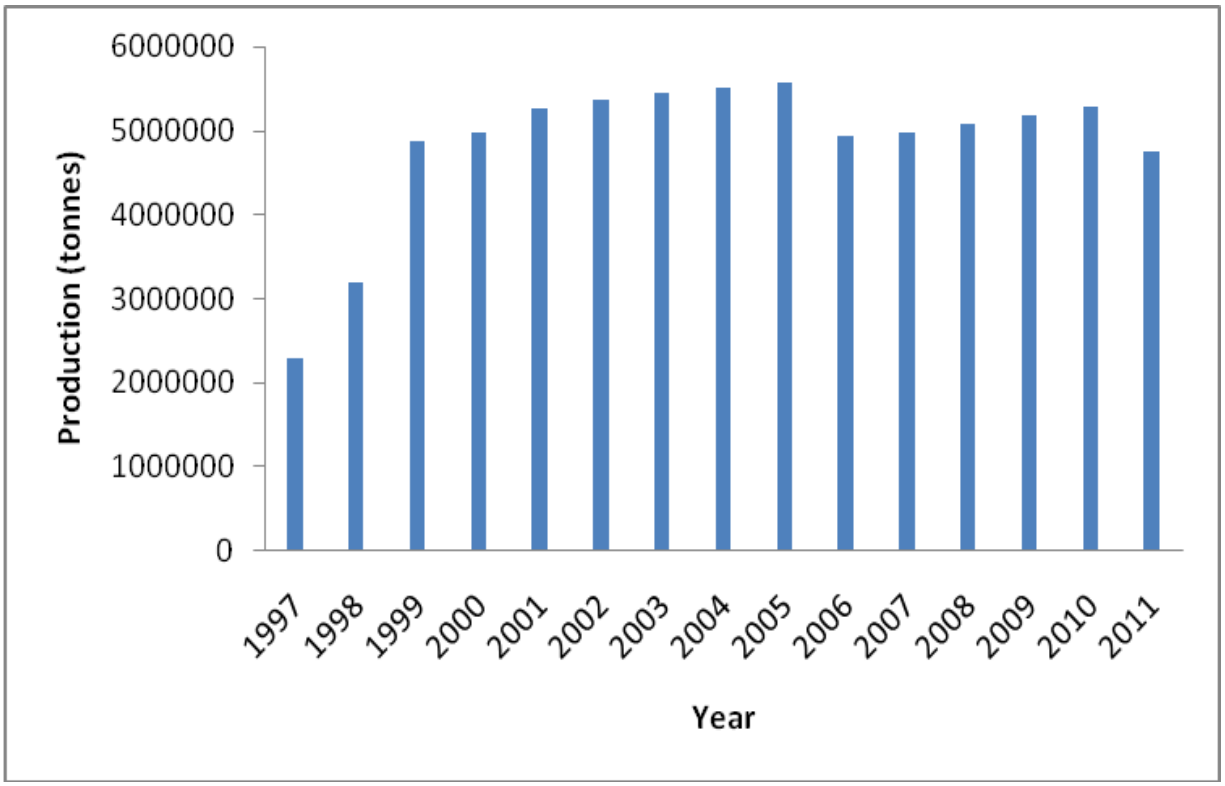

Figure 1: Production of cassava in Uganda for the last 15 years (FAOSTAT, 2011)

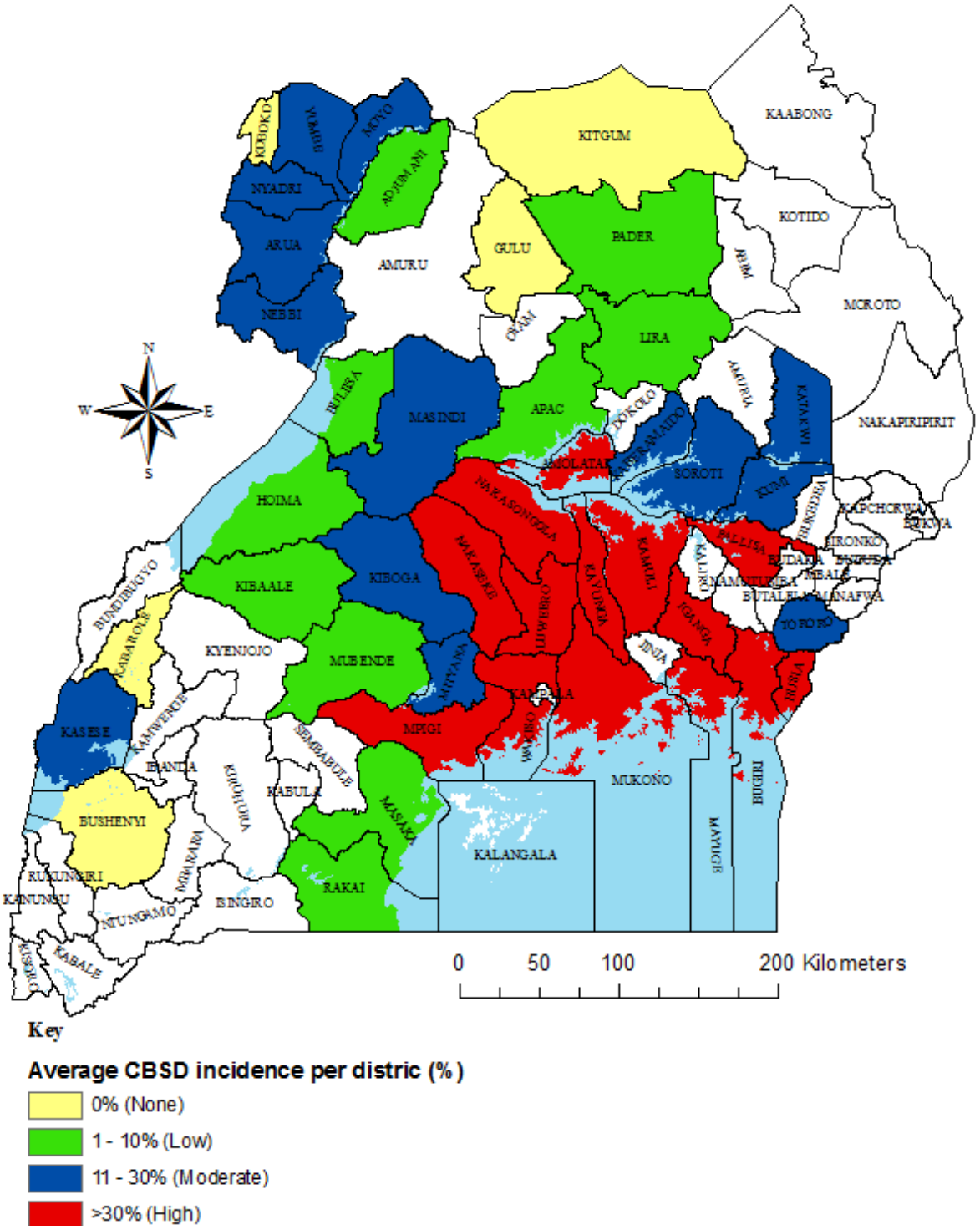

Figure 2: Incidence of CBSD in Uganda based on survey conducted by Eastern Africa Agricultural Productivity project (EAAPP) in 2011 (EAAPP Survey Report, 2012)

Recently, a survey conducted in Uganda by the Eastern Africa Agricultural Productivity Project (EAAPP) in 2011 in Uganda revealed that 38 of the 42 districts surveyed had CBSD, with varying incidence levels. Of these, 13 districts had comparatively high incidence of CBSD. These included; Amolatar (48.5%), Bugiri (59.3%), Busia (41.4%), Iganga (77.3%), Kamuli (93%), Kayunga (58%), Luwero (75.8%), Mayuge (68%), Mukono (52.7%), Nakaseke (73.5%), Nakasongola (80.3%), Pallisa (60.9%) and Wakiso (52.5%) (Figure 2).

In many CBSD affected countries, the spread of the disease has been controlled by 1) sensitizing farmers about the disease, 2) rouging, 3) use of CBSD-tolerant planting materials and 4) implementing strict quarantine (Hillocks, 2002a). In Uganda CBSD affects many improved CMD resistant and highly adopted cultivars like TME 204 and TME 14 (Alicai *et al.,* 2007) and thus pose a great threat to food security. The rapid spread of the disease in Uganda requires that efforts be made to mitigate its further spread and destruction to cassava. To attain this goal, reliable sources of tolerance to cassava brown streak disease (CBSD) should be sourced in both local and introduced cassava germplasm, and when identified, should be used as breeding stocks in the improvement of preferred cassava varieties in Uganda. This study was initiated to screen cassava genotypes introduced in Uganda from the Tanzania breeding programme for resistance or tolerance to CBSV and UCBSV. In addition, this study intended to generate progeny from these genotypes and further evaluate them for tolerance or resistance to CBSD.

1.3 Justification of the study

Demand for food is quickly rising and will continue to rise due to increase in population and reduction in arable land. In Africa, cassava has been identified as a major food security crop due to its adaptability to marginal areas. As a result, it has been widely grown in tropical and sub tropical areas. However, the role of cassava as a food security crop especially to the poor is under threat due to an increase in both biotic and abiotic factors that limit attainment of optimal yields. Various control strategies have been devised to combat the two major viral diseases CMD and CBSD. Significant progress has been made towards the control of CMD, but there has been limited progress for CBSD. For instance, between 1990 and 2003, 12 high yielding and CMD resistant varieties were released. But unfortunately all these are susceptible to CBSD, as they were not selected for CBSD resistance then.

Among the control strategies of CBSD, breeding for resistance offers the most efficient and sustainable management strategy (De Veries and Toenniesen, 2001). However before a breeding program is initiated, there is need to screen both local and introduced germplasm to identify possible sources of resistance or tolerance.

In other CSBD affected countries especially Tanzania and Mozambique, some cultivars like Kibaha, Namikonga, Kigoma Red, Nachinyaya, Kiroba, Kalulu and Kitumbua have been screened and identified to be tolerant to CBSD (Hillocks, 2006). These cultivars have potential for use as parental genotypes in the breeding program in Uganda.

Recent studies have shown that there are two species of the CBSD causal virus, cassava brown streak virus (CBSV) and Uganda cassava brown streak virus (UCBSV) and that both are present in Uganda (Mbanzibwa *et al.,* 2009a). Earlier reports show that the Ugandan strain was prevalent in cassava growing areas in high altitudes areas (1000m asl) in Uganda, north western Tanzania and western Kenya (Mbanzibwa *et al.,* 2009a). There is a need therefore to determine the response and specificity of resistance/tolerance of the introduced genotypes against these virus species under Ugandan conditions before they can be used as parental lines.

Since cassava is highly heterozygous, inbreeding has been suggested as a very useful strategy in exploring some of the hidden diversity in breeding program. According to Ceballos *et al.* (2007a), inbreeding enables selection of superior and genetically stable progenitors whose additive variance has been increased thereby improving its performance as a parent in hybrid combinations (forming better gene combinations) during the breeding process. Moreover, inbreeding is also associated with other benefits notable of which is the reduction of undesirable and deleterious genes (genetic load) which usually prevents the selected cultivars from fully expressing their genetic potential (Hedrick, 1983). Thus, an additional objective of this study was initiated to generate partial cassava inbreds for evaluation for resistance and/or tolerance to CBSD.

1.4 Objectives of the study

The overall objective of this study was to identify and generate sources of resistance and/or tolerance to CBSV and UCBSV from genotypes introduced to Uganda and in addition explore the inbreeding strategy as a way of generating new sources of resistance.

1.4.1 Specific objective

The specific objectives of the study are to:

1. Identify resistant genotypes for both CBSV and UCBSV in Uganda
2. Develop and screen generated partial inbred lines for resistance to CBSV and UCBSV in Uganda

1.5 Hypothesis

The above objectives are based on the following hypotheses:

1. Cassava genotypes tolerant to CBSV in Tanzania are also tolerant/resistant to combined CBSV and UCBSV species in Uganda
2. Cassava inbreds have higher levels of tolerance to CBSD than their respective non-inbred parents

CHAPTER TWO

LITERATURE REVIEW

2.1 The cassava brown streak disease

Cassava brown streak disease (CBSD) is a major disease affecting cassava causing yield losses of 50-70% among susceptible varieties (Legg and Hillocks, 2003). The disease is caused by two virus species, CBSV and UCBSV. CBSD is a systemic disease and thus its symptoms manifest on leaves, stem and roots of infected cassava varieties. On the leaves, it causes chlorotic leaf mottle initially along margins of secondary veins later affecting tertiary veins causing chlorotic blotches. On very sensitive varieties, the disease causes purple/brown elongate necrotic lesions on the exterior surface of the stem. Another notable symptom is the death of nodes and internodes that results into the so called dieback (Hillocks and Thresh, 2000).

On the roots, the disease causes yellow and/ or brown, corky necrosis within the starch bearing tissues along with black streaks which renders roots unusable. In advanced stages, the necrotic symptoms are combined with soft rot caused by invasion by secondary organisms. In some cases, infected roots develop fissures and pits in the surface bark rendering those roots unmarketable (Hillocks *et al.*, 2001). Root necrosis studies conducted in Uganda, indicate that symptoms can be expressed as early as four months in sensitive varieties (Abaca *et al.*, 2012). Initially, tolerance levels of any given cassava cultivar was determined by severity of CBSD symptoms as observed on the roots. However, today, tolerance is defined by both severity and incidence of CBSD. Incidence is the proportion of cassava plants of a given cultivar expressing CBSD symptoms while severity is the degree of CBSD infection on individual plants of a given genotype. Hillocks *et al.* (1996) reported that some cultivars may not develop root necrosis until eight months after planting, while others develop root necrosis as early as five months.

Factors that affect CBSD symptoms expression include plant age, genotype, soil nutrients, pests and environmental conditions (Hillocks and Jennings, 2003). As a result, foliar symptoms are variable, irregular and sometimes transient. In case of mixed infection of both CBSD and CMD, symptom specific to each virus are observed, except that distortion of leaves caused by CMD often masks CBSD symptoms. This presents a limitation in using symptoms for disease

diagnostics and thus phenotypic screening should be complimented with optimized diagnostic methods that employ either molecular or biological techniques.

2.2 Historical perspective and distribution of cassava brown streak disease

CBSD was first reported by Storey in 1936 near the coast of northern Tanzania. Since then the disease has been reported in coastal areas of Kenya, northern Mozambique, Zanzibar and areas close to the shores of Lake Malawi (Hillocks *et al.,* 2001; Gondwe *et al*., 2002). Recently reports have documented the presence of CBSD especially that caused by UCBSV in the Democratic Republic of Congo, Rwanda, Western Kenya, Rwanda, Burundi and Lake Victoria region of Tanzania (Abarshi *et al.,* 2010; Bigirimana *et al.*, 2011; Legg *et al*., 2011). In Uganda, CBSD was first observed in the 1940s on cassava genotypes introduced from Tanzania. The disease was however eradicated completely by the Ugandan Government (Jameson, 1964) but re-emerged in 2005 (Alicai *et al*., 2007). According to the survey carried out in Uganda in 2005, CBSD prevalence was first reported in four districts which included Mukono, Wakiso, Luwero (located in central Uganda) and Kaberamaido located in eastern part of the country. The disease was later reported in 12 other neighbouring districts (NARO, 2006). Today the disease is reported in over 37 districts, some of which exhibit mixed infection of both CBSV and UCBSV (EAAPP Survey Report, 2012).

2.3 Transmission of cassava brown streak virus (CBSV)

Transmission studies on CBSV show that the cassava brown streak virus is insect transmitted (Maruthi *et al.,* 2005), which could partly explain the rapid spread of the disease in Uganda. Two whitefly species are reported to transmit CBSV, and these include *Bemisia afer* and *Bemisia tabaci* (Gennadius) (Hemiptera: *Aleyrodidae*). Recent transmission studies confirmed *Bemisia tabaci* as the vector of CBSV (Maruthi *et al.,* 2005). It suffices to note that though this study (Maruthi *et* al., 2005) reported low transmission rate (20-22%), other studies have reported higher incidence of CBSD (64%) in areas with high *B. tabaci* population (Alicai *et al.,* 2007). Recently, a new whitefly species *Aleurodicus dispersus* was reported and associated to the spread of CBSD in the coastal region of Kenya (Mware *et al.,* 2010). Indeed high incidence of CBSD (74.4%) were recorded in Msambwani, a region with high *A. dispersus* population (mean

of 40.2 flies) as compared to Kilifi with CBSD incidence of 20% and *A dispersus* population of 0.6 (Mware *et al.,* 2010). Mware (2009) further compared the efficiency of transmission of the two reported species of whitefly (*B. tabaci* and *A. dispersus*) where *B. tabaci* was found to have higher transmission efficiency (40.7%) compared to *A. dispersus* with 25.9% concluding that *Bemisia tabaci* is the key vector of CBSVs.

2.4 Diversity of cassava brown streak virus

There are two genetically distinct species of CBSV in East Africa which include CBSV and UCBSV (Winter *et al*., 2010). Both of which are monopartite, positive-sense, single stranded RNA viruses characterised by an elongate flexuous filaments 650-750 nm long (Monger *et al.,* 2001, Winter *et al*., 2010). Both species are members of *Ipomovirus* belonging to family *Potyviridae*. According to Lecoq *et al.* (2000), this family contains monopartite viruses which include *sweet potato mild mottle virus, cucumber vein yellowing virus* and *squash vein yellowing virus*. The brown streak viral genome is monopartite 9.1kb in size (Mbazibwa *et al.,* 2009a) with only one particle size of linear positive sense single stranded RNA (ssRNA) encoding for structural and non – structural proteins.

The first reports showed that CBSV was only prevalent in low altitudes (200 – 600 m asl) in Tanzania, Kenya, Zanzibar and Mozambique (Hillocks, 1997; Hillocks, 2002a; Gondwe *et al.,* 2002). It was earlier thought that the two CBSV species were limited by altitude. Recently CBSV was reported in cassava growing area in Uganda at altitude above 1000 m asl (Alicai *et al.,* 2007). Therefore based on altitude, the strain found in coastal areas of Tanzania, Kenya, Zanzibar and Mozambique has been named CBSV while the Ugandan strain has been named UCBSV which is a mid altitude strain. Based on coat protein and amino acid comparison, Mbanzibwa (2009a) compared six isolates of brown streak virus obtained from Uganda and the Indian Ocean coastal area with eight isolates from coastal lowland of Tanzania and Mozambique. Results from that study concluded that the six isolates had identical though higher coat protein nucleotide sequence and amino acid sequence (90.7-99.5% and 93-99.5% respectively) as compared to 75.8-77.5% and 87-89.9% of the eight isolates. Thus, the study therefore concluded that there are only two genetically distinct and geographically separated population of CBSV in

East Africa. This however is no longer true. According to the recent reports and surveys (EAAPP, 2012), the two virus species have been found in different areas with varying altitude.

Major differences that contributed to this variability were located in *Ham1h pyrophosphatase* gene (Mbanzibwa, 2009b) and *P1* gene (Winter *et al.,* 2010). Reports of the occurrence of both species in Uganda have been documented (Mbanzibwa *et al.,* 2011). What is uncertain however is whether or not the species have the same virulence. It is against this background that this study sought to quantify the response of CBSD tolerant varieties from Tanzania against the UCBSV and/or their response to mixed infection of both CBSV and UCBSV.

2.5 Diagnostics methods used for detection of cassava brown streak virus

A number of conventional RT-PCR assay have been developed for detection of CBSV and UCBSV (Monger *et al*., 2001; Mbanzibwa *et al*., 2011). Primers that are specific to each species of CBSV have been developed; therefore simultaneous detection of both viruses in a sample is possible through a two step RT-PCR procedure. Recently Adams *et al*., (2013) developed a real time RT-PCR assay that can be used for detection and quantification of both CBSV and UCBSV even in very small quantities. However, the above methods require thermal cycling equipment and take a relatively long time. These limitations have been solved by the development of a Loop-mediated isothermal amplification (RT-LAMP) assay, a rapid detection system for both CBSV and UCBSV (Tomlinson *et al*., 2013). In comparison with RT-PCR and real time RT-PCR methods, RT-LAMP amplification is completed in 40 minutes and doesn't require a thermal cycler. In this thesis RT-PCR was used to monitor virus titre in candidate genotypes under field evaluation (Chapter four).

2.6 Differential reaction of cassava varieties to different CBSV strains

Different cassava cultivars are reported to exhibit different symptoms when affected with CBSD (Monger *et al.,* 2001). So far, no work has been done to determine the differential reaction of different cultivars of cassava to the two species in East Africa and thus, no standard differentials are available. Nevertheless, evaluation and screening of different cultivars has been done in Tanzania and Mozambique to select tolerant varieties to CBSD (Hillocks, 2002a). According to

that study conducted in Mozambique, six local and two improved cultivars with some levels of tolerance to CBSD were identified.

According to a survey carried out in 2005 in Uganda, CBSD was mainly observed on improved CMD resistant cultivars (TME 204 and TME 14) and one landrace (Kabwa) (NARO, 2006). Later on, Alicai *et al.* (2007) reported high incidence of CBSD on five other cultivars; TME 14 (64%), NASE 10 (40%), NASE 12 (22%) and TME 204 (16%). This was an unfortunate development as most of these were CMD resistant officially released varieties. In response to the challenge, it was highly recommended to screen local as well as introduced cassava germplasm against CBSD so that resistant genotypes and/or genes for resistance to CBSD can be identified and thereafter introgressed into preferred cultivars as a way of developing multiple resistance to CBSD, CMD and whiteflies (NARO, 2006). These virus resistant or tolerant clones would then be tested against both species of CBSV in order to determine whether their resistance is specific or comprehensive (nonspecific).

2.6.1 Mechanism of resistance to cassava brown streak virus

Understanding the resistance mechanism of a disease is critically important towards the development of a disease management package. Resistance to viruses in plants has been categorized into seven types based on how the resistance mechanism interferes with different stages of virus cycle in the host plant (Lecoq *et al.,* 2004). These include: (i) resistance to virus inoculation by vector, (ii) mature plant resistance (tendency to escape infection), (iii) immunity and extreme resistance due to lack of some factors necessary for virus pathogenesis, (iv) resistance to virus movement between the cells (hypersensitive reaction), (v) resistance to long distance movement of virus within the plant (virus invade only part of the plant), (vi) resistance to virus multiplication; and (vii) resistance to virus acquisition by a vector due to decreased virus multiplication and reduced virus availability to vector. These resistance mechanisms may operate singly or in combinations.

Resistance and/ or tolerance to CBSD has been identified in some genotypes in Tanzania and Uganda however its mode of action and other factors like its specificity, stability and durability among those clones have not been investigated. None of types listed above has been categorized

for CBSD resistance in any cassava genotype. It is possible that different genotypes exhibit different types of resistance and therefore different mechanism. This is because every genotype exhibits different symptom expression for CBSD (Abaca *et al.,* 2012). Therefore, mechanism of resistance to CBSD should be studied on genotype basis. In chapter IV, 11 cassava genotypes are evaluated in the field for reaction to CBSD. In addition, virus accumulation was monitored over 12 month evaluation period.

2.7 Management of cassava brown streak disease

CBSD is spreading rapidly in all major cassava growing areas in East Africa. The rate of spread and effects of the disease on cassava as a food security crop warrants implementation of effective disease management strategies. In terms of control, several options have been suggested, but with varying levels of success. Use of clean planting materials, rouging of infected plants, control of insect vectors, use of tolerant varieties and quarantine are some options that have been tried (Alicai *et al.,* 2007). For example, the first case of CBSD in Uganda in the 1940s was managed by destruction of all cassava with symptoms coupled with strict quarantine measures (Jameson, 1964). This demonstrated the ability of managing CBSD through phytosanitary measures. However for such programmes to be effective, three factors must come into play. These include: commitment by all stakeholders to combat the disease, major educational and training input to all stakeholders and the capacity for farmers or even the researchers to correctly identify CBSD-free materials.

For the case of Uganda, the use of stakes from asymptomatic tolerant varieties has accelerated the spread of the disease from one area to another. It is therefore imperative that strict quarantine procedures are implemented so that movement of germplasm in vegetative form from one area to another should be strictly controlled. This can be coupled with strengthening tissue culture and virus indexing capacity in all affected areas. Thermotherapy could also be considered in varieties were degeneration has been observed.

Host plant resistance is the most efficient and sustainable approach towards the management of CBSD in East Africa (Mahungu *et al.,* 1994). Indeed, breeding for resistance to CBSD is a major breeding objective of the Uganda National Cassava breeding programme. For host plant

resistance and/or tolerance, a number of CBSD tolerant varieties like *Nachinyaya, Kiroba, Kigoma red* and *Namikonga* have been identified among local varieties in Tanzania and Mozambique (Hillocks, 2002a). Virus free stocks of some of these identified cultivars have been multiplied and sent to other East African countries for evaluation and inclusion in their respective hybridisation schemes. Most of the current work is focusing on sourcing for resistance genes. The long term objective is to develop cultivars with multiple resistance to CBSD, CMD and whiteflies (Mahungu *et al.*, 1994; NARO, 2006).

Recently, Yadav *et al.* (2011) demonstrated that UCBSV can be effectively controlled using RNA interference (RNAi) technology in cassava. This was done by expressing constrictively an RNAi construct targeting the near full-length coat protein (FL-CP) of UCBSV as a hairpin construct in cassava. The transgenic cassava line expressing small interfering RNAs (siRNA) against this sequence showed 100% resistance to UCBSV across replicated graft inoculation experiment. Currently efforts of this approach are now testing CBSD transgenic line (with UCBSV and CBSV constructs) under confined field trials (CFT) at National Crops Resource Research Institute (NaCRRI), Uganda. According to Taylor *et al.*, (2012) a proof of concept was achieved by production and testing of virus resistant plants under greenhouse and confined field trials in Namulonge during the phase one of VIRCA (Virus Resistant Cassava for Africa) project. Under phase two of the project currently ongoing at NaCRRI, a farmer preferred, CMD resistant but CBSD susceptible line (TME 204) has been modified and is undergoing screening in a CFT at NaCRRI.

2.8 Breeding for resistance to cassava brown streak disease

CBSD breeding has been a major breeding objective in the Tanzania cassava programme since the 1930s. The pioneer breeding programme that responded to the CBSD problem was initiated at Amani Research Station, Tanzania. According to Jennings (1957), several cultivars both local and introduced were screened for resistance to CBSD. Of these, only two cultivars; *Macaxeira aipin* and *Aipin valenca* of Brazilian origin were resistant to CBSD. These two cultivars were selected as breeding stocks for resistance to CBSD to which limited progress was made (Jennings, 1957).

Wild relatives were also proposed as potential sources of CBSD resistance genes and indeed, several crosses were made between wild *Manihot* species (*M. glaziovii, M. melanobasis, M. dichotoma, M. cathartica, M. dulcis* and *M. saxicola*) in order to introgress resistance genes into preferred *M.esculenta* cultivars (Storey, 1939; Hillocks and Jennings, 2003). Crosses between; 1) *M. glaziovii*×cassava, *M. dichotoma*×cassava and *M. melanobasis*×cassava resulted into hybrids with resistance to CBSD showing only occasional, mild leaf symptoms. These interspecific hybrids were further intercrossed and backcrossed at East African Agriculture and Forestry Research Organisation (EAAFRO) Kenya, to generate clones resistant to CBSD (Jennings and Iglesias, 2002).

About 91 clones were generated and tested in different ecological zones in different countries. In Kenya for example, none of these clones developed CBSD after evaluation (Doughty, 1958). According to Hillocks and Jennings (2003), some of the Amani hybrids have remained resistant to CBSD since their evaluation in the 1950s. Breeding for CBSD resistance was re-initiated in 1980 at Agricultural Research Institute (ARI) Naliendele Mtwara and in 1994 at ARI (Kibaha) (Kanju *et al.,* 2003). Through these efforts a number of cultivars notable of which Kigoma red, Nachinyaya, Namikonga, Kiroba, Mzungu and NDL90/034 were identified to have high tolerance/resistance to CBSD though only Namikonga and NDL90/034 were recommended for release. Currently hybrids showing durable resistance to CBSD like *Namikonga* also refered to as (46106/27); a cross between *M. esculenta*×*M. glaziovii)* are used as breeding stocks for resistance to CBSD in different countries in East and Southern Africa.

Recent CBSD disease genetic studies by Kulembeka (2010), reported CBSD to be polygenic and that additive genetic effects are critical for its expression. This implies that many genes are involved in resistance or tolerance to CBSD and these genes act additively. However the number of quantitative trait loci (QTLs) and genes involved is not known. Only one study so far has been done on the identification of QTLs responsible to CBSD resistance. According to that study (Kulembeka, 2010), only one QTL was detected with a LOD score of 3.56 explaining 22.9% phenotypic variance in one location (Chambezi) and 19.2% of the phenotypic variance in another location (Naliendele). This however is a low estimate signifying that more work is needed to determine more QTLs that can explain a significant percentage of phenotypic variance.

Ever since the reemergence of CBSD in Uganda (Alicai *et al.,* 2007), attempts to control CBSD have been taken, especially sensitizing farmer about the disease and it spreading mechanism. Breeding for resistance to CBSD at the National Crops Resources Research Institute (NaCRRI) is still in its early stages and hence the need to screen tolerant genotypes to establish their adaptability and reaction to CBSD in Uganda.

2.8.1 Inbreeding in cassava

Cassava breeding, is mainly based on the use of heterozygous parents in inter and intraspecific crosses. During the breeding process, both additive and dominance effects influence heterosis thereby affecting the phenotypic selection process considering that cassava is vegetatively propagated, both additive and dominance effects have been perpetuated from one generation to another. However in breeding programs based on intercrossing, the dominance effects cannot be transmitted to the progeny but only additive portion of the total genetic variance is passed on to the next generation when the same clone is used as a parent. Therefore through inbreeding, dominance effects are erased from the selection process and additive variance is doubled or expanded thereby facilitating the selection of those additive effects that define a superior progenitor.

Although inbreeding doesn't change the frequency of alleles in a population, it does redistribute the frequency of genotypes, increasing the proportion of homozygote and correspondingly decreasing the proportion of heterozygote leading to a 50% transmission advantage. This, increased homozygosity increases the expression of the genetic load, resulting in inbreeding depression. Alternatively if a heterozygote is superior to homozygote, the reduced frequency of heterozygotes will reduce opportunities to express this over dominance.

It suffices to note that, inbreeding can be used as a strategy of reducing genetic load in a crop and thus enabling use of backcrossing in transferring desirable genes (Ceballos *et al.,* 2004). Inbreeding increases the frequency of the deleterious homozygous genotype therefore selection against deleterious alleles is also increased during inbreeding thereby providing an opportunity for the population to be purged of its mutational load.

More so, due to high heterozygous nature of cassava, useful recessive traits are masked. However, through inbreeding a number of useful recessive traits have been discovered which could have huge benefits for the crop. A good example is the discovery of amylose free waxy starch mutants in cassava (Ceballos *et al.,* 2007b).

Though inbreeding offers numerous advantages, it has hardly been utilized in cassava breeding. It has been generally believed that since cassava is highly heterozygous, inbreeding would not be feasible because of inbreeding depression. Inbreeding depression refers to the reduced fitness of offspring resulting from selfing relative to the fitness of offspring of randomly mated individuals. In this case, fitness refers to the relative ability of different genotypes to contribute individuals to the next generation. Inbreeding depression is thought to be caused by two main non mutually exclusive genetic mechanisms explained by two hypothesis; partial dominance hypothesis and over dominance hypothesis. Partial dominance posits that inbreeding depression is caused by reduced fitness in homozygosity of deleterious recessive alleles. Therefore partial dominance occurs when the effects of the recessive allele on the phenotype are only partially masked by the effect of dominant alleles. Over dominance is when the heterozygous genotypes have higher fitness than the homozygous genotypes. Therefore inbreeding depression is caused by reduction in the frequency of heterozygous genotypes as the result of inbreeding.

Kawano *et al.* (1978) conducted a study to analyze factors affecting efficiency of hybridization where he evaluated S_1 clones from 12 genotypes. In that study, they reported an average inbreeding depression (ID) for fresh root yield of 51.8%. Later on Rojas *et al.* (2009) conducted a similar study on eight S_1 cassava families where they reported a higher ID of 63.9%. Though these values seem high, a comparison with average ID of 50% for grain yield (Garcia *et al.,* 2004) suggests that ID in cassava are not unacceptably high, therefore, inbreeding is a feasible approach. However, not much work has been done in search of disease resistant genotypes in cassava through inbreeding.

Summary of literature review

A lot has been done to understand the etiology of CBSD, diversity of causative viruses, and the transmission of the disease. However a lot of information is still required to understand the

resistance to CBSD. Consequently, spread of the disease has kept ahead of breeding efforts. Currently no study has been conducted to understand or characterize resistance or tolerance to CBSD in parental genotypes used in breeding programs in East Africa. Efforts are needed also in exploiting other strategies like inbreeding in order to generate new sources of resistance and/or improved resistance since additive effects predominate the non-additive effects. This information gaps formed the basis of this thesis. This study was conducted to characterize resistance or tolerance to CBSD in selected genotypes sourced from Tanzania and Uganda and also to explore inbreeding as a strategy of generating new sources of resistance to CBSD. Information from this study will help cassava breeding programs especially in efforts to combat cassava brown streak disease through breeding.

CHAPTER THREE

SCREENING CASSAVA PARTIAL INBREDS FOR RESISTANCE TO CASSAVA BROWN STREAK DISEASE IN UGANDA

3.1 INTRODUCTION

Cassava (*Manihot esculenta* Crantz.) is one of the most important root crops grown widely in tropical countries. In recent years, cassava production has been greatly hindered by a myriad of abiotic and biotic stresses especially pests and diseases. Of these, cassava brown streak disease (CBSD) is currently the major disease affecting its production in many cassava growing areas within east and southern Africa (Pennisi, 2010). The disease is caused by two virus species, cassava brown streak virus (CBSV) and Uganda cassava brown streak virus (UCBSV), both are *Ipomoviruses* of family *Potyviridae* characterized by an elongate flexuous filament 650-690 nm long (Monger *et al.,* 2001, Mbanzibwa *et al*., 2011). The presence of two distinct species of viruses that cause CBSD has posed a great challenge in breeding for durable resistance in cassava. These viruses are distributed in Tanzania, Kenya, Uganda, Democratic Republic of Congo, Rwanda, Malawi and Mozambique. Undocumented reports of CBSD outbreak in Burundi and Zambia have also been made. Thus, the spread of CBSD is keeping ahead of breeding efforts tailored towards its control.

Recently, Kulembeka (2010) reported that CBSD resistance is more controlled by additive genetic effects than dominant effects. Understanding this gene action and/or the inheritance of CBSD resistance presented an opportunity for generating higher resistance levels through inbreeding of tolerant genotypes. Interestingly, tolerant genotypes have been identified in Tanzania (Edward Kanju, *Personal Communication*) and these can be used as a starting point in the generation of new sources of resistance. According to Walsh (2005), inbreeding allows "concentration" of desirable genes originally present in the elite clone. By forcing an average of half of the loci to become homozygous, the additive value in a self is increased while the genetic load (deleterious alleles) is reduced thereby allowing selection of superior progeny (Barrett and Charlesworth, 1991). As a result, inbreeding

results in high proportion of homozygous loci thereby producing progeny at both fitness extremes i.e. extremely high fitness with many homozygous with few deleterious mutations and extremely low fitness with many homozygous deleterious mutation. Indeed, a recent study on segregation of selected agronomic traits in cassava inbreds (Kawuki *et al.*, 2011) showed an increase in performance in agronomic traits (harvest index and root dry matter content) in some inbreds compared to their respective non-inbreds parents. Thus, in this paper, it was hypothesized that generated partial inbreds will not only be better progenitors but will also possess higher resistance to CBSD than their respective non inbred parents. This study was initiated to generate and screen cassava partial inbred for resistance to CBSD in Uganda.

3.2 MATERIALS AND METHOD

3.2.1 Generation of S_1 families from S_0

Eight cassava progenitors (S_0): Namikonga, 182/006661, Kigoma Red, TZ/130, TZ/140, 130040, 0040 and I00142 from Tanzania were established in isolated plots at National Crops Resources Research Institute, Central Uganda. With the exception of TZ/130 and TZ/140, which were selected from open pollinated seeds introduced in 2005, all the others progenitors were introduced as stem cutting in 2009. Each parental line contained 20 plants which were established in two row plots of 10 plants. At flowering, selfing was done by hand following standard procedures to generate partial inbred lines (S_1). Within a cassava field, it is possible to get mature pollen and mature female flowers of the same clone (from different branches) and thus selfing is possible. The number of flowers selfed and number successful selfed flowers (fruits per plant) were recorded and labelled appropriately. Three months later, the mature fruits were harvested and numbers of seeds harvested recorded. Open pollinated flowers were removed to avoid mixture between selfed seeds and open pollinated seeds. The harvested seed were established in a nursery after a two months period to break dormancy. The seeds from all the selfed cassava families were germinated at NaCRRI. After two months in the nursery, the seedlings were transplanted in a well prepared field, and screened for CBSD resistance for nine months.

3.2.2 S_1 Seedling evaluation trial

A total of eight S_1 families were evaluated. Family size varied between 17 and 280 seedlings. All seedlings belonging to a single family were established in the same block. Spreaders using a CBSD – susceptible variety (TME 204) were planted to augment the CBSD pressure in the evaluation plots. This trial was planted during the first rains (March) of 2011. Data for CBSD were collected on individual seedlings on two month interval after the third month after planting. Cassava raised from seed usually produces a few roots (1-10) which also provide an opportunity for CBSD root necrosis evaluation. However, to reduce error variance, cloning is required to provide more sample size. Thus, after nine months, each plant in the seedling evaluation trial (SET) was individually harvested and data taken for: foliage yield, root yield, CBSD root severity and CBSD root incidence. Thereafter, 8-12 cuttings were taken from each parents (S_0) and selfs (S_1) to generate clones for further evaluation.

3.2.3 Field establishment of cloned S_1 seedlings

A clonal evaluation trial (CET) was established at NaCRRI using single rows of six plants per genotype. Both S_1 progeny and the non-inbred parent (S_0) were established in the CET. This was done during the first rains of 2012. Each row represented a single clone and the spacing was 1m within and between the rows. In order to control variability in the field, clones from a given family were separated into three groups of roughly equal size and each group of a family was randomly allocated to one of the blocks along with respective parental genotypes for comparison. No selection was done. Spreader rows of TME 204 were established between rows to augment CBSD pressure. The genotypes were grown for 12 months under rain fed conditions with no fertilizer or herbicide applied.

3.2.4 Evaluation of S_1 clones for CBSD resistance

Above-ground CBSD symptoms (on leaves and stem) were assessed visually on every plant in each plot. Both incidence (proportion of cassava plants in a plot expressing CBSD symptoms) and severity (degree of infection of CBSD on individual plant) were used to quantify the disease. Five data sets at three, five, seven, nine and eleven months after planting (MAP) were collected. A severity score of 1-5 (Gondwe *et al.,* 2002) was adopted where 1- no symptom, 2- mild

symptom (1-10%), 3- pronounced foliar chlorotic mottle and mild stem lesion (11-25%), 4- severe chlorotic mottle and stem lesion (26-50%) and 5- very severe symptoms (>50%). Severity scores for root necrosis were also taken on all roots harvested at 12 months after planting. Severity scores for root necrosis were based on a 1-5 scale where 1- no necrosis, 2- mild necrotic lesions (1-10%), 3-pronounced necrotic lesion (11-25%), 4-severe necrotic lesion (26-50%) combined with mild root constriction and 5- very severe necrotic lesion (>50%) coupled with severe constriction. Root severity scores were converted into disease severity mean (DSM) using the following formula

$$DSM = \frac{\sum (\text{severity scores for all affected roots on the infected plant})}{\text{Total number of infected roots on the infected plant}}$$

Disease incidence (DI) of CBSD in harvested roots was quantified as a ratio of the number of roots showing roots symptoms to the total number of roots harvested per plant per genotype. Disease index of every clone was derived as a product of DI and DSM.

In addition, plants in each plot were uprooted and used for assessment of harvest index. This was done at harvest, 12 MAP. Fresh foliar weight (stems and leaves) and weight of roots per plot were separately measured and harvest index was calculated as the ratio of storage root weight to total plant biomass (Alves, 2002).

3.2.5 Estimation of heritability of CBSD resistance

To measure the heritability of resistance or tolerance to cassava brown streak disease, a parent-offspring regression was made with the offspring in one environment (NaCRRI) using mean values of disease index of parents and offspring based on root necrosis. The offspring were regressed on that of their parent using standard linear regression model $y_1 = b_0+b_1x_1 +e$ where y_1 is the mean of offspring of the i^{th} family, b_0 is the intercept, b_1 is the regression coefficient and x_1 is the parent of the i^{th} family and e is the random error. The expression $h^2=2b_1$ was used since partial inbred families are regressed on a single parent.

3.2.6 Determining effect of inbreeding on selected growth and reproductive traits

Growth traits like vigor, height and yield related traits (harvest index and dry matter content) along with reproductive traits (flowering/inflorescence) were evaluated both for parents and partial inbreds. Since flowering is highly associated to branching, inflorescence was evaluated by counting the number of branches containing flowers. These traits were compared between inbreds and parents to determine the effect of inbreeding on these traits. The effect of inbreeding was evaluated by calculating inbreeding depression using the formula below according to Kawuki *et al*. (2011).

$$\text{Inbreeding depression} = \left(\frac{So\ means - S1\ means}{So\ means}\right) 100$$

Where S_0 – parent and S_1 – Inbreds.

3.2.7 Data analysis

Data on disease index, vigor, inflorescence, harvest index and height were subjected to one way analysis of variance using Genstat version 14 at a level of significance level of 5% to compare families. The field reaction of each generated partial inbreds to CBSD was compared to that of the respective progenitors (S_0) by subjecting disease index data for the family (S_0 progenitor and its S_1 inbreds) to analysis of variance using Genstat 14th edition. Parent-offspring regression analysis was performed using Genstat version 14.

3.3 RESULTS

3.3.1 Generation of partial inbreds from selected cassava genotypes

The S_0 progenitors (0040 and I00142) produced the highest number of seeds (418 and 396 respectively) while the lowest number of seeds was obtained from Tz/140 (Table 1). At

germination, family I00142 produced the highest number of seedlings (280), followed by 0040 (200), and 130040 (200). The highest germination rate was recorded in family Tz/130 (73.2%), while the lowest was in 0040 (47.8%) (Table 1)

At the end of the seedling evaluation trial (SET), different families showed varying survival rates. TZ/130 had the highest survival rate of 87.8% followed by Namikonga (76.7%) and lowest in 0040 and Kigoma (50%). In the CET, family 100142 which had the highest number of seeds generated and seedling established at SET had the lowest survival rate of 6.1% while family TZ/130 maintained the highest rate of survival even at CET (66.7%) followed by TZ/140 (41.2%) (Table 1).

3.3.2 CBSD response of partial inbreds at seedling stage.

Frequency distribution based on the maximum severity score of root necrosis for the different families is presented in Figure 3. Most of the genotypes generated in different families had a maximum score of 1 for root necrosis. Family 100142 had the highest number of genotypes (84) with maximum severity score of 1 followed by 130040 and 0040 with 79 and 77 genotypes respectively. Family 182/00661 had the lowest number of genotypes (3) with maximum severity score of 1 for root necrosis.

Furthermore it was evident that, the frequency distribution of genotypes within different families was more skewed to the left meaning that most genotypes had the severity score of 1 and or 2 for root necrosis. However, the different phenotypic classes (1, 2, 3, 4 and 5) for CBSD were observed within Kigoma Red and 182/00661 showing segregation (Figure 3).

Table 1: Number of S_1 seeds generated, seedling established and clones generated at NaCRRI

S_0 progenitor	Seeds generated	Seedling germinated	clones generated [2]	clones established[3]	Survival (%)[4]
182/00661	79	40 (50.6)	24	6	15.0
Kigoma Red	60	40 (66.7)	20	7	17.5
TZ/130	123	90 (73.2)	79	60	66.7
I00142	396	280 (70.7)	160	17	6.1
130040	353	200 (56.7)	104	40	20.0
Namikonga	123	60 (48.8)	46	15	25.0
TZ/140	25	17 (68.0)	11	7	41.2
0040	418	200 (47.8)	100	27	13.5

Figures in parentheses indicate % germination; [2] Number of clones that survived per family at 11 MAP in 2011; [3] Number of clone that established. On which clonal evaluation data was taken during 2012; [4]% survival to the end of clonal evaluation trial.

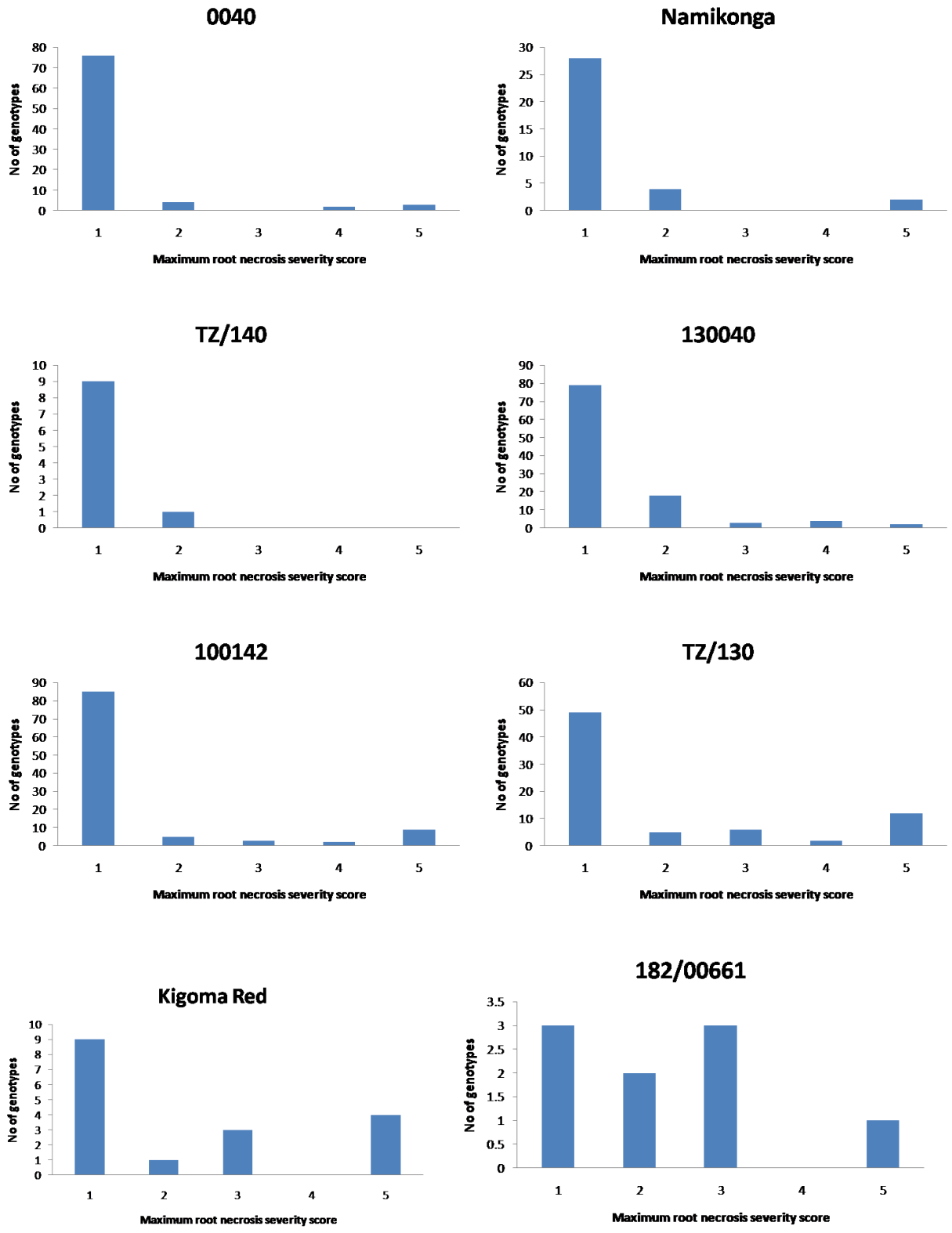

Figure 3: Frequency distribution showing variation in CBSD root necrosis based on maximum disease severity for S_1 inbreds raised under seedling evaluation trial.

3.3.3 Field reaction of cloned S_1 inbreds to CBSD

3.3.3.1 CBSD reaction based on foliar symptoms

Variation in susceptibility to CBSD among different clones of different cassava families was striking. It ranged from 0 – 100% incidence with severity scores of 1 – 4.5 (Table 2). On family basis, Namikonga had the lowest incidence (53.3%) followed by TZ/130 and TZ/140 having 56.7% and 57.1% respectively. Family 182/00661 had the highest incidence (83.3%). For parents, a similar trend was observed with Namikonga having the lowest incidence of 3.6% followed by TZ/130 (30.9%). S_0 progenitors TZ/140, Kigoma Red and 182/00661 had CBSD incidence of 100%. Parent 0040 and 100142 were lost during seedling evaluation and were therefore not included in clonal evaluation trial.

In comparison to S_0 progenitors, there was a reduction in incidence among inbreds of 130040, TZ/140, Kigoma Red and 182/00661 though the reduction was not significant at 5% level, while significant increase was observed in incidence among partial inbreds for TZ/130 and Namikonga. The key finding was that irrespective of the susceptibility level of the parental lines, some progeny had higher levels of resistance after 2 years of evaluation.

Considering the severity of CBSD, family 0040 had the lowest mean severity (2.3) with clone performance ranging from 1 – 3, followed by family 130040 (2.5). Family 182/00661 and TZ/140 had the highest severity of 3.4 and 3.1 respectively. Parental line, Namikonga had the lowest severity of 2.0 followed by TZ/130 (2.6). Like the inbreds, the highest severity score (3.7) among the parents was recorded in 182/00661 (Table 2). In general, there was no significant difference in performance based on severity of CBSD between parents and their respective partial inbreds except for Namikonga.

Though there was no significant difference in general between parents and their respective partial inbreds in their response to CBSD foliar severity, there was a varying number of symptomless clones generated in different cassava families involved. Of all the families, Namikonga had the highest percentage of clones that remained symptomless (46.7%) followed by TZ/130 and TZ/140 with 43.3% and 42.8% respectively. The lowest percentage of symptomless clones was recorded in family 182/00661 (16.7%).

3.3.3.2 CBSD reaction at clonal stage based on root symptoms

In general, there was no significant difference in response to CBSD under field conditions between generated partial inbreds and their respective progenitors except for Namikonga. However, non inbreds (S_0) had a lower disease index as compared to the respective inbred progenies. Of all the inbreds, family 0040 had the lowest disease index (1.48) followed by 130040 and TZ/130 with 1.52 and 1.76 respectively while Kigoma Red had the highest disease index (2.75) (Table 3). Among the parents, TZ/140 had the lowest disease index of 0.18 followed by Namikonga with disease index of 0.2. Similar to partial inbreds, Kigoma Red had the highest disease index of 2.44 among the parents.

Based on maximum severity scores for CBSD root necrosis, the genetic potential of all evaluated S_1 inbreds was assessed. Of all the evaluated parents (S_0), Namikonga and TZ/140 had the lowest score of 2, while other parents had a score of 5. According to frequency distribution of partial inbreds in different families, most of the genotypes generated had a score of 5 (Figure 4). Inbreds derived from Namikonga exhibited two extremes expected of a population derived through inbreeding. i.e, 42% had score 1, (resistant) while 42% had score 5 (susceptible) (Figure 4).

Though there was no significant difference, a varying number of clones/genotypes that remained symptomless for root necrosis were generated. Of all the families evaluated, TZ/130 produced the highest percentage of asymptomatic clones (15%) followed by 0040 and Namikonga with 7% and 5% respectively.

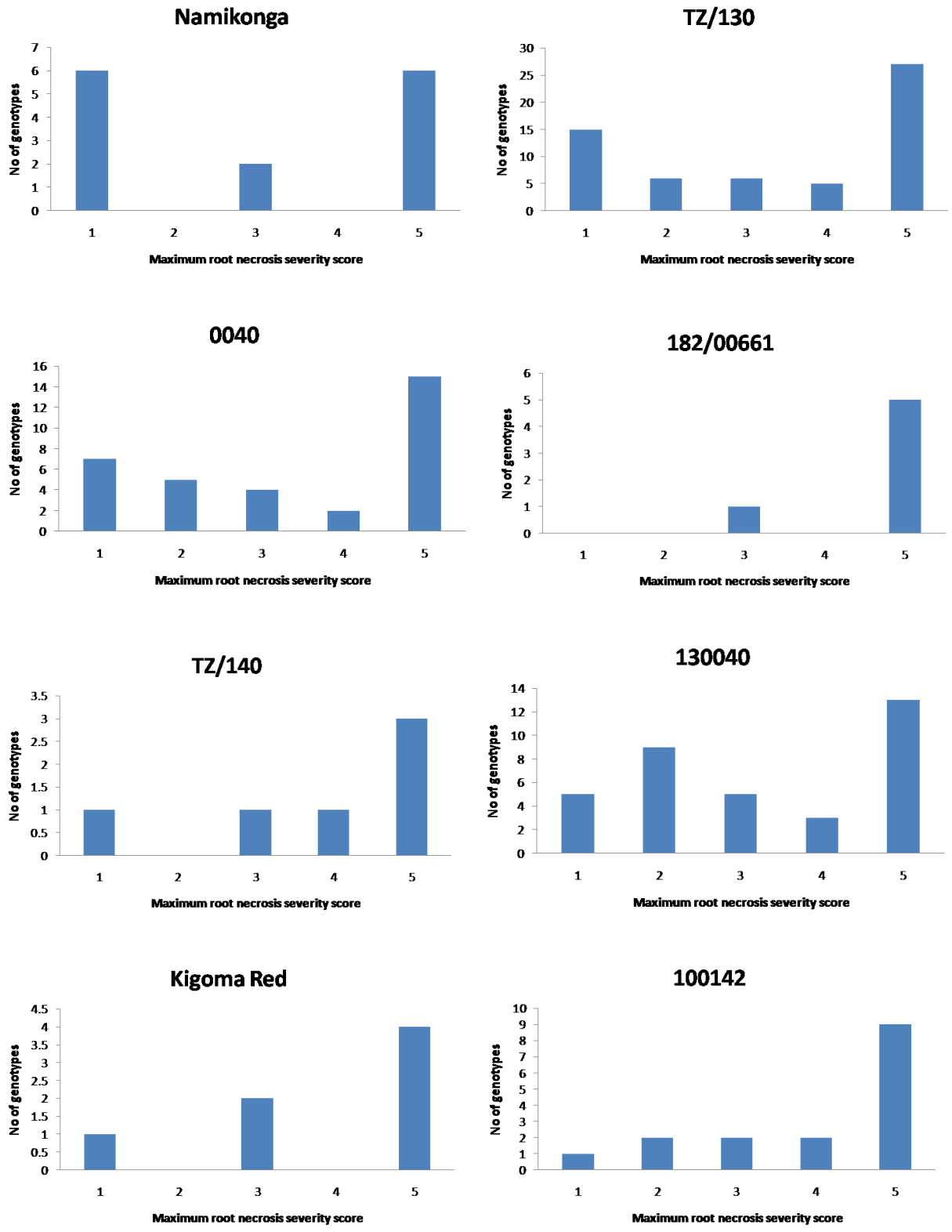

Figure 4: Frequency distribution showing variation in CBSD root necrosis based on maximum disease severity for S_1 inbreds under clonal evaluation trial

Table 2: Field reaction of partial inbreds (S_1) and their respective parents (S_0) against CBSD in Uganda based on foliar symptoms

Family	Partial inbreds (S_1)				Parent (S_0)			
	No of clones	Incidence	severity	Symptomless S_1 clones (%)	Incidence	Severity	Min	Max
TZ/130	60	56.7	1.99±0.1	26	30.9	1.59±0.1	1	3
Namikonga	15	53.3	2.16±0.1	7	3.6	1.04±0.1	1	2
130040	37	72.9	2.41±0.1	10	76.4	2.64±0.2	1	4
TZ/140	7	57.1	1.79±0.2	3	100	3.67±0.2	2	3
Kigoma	6	66.7	2.60±0.2	2	100	3.33±0.2	3	3
182/00661	6	83.3	4.05±0.2	1	100	3.92±0.2	3	4
0040	27	74.0	2.01±0.1	7	-	-	-	-
100142	15	66.0	2.48±0.1	5	-	-	-	-
LSD			0.44			0.48		
CV%			47.1			30.6		

[1]Sev (F) –Mean disease severity at family basis; [2]Sev (range) based on clone performance; **significant difference at (5%) Incid = Incidence; Sev = Severity;min = minimum; Max = maximum

Table 3: Field reaction of partial inbreds (S_1) and their respective parents (S_0) against CBSD in Uganda based on root symptoms

Family	Partial inbreds S_1					Parents S_0				
	Clones	Incid	DSM	D. Index	DSM (range)	symptomless (%)	incid	DSI	D. index	Min	Max
TZ/130	60	0.46±0.1	3.48±0.2	1.76±0.22	2.00—5.00	15	0.34±0.3	3.16±0.3	1.21±0.5	1	5
Namikonga	15	0.63±0.1	3.26±0.3	2.19±0.5**	2.00 – 4.90	5	0.10±0.3	2.00±0.9	0.20±1.4	1	2
130040	37	0.45±0.1	3.02±0.2	1.52±0.3	2.00—5.00	4	0.18±0.8	2.88±0.5	0.49±0.5	1	5
TZ/140	7	0.72±0.1	3.36±0.4	2.56±0.6**	2.30—5.00	1	0.09±0.2	2.00±0.7	0.18±1.0	1	2
Kigoma red	6	0.84±0.1	3.23±0.4	2.75±0.5	2.10—5.00	1	0.92±0.2	2.62±0.5	2.44±0.7	1	5
182/00661	6	0.74±0.2	2.85±0.5	2.12±0.8	2.30—3.80	0	0.47±0.2	3.39±0.5	1.70±0.7	4	5
0040	27	0.48±0.1	2.98±0.2	1.48±0.3	2.00—4.60	7	-	-	-	-	-
100142	15	0.51±0.1	3.64±0.2	2.06±0.4	2.00—5.00	1	-	-	-	-	-
LSD		0.31	0.89	1.35			0.35	1.45	1.69		

DSM – Disease severity means D.Index – Disease index Incid – incidence **significant difference at (5%) ; Incid – Incidence; Sev - Severity; min – minimum; Max – maximum; LSD – Least significant difference;

3.3.4 Heritability of CBSD resistance based on parent offspring regression

According to the Fig 5, the regression of field response to CBSD of generated partial inbreds on that of their corresponding parent resulted in a linear model (y=0.216x + 1.752). A moderate estimate of heritability for CBSD resistance of 0.43 among generated partial inbreds was obtained by parent offspring regression.

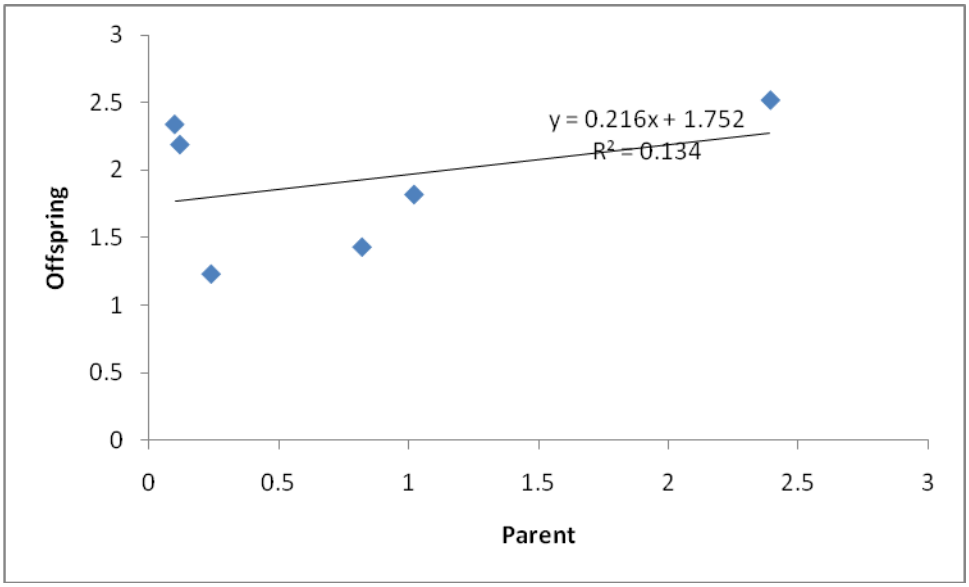

Fig 5: Heritability of CBSD resistance based on parent-offspring regression

3.3.5 Effect of inbreeding on growth, reproductive and production traits

Though this study intended to explore the benefits of inbreeding in search of resistance to CBSD, a large proportion of seedlings generated were characterized by loss in vigour, height and reduction in growth, and therefore did not survive to make it for the clonal evaluation trial (Table 1). Only data for the surviving clones at clonal stage is presented in Table 5.

Based on sprouting, there was no noticeable inbreeding depression (ID) in S_1 progeny derived from parental lines TZ/130, 130040 and Kigoma Red all having progeny with ID values less than zero (Table 5). However, ID was observed in Namikonga, 182/00661 and TZ/140 with ID values of 20.8, 36.7 and 42.9 respectively. S_1 progeny derived from parental line TZ/130 had the highest

percentage of sprouting (74.3%) followed by family 130040 with 64.1% while TZ/140 had the lowest percentage of sprouting (57.1%).

Based on growth vigour, there was no observable difference between parents and partial inbreds (Table 5). This was expressed in the absence of inbreeding depression in Namikonga (-14.2), TZ/130 (0), 130040 (-3.8) and 182/00661 (0). Marginal ID was observed in S_1 derived from Kigoma Red and TZ/140 with 10.8 and 12.9 respectively. Highest growth vigour was recorded in family TZ/140 with 6.1, while Namikonga had the lowest growth vigour of 4.8 (Table 5).

The height of partial inbreds among different families varied from 145.4 – 198.9 cm. In general, there was no significant difference in height between parents and corresponding partial inbreds except for family TZ/130 (Table 5). Though there was no significant difference in height, the partial inbreds were taller than their respective parents among Namikonga, 130040, TZ/140 and 182/00661. However, progeny from family Kigoma Red and TZ/130 showed a low inbreeding depression of 12.1 and 22.5 respectively. Among the partial inbreds, TZ/140 had the highest mean height (198.9 cm), while Kigoma Red had the lowest mean height (145.4 cm).

There was no significant effect on flowering among generated partial inbreds (Table 5). The partial inbreds had higher number of inflorescence than their respective parents and thus no noticeable ID was observed. Of all the inbreds, family 0040 had the highest number of inflorescence (63.6) followed by 130040 with 60.9, while family 182/00661 had the lowest number of inflorescence (30.9) among the partial inbreds (Table 5). Among the parents, 130040 had the highest number of inflorescence (56.2) followed by Kigoma Red with 51.0. A significant difference in the number of inflorescence produced between S_0 and S_1 was only observed in TZ/140.

Further results indicated no significant effect of inbreeding on root dry matter content (Table 5). A comparison between the dry matter content (DMC) of partial inbreds and their respective parents shows that inbreds generally had higher DMC than their respective parents though the difference was not significant at 5% significant level (Table 5). This is with exception of only two parents Namikonga and 130040 which had higher DMC than their respective inbred progeny. As a result, inbreeding depression was not observed except for two families

(Namikonga and 130040) where low inbreeding depression values of 2.5 and 8.9 respectively were recorded.

The yield potential of both parents and partial inbreds in this study was determined by the Harvest Index (HI). There was an increase in HI among partial inbreds of Namikonga. Of these only Namikonga showed a significant difference at 5% significant level between parents and their respective partial inbreds. The HI of 182/00661 parent was exactly similar to that of partial inbreds derived. However there was a yield reduction among the inbreds of TZ/130 and TZ/140 though the difference was only significant in TZ/140 (Table 5). As a result, inbreeding depression with respect to yield/HI was only recorded in family TZ/130 and TZ/140 (Table 5).

Table 4: Effect of inbreeding on sprouting, growth vigor and height of cassava in selected genotypes

	Parent (S_0)		Partial Inbreds				ID*
	Means	**No of plants**	**Means**	**No of genotypes**	**Min**	**Max**	
Sprouting %[1]							
Namikonga	80.0	21	63.3	15	16.7	100	20.8
Tz/130	73	43	74.34	60	16.7	100	-1.0
130040	61.9	25	64.1	40	16.7	100	-3.6
Tz/140	100	6	57.1**	7	16.7	100	42.9
Kigoma Red	45.8	8	63.3	7	16.7	100	-38.2
182/00661	100	18	63.9**	6	16.7	100	36.1
0040	-	-	62.1	27	16.7	100	-
100142	-	-	63.2	17	16.7	100	-
	LSD = 34.9	Cv% = 30.1	LSD = 29.3	CV% = 43			
Vigor[2]							
Namikonga	4.2	21	4.8	15	3	7	-14.2
Tz/130	5.5	43	5.5	60	3	7	0
130040	5.3	25	5.5	40	3	7	-3.8
Tz/140	7.0	6	6.1	7	3	7	12.9
Kigoma Red	6.5	8	5.8	7	3	7	10.8
182/00661	5.0	18	5.0	6	3	7	0
0040	-	-	5.6	27	3	7	-
100142	-	-	5.1	17	3	7	-
	LSD = 1.93	CV%=23.7	LSD = 1.13	CV%= 28.3			
Height[3]							
Namikonga	142	21	163.9	15	54	242	-15.4
Tz/130	198.5	43	153.9**	60	35	321	22.5
130040	142.6	25	174.7	40	62	319	-22.5
Tz/140	172.7	6	198.9	7	64	301	-15.2
Kigoma Red	165.5	8	145.4	7	58	224	12.1
182/00661	133.6	18	150.4	6	86	251	-12.6
0040	-	-	154.9	27	69	311	-
100142	-	-	145.4	17	54	311	-
	LSD = 33.8	CV% = 20.4	LSD = 49.6	CV%=35.9			

ID*- Inbreeding depression **Significant difference at 5% level

[1]sprouting was assessed as proportions of plants that sprouted/plot at 1MAP; [2] Plant vigour scored on a scale of 3,5 and 7 with 7 = most vigorous, 3 poor vigour, and 5 = intermediate vigour; [3] height measurements taken at 12 MAP as length from ground to plant apex on plant basis.

Table 5: Effect of inbreeding on flowering and yield of cassava in selected genotypes

	Parent (S_0)		Partial Inbreds				ID*
	Means	**No of plants**	**Means**	**No of genotypes**	**Min**	**Max**	
Inflorescence							
Namikonga	34.0	21	49.3	15	0	195	-45
Tz/130	51.0	43	50.8	60	0	288	0.39
130040	56.2	6	60.9	40	0	252	-8.4
Tz/140	10.8	8	36.3**	7	0	130	-236.1
Kigoma Red	38.1	18	38.2	7	3	123	-0.3
182/00661	32.9	25	30.9	6	0	150	6.1
0040	-	-	63.6	27	0	270	-
100142	-	-	51.6	17	0	210	-
	LSD = 23.6	CV% =71.8	LSD = 24.4	CV% = 99			
Harvest Index							
Namikonga	0.16	5	0.25**	13	0	0.14	-56.3
Tz/130	0.34	10	0.32	45	0.14	0.42	5.9
130040	0.28	6	0.29	35	0.04	0.40	-3.6
Tz/140	0.35	8	0.28**	6	0.17	0.40	20
Kigoma Red	0.29	10	0.33	7	0.26	0.38	-13.8
182/00661	0.39	15	0.40	3	0.36	0.43	-10.3
0040	-	-	0.33	20	0.22	0.37	-
100142	-	-	0.32	16	0.22	0.40	-
	LSD = 0.08	CV%=17.4	LSD = 0.06	CV%= 21.4			
Dry matter content							
Namikonga	40.8	5	39.3	13	21.7	44.4	2.5
Tz/130	33.1	10	33.4	45	16.1	51.4	-0.9
130040	36.9	6	33.6	35	22.4	41.0	8.9
Tz/140	30.1	8	31.7	6	19.5	41.5	-5.3
Kigoma Red	29.1	10	32.3	7	25.9	41.8	-10.9
182/00661	35.9	15	36.8	3	33.45	40.22	-2.5
0040	-	-	31.2	20	18.5	43.11	-
100142	-	-	30.5	16	20.4	37.1	-
	LSD = 5.81	CV% = 10.3	LSD = 5.93	CV% = 18			

ID*- Inbreeding depression **Significant difference at 5%

3.4 Discussion

The objective of this study was to develop and screen partial inbreds for resistance to CBSV and UCBSV in Uganda. Evaluations were done twice, using seedling and at clonal stage in a CBSD hotspot at Namulonge. Thus we generated sufficient data to have a good categorization of the evaluated populations in respect to CBSD resistance and/or susceptibility. Though there was no significant difference between S_1 inbreds and their respective progenitors S_0, a varying number of the generated clones that remained symptomless for both UCBSV and CBSV (on roots) for the two seasons evaluated in a "hotspot" zone (Table 3). These clones are potential sources of resistance to CBSD. Indeed, the generation of field resistant clones i.e., from TZ/130 (Table 3) shows that higher level of resistance can be generated through inbreeding. At a family level, the effect of these resistant clones generated in this study was not significant to influence the overall mean. It can therefore be envisaged that more cycles of inbreeding for these clones that remained symptomless will lead to generation of more new sources of resistance and significant increase in resistance to CBSD among the inbreds than their respective progenitors.

The heritability estimate of 0.43 obtained in this study implies that only 43% of the observed phenotypic variance in response to CBSD among inbreds is due to additive genetic effects. This is a modest estimate which implies that the response of generated partial inbreds to CBSD can fairly be predicted by severity or disease index of parental genotypes. Moderate estimates obtained in this study also suggest that substantial genetic gain would be obtained when selecting for resistance in partially inbred cassava families though selection would be more effective in later generation (S_3 or S_4). Provided that flowering is possible and that tolerance to ID has been built.

Previous studies conducted in Tanzania (Kulembeka, 2010) indicate that CBSD resistance is largely under control of additive effects. Additive effects can easily be exploited with inbreeding. This, thus explains the higher levels of CBSD resistance observed in the few S_1 clones.

The reduction in fitness components (germination and survival) of generated partial inbreds especially in the seedling evaluation trial and low survival in CET was attributed to inbreeding depression. According to Charlesworth and Charlesworth (1987), there are two competing hypotheses that describe the decline in fitness with inbreeding; partial dominance hypothesis and

over dominance hypothesis. Overdominance hypothesis predicts that inbreeding depression will increase as a population becomes more inbred due to loss of heterozygote advantage. The data generated in this study indicated a general increase in mean performance for sprouting, vigour, height, flowering, dry matter content and harvest index among some partial inbred families at the CET. This means that Overdominance hypothesis could not explain inbreeding in cassava inbreds generated. When the surviving clones were evaluated for inbreeding depression (Table 4 and 5), it was found that some families did not exhibit inbreeding depression for the evaluated fitness traits while others showed a low inbreeding depression.

This phenomenon suggests that through the two trials (SET and CET) there was purging of the deleterious alleles leading to mortality of a large portion of generated partial inbreds before/and in the CET. This suggests that partial dominance hypothesis best explains inbreeding depression in the partial inbreds evaluated. It also suggests (based on the degree at which inbreeding depression is purged), that inbreeding depression is caused by genes of major effects. Tolerance to inbreeding depression is another possibility that could have been observed at CET. Partial dominance posts that inbreeding depression is the result of an increase in the frequency of deleterious alleles. With selfing, recessive deleterious alleles, once masked by dominance effects in the heterozygous form become homozygous and express these effects on the components of fitness. It can therefore be predicted that inbreeding depression will decrease with selfing due to purging process. According to Kawuki *et al* (2011), there was an increase in mean performance in amylose content among six cassava S_1 families generated at NaCRRI. This means therefore that such traits and others which are enhanced through inbreeding can be further improved by more cycles of inbreeding without effect on fitness. Flowering which is critical in advancing generations of selfing appears not to be restrained by inbreeding. This provides further motivation to explore inbreeding in cassava.

The CBSD phenotypic class frequency distribution observed in different families in this study showed that there was segregation though to varying degree. There was significant segregation observed in all families except for Namikonga which had two distinct classes (susceptible and resistant). This means that some resistant genes in those segregating families were in heterozygous state in the $S_{0,}$ while the resistant genes in Namikonga were probably in

homozygous state. In general, segregation implies that some resistance genes are in the heterozygous state. If they were all in the homozygous state, then there would be no segregation in S_1. This means that there is an opportunity for inbreeding to improve resistance. Breeding will be easier if we know that resistance genes are fixed in source genotype.

This is one of the few studies that have explored inbreeding for purposes of getting new resistance and/or higher resistance levels to CBSD. These findings are encouraging and thus justify the use of inbreeding in cassava, a highly heterozygous crop. The anticipated ID in outcrossing species, isn't pronounced as shown in Table 5 and 6.

3.5 Conclusion

This study was initiated with a premise that inbreeding would significantly improve resistance to CBSD among partial inbreds as compared to their respective non inbred progenitors. From the results obtained in this study, it can be concluded that:

i. Within each family, a few S_I inbreds (1-15) showed higher levels of resistance than the S_0 progenitors, with the highest number being observed in TZ/130. It is possible therefore to get higher levels of resistance in S_1 than their respective S_0 only that it is probable that a higher number of selfs has to be made in order to increase chances of getting resistant clones.

ii. There was little or no inbreeding depression recorded for key traits in the clones that survived up to the time of evaluation in the CET. This is because very high inbreeding depression was exhibited in seedling evaluation trial and thus clones evaluated in CET had some tolerance to ID.

iii. High mortality in early cycles of evaluation due to inbreeding depression suggested high rate of purging and also that inbreeding depression is as a result of major genes.

It can therefore be generally concluded that inbreeding can be used to generate new genetic stocks for CBSD resistance. Based on the findings, resistance to CBSD could be improved greatly by conducting more selfing cycles of the newly generated resistant clones to S_3 or S_4 which would lead to increased resistance on a population level. Alternatively, the generated S1 can be crossed in different combination enable the exploitation of both additive and non-additive

genetic effects of CBSD. Its is further thought that flowering will unlikely be constrained by further generations of inbreeding.

CHAPTER FOUR

SCREENING OF SELECTED CASSAVA GENOTYPES FOR RESISTANCE TO BOTH SPECIES OF CASSAVA BROWN STREAK VIRUSES IN UGANDA

4.1 INTRODUCTION

Cassava brown streak disease (CBSD) has been identified among the seven most serious threats to world food security (Pennisi, 2010). The disease is currently managed by cultivation of symptom free planting material of tolerant varieties and destruction of affected plants. These are short term measures, as breeding efforts to develop resistant varieties continue. There is a need to identify genotypes that have immunity or resistance to both UCBSV and CBSV. Screening of local and introduced germplasm is going on in Tanzania, Kenya, Uganda, Malawi and Mozambique to identify robust resistance to CBSD. The challenge to resistance breeding however, is the presence of two distinct virus species that cause the disease; cassava brown streak virus (CBSV), and Uganda cassava brown streak virus (UCBSV), both picorna-like (+) ssRNA viruses genus *Impomovirus* from genus *potyviridae* which are prevalent in the region (Mbazibwa *et al.,* 2009a). According to Mbazibwa *et al.* (2011), these species are widely distributed within East Africa implying that resistance reported for a given genotype in one area isn't necessarily present in another region with a different strain/species i.e., their exits significant genotype X strain interaction. A number of CBSD resistant/tolerant cassava genotypes have been selected in Tanzania, Kenya, Mozambique and Uganda. The effectiveness of these genotypes against the two species of CBSV is however not known. It is not clear whether this resistance or tolerance is specific or comprehensive to both UCBSV and CBSV.

According to Hillocks *et al.,* (2002b), cassava varieties differ in their symptom expression. Some show both foliar and root symptom while other may show either foliar or root symptoms with varying severity levels. In the field, the level of resistance of a given genotype is dependent on the symptom expression on the host plant. Politowski and Browning (1978) also reported that a genotype with a susceptible infection type can be considered to have some resistance or tolerance depending on the level of pathogen development within host plant tissues. Therefore term "resistance" used here is used in a similar sense of Fraser (1986) for any inhibition of virus

multiplication and of its pathogenic effects on cassava. It is therefore a combination of two different components of resistance: virus content (virus resistance) and symptom expression (disease resistance). Few cases of plants exhibiting resistance to virus multiplication or virus accumulation have been documented (Lecoq *et al.*, 1982, 2004). According to Moreno *et al.* (2011), there was an association between virus load and symptom expression for CBSD though it was cultivar specific. This therefore means that some genotypes resistant or tolerant to CBSD limit virus acquisition, accumulation or movement within their tissue. In search for these types of resistance, this study was initiated to detect, specify and compare titre levels of CBSV and UCBSV in cassava parental genotypes considered to be tolerant or resistant to CBSD in Uganda and/or in Tanzania in order to get insight in the resistance mechanisms to both CBSV and UCBSV in cassava.

4.2 MATERIALS AND METHOD

4.2.1 Selection and field establishment of selected cassava genotypes

Eleven cassava genotypes selected from Uganda and Tanzania were screened for field resistance to both UCBSV and CBSV in Uganda. These included AR40-6, NDL06/132, Kiroba and Namikonga (reported to be CBSD tolerant) in Tanzania and MM96/4271 (NASE 14), 72-TME 14 (NASE 19), NASE 1 and TZ/130 (tolerant in Uganda). Tanzanian genotypes 'Albert' and 'Kibaha' and TME 204 from Uganda were added as susceptible controls. Materials from Tanzania were obtained as tissue culture material while materials from Uganda were sourced from disease-free areas. Thus, CBSD-free stakes/plantlets were used for this experiment. Field trials were set up in the first rains of 2012 at National Crops Resources Research Institute (NaCRRI), Central Uganda, an area with high CBSD pressure and high whitefly population. At this site, CBSD root symptoms are normally observed on susceptible varieties as early as four months after planting (Abaca *et al*., 2012). Due to limited planting materials, the test genotypes were established in two row unreplicated plots each containing 10 plants with a spacing of 1m×1m. Each plot was separated by a CBSD - spreader row of TME 204 to augment CBSD pressure. The genotypes were grown for 12 months under rain fed conditions and no fertilizer or herbicide was applied.

4.2.2 Sample collection and RNA extraction

Mature leaf samples were taken from six plants from each genotype and pooled to form a uniform sample (Adams *et al*., 2012). Thus, a total of 11 samples were collected, transferred to the laboratory and immediately stored at -84^{0}C. This was done at three, five, seven, nine and 11 months after planting. Sample tissue (approximately 100 mg) was ground into fine powder using liquid nitrogen and a small hand roller. CTAB grinding buffer (1 ml) containing 2% CTAB, 100mM Tris – HCl, pH 8.0, 20mM EDTA and 1.4M NaCl was mixed with a smooth paste of ground sample tissue of each sample to generate sap that was used for RNA extraction. The sap (0.7 ml) was incubated at 65^{0}C for 15 minutes after which 700µl of chloroform: Isoamyl alcohol I.A.A (24:1) was added and centrifuged at maximum speed in a microfuge for 10 minutes at room temperature. The aqueous layer formed was removed and transferred into clean nucleases free 1.5 ml microfuge tubes to which an equal volume of 4M LiCl was added and incubated overnight. The mixture was centrifuged for 30 min at maximum speed of 13,000 g at 4^{0}C to pellet the nucleic acids.

The pellet was re-suspended in 200µl of TE buffer containing 1% SDS to which 100µl of 5M NaCl and 300µl of ice cold iso-propanol was added and the mixture incubated at -20^{0}C for 30 min. After incubation, the mixture was centrifuged for 10 minutes at 13,000 g to pellet the nucleic acid. The pellet was then washed by adding 500µl of 70% ethanol and spinning for 4 min at 4^{0}C. The ethanol was decanted off and pellet was dried and re-suspended in 50µl of nuclease – free sterile water. The quality and quantity of each sample was checked using the micro volumetric Nanodrop ND-1000 using a dilution factor of 40. Due to differences in RNA quantity, the samples were normalized to a working concentration of 100 ngµl^{-1} by addition of an appropriate amount of sterile water.

4.4.3 Real time quantification of CBSV and UCBSV during crop growth

The RT-PCR assay used was based on TaqMan chemistry using primer and probe sequences reported by Adams *et al.* (2012). RNA was analysed using two primer/probe sets from Eurofin (Table 1). For each RNA sample, two replicate reaction were run in 25 µl reaction containing 12.5µl of Maxima Probe qPCR Master Mix (2X) (Fermentas), 7.5µM of each forward and reverse primer, 5µM Taqman probe, 100ng of template and nuclease free sterile water to volume

of 25µl. The reaction were incubated for 60 min at 42^0C then initial denaturation step run for 10 min at 95^0C followed by 40 cycles of denaturation for 15 sec at 95^0C, annealing for 30 sec at 60^0C and extension for 30 sec at 72^0C.

All real-time PCR reactions were performed on a Applied Biosystems' One Step Plus® sequence detection system using micro Amp optical 96 well reaction plates (Applied Biosystems). In addition, non-template water control (NTC) was included on every plate. The Ct values were used to determine the fold change in expression of a target gene for both CBSV and UCBSV in selected cassava parental genotype using comparative $2^{-\Delta\Delta Ct}$ method as described by Livak and Schmittgen (2001) where $\Delta\Delta Ct = (Ct_{target}\text{-}Ct_{Cox})_{time\ x} - (Ct_{target}\text{-}Ct_{Cox})_{3\ months}$ Where x is time (5, 7, 9, 11 months after planting). The virus titres were transformed to log10 and plotted against time (MAP) in order to monitor the accumulation of virus in different genotypes with time.

Table 6: Primers and probes used in the RT-PCR assay for CBSV and UCBSV

Primer	Sequence
CBSV-TzCP-66F	5'-GCCAACTARAACTCGAAGTCCATT-3
CBSV-TzCP-152R	5'-TTCAGTTGTTTAAGCAGTTCGTTCA-3'
CBSV-TzCP-92T	5'-[FAM]-AGTCAAGGAGGCTTCGTGCYCCTC-[TAMRA] -3'
CBSV-UgCP-381F	5'- GATYAARAAGACITTCAAGCCTCCAAA-3'
CBSV-UgCP-474R	5'AATTACATCAGGRGTTAGRTTRTCCCTT-3'
CBSV-UgCP-418T	5'- [VIC]-TCAGCTTACATTTGGATTCCACGCTCTCA- [TAMRA] -3'
COX-F	5'- CGTCGCATTCCAGATTATCCA-3'
COX-R	5'- CAACTACGGATATATAAGRRCCRRAACTG-3'
COX Pe	5'- [FAM]-AGGGCATTCCATCCAGCGTAAGCA-[TAMRA]-3'

4.2.5 Field evaluation of selected cassava genotypes for CBSD

Both incidence (proportion of cassava plants of a given cultivar expressing CBSD symptoms) and severity (degree of CBSD infection on individual plants) were used to quantify the disease. Four data sets at three, five, seven and nine MAP were collected on foliar disease severity. A severity score of 1-5 (Gondwe *et al.,* 2002) was adopted where 1- no symptom, 2- mild symptom

(1-10%), 3- pronounced foliar chlorotic mottle and mild stem lesion (11-25%), 4- severe chlorotic mottle and stem lesion (26-50%) and 5- very severe symptoms (>50%). Severity scores for root necrosis were also taken on all roots harvested per plant at 12 MAP. Severity scores were taken for each root based on the necrotic proportion where 1- no necrosis, 2- mild necrotic lesions (1-10%), 3-pronounced necrotic lesion (11-25%), 4-severe necrotic lesion (26-50%) combined with mild root constriction and 5- very severe necrotic lesion (>50%) coupled with severe constriction. The disease severity means (DSM) were calculated using the following formula

$$DSM = \frac{\sum (\text{severity scores for all affected roots on the infected plant})}{\text{Total number of infected roots on the plant}}$$

Disease incidence (DI) on a per plant basis was quantified as a ratio of the number of roots showing roots symptoms to the total number of roots harvested per plant. Disease index of every clone was derived as a product of DI and DSM. Since the genetic structure of the test germplasm was not known, the rank sum method based on disease incidence and mean severity score of root necrosis was used to separate genotypes into specific resistance groups. This was done by ranking both disease incidence and mean severity score for root necrosis on a per plant basis using Wilcoxon ranking method (Wilcoxon, 1945; Onyeka *et al.*, 2005). Rank sum was derived as sum of incidence and severity for each plant and was used to obtain a mean of all rank sum scores (P_n) for all genotypes screened on plant basis. The mean rank sum per genotype (X_n) was compared with the grand means of the ranks sum across all the cassava genotypes (P_n) to determine the deviation of each cassava genotype from the grand mean. Deviation of each cassava genotype from the grand mean was calculated as

$$di = \left\lceil \frac{(Xn - Pn)}{\text{Standard deviation}} \right\rceil$$

Negative deviations from the grand mean were rated resistant/tolerant while positive deviation were rated susceptible. Genotypes with -1, -2 and -3 deviation to the left of P_n were classified as

moderately tolerant, tolerant and resistant respectively while those with 1, 2 and 3 deviation to the right of P_n were classified as moderately susceptible, susceptible and highly susceptible.

Above ground symptoms were compared with symptom expression in the roots. Virus accumulation and symptom assessment (using 1-5 scales described above) were used to distinguish and characterize different types of CBSV/UCBSV resistance or tolerance in the test cassava genotypes.

4.3 RESULTS

4.3.1 Detection and accumulation of UCBSV or/and CBSV in selected cassava genotypes

Both UCBSV and CBSV species were detected in all genotypes (Table 2). Though both species were detected in all the genotypes, the viral load differed among genotypes. Cassava genotype MM96/4271 (also referred to as NASE 14), had the lowest virus titre for both UCBSV and CBSV of 1.16 and 0.00071 folds respectively (Table 2). Other genotypes with comparatively low virus titre for UCBSV include Kiroba (0.7 folds), AR40-6 (0.026 folds), TZ/130 (1.72 folds), Namikonga (9.25 folds) and NASE 19 (16.11 folds) while NDL06/132 had the highest virus titre (353169.2 folds). For CBSV, Kiroba, NASE 19 and Namikonga also had a comparatively low virus titre of 30.1 folds, 165.4 folds and 199.5 folds respectively, while NDL06/132 had the highest virus titre of 294927.33 folds. Thus among the presumed tolerant varieties, NDL06/132 had the highest viral load for both species.

In cases of mixed infection, the concentration of CBSV was significantly higher than the concentration of UCBSV. To cite a few cases, CBSV concentration in TZ/130 and AR40-6 was respectively 143431.3 folds and 294927.33 folds, as compared to 1.72 folds and 0.026 folds of UCBSV in the same genotypes respectively. However, there were genotypes like Kibaha and NDL06/132 which had higher virus titre for UCBSV than CBSV (Table 7). The already-known susceptible genotypes (Albert and TME 204) had consistently higher viral loads for both UCBSV and CBSV.

Results further indicated that genotype NASE 14 exhibited a very low change in virus titre (from 1.77 folds at 5 MAP to 1.16 folds at 11 MAP for UBSV and 6.54 folds at 5MAP to 0.00071

folds at 11 MAP for CBSV). This shows that the two virus species were present in the plant tissue but the plant had a mechanism of limiting virus accumulation within the plant. A special case was observed in Kiroba, where UCBSV was detected at 5 and 7 MAP and thereafter, the virus was eliminated and/or remained undetected at 9 and 11 MAP. Based on Figure 6, there was a high accumulation of CBSV in all tolerant as compared to that of UCBSV. This is also shown by the amplification plots (Figure 7) where for most genotypes, CBSV begins amplification at relatively low Ct values than UCBSV.

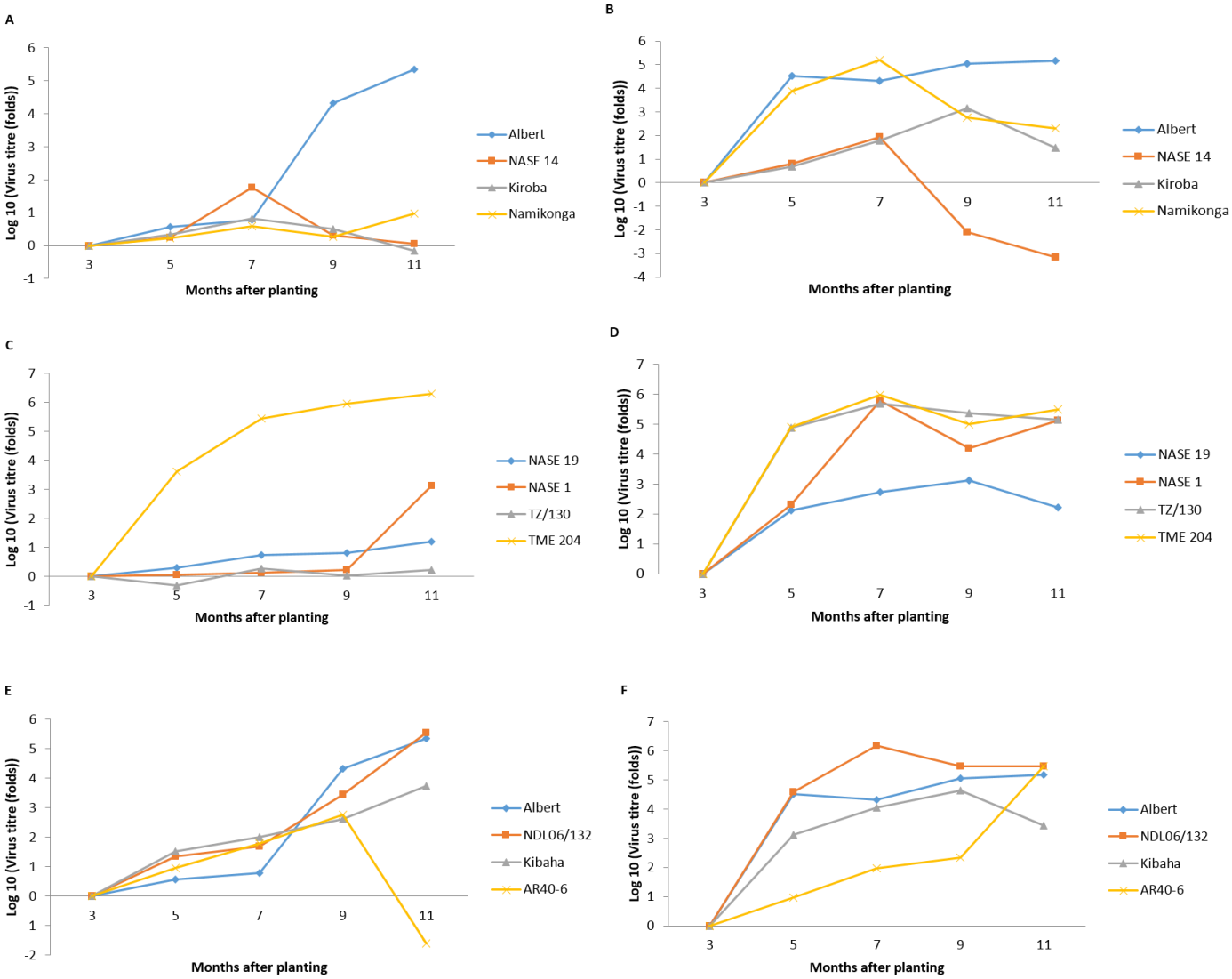

Figure 6: Accumulation of both UCBSV (A, C and E) and CBSV (B, D and F) with time in selected genotypes with different resistance categories for CBSD screened at NaCRRI, Uganda

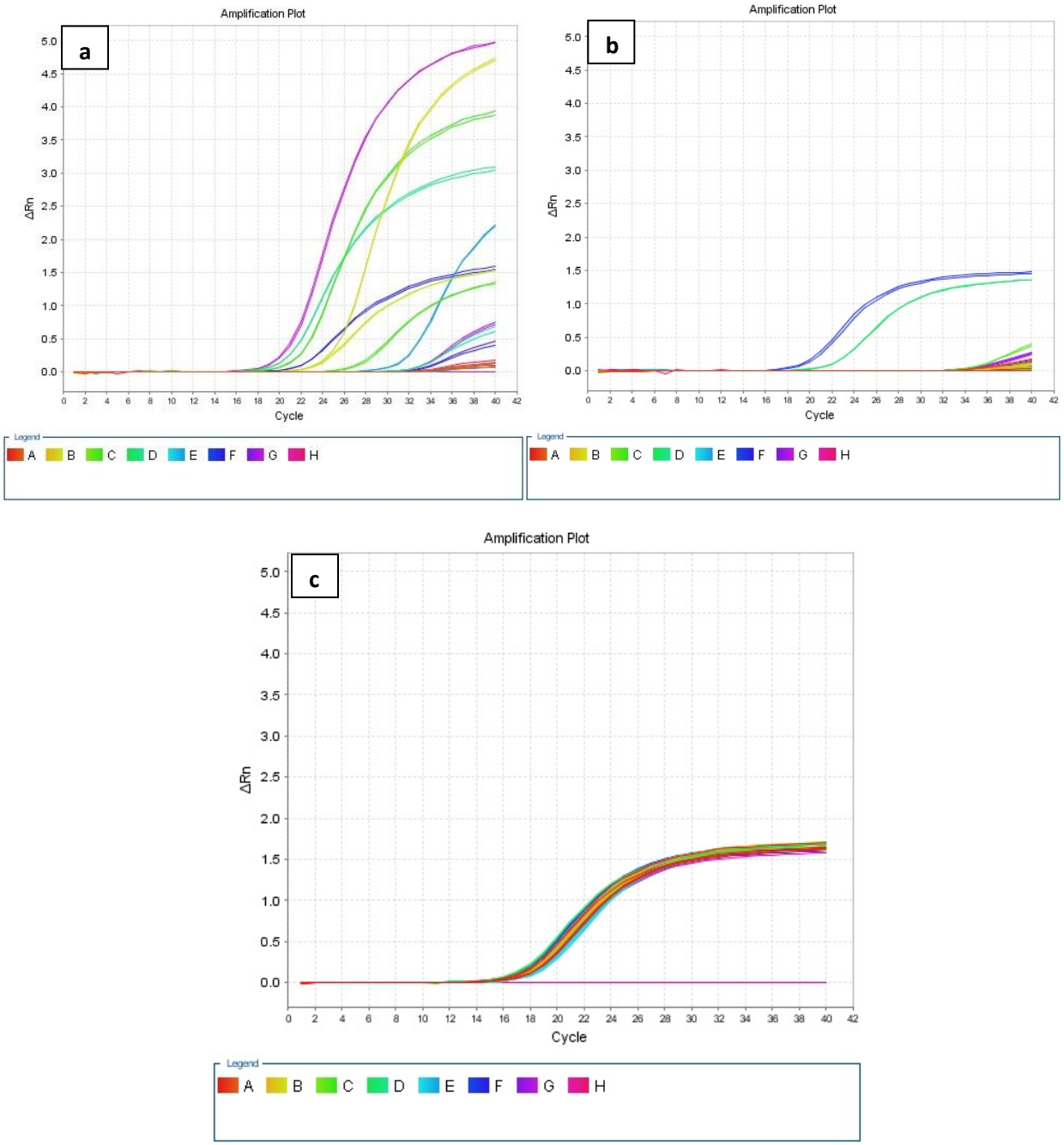

Figure 7: Amplification plots for CBSV (a), UCBSV (b) and indigenous control (COX) (c) for selected cassava parental genotypes evaluated at NaCRRI at 11 MAP

Table 7: Presence of CBSV species and their titre in selected cassava genotypes at 3,5,7,9 and 11 MAP at NaCRRI, Uganda, 2011 - 2012

Genotype	Specificity of resistance											
	3MAP*		5MAP		7MAP		9MAP		11MAP		Virus titre at 11 MAP (folds)	
	UCBSV	CBSV	UCBSV	CBSV	UCBSV	CBSV	UCBSV	CBSV	UCBSV	CBSV	UCBSV	CBSV
NASE 14	+	+	+	+	+	+	+	+	+	+	1.16	0.00071
Kiroba	-	-	+	+	+	+	-	+	-	+	0.7	30.1
NASE 19	+	-	+	+	+	+	+	+	+	+	16.11	165.42
Namikonga	+	+	+	+	+	+	+	+	+	+	9.25	199.5
TZ/130	+	+	+	+	+	+	+	+	+	+	1.72	143431.3
NASE 1	-	+	-	+	-	+	+	+	+	+	133.4	133826.1
Kibaha	-	+	+	+	+	+	+	+	+	+	5634.21	2836.44
Albert	+	+	+	+	+	+	+	+	+	+	220435.95	148489.36
AR40-6	+	+	+	+	+	+	+	+	+	+	0.026	294927.33
NDL06/132	+	+	+	+	+	+	+	+	+	+	353169.2	297978.71
TME 204	+	+	+	+	+	+	+	+	+	+	2039805.3	318293.9

*MAP – Months after planting

4.3.2 Assessment of CBSD resistance in selected cassava genotypes in Uganda

Based on CBSD foliar symptoms

Cassava genotypes screened in this study showed varied response to CBSD foliar symptoms. Of the 11 genotypes, four did not show foliar symptoms. These include NASE 14, NASE 1, Kiroba and NASE 19, while Albert and TME 204 showed severe foliar symptoms during the 9 month evaluation period. Among the symptomatic genotypes, Namikonga and TZ/130 had the lowest incidence of 9% and 17%, and severity of 1.09 and 1.17 respectively, in comparison to TME 204 which had a severity of 4.07 and incidence of 100 (Table 8).

In general the virus titre in asymptomatic plants was lower than that of symptomatic plants except for NASE 1. Though NASE 1 did not show foliar symptoms, it allowed accumulation of CBSV by 133826.1 folds as compared to other genotypes like NASE 14, Kiroba and 72 –TME 14. Among the symptomatic, Namikonga had a comparatively low virus titre of both UCBSV (9.25 folds) and CBSV (199.5 folds). However, the virus titre of 199.5 folds was relatively high as compared to that of NASE 14 and Kiroba.

Based on CBSD root symptoms

The selected cassava genotypes were screened and categorized in different resistance categories according to root symptoms. According to Table 9, only Namikonga was found to be tolerant to CBSD under field conditions with a deviation of -2.38. other genotypes like NASE 1, AR40-6, Kiroba, TZ/130 and NASE 14 were found to be moderately tolerant to CBSD. In addition, some genotypes had varying levels of susceptibility. For instance NASE 19 and NDL06/132 were found to be moderately susceptible to CBSD, in comparison to Kibaha and TME 204 which were highly susceptible to CBSD (Table 9).

This trend was also shown by the number of plants per genotypes that remained symptomless for the entire period of evaluation (12 MAP). Among all the evaluated genotypes, Namikonga had the highest number (83.3%) of plants that remained symptomless followed by NASE 1 (73.3%) and AR40-6 (63.6%) (Table 9). Similarly Namikonga had the lowest disease index (0.20) followed by Kiroba and NASE 1 with 0.29 and 0.36 respectively. The three genotypes also had a low incidence and maximum severity of the disease compared to Kibaha and TME 204.

In comparison with virus titre/accumulation, NASE 14 which had the lowest virus titre for both UCBSV and CBSV was characterized as a moderately tolerant genotype to CBSD with a maximum severity of 5. This can be compared to Namikonga and NASE 1 which had a virus titre of 199.5 and 133826.1 folds for CBSV but ranked as tolerant and moderately tolerant genotype respectively. Though these genotypes (Namikonga and NASE 1) were not able to restrict virus accumulation, they were able to restrict symptom expression and symptom development in the roots giving a high percentage of asymptomatic plants (83.3%) and (73.3%) for Namikonga and NASE 1 respectively as compared to 46.7% of NASE 14 (Table 9)

Table 8: Comparison between CBSD foliar symptoms and viral load in selected genotypes

Genotypes	Incidence(%)	Severity	Min	Max	D. index	Virus Titre (folds)	
						UCBSV	CBSV
NASE 14	0	1.00	1	1	0	1.16	0.00071
Kiroba	0	1.00	1	1	0	0.7	30.1
NASE 1	0	1.00	1	1	0	133.4	133826.1
NASE 19	0	1.00	1	1	0	16.11	165.42
Namikonga	9	1.09	1	2	0.09	9.25	199.5
TZ/130	17	1.17	1	2	0.19	1.72	143431.3
AR40-6	52	1.61	1	3	0.84	0.026	294927.3
Kibaha	75	2.25	1	3	1.69	5634.2	2836.44
NDL06/132	67	2.30	1	4	1.56	353169.2	297978.7
Albert	100	3.00	3	3	3	220435.9	148489.4
TME 204	100	4.07	3	5	4.07	2039805.3	318293.9

Foliar assessment done at 9 MAP; Virus quantification done at 3,5,7,9 and 11 MAP

Table 9: Disease rating of selected cassava parental genotypes to cassava brown streak disease as determined by ranking

Genotypes	CBSD Severity		Symptomless %	DI	DSM	D.Index	d_i	RC	Virus titre (folds)	
	Min	Max							UCBSV	CBSV
Namikonga	1	2	83.3	0.1	2.0	0.20	-2.38	T	9.25	199.5
NASE 1	1	2	73.3	0.2	2.0	0.36	-1.16	MT	133.4	133826.1
AR40-6	1	3	63.6	0.3	2.0	0.58	-0.73	MT	0.026	294927.33
Kiroba	1	3	42.8	0.1	2.0	0.29	-0.66	MT	0.7	30.1
TZ/130	1	4	52.4	0.4	2.5	1.12	-0.34	MT	1.72	143431.3
NASE 14	1	5	46.7	0.3	2.3	0.94	-0.35	MT	1.16	0.00071
NASE 19	1	5	18.2	0.7	3.4	2.45	0.69	MS	16.11	165.42
NDL06/132	2	3	0	0.4	2.4	0.95	1.07	MS	353169.2	297978.7
Albert	1	5	5	0.7	3.5	2.45	1.54	MS	220435.9	148489.4
Kibaha	3	5	0	0.7	3.5	2.49	4.27	HS	5634.2	2836.44
TME 204	4	5	0	1.0	4.7	4.66	20.62	HS	2039805.3	318293.9

DI – Disease incidence; DSM – Disease severity mean; D.Index – Disease Index; RC – Resistance category; d_i – Deviation ; R – Resistant;

T – Tolerant; MT – Moderately tolerant; MS – Moderately susceptible; S – Susceptible; HS – Highly susceptible

Symptomless = proportion of plants that don't have root symptoms.

4.3.3 Root dry matter and harvest index performance of the test genotypes at NaCRRI

There was a great variation in harvest index among the screened genotypes ranging from 0.15 – 0.49 (Table 10). Genotypes AR40–6 and NDL06/132 had the highest harvest index of 0.49, followed by TZ/130 and Kiroba with 0.46 and 0.39 respectively, while 72–TME 14 and Namikonga had significantly low values of harvest index of 0.26 and 0.15 respectively. Thus, the higher levels of resistance in Namikonga don't translate into higher harvest index. On the contrary, a reverse was observed for dry matter content where Namikonga, 72–TME 14 and Kiroba had significantly high dry matter content of 49.8%, 40.8% and 39.5%, as compared to other parental genotypes while, TZ/130 had the lowest dry matter content of 32.9%

Table 10: Harvest index and dry matter content of cassava genotypes screened at NaCRRI

Parental genotypes	Harvest index	Dry matter content (%)
Namikonga	0.15±0.04	49.8±2.09
AR40-6	0.49±0.06	38.1±2.96
Kibaha	0.37±0.08	35.4±4.19
NASE 14	0.37±0.06	37.7±2.96
72 – TME 14	0.26±0.06	40.8±2.96
TZ/130	0.46±0.06	32.9±2.96
NDL06/132	0.49±0.09	38.1±4.18
Kiroba	0.39±0.09	39.5±4.18
Albert	0.32±0.06	37.8±2.98
NASE 1	0.35±0.06	39.9±2.96
Grand mean	0.33±0.09	38.5±4.19
LSD	0.2	10.4
CV%	26	10.9

4.4 Discussion

The objective of this study was to screen cassava genotypes reported to be tolerant or resistant to CBSD in Tanzania and Uganda for their field reaction to both UCBSV and CBSV in Uganda. CBSD tolerant materials were sourced from breeding programme in Tanzania (AR40-6, NDL06/132, Kiroba and Namikonga) and Uganda (NASE 1, NASE 14, NASE 19 and TZ/130). It suffices to note that selection of these respective materials was done before 2010, and by then selection was based on symptom expression from only one species, CBSV or UCBSV, and not a mixture. Thus, this study re-evaluated these materials under conditions that ensured presence of two viral species though results in this study suggest that CBSV is more aggressive than UCBSV.

Based on both field evaluation and molecular diagnostic results, no immunity rather mixed infection of both UCBSV and CBSV was observed in all screened parental cassava genotypes. This means that all the screened cassava genotypes possessed factors necessary for both UCBSV and CBSV pathogenesis. Though mixed infection was observed in all screened genotypes, all genotypes had different virus titre of UCBSV and CBSV. Of all the screened parental genotypes, Namikonga and NASE 1 allowed accumulation of virus especially CBSV while restricting the effects of the viruses especially in terms of symptom expression. This means that Namikonga possesses genes that work together to limit root necrosis. It is worth noting that only leaf samples were used for analysis of virus accumulation. Therefore there is a possibility that Namikonga allows virus accumulation in the leaves but possesses resistance to long-distance movement of the virus. This may explain the very low disease index in Namikonga based on root symptoms. It can therefore be concluded that Namikonga has a comprehensive or non specific tolerance to CBSD. It therefore means that though Namikonga has a susceptible infection type and is able to support the development of UCBSV (9.25 folds) and CBSV (199.5 folds) which are almost the same as NASE 19, however Namikonga has significantly low disease index. In a related study, Namikonga was crossed to a CBSD tolerant variety (NASE 14) and to a CBSD susceptible variety NASE 13, and progeny evaluated for field resistance to CBSD. It was observed the progeny involving NASE 14 had higher levels of resistance than progeny involving NASE 13 as

the parent (Kyaligonza Vincent personal communication). This further confirms the observed tolerance and moderate tolerance respectively in genotypes Namikonga and NASE 14.

Based on virus titre of both CBSV and UCBSV with time, NASE 14 possesses a mechanism that suppresses virus multiplication within the plant. It can be concluded from the results that NASE 14 possesses resistance to virus accumulation while AR40-6 and TZ/130 possess specific resistance to UCBSV based on virus accumulation. Furthermore, NASE 19 possessed specific tolerance to UCBSV based on virus accumulation in tissue. According to Politowski and Browning (1978), this kind of resistance can be termed as dilatory resistance since it enables the plant to reduce the rate of pathogen development. Accumulation of virus particles inside the plant cell involves translation, replication, cell- to – cell and long distance movement of viral sequences. This means that genotypes that have resistance to virus accumulation impede one or several of these stages involved in virus accumulation. It is therefore very important to further investigate these processes in these genotypes and also to identify genes that are responsible for these limitations.

While some genotypes exhibited resistance to virus accumulation, a special case observed in Kiroba suggests reversion or 'recovery' as a mechanism of resistance to UCBSV. Kiroba tested positive for UCBSV at 5, and 7 MAP, but negative at 9 and 11 MAP (Table 2). According to Van den Bosch *et al* (2007), the reduction in virus titres in resistant plants was termed 'reversion'. However the case of Kiroba was beyond merely reducing the titre values but also led to UCBSV suppression making resistance in Kiroba specific to UCBSV. It is possible that Kiroba has resistance to virus movement, post transcriptional virus silencing or virus – induced gene silencing as a mechanism of resistance to UCBSV. These are aspects are currently investigated in another study (Morag Ferguson personal communication). According to Baulcombe (1999) some plants naturally exhibit antiviral defence system where viral RNA in plant mediate defence mechanism thus slowing down and stopping the virus accumulation at a certain stage of infection and/or plant growth. The fact that this mechanism allows the virus to survive in the plant for some time before it is eliminated means that this mechanism is not constitutive particularly in situations where vectors transmit the virus at ease.

Though NASE 14 exhibits resistance to virus accumulation, it possesses an "All or nothing" response where most of the plants remain asymptomatic for both foliar and roots while the few that succumb show very high severity (4 or 5) and incidence (90 -100) on roots. This is coupled with reduction in growth and in some cases dieback. This suggests that NASE 14 confers partial resistance that breaks down under high inoculum pressure and/or with degeneration with which stakes are recycled during each growing season. It can be hypothesized that there could be a threshold at which the virus, based on accumulation level overcomes the plant defence mechanism thereby causing necrosis in affected plants. The practices of establishing plants in areas of high disease pressure or by re-planting the same stakes for a number of seasons are some avenues that allow the virus load to accumulate beyond the threshold. It is therefore important to monitor virus accumulation especially in plants that have showed foliar symptom in the early stages of plant development and compare with those that are asymptomatic in the same environment to verify this hypothesis and also determine this threshold. Information from such studies will be important in determining resistance durability and in designing seed systems for cassava planting materials.

A difference in symptom expression would be expected if virus titre is positively correlated to symptom development. It can be easily assumed that asymptomatic plants would always have lower virus titre. However the case of NASE 1 in this study is interesting. NASE 1 had virus titre of 133.4 and 133826, respectively for UCBSV and CBSV; this was associated with disease index of 0.21 and 0, respectively for roots and foliar (Table 3 and 4). This shows that the relationship between virus accumulation and symptom expression is very poor in NASE 1. It could also indicate that observed symptoms were largely due to CBSV infection. This finding differs from that reported by Moreno *et al*. (2011) and Mohammed (2012) who described the relationship to be always positive. This study used similar varieties; Kaleso (Highly identical to Namikonga), Kiroba and Albert however the difference may be due to the different environments in which these evaluations were conducted. The two studies that reported positive relationship were conducted in controlled environment (screen house) using grafting as the inoculation method, while the current study was conducted in the field. Therefore some genotype may allow accumulation of virus while they restrict the effects of the virus (as revealed by symptoms). This

could means that there are distinct genes that govern symptom expression and virus accumulation and these gene clusters are not closely linked.

Though Namikonga was very effective in resisting the effects of both CBSV and UCBSV, its yield is very low compared to other parental genotypes. The low yield for Namikonga could be due to yield cost on the plant due to resistance to CBSD. Brown (2002) reported that resistance genes in some plant might impose a metabolic cost on plant thereby reducing the fitness of the host. In order for resistant plants to maintain defence levels under biotic stress, growth and other competing physiological processes decrease in accordance with optimal allocation theory for plant defence (Zangerl and Bazzaz, 1992). Secondly the low yield may be attributed to the fact that Namikonga is a BC_2 hybrid having *M.glaziovii* as its parent and thus it still has *M.glaziovii* characteristics including low yield.

5.0 Conclusion

In conclusion, none of the genotypes screened was found to be immune against both viruses. Based on virus accumulation with time, only one genotype (NASE 14) was found to possess comprehensive resistance while AR40-6, Kiroba and Tz/130 had specific resistance against UCBSV. On the other hand, only one genotype (Namikonga) was found to be tolerant to CBSD in the field. This means that a lot of efforts are still needed in the generation of new sources of resistance to both UCBSV and CBSV and field resistance. This however needs to take into consideration that different genotypes exhibit different resistance mechanisms to the viruses either through controlled/reduced virus multiplication or symptom expression and recovery. For enduring resistance, various mechanism can be combined or exploited by considering both virus and disease resistance in different genotypes.

CHAPTER FIVE

GENERAL DISCUSSION, RECOMMENDATIONS AND CONCLUSIONS

This study was undertaken to respond to the CBSD epidemic in Uganda. CBSD has so far spread to over 42 districts since the outbreak in 2005. Specifically, this study; 1) generated and evaluated S_1 inbreds for CBSD resistance or tolerance and 2) quantified tolerance or resistance to CBSD in selected cassava genotypes sourced from Uganda and Tanzania breeding programmes. Field trials to determine resistance to CBSD in selected cassava parental genotypes was done to obtain results that are relevant to the crop situation. As reported by both Kawuki *et al*. (2011) and Ceballos *et al*., (2007a), inbreeding is very effective in improving the expression of some traits especially those that are recessive in nature and/or those with additive effects. Significant effect of inbreeding on resistance to CBSD in one inbreeding cycle would be possible if heritability of CBSD resistance within the selected genotypes was high.

Based on this study, the heritability of CBSD resistance in the selected S_0 genotype was moderate which provides the premise that gains can be got through inbreeding. The generating of new sources of resistance to CBSD (clones that remained asymptomatic both for foliar and root for the two seasons of evaluation in hot spot) prove that inbreeding can be used in resistance breeding. It is therefore recommended that clones with the maximum severity score of 1 and 2 should be further selfed to S_3 or S_4 in order to generate significant level of resistance among the inbred population as compared to their respective progenitors. Since low or no inbreeding depression was recorded, more cycles of inbreeding will have no effects on growth, production and fitness traits rather this will lead to purging of inbreeding depression within the selected and generated inbred population. This will increase the sources of resistance to CBSD especially for resistance breeding in the region.

Furthermore, according to the study, none of the genotypes screened exhibited resistance to infection to both UCBSV and CBSV. However some of the infected plants remained symptomless or had reduced symptom expression in the presence of the disease. Therefore some genotypes exhibited resistance to the disease (the effects of UCBSV and CBSV) while showing susceptibility to infection and multiplication of the two virus species. The use of such genotypes

has accelerated wide spread of virus from infected symptomless stocks grown in proximity to healthy plants.

The fact that no genotype was identified with resistance to CBSV and UCBSV infection among the selected genotypes from Tanzania and Uganda means that a lot of work is still required. It is possible that such genotypes can be identified in wider collection of germplasm of local and introductions from other countries and international research centres like IITA and CIAT using high precision techniques like real time PCR in diagnostics. It is very important also for breeding programmes to generate new genetic stocks for CBSD resistance breeding. This study has demonstrated that new genetic stocks can be generated through inbreeding of highly tolerant and resistant genotypes. Selected inbreds from different families can also be crossed within and among themselves to concentrate CBSD resistance alleles. In addition, new genetic stocks will aid in conducting future studies on the genetics of CBSD to address questions on the number of QTLs that are required to control CBSD resistance, number of genes and mechanism of symptom suppression in both leaves and roots. Such studies require genotypes with various backgrounds like those with resistance to infection, those that show foliar symptoms but no root symptoms and those that show root symptoms with no foliar symptoms.

Furthermore, deeper studies should be done to explore the resistance mechanism especially in the available sources of resistance. It is therefore recommended that transcriptome profiles of Namikonga are generated in order to identify key resistant genes from differently expressed genes and gene clusters in order to understand resistance in Namikonga. More so, genes controlling resistance to virus accumulation in NASE 14 should be identified so that they can be transferred into Namikonga background in order to take advantage of both virus and disease resistance. Efforts to undertake/explore this approach have been initiated and preliminary results are encouraging (unpublished data). Furthermore due to varying mode of resistance in different genotypes, number of QTLs (possibly) for CBSD resistance and expression of other economically important traits, it is recommended that category of resistance, specificity of resistance and other economically important traits are put in consideration at selection stage.

Based on the results obtained in study two (Chapter 4), Namikonga had low yield as compared to other cassava parental genotypes. This low yield can be attributed to a yield cost of resistance to

CBSD. In comparison to study 1(Chapter 3), the breeding value (for yield) of Namikonga was highly improved through inbreeding. According to results shown in Table 6, Namikonga derived progeny had the high harvest index as compared to S_0 parent thus through inbreeding, there was a concentration of alleles that are affect yield in this genotype.

In most cases the search for resistance to CBSD has dominated the selection process in most cassava breeding programs in CBSD affected areas and as a result this may cause a yield penalty. If resistance is indeed costly, the most effective strategy may not be to select for excellent resistance (if that means sacrificing yield and quality), but to select for at least moderate resistance. This however may accelerate the spread of the disease from one area to the other. It is thus recommended that approaches that gives high precision for selecting multiple traits like genomic selection and molecular approaches like real- time PCR analysis should be included in the selection process.

Breeding for resistance to cassava virus especially CBSD is posed with the problem of researchers not having standard methods and terminologies in evaluating for resistance. While breeders evaluate based on the impact of virus on yield and quality; pathologists consider the fate of virus in the plant. More so, other factors like differential expression of both foliar and root symptoms in different genotypes have further complicated the evaluation of CBSD resistance. The method of using disease index and categorizing different genotypes into different categories used in this study came in at a right time to solve this problem. This method will be very useful in selection of parental genotypes and or breeding population/clones for advancement. This is because the method takes care of the response of each genotype to the viruses in terms of the incidence and severity thereby taking advantage of different mechanisms of resistance to CBSD. Since CBSD resistance is polygenic in nature, it can be hypothesized that different genotypes in different resistance categories carry varying numbers of QTLs responsible for resistance to CBSD. It can therefore be envisaged that crosses between and within genotypes in high resistance categories (resistant and tolerant) like Namikonga and NASE 1 will increase resistance to CBSD by shifting the deviation (*d*) more to the left thereby increasing resistance to CBSD on population basis rather than individual basis.

In conclusion, from the best genotypes selected from both Tanzania and Uganda, only one was found resistant to CBSD. Therefore some effort are needed to generate new sources of resistance with a target of generating genotypes with resistance to infection of both UCBSV and CBSV, exploit different mechanism of resistance by pyramiding them in elite clones and understanding resistance mechanisms on both genomic and metabolic level. Inbreeding is one of the approaches that can be used to generate new sources of resistance to CBSD and other complex traits especially in highly heterozygous crops. The 44 clones with CBSD root score of 1 or 2 (and classified as resistant) through inbreeding, have been replanted in replicated trials for further screening for both virus and disease resistance. Efforts will also be made to cross among themselves and resultant progeny evaluated alongside the S_2, S_3 and S_4 derived progeny for CBSD resistance.

REFERENCES

Abaca A, Kawuki R, Tukamuhabwa P, Baguma Y, Pariyo A, Alicai T, Omongo C.A and Bua A (2012). Evaluation of local and elite cassava genotypes for resistance to cassava brown streak disease in Uganda. *Journal of Agronomy*, 11: 65-72.

Abarshi M.M, Mohammed I.U, Wasswa P, Hillocks R.J, Holt J, Legg J.P, Seal S.E., Maruthi M.N (2010). Optimization of diagnostic RT-PCR protocol and sampling procedure for the reliable and cost-effective detection of cassava brown streak virus. *Journal of Virological Methods* 163, 353-359.

Adams I.P, Abidrabo P, Miano D.W, Alicai T, Kinyua Z.M, Clarke J, Macarthur R, Weekes R, Laurenson L, Hany U, Peters D, Potts M, Glover R, Boonham N and Smith J (2013). High throughput real-time RT-PCR assay for specific detection of cassava brown streak disease causal viruses and their application to testing of plant materials. *Plant Pathology* 62(1):233-242.

Alicai T, Omongo C.A, Maruthi M.N, Hillocks R.J, Baguma Y, Kawuki R, Bua A, Otim-Nape G.W, Colvin J (2007). Re-emergence of cassava brown streak disease in Uganda. *Plant Disease* 91:24-29.

Alves C.A.A (2002). Cassava botany and physiology. Pg 67-89. In: R.J. Hillocks *et al.*, (ed) Cassava: Biology, Production and Utilization. CABI. Wallingford, UK

Baulcombe D.C (1999). Fast forward genetics based on virus-induced gene silencing. *Current Opinion Plant Biology*. 2:109 – 113.

Barrett S.C.H, and Charlesworth D (1991). Effect of a change in the level of inbreeding on the genetic load. *Nature* 352:522-524.

Bigirimana S, Barumbanze P, Ndayihanzamaso P., Shirima R, Legg J.P, (2011). First report of cassava brown streak disease and associated Ugandan cassava brown streak virus in

Burundi. *New Disease Reports* 24, 26, http://dx.doi.org/10.5197/j.2044-0588.2011.024.026.

Brown J.K.M. (2002). Yield penalties of disease resistance in crops. *Current. Opinion. Plant Biology*. 5, 339–344.

Ceballos H, Iglesias C.A, Perez J.C, Dixon A G. O (2004). Cassava breeding opportunities and challenges. *Plant Molecular Biology* 56:503-515.

Ceballos H. Fregene M, Perez J.C, Morante N and Calle F (2007a). Cassava genetic improvement. In: (Kang M.S, Priyadarshan P.M, (eds) Breeding major food staples *Blackwell Publishing*, Ames. IA USA Pg 365-391.

Ceballos H., Sanchez T, Morante N, Fregene M, Dufour D, Smith A.M, Denyer K, Perez C.J, Calle F and Mestres C (2007b). Discovery of an Amylose – free starch mutant in cassava (*Manihot esculenta Crantz*) *Journal Agricultural Food Chemistry* 55:7469 – 7476.

Charlesworth D and Charlesworth B (1987). Inbreeding depression and its evolutionary consequences *Annual. Review Ecological Systems* 18: 237-268.

Devries J and Toenniessen G (2001). Securing the harvest: Biotechnology, breeding and seed systems for African Crops. *African Journal of Root and Tuber Crops* 2:181-186.

Doughty L.R. (1958). Cassava breeding for resistance to mosaic and brown streak disease viruses; A review of twenty-one years work. In: East African Agriculture and Forestry Research Organisation, Annual Report. Muguga, Kenya, EAAFRO. Pg 48-53.

East African Agricultural Productivity Project (EAAPP) (2012). Status of cassava pest and diseases in Uganda. A survey conducted in 2011. Cassava Regional Centre of excellence (CRCoE)

FAOSTAT (2011) http://faostat.fao.org/site/339/default.aspx. Retrieved on 11/07/2013.

FAOSTAT (2010) http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor Retrieved on 12/10/2012.

FAOSTAT (2009) http://www.faostat.fao.org/faostat/servlet/xteservlet3 Retrieved 0n 8. Aug.2010.

Fraser R.S.S (1986) Gene for resistance to plant viruses. CRC. *Critical. Review. Plant Science* 3: 257-294

Fregene M, Matsumura H, Akano A Dixon A and Terauchi R (2004). Serial analysis of gene expression (SAGE) of host-plant resistance to the cassava mosaic disease (CMD) *Plant Molecular Biology* 56:563-591.

Garcia P, San Vicente F, Bejarano A, and Quijada P (2004). Inbreeding depression in tropical maize populations before and after recurrent selection of full-sib families. Bioagro, Western University Lisandro Alvarado Center-Cabudare Barquisimeto, Venezuela. 16 (1):17-25.

Gondwe F.M.T, Mahungu N.M, Hillocks R.J, Raya M.D, Moyo C.C., Soko M.M., Chipungu F.P. and Benesi I.R.M. (2002). Economic losses by small scale farmers in Malawi due to cassava brown streak disease. 'Development of a co-ordinated plan of action for CBSD Research in Eastern and Southern Africa' – Proceedings of a Workshop held at Whitesands Hotel, Mombasa, Kenya, 27-30 October 2002. Aylesford, UK, DFID Crop Protection Programme.

Harrison B.D, Swanson M.M, and Robinson D.J (1995). Cassava viruses in the old world. In Proceeding of the second international scientific meeting of the cassava Biotechnology network. Bogor, Indonesia 3:463-472.

Harrison B. D (2002). Virus variation in relation to resistance-breaking in plants. *Euphytica* 124: 181–192.

Hedrick P.W, (1983). Genetics of populations. Science Books International. Van Nostrands Reinhold company. New York pages 629.

Hershey C.H, (2010). Cassava genetic improvement: Theory and practice. CIAT/Rockfeller

Hershey C, (2011). Cassava breeding: theory and practice. A publication by Food and Agriculture Organisation (FAO), In press.

Hillocks R.J, Raya M and Thresh J.M (1996). The association between root necrosis and above ground symptoms of brown streak virus infection of cassava in southern Tanzania. *International Journal of Pest Management* 42.285-289.

Hillocks R. J (1997). Cassava virus diseases and their control with special reference to southern Tanzania. *Intergrated Pest Management Reviews* 2: 125-138.

Hillocks R.J and Thresh J.M (2000) Cassava mosaic and cassava brown streak viruses in Africa: A comparative guide to symptom and aetiologies. *Roots* 7 (1):1-8.

Hillocks R.J, Thresh J.M, Tomas, J, Botao M, Macia R and Zavier R. (2001). Cassava brown streak disease in northern Mozambique. *International Journal of Pest Management* 48:179-182.

Hillocks R.J. (2002a). Control of cassava virus diseases in eastern and southern Africa. Department for International Development (DFID) Crop Protection Programme, Final Technical Report, Project RJ563. Natural Resources Institute (NRI), University of Greenwich, Chatham, Kent, UK. Pg 48.

Hillock R.J, Thresh J.M and Belloti A.C (2002b). Cassava: Biology, production and utilization. CABI pg 76, 77.

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

Hillocks R.J (2006). Promotion of control measures for cassava brown streak disease. DFID Crop Protection Programme, Final Technical Report, Project R8404. Natural Resources Institute (NRI), University of Greenwich, Chatham, Kent, UK. Pg 24.

Jameson J.D (1964). Cassava mosaic disease in Uganda. *East Africa Agriculture and Forestry Journal* 30: 208-213.

Jennings D.L (1957). Further studies in breeding cassava for virus resistance. *East African Agricultural Journal* 22:213-219.

Jennings D.L and Iglesias C. (2002). Breeding for crop improvement. In: R.J.Hillocks (ed) Cassava: Biology, production and utilization CABI, Wallingford, UK. pg 149-166

Kawano K, Amanya A, Daza P and Rios M (1978). Factors affecting efficiency of hybridization and selection of cassava (*Manihot esculenta Crantz). Crop Science*. 18:373-376

Kawuki R.S, Nuwamanya E, Labuschagne, M.T, Herselman L and Ferguson M.E (2011). Segregation of selected agronomic traits in six S_1 cassava families. *Journal of Plant Breeding and Crop science* 3(8): pp. 154-160

Kulembeka H.P.K (2010) Genetic linkage mapping of field resistance to cassava brown streak disease in cassava (*Manihot esculenta Crantz*) landraces from Tanzania. PhD Thesis, University of Free State, Bloemfontein. South Africa. p 134.

Lecoq H, Pochard E, Pitrat M, Laterrot H, and Marchoux G (1982). Identification and exploitation of resistance to viruses in plant vegetable. *Cryptogam Mycology*. 3: 333-345.

Lecoq H, Desbiez C, Belecolle B, Cohen S and Mansour A (2000). Cytological and molecular evidence that the whitefly-transmitted cucumber vein yellowing virus is a tentative member of family *Potyviridae*. *Journal of General Viology* 81: 2289.

Lecoq H, Moury B, Desbiez C, Palloix A and Pitrat M (2004). Durable virus resistance in plants through conventional approaches:a challenge. *Virus Research* 100: 31-39.

Legg P. and Hillocks R. J. (2003). Cassava brown streak virus disease: Past, Present and Future. Proceedings of an International Workshop Mombasa, Kenya, 27-30 October 2002 pg 12-13, NRI and Bristol University.

Legg J.P, Jeremiah S.C, Obiero H.M, Maruthi M.N, Ndyetabula, I, Okao-Okuja G, Bouwmeester H, Bigirimana S, Tata-Hangy W, Gashaka G, Mkamilo G, Alicai T, Lava Kumar P, (2011). Comparing the regional epidemiology of the cassava mosaic and cassava brown streak virus pandemics in Africa. *Virus Research* 159: 161–170.

Livak K, and Schmittgen T, 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta}C_t$ method *Methods* 25: 402–408.

Mahungu N.H., Dixon A.G.O, Mkumbira, J.M (1994). Breeding cassava for multiple pest resistance in Africa. *African Crop Science Journal* 2:539-552.

Maruthi M.N, Hillocks R.J, Rekha A.R, Colvin.J (2005). Transmission of cassava brown streak virus by whiteflies. *Journal of Phytopathology* 153: 307-312.

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

Mbanzibwa D.R, Tian Y, Mukasa S.B, Valkonen J.P.T (2009b). Cassava brown streak virus (*Potyviridae*) encodes a putative Maf/HAM1 *pyrophosphatase* implicated in reduction of mutations and a *P1 proteinase* that suppresses RNA silencing but contains no HC-Pro. *Journal of Virology* 83:6934-6940.

Mbanzibwa D.R, Tian YP, Tugume A.K, Mukasa S.B, Tairo F, Kyamanywa S, Kullaya A, Valkonen J.P.T (2011). Simultaneous virus-specific detection of the two cassava brown streak associated viruses by RT-PCR reveals wide distribution in East Africa, mixed infections, and infections in *Manihot glaziovii*. *Journal of Virological Methods* 171:394-400.

Mohammed I.U (2012). Virus – host interaction in the cassava brown streak disease pathosystem. PhD Thesis, University of Greenwich.

Monger W.A, Seal S, Cotton. S and Foster, G.A (2001). Identification of different isolates of cassava brown streak virus and development of a diagnostic test. *Plant Pathology* 50:768-775.

Moreno I, Gruissem W, Vanderschuren H, (2011). Reference genes for reliable potyvirus quantitation in cassava and analysis of cassava brown streak virus load in host varieties. *Journal of Virological Methods* 177: 49–54.

Mware B, Narla R, Amata R, Olubayo F, Songa J, Kyamanyua S and Ateka E.M (2009) Efficiency of cassava brown streak transmission by two whitefly species in coastal Kenya. *Journal of General and Molecular Virology* 1(4): 040-045.

Mware B., Olubayo F Narla R, Songa J, Amata R, Kyamanywa S and Ateka E.M (2010). First record of spiraling whitefly in coastal Kenya: emergence, host range, distribution and association with cassava brown streak virus disease. *International .Journal of Agricultural Biology*. 12:411-415.

NARO (2006) Occurrence of cassava brown streak disease in Uganda. *Project Report A1150.*

Nassar N.M.A and Ortiz R (2007). Cassava improvement: challenges and impact. *Journal of Agricultural Science* 145, 163-171.

United States Department of Agriculture (USDA) "Nutrient data laboratory". Retrieved January 2012 from http://fnic.nal.usda.gov/.

Okigbo B.N (2000). Nutritional implication of projects giving high priority to the production of staple of nutritive quality; the case for cassava (*Manihot esculenta* Crantz) in humid tropics of West Africa. *International Institute of Tropical Agriculture*, Ibadan Nigeria

Olsen K.M (2004). SNPs, SSRs and inferences on cassava's origin. *Plant Molecular Biology* 56:517-526

Onyeka T. J., Dixon A. G. O., and Ekpo E. J. A (2005). Identification of levels of resistance to cassava root rot disease (*Botryodiplodia theobromae*) in African landraces and improved germplasm using in vitro inoculation method. *Euphytica* 145:281-288.

Pennisi E (2010). Armed and dangerous. *Science,* 327, 804-805.

Rickard J.E, Asaoke, M., Blanshard, J.M.V (1991). The physiochemical properties of cassava starch. *Tropical Science* 31: 189-207.

Rogers D.J and Appan S.G (1973). *Manihot, manihotoides*. Flora Neotropica. Hafner press, Newyork pg 272-274.

Rojas M.C, Perez J.C, Ceballos H, Baena D, Morante N, Calle F (2009). Analysis of inbreeding depression in eight S_1 cassava families. *Crop Science*. 49: 1-6.

Salcedo A, Valle A.D, Sanchez. B, Ocasio V, Ortiz. A, Marquez P, Siritunga D (2010). Comparative evaluation of physiological post harvest root deterioration of 25 cassava (*Manihot esculenta*) accessions: visual vs hydroxycoumarins fluorescent accumulation analysis. *Africa Journal of Agricultural Research* 5:3138-3144.

Scott G.J, Rosegrant M.W and Ringles M.W (2000). Roots and tuber for 21st Century: Trends, projection and policy options. *Food, Agriculture and Environment Discussion Paper 31*. International Food Policy Research Institute.

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

Storey H.H (1939). Report of the plant pathologist. *Annual Report from East African Agricultural Research Station for 1939.*

Taylor N.J, Halsey M, Gaitan-solis.E, Anderson D, Gichuki S, Miano D, Bua A, Alicai T and Fauquet C.M (2012). The VIRCA Project: Virus resistant cassava for Africa. GM crops and food: Biotechnology in Agriculture and the food chain. *Lande biosciences*. 3:2, 1-11.

Tomlinson J.A, Ostoja-Starzewska S, Adams I.P, Miano D.W, Abidrabo P, Kinyua Z, Alicai T, Dickinson M.J, Peters D, Boonham N and Smith J (2013). Loop-mediated isothermal amplification for rapid detection of the causal agents of cassava brown streak disease. *Journal of Virological Methods*. 191 (2): 148–154.

Van den Bosch F. Jeger M.J and Gilligan C. A (2007) Disease control and its selection for damaging plant virus strains in vegetatively propagated staple food crop, Proceedings of the Royal society: *Biological Journal* 274: 11 – 18.

Walsh B (2005). The struggle to exploit non-additive variation. *Australian Journal of Agricultural Research*, 56:873-881.

Wilcoxon F (1945) Individual comparison by ranking method. *Biometrics Bulletin* 1 (6): 80 – 83

Winter S, Koerbler M, Stein B, Pietruszka A, Martina Paape and Butgereitt A (2010). The analysis of cassava brown streak viruses reveals the presence of distinct virus species causing cassava brown streak disease in East Africa. *Journal of General Virology* 91:1365.

Yadav J, Ogwok E, Wagaba H, Patil B. L, Bagewadi B, Alicai T, Gaitan-solis E, Taylor J and Fauquet M. C (2011). RNAi-mediated resistance to cassava brown streak Uganda virus in transgenic cassava. *Molecular Plant Pathology* 12(7):677-687.

Zangerl A. R and Bazzaz F. A (1992) Theory and pattern in plant defence allocation. In R. S. Fritz and E. L Simms (eds) Plant resistance to herbivores and pathogens. *Ecology, Evolution and Genetics.* Chicago press. Chicago.