EVALUATING RESPONSE TO CASSAVA VIRAL DISEASE INFECTIONS AMONG SELECTED CASSAVA GENOTYPES UNDER FIELD AND GREENHOUSE ASSAYS IN LOWER EASTERN KENYA.

BY

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Declaration

This thesis is my original work and has never been presented for the award of degree in any other university.

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Abbreviations and Acronyms

CMD- Cassava mosaic disease.

CBSD- Cassava brown streak disease

CBSV- Cassava brown streak virus

CMBs- Cassava mosaic Begomoviruses

SAS- statistical Analysis System

LSD- Least Significant Difference

DSM- Disease Severity Mean

RT-PCR- Reverse transcriptase polymerase chain reaction

KALRO- Kenya Agriculture and Livestock Organization

ELISA- Enzyme linked Immunosorbentassy

ACMV- African cassava mosaic virus

EACMV- East African cassava mosaic virus

DNA- Deoxyribonucleic acid

EDTA- Ethylene-diaminetetraacetate.

SDS- Sodium dodecyl sulphate

TAE- Tris- acetate

µM- micromolar

Ml- Milliliters

WAG- Weeks after grafting.

Abstract

Cassava mosaic geminiviruses (CMGs) and cassava brown streak viruses (CBSVs) respectively cause cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) which significantly inhibit cassava production in Kenya. This study aimed at determining the prevalence and incidence of CMD and CBSD in lower eastern Kenya and resistance response of new improved cassava genotypes. To determine the prevalence and incidence of CMD and CBSD in lower eastern Kenya, survey was conducted in Machakos, Makueni and Kitui and thirty farmer's field were sampled. Sampling procedure involved stopping at regular intervals of about 15 to 20km between farmer's fields along transect in each sampling location and sampled farms purposively selected. To evaluate the resistance response of fifteen improved genotypes and two local susceptible controls, field experiment was laid out in randomized complete block design with four replicates. Three replicates from each improved and susceptible controls were further chip bud grafted with CMBs and CBSVs infected scions under greenhouse conditions in a complete randomized design. Results revealed 51% CMD and 17.67% CBSD incidences in lower Eastern Kenya. Both diseases exhibited wider incidence distributions with 59%, 63% and 31% CMD recorded in Makueni, Kitui and Machakos respectively, while 31%, 15% and 7% CBSD was observed in the same order. *Bemisia tabaci*, exhibited wider distributions with an average of three flies per plant in Makueni, two in Kitui and one in Machakos. All fifteen improved genotypes did not show any viral disease symptoms while 93.7% and 100% CMD and CBSD incidences respectively, were analyzed in susceptible 990072, while 990067, recorded 100% CMD and 81.3% CBSD incidences under field conditions. Variations in CBSD and CMD symptom were observed under greenhouse conditions, For example only four of the improved genotypes (Kiboko281, Thika280, Kiboko277 and Kiboko276) and two controls (990072 and 990067) expressed between 20 – 90% CBSD incidences from 6 – 8 weeks after grafting. In contrast, ten genotypes and two controls (990072 and 990067) exhibited 10 – 60% CMD incidences between 2 – 8 weeks after grafting. The study gives evidence of the presence of both viral diseases in lower eastern Kenya and recommends that the five (Kiboko300, Kiboko297, Thika272, Thika279 and Kiboko275) improved genotypes found to be CMD and CBSD free be adopted by farmers in lower eastern Kenya.

CHAPTER ONE

1.0 Background information

Cassava (*Manihot esculenta Crantz*) originated from Central and South America and was introduced to the East African coast in the eighteenth century by Portuguese sailors (Olsen, 2004). The crop has spread and gained prominence as a major staple food for many communities in sub Saharan Africa (Fauquet and Fargette, 1990). Cassava is a staple food for over 700 million people worldwide and is the fourth most important food source of carbohydrates after rice, sugar cane and maize (Fregene and Pounti-Kaerlas, 2002).

About 54% of world cassava production comes from Africa where about 40% of the population in sub-Saharan African depends on it as staple food (Nweke *et al.,* 2002). Production and consumption of the crop has risen due to climate change, population growth, low and stagnant per capita incomes and rapid urbanization all of which generate demand for cheap sources of starch to feed the poor rural and urban consumers (Scott *et al.,* 2000)

Cassava can be grown in a wide range of environments and can withstand long periods of drought. Its growth is indeterminate and the storage roots can be left in the ground for many months until required for consumption or processing (Irungu, 2011). Moreover, cassava will produce some yields even in very marginal soils where it can play a key role in food security (Okuja *et al.,* 2004). Traditional cassava utilization in Kenya is limited to roasting and boiling of fresh roots as well as processing into flour for consumption in major production regions. The crop is grown by small poor households for subsistence and forms an important food security crop (Irungu, 2011).

Biotic constraints remain a major hindrance to cassava production of which nine viruses isolates have been isolated from cassava in Africa (Calvert and Thresh, 2002). Of these, cassava mosaic Gemini viruses (CMGs) and cassava brown streak viruses (CBSVs) cause diseases of major economic significance. Cassava mosaic geminiviruse cause Cassava Mosaic disease while CBSVs cause Cassava Brown Streak disease. In recent years, both CMGs and CBSVs have come under increasing scrutiny; because of the devastating losses they cause (Fauquet, 2008). In Kenya, an estimated loss of over US $14million per annum for CMD (Legg and Okuja, 1999)

and weight loss of produced roots of up to 70% for CBSD (Pheneas and James, 2007) has been reported.

Both viruses are transmitted by whitefly vector (*Bemisia tabaci)*. Cassava brown streak disease was previously thought to be limited having been reported in East African coastal areas (Storey, 1936; Thresh, 2002). Jennings (1960) considered CBSD as a serious problem in all cassava growing areas of the East Coast of Africa and it was confirmed as major constraints to cassava production in Northern Mozambique (Hillocks *et al.,* 2002). Cassava brown streak disease and CMD have been reported at high altitude areas around Lake Victoria in Uganda, Tanzania and Western Kenya (Alicai *et al.,* 2007; Mware *et al*., 2009).

During viral co-infection, the viruses involved enjoy mutual benefits thereby enhancing titre levels and severity (Savenkov and Volkonen, 2001). In cassava, the occurrence of synergistic interaction between Cameroon isolates of ACMV and EACMV (Fondong, 2000) and between ACMV-UG and EACMV UG2 (Pita *et al.,* 1999) or even between viruses and viroid's (Volkonen, 1992) have been reported.

Both viral diseases are symptomatic and hence their symptoms manifest on leaves, stem and roots of infected cassava plant. Cassava brown streak disease symptoms includes: chlorotic leaf mottle along margins of secondary veins at initial stage, later affecting tertiary veins causing chlorotic blotches, purple/ brown elongated necrotic lesion on the exterior surface of the stem, dieback and yellow and/or brown, corky necrosis along with black streaks on roots (Hillocks *et al.,* 2002). The CMD symptoms are; yellow mosaic, setting up unequal expansion in affected areas causing twisting, narrowing and malformation of the leaf (CABI, 2004).

Cassava Mosaic and Cassava Brown Streak Disease infected planting materials is an effective means of perpetuating and disseminating both diseases (Legg and Hillocks, 2003). Wagaba *et al.,* (2013) demonstrated that CBSV and CMV was graft transmissible whereby auxiliary buds excised from infected plant and inserted into a rootstock of a healthy plant invariably gave rise to plants showing CBSD and CMD symptoms. Also there has been evidence of natural spread between plants as clones introduced from West Africa or other areas that are free from CMD and CBSD have become infected when grown at sites in Mozambique, Malawi, Kenya and Tanzania (Bock, 1994). Storey (1936) speculated that this natural spread was most likely to be mediated

by the whiteflies (*B. species*). However, until recently, only a few transmission experiments had been conducted. Transmission of CMGs and CBSV was successful with the whitefly vector (*B. tabaci)* and with six species of aphids in an experiment conducted in Kenya (Mware *et al.,* 2009).

In many CBSD and CMD affected countries, the spread of the disease have been controlled by sensitizing farmers about the disease, rouging off infected plants, use of tolerant genotypes, use of clean planting materials and implementing strict quarantine (Hillocks, 2002). Genotypes resistant (TME204 and TME 14) to CMD have been identified but these are susceptible to CBSD (Alicia *et al.,* 2007). However, new genotypes have been bred recently for CBSD resistance but have not been evaluated for CMD and CBSD resistance. The purpose of the present study was to assess the prevalence and incidence, of CMD and CBSD in Machakos, Kitui, and Makueni counties followed by evaluation of the response to cassava virus disease infections among selected cassava genotypes under field and greenhouse assays.

1.1: Statement of the problem.

Pest and diseases are the major cassava production problem causing the highest yield losses and in turn render many areas nonproductive. Diseases like cassava mosaic disease, cassava brown streak disease, cassava bacterial blight and insect pests like green mites (*Mononych ellustanojoa*) and *B. tabaci* have devastating effects on cassava production.

However, cassava mosaic and cassava brown streak diseases are the most problematic (Fauquet, 2005). This is because the rate of evolution among viral populations is high. For instance, cassava mosaic disease has been reported to be caused by nine viruses of genus Begomoviruses (Fregene *et al.,* 2004), while cassava brown streak disease is caused by two viruses (Uganda cassava brown streak virus and Cassava brown streak virus).

The two diseases are spreading extensively both in lowlands and highlands (Mware *et al.,* 2009). In Tanzania, CMD has been reported from many locations (Ndunguru *et al.,* 2005). Comprehensive characterization by Ndunguru *et al.,* (2005) showed seven cassava mosaic geminiviruses species occur in Tanzania. Mbanzibwa *et al*., (2009a) reported prevalence of two potyvirus species causing CBSD in the Lake Victoria basin and along the coastal belt of Indian Ocean.

A countrywide survey of all major cassava-growing areas in Kenya by Bull *et al.* (2006) reported presence of six CMG species with novel Begomoviruses and a new recombinant strain of East Africa Cassava Mosaic Virus, demonstrating increasing diversity and geographical distribution of CMGs. Similarly, recent reemergence of CBSD has been reported in many districts in Uganda (Alicai *et al*., 2007) as well as from Malawi (Winter *et al.*, 2010), Rwanda (Shirima *et al*., 2012) and Kenya (Mware *et al*., 2009).

Although resistant genotypes to cassava mosaic disease such as (TME204 and TME14) have been identified, they are susceptible to cassava brown streak disease (Alicai *et al.*, 2007). Cassava genotypes have been bred for resistance against CBSD at KALRO- Kiboko sub-center in Kenya but have not been evaluated for their response to CMD and CBSD. Evaluation of these genotypes may lead to identification of new sources of resistance.

1.2: Broad objective

To assess the prevalence and incidence of CMD and CBSD in Machakos, Kitui and Makueni counties and resistance response of new improved cassava genotypes under field and greenhouse assays in lower eastern Kenya.

1.3: Specific objectives.

i. To evaluate the prevalence and incidence of CMD and CBSD in Machakos, Kitui and Makueni counties of lower Eastern Kenya.
ii. To assess the morphological response of selected cassava genotypes against cassava brown streak and cassava mosaic virus infections under field conditions.
iii. To evaluate response of selected cassava genotypes to cassava brown streak and cassava mosaic viruses under greenhouse assays.

1.4: Hypothesis

i. There is no significant difference in CMD and CBSD Prevalence and incidence in Machakos, Kitui and Makueni counties of lower Eastern Kenya.
ii. There is no significant difference in virus-infection response amongst cassava genotypes subjected to cassava brown streak and cassava mosaic viruses under field conditions.

iii. There is no significant difference in virus infections response among selected cassava genotypes to cassava mosaic and cassava brown streak under greenhouse assays.

1.5: Research justification.

Demand for food is quickly rising and will continue to rise due to increase in population and reduction in arable land (FA0, 2009). In Africa, cassava has been identified as a major food security crop due to its adaptability to marginal areas (Okuja *et al.,* 2004). 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.

Among the biotic factors, CMD and CBSD are the most devastating (Fauquet, 2009). Cassava mosaic disease is caused by CMGs while CBSD is caused by CBSV and Uganda cassava brown streak virus (UCBSV). Both cassava viruses have spread extensively in all cassava growing areas (Munga *et al.,* 2002).

Various control strategies have been devised to combat the two diseases, significant progress has been made towards control of CMD, For example the genotypes TME 204 and TME 14 were identified to be CMD resistant but unfortunately they are susceptible to CBSD, as they were not selected for CBSD resistance (Alicai *et al.,* 2007).

The genotypes from KALRO Kiboko sub-center were bred for CBSVs resistance using parents from Zanzibar and needs to be evaluated to confirm their resistance against CBSV and CMGs. Evaluating these genotypes may lead to identification of new sources of resistance.

The cassava genotypes that will be found to be tolerant or resistant to CMD and CBSD will benefit farmers involved in cassava production, where the two viral diseases have been a major threat. This may lead to increased output since more cassava root tubers will be produced with low or no necrosis and hence more income will be generated. Information obtained from this study will also benefit researchers and policy makers as more knowledge will be added to the scientific world.

CHAPTER TWO

2.0: Literature review

2.1: Description of Cassava

Cassava is a tropical perennial shrub that can grow to a mature plant height ranging from one to four meters depending on the environment and genotype. It has erect smooth stems radiating from the roots. The stems contain nodes at intervals that give rise to new plants. Leaves are large lobed, borne on a long, slender stalk joining a leaf (Irungu, 2011). The color of leaves appear dark green but in some genotypes yellow or purple pigmentation may occur (Purseglove, 1968). Male and female flowers are found on the same plant. In some genotypes of cassava cyanide producing sugar derivative occurs in varying amounts (Ng and Ng, 2002). There are many wild relatives of cassava However, based on morphological, ecological, and geographical evidence, listed *M. carthaginensis, M. aesculifolia, M. grahami*, *M. flabellifolia*, and *M. saxicola*as the most closely related species to cultivated cassava Rogers, (1963).

Cassava is known to be drought tolerant crop. Apart from the first few weeks after planting, cassava tolerates drought and can perform with an annual rainfall of less than 600mm as in the semi-arid tropics (De Tafur *et al.*, 1997) to more than 1000mm in the sub-humid and humid tropics (Pellet and El-Sharkawy, 2001). Cassava is one of the most adopted and or adapted crops in African Agriculture and is cultivated in about 40 African countries (Aloyce *et al.*, 2013). Wide spread adaptations of cassava in different soil and environmental conditions is caused primarily by the physiological traits possessed (Okuja *et al.*, 2004).

El-Sharkawy, (2007) reported some of these characteristics such as: the high photosynthetic capacity of cassava important for high productivity; possession of a tight stomatal control over leaf gas exchange and ability to shade leaves, reduces water losses during dry spell; ability to extract water from deep soils which enables plant to extract water in seasonally dry and semi-arid environments and, ability to rapid multiply through cuttings. Additionally, Jarvis *et al*., (2012) pointed out that, cassava is a crop with high flexibility in adjusting to future climatic changes and therefore has a potential to become a crop of choice when other food crops are challenged.

2.2: Importance of cassava

Cassava has a reputation as a poor person's crop, i.e. a crop of last resort (Hillocks *et al*., 2001). Generally the crop is used as food, as an industrial raw material and substitute in animal feeds. About 90% of cassava root production is utilized as food and it is an important source of carbohydrates. In Africa cassava is produced mainly by small-scale farmers on marginal and sub-marginal lands (Hillocks, 2000). The bulk of cassava grown in Africa is utilized as food in the form of fresh roots and processed products such as flour and preparations of fermented meals (Kawano, 2003). Furthermore, cassava leaves are consumed as vegetables and are the source of proteins and minerals (Lancaster and Brooks, 1983). Cassava leaves contain an average of 21% protein, which is high among non-leguminous plants (Ravindran, 1993).

A cassava root is transformed into a wide range of traditional products. The world production of cassava roots has reached 250.2 million metric tons of which trade accounts for about 10% of the total production (Aloyce *et al.,* 2013). Trade involves cassava for both human consumption and industrial use. Industrial use of cassava involves production of such commodities as ethanol, binding agent, paper, and textiles and flavoring agent in Asian cooking and starch (Balagopalan, 2002).

2.3: Production constraints

Cassava production in sub-Saharan Africa is particularly exposed to numerous biotic stresses. Common constraints include pests and diseases, poor agronomic practices, high cyanide levels, lack of clean planting materials, low yielding genotypes, and long maturity periods (Thresh *et al*., 1994). Pests and diseases are the most economically important constraints (Herren, 1994). Pests infesting cassava include mealy bugs (*Phenacoccus manihotae*), cassava green mite (*Mononych ellustanajoa*), cassava hornworm (*Erinnyisello*), scales, thrips and whitely (*Bemisia tabaci) (*Montero, 2003*).* Diseases among others include cassava bacterial blight, cassava virus diseases, cassava anthracnose disease, cassava bud necrosis, and root rots (Calvert and Thresh, 2002).

Economic importance of cassava diseases depends on the extent of damage a disease causes to the productive part of cassava. In sub-Saharan Africa virus diseases of cassava are the most

important (Taylor and Fauquet, 1997; Thresh *et al*., 1994; Thresh *et al*., 1997). Cassava is reported to be vulnerable to at least 20 different viral diseases among which CMD and CBSD are the most devastating (Patil and Fauquet, 2009). Sources of CMD and CBSD in cassava are believed to be viruses already present in the indigenous African flora (Legg and Hillocks, 2003). Factors influencing perpetuation of the virus diseases in cassava plant includes: abundance of efficient insect vectors for transmission, planting of susceptible genotypes and continuous use of unclean planting materials normally selected from the previous seasons (Legg and Thresh, 2003). With the evident success on biological control of cassava mealy bug, cassava green mite, CMD and CBSD remained the challenge. More information on the causative pathogens and efficient diagnostic tools are the prerequisite for the formulation of sustainable management approaches. Thus, CMD and CBSD are now one of research priority of many root and tuber crop programs in many African countries (Legg and Thresh, 2003).

2.4: Cassava Viruses

2.4.1: Cassava Mosaic Begomoviruses

Cassava mosaic disease (CMD) is caused by cassava mosaic Begomoviruses (CMBs), and was first described from what is now Tanzania towards the end of the 19th century (Warburg, 1894), and constitutes one of the most widespread and devastating diseases of cassava in Africa (Bock and Woods, 1983; Thresh *et al*., 1998). Early studies by Zimmerman (1906) suggested that CMD is caused by a virus; however for many years viral etiology of CMBs remained unclear until 1938 when another study by Storey *et al.,* (1936) from Amani research station in North Eastern Tanzania confirmed that the disease is caused by cassava mosaic geminiviruses (CMGs) (family: *Geminiviridae*: genus; Begomoviruses).

The virus is systemically transmitted in a persistent manner by whitefly (*Bemisia tabaci* Gennadius) (Homoptera: *Aleyrodidae*) (Dubern, 1994). Cassava mosaic begomoviruses greatly reduce the growth and yield of cassava particularly local unimproved genotypes (Thresh *et al*., 1997). Cassava mosaic begomoviruses spread easily from one field to another through planting of infected stem cuttings from the previous crop (Fauquet *et al*., 1988). Incidence, spread, severity and the extent of yield loss depend on the varietal susceptibility and stage of plant growth at which infection occurs. Recently, it was established that the severity of CMD is

influenced by synergistic effects of co-infection of CMBs and its associated DNA satellites (Ndunguru *et al.*, 2008).

An estimated loss of over US $14million per annum caused by CMD has been reported (Legg and Okuja, 1999), losses are attributed to damage on leaves and stems, which interfere with the way in which the plant makes food for storage in the roots. The damaged photosynthetic areas reduce the growth of the plants, number of storage roots and the ability of the storage roots to enlarge and mature. Loss of planting material also occurs in infected cassava, where stem cuttings are unhealthy and unsuitable for planting (Aloyce *et al.,* 2013).

2.4.1.1: Cassava mosaic Begomoviruses structure

Viruses of the family *Geminiviridae* comprise a single–stranded DNA genome that is encapsidated in characteristic twinned (so called geminate) particles (Bull *et al*., 2006). The genome consist of two parts namely DNA-A and DNA-B components (Bull *et al.*, 2006). DNA-A component replicates autonomously (Stanley, 1990) and comprises of six specific protein encoding open reading frames (ORFs), AV1 & AV2 on the virion-sense strand, and AC1-AC4 on the complementary sense strand.

Replication associated protein (Rep) is encoded by AC1 required for initiation of viral DNA replication, AC2 gene encodes for transcriptional activator protein (TrAP) that control gene expression, AC3 encodes for replication enhancer protein (REn) while RNA silencing suppressor protein is coded by AC4 gene (Stanley and Gay, 1983). Virion-sense strand on AV1 codes CP responsible for virus transmission from plant to plant by whitefly (*Bemisia tabaci)* and AV2 for pre coat protein (Patil and Fauquet, 2009). Replication of DNA-B depends on DNA-A. Deoxyribonucleic acid (DNA) B has two ORFs one each on the virion and complementary strand; BV1 is a shuttle protein encoder (NSP) while BC1 is responsible for movement protein (MP) encoding (Chatterji *et al.*, 1999).

The virus moves within and between cells of host plants by a co-operative action of the two genes (Hanley-Bowdoin *et al*., 2004). Virus infection and subsequent symptom development in host plant requires presence of both virus components (DNA-A and DNA-B) (Stanley and Gay, 1983).

2.4.1.2: Cassava mosaic disease symptoms

Infected cassava plants exhibit a range of symptoms variation. Gibson and Otim-Nape, (1997) reported factors contributing to the variation in symptoms to include: types of virus strain, age of plant, host plant sensitivity and environmental factors such as moisture availability in the soil, fertility of the soil, solar radiation and temperature. However, characteristic symptoms of CMD infected plants are infected leaves that show green to yellow mosaic, setting up unequal expansion in affected areas causing twisting, narrowing and malformation of the leaf. In condition of severe infections young leaves abscise and affected plant appear stunted and produce small fewer tubers (Legg and Thresh, 2003). These morphological alterations in cassava plants result in significant losses in storage root yield (CABI, 2004).

2.4.2: Cassava Brown Streak Virus (CBSV)

Cassava brown streak disease (CBSD) was first described in the Amani district in Tanzania in 1930 (Storey, 1936). CBSD was reported to be a major threat to food security in the coastal regions of Tanzania (Legg and Raya, 1998; Hillocks *et al*., 1996), Northern Mozambique (Hillocks *et al*., 2002) and in the coastal strip of Lake Malawi. Cassava brown streak disease is caused by *Cassava Brown Streak Viruses* in the genus *Ipomovirus* the family *Potyviridae* (Monger *et al*., 2001a).

2.4.2.1: Genome structure and organization of CBSV

The genome of CBSV is about 9kb composed of positive sense single stranded linear ssRNA, and a poly (A) tail at the 3´end (Mbanzibwa *et al.*, 2009b). Unlike members of specie of Genus *Ipomovirus* (Collins *et al.*, 1998) CBSV genome lacks helper component proteinase but contain PI serine proteinase that strongly suppress RNA silencing (Mbanzibwa *et al*., 2009b). CBSV genome contains a single ORF, with UTR at the 5´ and 3´ends.

It encodes a large polyprotein ca. (2902 aa) that is processed by virus-encoded proteases into mature proteins namely P1 proteinase (P1-Pro), the third protein (P3), 6kDa protein 1 (6K1), cylindrical inclusion protein that is an RNA helicase (CI), 6kDa protein 2 (6K2), nuclear inclusion protein a (NIa), which can be further processed into the viral protein genome-linked (VPg) and NIa proteinase (Pro) (Aloyce *et al.*, 2013). Beside exceptional structure of the 5'-proximal part of the genomes CBSV also contained a Maf/HAM1-like sequence recombined

between the replicase (Nib) and the coat protein domains in the 3'proxial part of the genome highly conserved in *Potyviridae* (Monger *et al.*, 2001a).

2.4.2.2: CBSV Transmission, spread and host range

The primary source of CBSV transmission to a new cassava plant is through the use of diseased planting materials. Insect transmission of CBSV has also been suggested and that the most likely vector is the whitefly *B. tabaci* (Gennadius) (Hemiptera: *Aleyrodidae*) (Bock, 1994). Unsuccessful transmission attempt was done by Lennon *et al*., (1986); Brunt *et al*., (1990) and Bock *et al*., (1994).

First whitefly transmission confirmation came from Maruthi *et al.,* (2005) whereby the whitefly *B. tabaci* transmitted CBSV at a very low rate (maximum of 22%). Contrary to this recent study by Mware *et al*., (2009) showed that CBSV is transmitted by *B. tabaci* and spiraling whitefly (*Aleurodicus dispersus)* Russell (Hom, *Aleyrodidae*) with transmission efficiencies of 40.7% and 25.9% respectively. Other means of transmission of the CBSVs includes grafting, cutting tools and leaf harvesting. Experimentally, CBSV can be transmitted to *Nicotiana benthamiana* and *N. rustica* (Alicai *et al.*, 2007). So far cassava brown streak disease is not known to attack other crops and no known host range for CBSV been reported to date (Chellapan *et al.,* 2004). However, it is believed that CBSV must have an indigenous host from which it spread to cassava after being introduced to Africa (Calvert and Thresh, 2002).

2.4.2.3: Cassava brown streak disease symptoms

The leaf symptoms includes: Yellow chlorosis on Secondary and tertiary veins and or general blotchy chlorotic mottle. Both symptoms appear on the lower mature leaves and vary from genotype to genotype. Symptoms may differ with genotypes and do not appear on newly formed foliage especially at high temperatures (Abaca *et al.,* 2012). Symptoms can also be transient when a period of active growth produces symptom-free tissues (Jennings, 1960). Unlike CMD, CBSD does not induce leaf distortion or size reduction (Irungu, 2011). However, CBSV induce brown necrotic streaks on the green portions of stems of cassava plants. The upper portion of the stem become necrotic and then dries out causing shoot dieback (Aloyce, 2013).The most economical importance of CBSD is the destruction of storage roots. Some genotypes do not show root necrosis until more than 8 months after planting using infected cutting despite the earlier presence of apparent foliar symptoms (Hillocks *et al*., 1996). Characteristic symptoms

begin with small yellowish or brown, corky specks that increase in size and number until the whole root becomes in-edible (Hillocks *et al.,* 2002).

Symptoms in roots become more intense as the crop matures particularly beyond physiological maturity at about 12 months post planting. Symptomatic roots also suffer from secondary infection caused by soil-borne pathogens and normally soft rot sets in (Hillocks *et al.*, 2001).

2.5: Diagnosis of cassava viruses.

Evolution of plant pathogens into different strains at different ecological and or geographical locations and endemic plant pathogens (Garcia and Fraile, 2008) continue to be a challenge towards safeguarding plant health. Moreover, climate change, globalization and increased human mobility have increased the spread of invasive plant pathogens (Irungu, 2011). Therefore, application of diagnostic techniques for accurate disease diagnosis is necessary for development and application of mitigation strategies. Different diagnostic techniques that have been used to detect cassava virus includes: serological assay, diagnostic hosts and Nucleic acid analysis.

A diagnostic host is a technique whereby, an indicator plant is used in a disease diagnosis for mechanical transmissible viruses (Bock, 1994). *Nicotiana benthamiana* have been used as an experimental host for CMGs and CBSV and therefore the best transmission host plant in assessing the interaction of the two viruses. (Monger *et al.,* 2001a).

Serological diagnostic technique is also used in cassava virus diagnosis whereby, the target virus is identified through antigen-antibody specific interaction. Tested virus is the antigen which reacts with the specific antibody (Aloyce, 2013). The commonly used serological assay is the enzyme linked immunosorbent (ELISA), screening kit for plant viruses have been developed (Prasangika *et al.,* 2008). Enzyme linked immunosorbent with monoclonal antibodies was developed to discriminate between ACMV and EACMV (Swanson and Harrison, 1994). Serology-based ELISA kit has also been developed for detection of several species and or serotypes of CBSVs (Winter *et al.,* 2010). Enzyme linked immunosorbent assays are cheap and quick technique for identification of cassava viruses but its sensitivity requires fresh leaves with clear symptoms (Adams *et al.,* 2012) and therefore, limited to virus detection at early stage of infection or in plants with latent symptoms.

Nucleic acid analysis is a technique in which DNA-based diagnosis is used. Polymerase chain reaction techniques are now being widely practiced for CMGs detection (Legg *et al.,* 2001). This diagnostic and detection technique requires the use of specific primers designed from full length sequence of DNA-A (Pita *et al.,* 2001b). In PCR with restriction fragment length polymorphism (RFLP) analysis, the CMG DNA-A primers are used to amplify near full length DNA-A fragments from whole plant DNA. Amplified full-length DNA-A products are then digested with restriction enzymes and the digests run on an agarose gel (Monger *et al.,* 2001a).

The characterization of CBSV and sequencing of 3' terminal region of its genome facilitated the development of Reverse transcriptase- polymerase chain reaction (RT-PCR) based diagnostic protocol using specific primers (Monger *et al.,* 2001a). Reverse transcriptase- polymerase chain reaction made it possible to generate good quantities of cDNA copies of a particular RNA molecule (Irungu, 2011).

2.6: Management of cassava virus diseases.

Maintenance of a CBSD and CMD-free crop through phytosanitation and the development of host plant resistance, are the two major approaches used to manage cassava virus (Thresh *et al.,* 1998). However, both techniques are difficult for farmers to apply because they have small plots of land, and even if they try to maintain a "clean" crop, it may become infected from external inoculums sources in neighbors"-fields (Thresh *et al.,* 1998). In addition, farmers are often unwilling to remove growing plants that might contribute to some yield. The reluctance is even greater when there is a high rate of disease spread leading to the infection of a substantial proportion, if not all, of the plants. Finally, regarding selection, either there may be an insufficient number of disease-free plants remaining at the end of the growing season from which to select, or, if there are sufficient plants, the conditions at harvest time may be unfavorable for symptom development (Irungu, 2011).

Genetic-derived resistance is exploited as cultivars vary in their response to CMD and CBSD. Programs to develop host plant resistance to CMD and CBSD began in the 1930s in Tanzania (Nichols, 1950). Through breeding, crosses between cassava and wild cassava species (Jennings, 1957) were used to develop resistant genotypes. Using backcrosses and intercrosses to develop good quality storage roots and resistance to viruses, high resistance to CBSD among the cassava

cultivars evaluated was rare but much more promising to CMD. The Progress with the breeding program achieved mixed success because the developed resistant varieties for CMD were susceptible to CBSD. However the use of resistant cultivars (Hillocks, 2000) combined with the release of virus-free planting material formed an effective control strategy.

Evaluation and selection for screening for resistance requires prior knowledge of the source of resistance which provides an indication on the resistance status of the cassava lines against a particular virus type or variant. Genetically transformation of cassava, with genes that confer resistance to the virus is a novel approach which has an advantage of keeping the traits such as taste and root quality that are considered of importance to cassava producers and consumers (Hillocks, 2000)

The challenge of developing a comprehensive integrated pest management (IPM) approach for cassava viruses remains unmet. New concerns about whitefly populations and emerging virus isolates therefore means that efforts are needed to extend and improve the control options available for farmers, and, together with the incorporation of genetic transformation-based control methods, will give conditions under which each is most appropriate and on how best to combine them into an integrated strategy (Irungu, 2011).

CHAPTER THREE

3.0: METHODOLOGY

3.1: MATERIALS AND METHODS

3.2: Experimental Site

A CBSD and CMD survey was conducted in three counties (Machakos, Kitui and Makueni) of lower Eastern Kenya. The greenhouse experiment was conducted at KALRO Katumani in Machakos (March to June 2017) while the field trials were conducted at SEKU from April 2016 to April 2017.South Eastern Kenya University is located at latitude 1.307689^{o}S and longitude 37.755011^{o}E 1157m above sea level. This site receives a bimodal annual rainfall ranging from 300mm - 1050mm with about 40% reliability and an annual temperature of 14^{o}C to 34^{o}C (Ministry Agriculture, 2010).

3.3: Cassava Genotypes

The field experiment involved seventeen (17) cassava genotypes. Seventeen genotypes were planted at South Eastern Kenya University's (SEKU) field.

Table 3.1: The list of cassava genotype

Genotypes	Parents	Genotypes	Parents
Kiboko 275	990067XSepinde	Thika 289	990183XKisimbani
Thika 279	990183X 990127	Kiboko 297	990183XKisimbani
Thika 272	990127X 990005	Kiboko 300	990183XKisimbani
Kiboko 281	990183X 990067	Kiboko 295	990183XKisimbani
Kiboko 277	990067X Sepinde	Thika 278	990183 X 990127
Thika 280	990183X 990127	Kiboko 276	990067 X Sepinde
Kiboko 271	990127X 990005	990072	Susceptible control
Thika 273	990127X 990005	990067	Susceptible control
Kiboko 274	990127X 990005		

Kisimbani and Sepinde cassava genotypes were sourced from Zanzibar and planted in the crossing blocks with local genotypes which include 990005, 990067, 990183 and 990127 at KALRO Kandara. Kisimbani is known to be resistant to CBSV but lacks in root quality (taste and texture) which Sepinde possesses. Seedling establishment was done at KALRO Kandara and the genotypes showing CBSV resistance were selected, seventeen of the selected genotypes were planted for evaluation at SEKU.

3.4: Weather conditions during survey and field experiment period.

To determine the weather conditions experienced during experimental period, Weather data of between March 2016 and May 2017 were obtained from the weather stations nearest to the study area in May 2017. For example, Rainfall and Temperature data of SEKU field trial was obtained from SEKU meteorological department in Kitui County, Rainfall and temperature data for Machakos County was obtained from KALRO meteorological department a station based at KALRO Katumani research centre in Machakos while in Makueni County, the rainfall and temperature data was obtained from KALRO Kiboko weather station based at KALRO kiboko research centre in Makueni.

3.5: Determination of CMD and CBSD prevalence, incidence and mean *B. tabaci* count.

The survey areas were selected based on available data on cassava production in Kitui, Makueni and Machakos Counties of Lower eastern Kenya and where the disease under study has caused serious problems. Survey was conducted in March 2016 at Kitui, Machakos and Makueni to determine the CMD and CBSD incidence, prevalence and whitefly (*B. tabaci*) counts in lower eastern Kenya. Sampling was done using procedures described by Mware *et al.,* (2009) following the existing county administrative boundaries which were further divided into divisions, locations and villages (Otim-Nape *et al.,* 2000) that were purposively selected. Farmers in each county were identified using systematic sampling. This involved stopping at regular intervals of about 15 to 20km (to allow for wide coverage of the survey area) between farmers' fields along transect that transverse each sampling location. A field with 6 to 9 months old cassava plants was purposively selected and sampled when both CBSD and CMD symptoms are clearly visible since *B. tabaci* has a preferential feeding habit on cassava plants at this stage (Mware *et al.,* 2009). During sampling, thirty cassava plants were selected along representative transects of the field at the opposite ends and the center of the diagonals of the cassava fields (Sseruwagi *et al.,* 2004). Sampling was conducted by visually inspecting cassava plants for presence of typical virus disease symptoms.

The CMD incidence was calculated according to method described by Hillocks *et al.,* (1996) as a percentage of plants showing CMD symptoms to the total number of plants sampled in a field. The same procedure was used in determining CBSD incidence. The prevalence was determined as the proportion in percentage of production unit (farmer field) in which the disease symptoms were observed. A total of thirty (30) plants were randomly sampled along the diagonals of each field to determine both CMD and CBSD incidence. Adult whitefly (*B. tabaci*) population was determined by counting the number of whiteflies on the top five fully expanded leaves of a representative shoot on each of the 30 cassava plants (Sseruwagi *et al.,* 2004).The severity of CMD was determined by scoring the shoot symptoms to a scale of 1 to 5 (Hahn *et al.,* 1989) while severity of CBSD on above ground shoot symptoms was scored following the scale of 1 to 5 (Hillocks *et al.,* 1996) as shown in table 3.2. Data on CBSD and CMD incidence, severity and *B. tabaci* population were subjected to analysis of variance and means separated by least significant difference (LSD) test at P= 0.05 using SAS software version9.0 and Correlations were performed using SPSS version 22.

Table 3.2: scoring scale for above ground symptoms for (CMD) and (CBSD)

Severity score	Severity rating	Qualitative description of symptoms	
		CMD (Hahn *et al.*, 1989)	**CBSD (Hillocks *et al.*, 1996)**
1	Healthy	No visible symptoms	No visible symptoms
2	Mild	A mild distortion only at the base of leaflets appearing green and healthy/mild chlorotic pattern over entire leaflets.	Foliar mosaic with mild stem lesions and no die back.
3	Moderate	Conspicuous mosaic pattern throughout leaf narrowing and distortion of lower a third of leaflets.	Foliar mosaic with mild stem lesions and no die back.
4	Severe	Severe mosaic, distortion of two-thirds of leaflets and general reduction of leaf size.	Foliar mosaic and pronounced stem lesions and no die back.
5	Very severe	Severe mosaic, distortion of three-thirds of leaflets, twisted and misshapen leaves	Defoliation with pronounced stem lesions and dieback.

3.6: Experimental Designs and Data collection

3.6.1: Field Trials

The field experiment was conducted at SEKU during April 2016 planting season. The selected cassava genotypes were randomized using a standard statistical table for random numbers and planted in randomized complete block design (RCBD) with four replicates at SEKU. A stem cutting of 20cm was planted in a hole of about 15cm depth at spacing of 1m by 1m (Ng and Ng, 2002) between the plants, five cuttings per genotypes were planted. The plot size was 13m by 7m, inter-plot space was 1m and space between the blocks was 2m.

Above-ground CBSD and CMD 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 and CMD symptoms) and severity (degree of infection of CBSD and CMD on individual plant) was

used to quantify the disease. Data on plant establishment, number of branches per genotype and height was also recorded. Three data sets was collected that is, at three, six, and nine months after planting (MAP).

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 recorded on all roots harvested 10 months after planting at SEKU field trial. Severity scores for root necrosis used was 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.

3.6.2: Greenhouse experiment

Fifteen improved cassava genotypes and two local susceptible controls that were evaluated at SEKU field during April 2016 planting season (Table 3.1) were planted in the pots under greenhouse conditions laid out in a complete randomized design. The netting around the greenhouse was whitefly or insect tight to prevent the exposure of the genotypes to the CMD and CBSD vectors. Sandy soil and manure were mixed and filled in each pot. Single cutting (10cm) of each variety was planted per pot. Watering was done to field capacity once per day until sprouting then twice per week.

Cassava genotypes to be tested were inoculated with the CMV and CBSV through chip bud grafting under greenhouse conditions following the method described by Kester *et al.,* (2002) where, auxiliary buds of between 3mm and 6mm width were obtained from non-lignified stems of symptomatic plants and used as source of virus inoculums. Buds with the petiole and leaves attached were excised four to twelve nodes below the apical point from virus- infected plants by making a triangular cut with a double-edged razor blade. The bud was excised to a depth of about 2mm, sufficient to expose the cambium layer. Auxiliary buds of equivalent size were excised from the rootstock of test plants six to eight nodes above soil level at eight weeks after planting.

The inoculum bud was inserted into the rootstock and secured to the test plant by wrapping it to the rootstock stem with parafilm. The petiole was then retained and the leaf blade removed from the scion bud. The grafted plant was then maintained in the greenhouse under growth conditions described by Wagaba *et al.,* (2013). The parafilm used to wrap the grafted plants were removed two weeks after bud graft insertion and success or failure of graft union recorded. A graft was determined to be successful if the scion bud retained its green color and had fused to the rootstock with visible callus tissue formed at the graft union edges (Wagaba *et al.,* 2013).

Two weeks after grafting, foliar symptoms development of CMD and CBSD was monitored once per week through visual observations and severity recorded using the scale of 1-5(Gondwe *et al.,* 2002) where 1- denotes no symptom, 2- shows mild symptom (1-10%), 3- indicate pronounced foliar chlorotic mottle and mild stem lesion (11-25%), 4- denote severe chlorotic mottle and stem lesion (26-50%) and 5- very severe symptoms (>50%).

3.7: Data analysis

Data on plant height, first branching height, number of branches, CMD and CBSD incidence and severity were subjected to ANOVA using Statistical Analysis System (SAS institute, 2004). Means were separated by least significant difference (LSD) $P< 0.05$ then correlation performed using statistical package for social sciences (SPSS) version 22.

CHAPTER FOUR

4.0: Results

4.1: Weather conditions recorded during survey and field experiment period.

The mean monthly rainfall recorded at SEKU Kitui County in the year 2016 was 1.1mm. The average minimum temperature of 14.76°C and maximum temperature of 28.51°C was recorded. No rainfall was recorded in months June, July, August and September. April 2016 recorded the highest rainfall amount of 5.0mm followed by November (2.7mm) and March (1.1mm). March was the hottest month with minimum temperature of 15.9°C and maximum temperature of 30.4°C while July was coolest (minimum temperature of 12.8°C and maximum temperature of 25.5°C) (Fig 4.1).

Mean monthly rainfall recorded at Machakos County in the year 2016 was 69.17mm. Average minimum temperature was 12.5°C and maximum temperature of 25.5°C. November was the wettest month during the year with rainfall amount of 191mm. March was the hottest month with minimum temperature of 14.1°C and maximum temperature of 27.3°C while July was coolest with minimum temperature (10.5°C) and maximum temperature of 22.5°C (Fig 4.2). Makueni County had mean monthly rainfall of 69.50mm. Minimum mean temperature recorded was 15.0°C and a mean maximum temperature of 28.0°C. November recorded the wettest month (221mm) while July was the driest (1mm). February was the hottest month in the year with minimum temperature of 15.5°C and maximum temperature of 30.5°C while July was the coldest (minimum temperature 13.2°C and maximum temperature 25°C) (Fig 4.3).

Very low amount of rainfall was recorded during field experimental period of April 2016 to April 2017 at SEKU in Kitui County (Fig 4.1). This contributed to reduction in growth rate of the cassava genotypes at SEKU field trial.

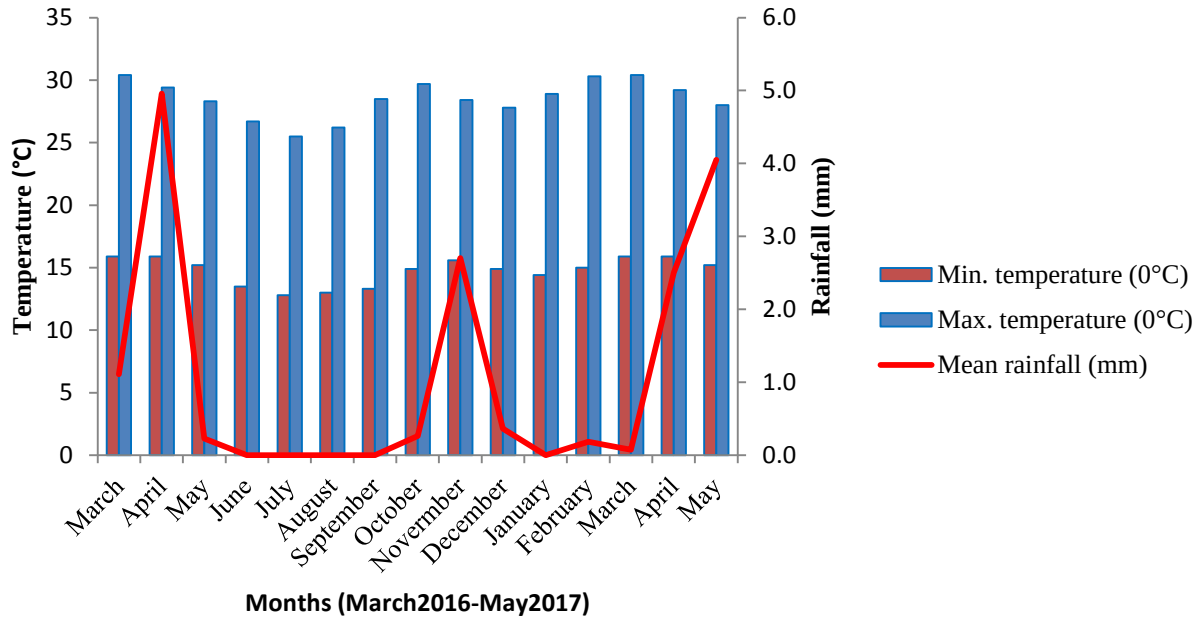

Figure 4.1: Average Rainfall (mm) and, Maximum and Minimum Temperature (0°C) during March 2016 to May 2017 in SEKU, Kitui County.

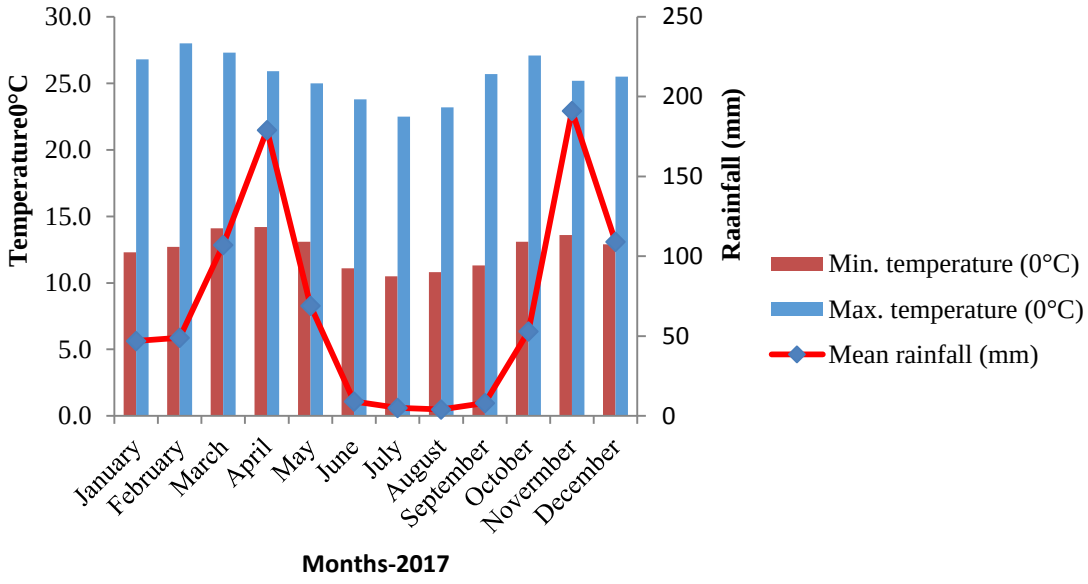

Figure 4.2: Average Rainfall (mm) and, Maximum and Minimum Temperature (0°C) in the year 2016 at Machakos County.

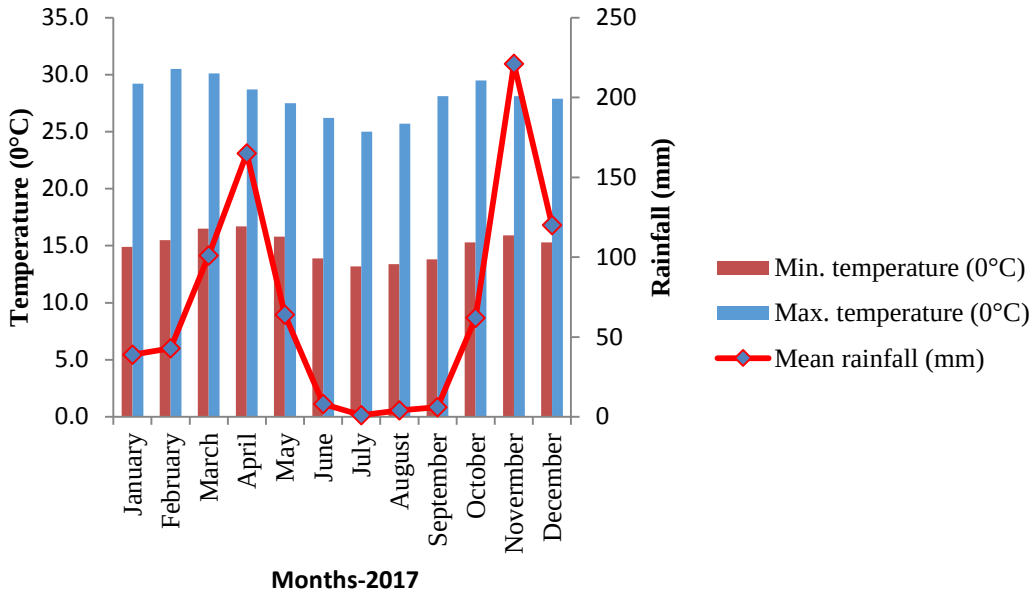

Figure 4.3: Average rainfall (mm) and, Maximum and Minimum Temperature (0°C) in the year 2016 in Makueni County.

4.2: Survey on CMD, CBSD and associated whitefly vectors.

4.2.1: Symptomatology

In the farmer's field, symptoms typical of CMD were observed on the leaves comprising yellow to pale green chlorotic mosaic, stunted growth on the severely affected plants and leaf curling. The CBSD symptoms observed were yellow vein banding, expressed mainly on the lower, older leaves and chlorosis which occurred along the secondary and tertiary veins, giving a feathery appearance (Plate 4.1). Generally, in all cassava fields, CMD symptoms were distinct from CBSD symptoms, as leaves from CBSD infected plants had little or no distortion (Plate 4.1) indicates cassava plants with mild to severe CMD and CBSD symptoms across the counties.

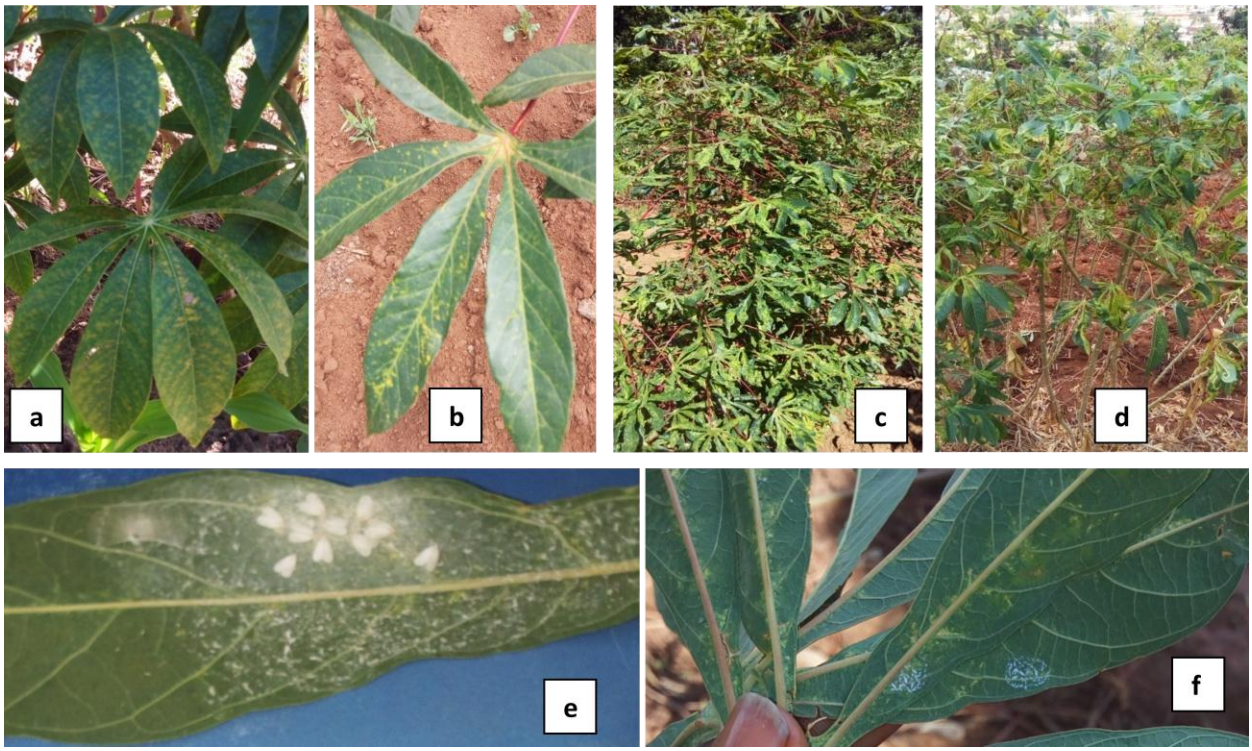

Plate 1.0: Plants showing CBSD and CMD symptoms in the farmers' field: *Fig. 1a & b: chlorotic spots along the secondary and tertiary vein of the leaves of a plant attacked by CBSD. Fig. 1c & d: pale green to yellow mosaic on the leaves of plants infected by CMD. Fig. 1e: spiraling whitefly (Aleurodicus dispersus) underside the leaf of a cassava plant and Fig. 1f: white spiral mark showing evidence of presence of the spiral whitefly.*

4.2.2: Prevalence of CMD and CBSD

Mean CMD prevalence recorded in lower eastern Kenya was 56.67% while mean CBSD prevalence was 18.33%. Varied disease prevalence per County (Kitui, Makueni and Machakos) was observed where, Makueni had 75%, followed by Kitui with 69% and Machakos 26% for CMD, while CBSD prevalence of 35%, 14% and 6% was respectively recorded in Makueni, Kitui and Machakos (Table 4.1). Analysis indicated significant difference ($P \leq 0.05$) in CMD prevalence between Kitui and Machakos and between Machakos and Makueni. Although CMD prevalence between Kitui and Makueni did not significantly vary, Makueni exhibited relatively higher prevalence at 75% compared to 69% of Kitui (Table 4.1). Cassava brown streak disease prevalence was significantly different ($P \leq 0.05$) between Kitui (14%) and Makueni (35%) and between Makueni (35%) and Machakos (6%). There was no significant difference in CBSD prevalence between Kitui (14%) and Machakos (6%) (Table 4.1).

Table 4.1: Cassava mosaic and cassava brown streak disease prevalence in Makueni, Kitui and Machakos of lower eastern Kenya.

Survey region			CMD	CBSD
County	**Farm #**	**Plant #**	**Prevalence (%)**	**Prevalence (%)**
Kitui	1	30	50	0
	2	30	0	0
	3	30	100	60
	4	30	50	0
	5	30	100	0
	6	30	100	20
	7	30	80	30
	8	30	100	10
	9	30	100	20
	10	30	10	0
Mean prevalence			**69**	**14**
Makueni	11	30	100	30
	12	30	100	50
	13	30	100	40
	14	30	100	60
	15	30	80	30
	16	30	20	0
	17	30	50	50
	18	30	80	40
	19	30	100	50
	20	30	20	0
Mean prevalence			**75**	**35**
Machakos	21	30	100	0
	22	30	40	0
	23	30	0	0
	24	30	20	20
	25	30	0	0
	26	30	0	0
	27	30	0	0
	28	30	0	0
	29	30	100	40
	30	30	0	0
Mean prevalence			**26**	**6**
Grand mean			**56.67**	**18.33**
Lsd			**15.67**	**9.3**

Least significant difference (Lsd) at P≤0.05

4.2.3: Disease incidence, severity and occurrence of *B. tabaci* in three Counties of lower eastern Kenya.

Mean CMD incidence recorded in lower eastern Kenya was 51% with severity of 3 while mean CBSD incidence was 17.67% with severity of 2 and mean *B.tabaci* count of two flies per plant (Table 4.2). Cassava mosaic disease and CBSD incidences and severity varied significantly ($P \leq 0.05$) in the three Counties surveyed. Generally, CMD, CBSD and whitefly population were found to be significantly higher ($P \leq 0.05$) in Kitui and Makueni and least in Machakos (Table 4.2). Specifically, significant variation ($P \leq 0.05$) in CMD incidence was noted between Kitui and Machakos. For instance, the mean CMD incidence recorded in Kitui was 63% with most of the farms surveyed recording incidences more than 60% and a severity of between 3.0 and 5.0 while, Machakos recorded mean CMD incidence of 31% with only three of the farms sampled recording CMD incidence ≥60% and severity of between 3.0 and 4.0 (Table 4.2). Significant variations between Makueni and Machakos was also observed with Makueni recording on average 59% CMD incidence with most farms showing more than 50% CMD and severity of between 3.0 and 5.0 while Machakos had CMD incidence of 31% and only two farms (#21 and 28) recording 100% with severity of 4.0 (Table 4.2). The 63% CMD incidence in Kitui was relatively higher than 59% of Makueni, although the two Counties did not substantially vary.

The CBSD incidence was not significantly different between Kitui (15%) and Machakos (7%) where, most areas surveyed in both zones recorded CBSD incidence ranging 20% and 30% with severity of 2.0 (Table 4.2.). Significant difference ($P \leq 0.05$) in CBSD incidence between Kitui and Makueni and Machakos and Makueni was analyzed. Most farms in Makueni showed 20% to 60% CBSD incidence while 50% (5) farms under Kitui and 70% (7) under Machakos were CBSD-free that is showed 0% incidence and mean severity of 1.0 (Table 4.2). Mean CMD severity was significantly different ($P \leq 0.001$) between Kitui (3.6) and Machakos (2.0). No significant difference was noted between Kitui (3.6) and Makueni (3.0). Also, a significant difference ($P \leq 0.05$) in mean CBSD severity was recorded between Makueni with mean CBSD severity score of 2.0 and Kitui with mean CBSD severity score of 1.6 and Machakos with mean severity score of 1.3 (Table 4.2.).

Three species of whitefly vectors were identified in most fields. These were *B tabaci, Bemisia afer and Aleurodicus dispersus* (Fig. 1e & f). Of the three, *B. tabaci* was the most abundant

vector in all fields. The mean total adult *B. tabaci* per plant differed significantly ($P \leq 0.01$) in the three counties of lower eastern Kenya with the vector being more abundant in Kitui and Makueni (2- 3 per plant), wider variations (0-3) were counted in Machakos (Table 4.2).

Table 4.2: Incidence, severity of CMD and CBSD and the mean *B. tabaci* count in relation to different Counties of lower eastern Kenya.

Survey region			CMD		CBSD		*B.tabaci* **Mean
County	**Farm #**	**Plant #**	**Incidence (%)**	**severity***	**Incidence (%)**	**severity***	**Count.**
Kitui	1	30	50	3	0	1	3
	2	30	0	1	0	1	0
	3	30	100	4	60	3	2
	4	30	50	3	0	1	1
	5	30	100	4	0	1	1
	6	30	100	5	20	2	3
	7	30	60	5	30	2	2
average	8	30	100	5	20	2	3
elevation	9	30	60	4	20	2	2
(892.58m)	10	30	10	2	0	1	0
	Mean		**63**	**3.6**	**15**	**1.6**	**1.7**
Makueni	11	30	100	4	30	2	3
	12	30	100	4	50	2	2
	13	30	100	4	40	2	3
	14	30	20	2	60	2	3
	15	30	0	1	0	1	0
	16	30	10	2	0	1	0
	17	30	50	2	60	3	2
Average	18	30	80	3	30	3	2
elevation	19	30	30	3	0	1	1
(949.75m)	20	30	100	5	40	3	3
	Mean		**59**	**3**	**31**	**2**	**1.9**
Machakos	21	30	100	4	0	1	1
	22	30	20	2	0	1	0
	23	30	0	1	0	1	1
	24	30	0	1	20	2	1
	25	30	0	1	0	1	1
	26	30	0	1	0	1	0
	27	30	0	1	0	1	0
average	28	30	100	4	20	2	3
elevation	29	30	60	3	30	2	3
(1175.78m)	30	30	30	2	0	1	0
	Mean		**31**	**2**	**7**	**1.3**	**1**
Grand	**Mean**		**51**	**2.87**	**17.67**	**1.63**	**1.53**
	Lsd		**15.7**	**0.52**	**7.9**	**0.37**	**0.37**

***** *Severity of foliar CBSD symptoms determined following a scale of 1 to 5 (Hillocks et al., 1996) and CMD foliar symptoms determined following scale of 1 to 5 (Hahn et al., 1989);* ****** *Figures are means of whitefly adults per plant; Lsd- Least significant difference.*

4.2.5: Correlations among field- surveyed data.

There was a significant (P≤ 0.01) and positive correlations between disease incidence, severity and whitefly population (Table 4.3). For example, the number of *B. tabaci* positively correlated with CMD severity (r= 0.535), CBSD incidence (r= 0.698) and CBSD severity (r= 0.671) (Table 4.3).

Table 4.3: Correlation analyses among CBSD and CMD incidence, severity and the number of adult whitefly (*B. tabaci*)

	CMDI	**CMDS**	**CBSDI**	**CBSDS**	***B.tabaci***
CMDI	1				
CMDS	.828**	1			
CBSDI	.095	.366	1		
CBSDS	.010	.286	.885**	1	
B. tabaci	.395	.535*	.698**	.671**	1

****.Significant** (0.01); ***.Significant** (P<0.05)

CMDI= cassava mosaic disease incidence, CMDS= cassava mosaic disease severity, CBSDI= cassava brown streak disease incidence, CBSDS= cassava brown streak disease severity.

4.3: Cassava genotypic response to CMD and CBSD infections at SEKU.

4.3.1: Cassava mosaic disease and CBSD shoot symptoms severity and incidence.

Seventeen cassava genotypes were evaluated for their response to CMD and CBSD infections under field conditions in SEKU. Duncan's multiple range test indicated significant (P≤0.001) variation in response to CBSD and CMD between the cassava genotypes (Table 4.4). Improved genotypes did not show both CMD and CBSD shoot symptoms compared to the two susceptible controls (990072 & 990067) that exhibited severe shoot symptoms with mean CMD shoot severity of 4.25 and 5 and, mean shoot incidence of 93.75% and 100% respectively (Table 4.4). Susceptible 990072 showed a mean CBSD shoot severity of 5 and mean incidence of 100% and 990067 had CBSD shoot severity of 3.75 and incidence of 87.5% (Table 4.4). Shoot symptoms on these susceptible genotypes were recorded as early as 3 MAP and persisted up to 12 MAP (Plate 4.2). Higher leaf abscission was also noted on susceptible 990072 which exhibited predominant CBSD symptoms.

4.3.2: Storage root necrosis.

Significant differences (P≤0.05) between genotypes were observed for storage root necrosis. Improved genotypes did not show root necrosis symptoms (Plate 4.5) while susceptible genotype control 990072 exhibited high proportions of plants with root necrosis (80%) followed by susceptible genotype 990067 (45%) (Table 4.5). Thirteen plants of 990072 showed root symptoms with a maximum score of 5 and, five plants of 990067 showed root symptoms with maximum score of 2. This was coupled with reduction of growth and in some cases dieback.

Table: 4.4: Foliar symptoms development

	CMD		**CBSD**		**CMD**		**CBSD**		**CMD**		**CBSD**	
genotypes	Inci. (%) 3M AP	Sev. (1-5) 3MAP	Inc. (%) 3M AP	Sev. (1-5) 3M AP	Inc. (%) 6M AP	Sev. (1-5) 6M AP	Inc. (%) 6M AP	Sev. (1-5) 6M AP	Inc. (%) 9M AP	Sev. (1-5) 9M AP	Inc. (%) 9M AP	Sev. (1-5) 9M AP
K.271	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}
T.278	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}
K.300	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}
K.297	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}
K.295	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}
T.289	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}
K.281	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}
T.280	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}
T.279	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}
K.274	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}
K.277	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}
K.276	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}
K.275	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}
T.272	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}
T.273	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}	0^{a}	1.0^{a}
990072	**65^{b}**	**3.25^{b}**	**65^{c}**	**4.25 c**	**93.75^{b}**	**4.25 b**	**100^{c}**	**4.75 c**	**93.75^{b}**	**4.25 b**	**100^{c}**	**5.00 c**
990067	**65^{b}**	**4.50^{c}**	**55^{b}**	**3.50 b**	**100^{c}**	**4.50 c**	**81.25^{b}**	**3.75 b**	**100^{c}**	**5.00 c**	**87.50^{b}**	**3.75 b**
Mean	**7.88**	**1.35**	**7.27**	**1.35**	**11.74**	**1.41**	**10.98**	**1.4**	**11.74**	**1.44**	**11.36**	**1.41**

Based on Duncan Multiple range test at P=0.05, means followed by the same subscript letters in the same column are not significantly different where MAP= Months After Planting, CMD= Cassava Mosaic Disease, CBSD= Cassava Brown Streak Disease, Inc.=Incidence, Sev=Severity, K.= Kiboko, T.= Thika.

Table 4.5: Root necrosis development

Genotypes	Mean Total Plants	Mean Total tubers	CBSDI (%)	CBSDS (1-5)
Thika272	14^{a}	60^{a}	0^{a}	1.0^{a}
Thika280	14^{a}	59^{a}	0^{a}	1.0^{a}
Thika289	15^{a}	57^{a}	0^{a}	1.0^{a}
Thika273	13^{a}	50^{b}	0^{a}	1.0^{a}
Kiboko295	13^{a}	49^{b}	0^{a}	1.0^{a}
Kiboko275	14^{a}	48^{b}	0^{a}	1.0^{a}
Thika278	13^{a}	47^{b}	0^{a}	1.0^{a}
Kiboko297	12^{a}	38^{b}	0^{a}	1.0^{a}
Kiboko271	11^{a}	35^{b}	0^{a}	1.0^{a}
Kiboko300	15^{a}	34^{b}	0^{a}	1.0^{a}
Thika279	10^{b}	32^{c}	0^{a}	1.0^{a}
Kiboko276	7^{b}	26^{c}	0^{a}	1.0^{a}
Kiboko277	13^{a}	26^{c}	0^{a}	1.0^{a}
Kiboko281	7^{b}	22^{c}	0^{a}	1.0^{a}
990072	**13^{a}**	**16^{c}**	**80^{c}**	**5.00^{b}**
Kiboko274	6^{b}	15^{c}	0^{a}	1.0^{a}
990067	**12^{a}**	**11^{c}**	**45^{b}**	**2.25^{b}**
Mean	**11.88**	**36.76**	**7.35**	**1.31**

Based on Duncan Multiple range test at P=0.05, means followed by the same subscript letters in the same column are not significantly different where CMDI= Cassava Mosaic Disease Incidence, CMDS= Cassava Mosaic Disease Severity, CBSDI= Cassava Brown Streak Disease Incidence, CBSDS= Cassava Brown Streak Disease Severity.

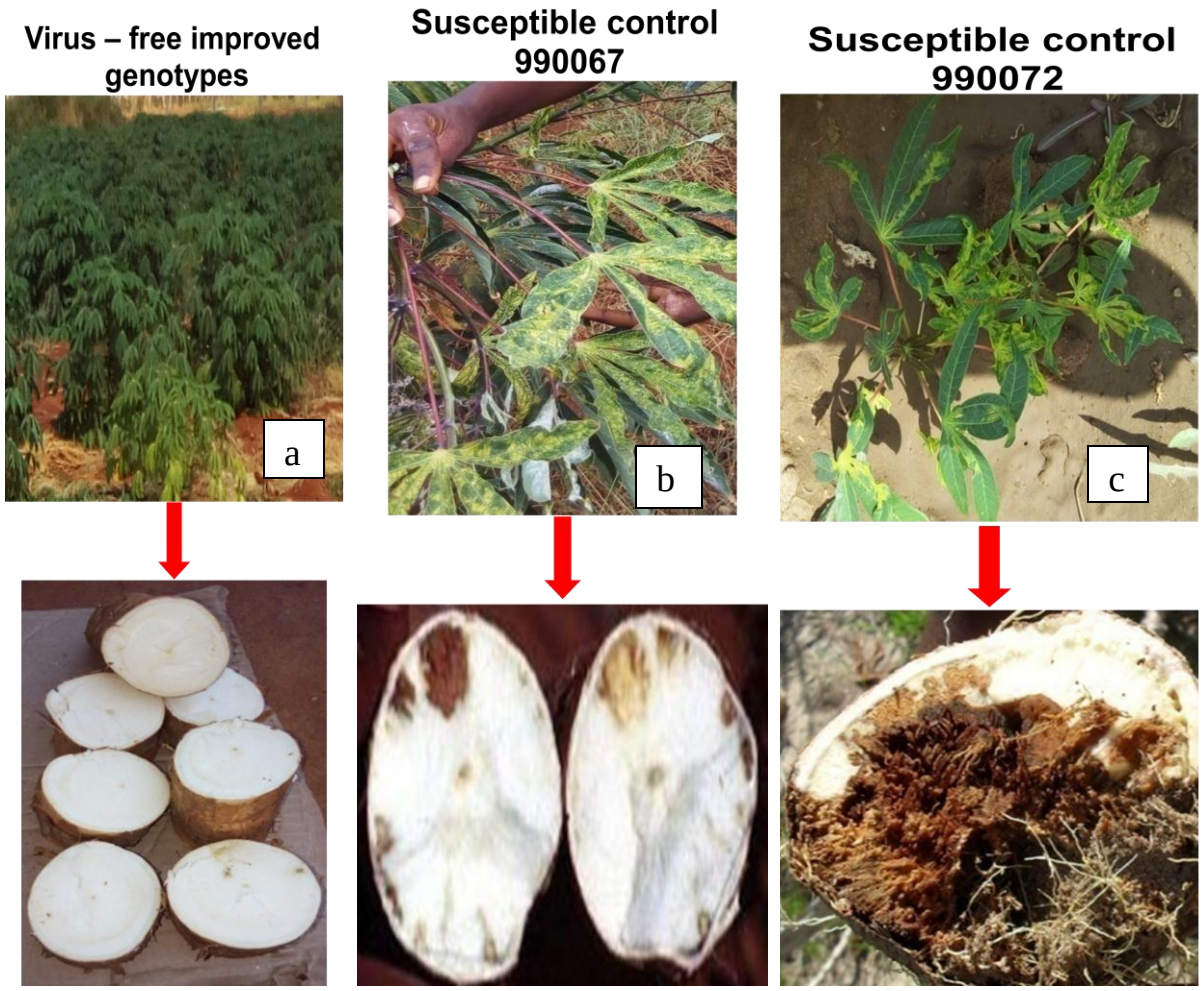

Plate 4.2: Cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) symptoms observed in the fields: (a) healthy plants and tubers on improved genotypes; (b) foliar and root necrosis on susceptible control 990067 and (c) severe foliar and root necrosis on susceptible control 990072.

4.4: Correlations between CBSD and CMD indices and growth parameters

There was a significant ($P \leq 0.01$) and positive correlation between shoot disease incidence and severity and the corresponding root disease incidence and severity. A positive correlation between CBSD and CMD was also recorded. For example, CBSD shoot incidence positively correlated with CBSD root incidence (r= 0.976) and CBSD root severity (r= 0.951). CMD shoot incidence positively correlated with CBSD root incidence (r= 0.914) (Table 4.6). CBSD shoot incidence positively correlated with CMD shoot incidence (r= 0.944) and severity (r= 0.984).

There was a negative correlation between the growth parameters (plant height, branching and number of branches) and disease indices and positive correlations between the plant establishment and disease indices. For example, plant height negatively correlated with CMD shoot incidence (r= -0.417), CMD severity (r= -0.418), CBSD incidence (r= -0.412) and CBSD

severity (r= -0.401). First branching height also negatively correlated with CMD severity (r= -0.448) and CBSD severity (r= 0.442), plant establishment positively correlated with CBSD incidence (r=0.168), CBSD severity (r=0.165), CMD incidence (r=0.169) and CMD severity (r=0.168) ((Table 4.6).

Table 4.6: Correlations between disease indices and growth parameters.

	Estab	Height	branching	No of branches	F. CBSD Inci..	Sev..	F. CMD Inci..	Sev..	Sev..	R. CBSD Inci..
Estab..	**1**									
Height	0.408	**1**								
branchng	0.221	0.228	**1**							
Nbranchs	.552*	0.482	0.414	**1**						
CBSDI	0.168	-0.412	-0.45	-0.217	**1**					
CBSDS	0.165	-0.401	-0.442	-0.266	.992**	**1**				
CMDI.	0.169	-0.417	-0.451	-0.171	.994**	.974**	**1**			
CMDS	0.168	-0.418	-0.448	-0.138	.984**	.954**	.997**	**1**		
RCBSDI	0.162	-0.388	-0.432	-0.302	.976**	.995**	.948**	.921**	.826**	**1**
RCBSDS	0.156	-0.371	-0.418	-0.335	.951**	.982**	.914**	.881**	.769**	.996**

***. Significant (P<0.05); **. Significant (P<0.01)**

Abbreviations in the table: 'Estab' Establishment, 'F.CBSDinci..'Foliar cassava brown streak disease incidence, 'F.CBSDsev..' Foliar cassava brown streak disease severity, 'F.CMDInc..' Foliar cassava mosaic disease incidence, 'F.CMDSev..' foliar cassava mosaic disease severity, 'R.CMDInci..' Root cassava mosaic disease incidence, 'R.CMDSev..' Root cassava mosaic disease severity, 'R.CBSDSev' Root cassava brown streak disease severity, 'R.CBSDInci' Root cassava brown streak disease incidence.

4.5: Foliar disease symptoms under green house assays

Successful graft contacts were observed ten days after grafting as buds were seen sprouting on the grafted scions (Plate 4.3). The grafted plants eventually developed mild to severe symptoms depending on the genotype.

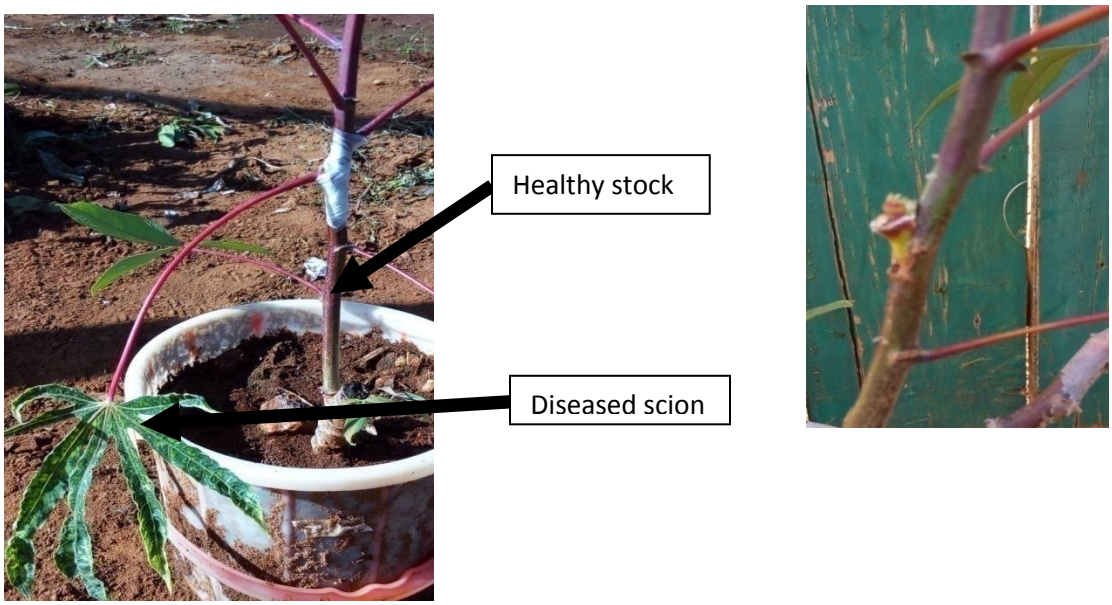

Plate 4.3: Successful graft contact initiated for virus transmission in cassava plants.

The CBSD symptoms were first observed at four weeks after grafting (WAG) in control genotype 990072 with CBSD incidence of 46.6% and severity score of 3 and six WAG, the same susceptible genotype 990072 recorded high CBSD incidence of 60% and severity score of 3. It was followed by Kiboko276 (20%) with severity of 2, and control 990067 (6.6%) with severity of 2 (Fig 4.4). At the end of eight weeks observation period, susceptible genotype 990072 showed a maximum CBSD incidence of 86.6% with severity score of 3.67. Kiboko276 showed mild symptoms with incidence of 33.4% and score of 2. Kiboko281 and 990067 exhibited mildest CBSD symptoms with incidence (26.6%) and severity of 2. (Fig 4.4)

CBSV- induced CBSD leaf symptoms first appeared two nodes below the grafting site as hundreds of small chlorotic spots spread over the entire lamina surface. As these leaves aged, chlorosis became more severe (Plate 4.4). Over a subsequent period of about five to six WAG, feathery chlorosis of veinal regions developed on two to three leaves above those leaves showing initial symptoms described above (Plate 4.4). Generally, eleven genotypes (Kiboko300, Kiboko297, Kiboko295, Thika289, Thika279, Thika278, Kiboko275, Kiboko274, Thika273, Thika272 and Kiboko271) did not show CBSD symptoms at the end of eight WAG period of

observation compared to four genotypes (Kiboko281, Thika280, Kiboko277 and Kiboko276) and two controls (990072 and 990067) that showed CBSD symptoms (Fig 4.4).

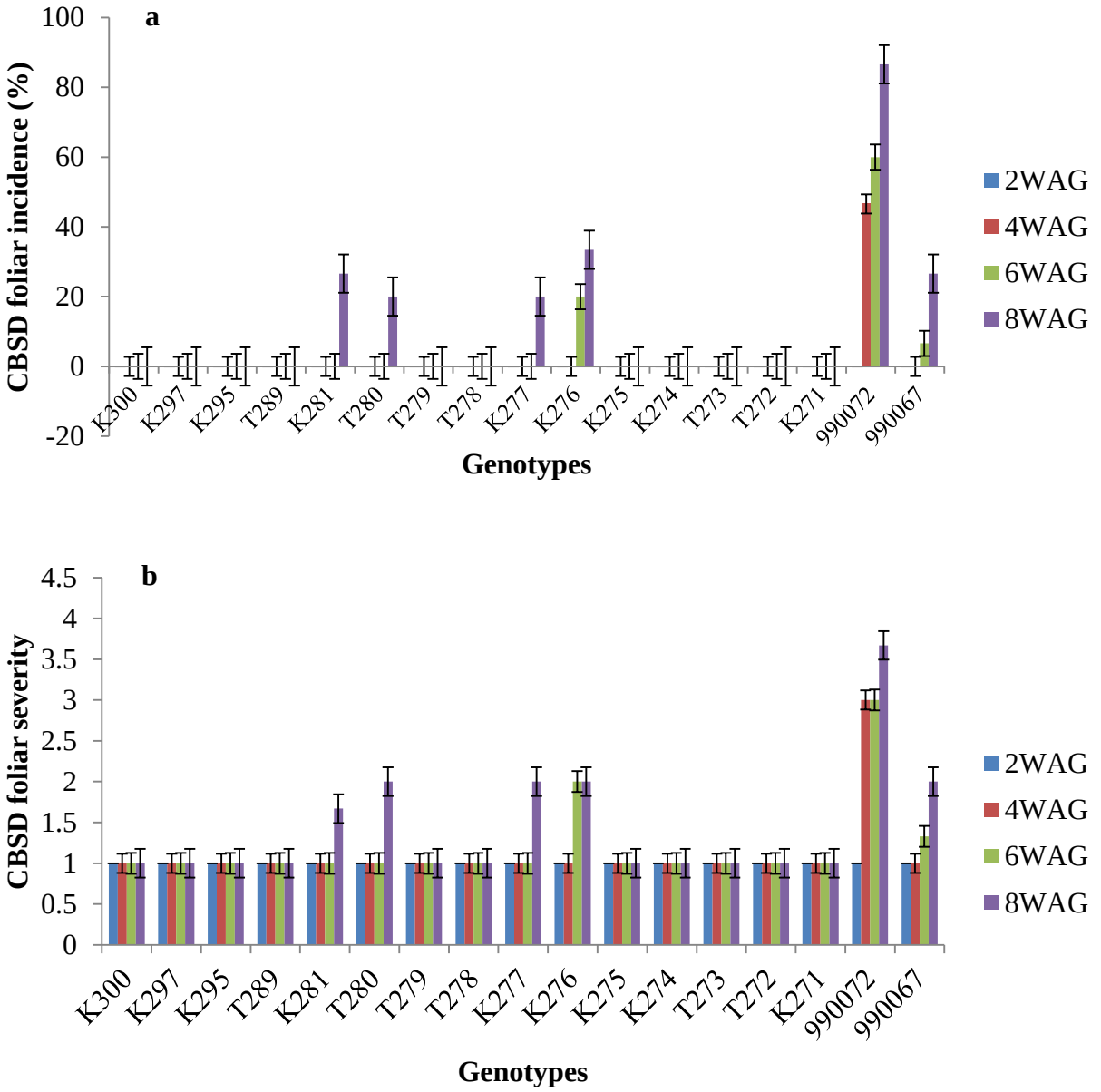

Figure 4.4: Means by weekly variations in CBSD symptoms development on seventeen genotypes artificially infected with CBSV by chip bud grafting: (**a**) percentage disease incidence development at 2, 4, 6 and 8 weeks after grafting; (**b**) foliar symptom development severity scores at 2, 4, 6 and 8 weeks after grafting. WAG= week after grafting, CBSD= cassava brown streak disease, CBSV= cassava brown streak virus, error bars= based on Duncan multiple range test.

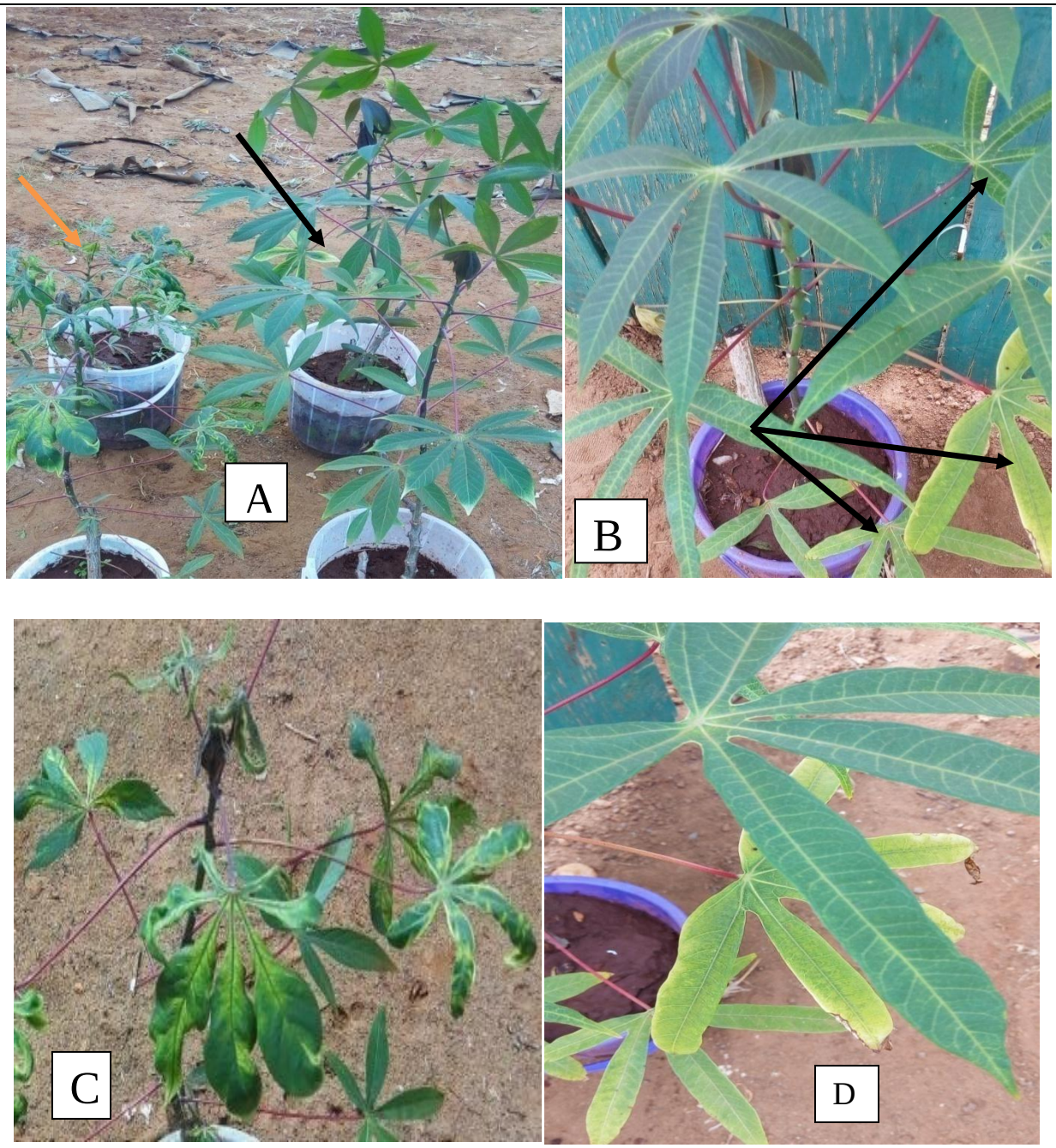

Plate 4.4: Disease symptoms on cassava plants following transmission by chip bud graft inoculations. (A) CMD symptoms on genotype Kiboko272 (brown arrow) and a healthy inoculated plant (black arrow), **(B and D)** feathery chlorosis on veinal regions and chlorotic spot on leaf of genotype 990072 showing evidence of CBSD (black arrows). **(C)** Yellow to pale green chlorotic mosaic and leaf curling on genotype 990067 as evidence of CMD.

In graft transmission study of CMV, CMD symptoms were first observed two weeks after bud inoculation (Fig 4.5). Susceptible genotype 990067 and Kiboko271 showed CMD incidence of 26.6% with severity of 3 at two WAG, followed by Thika280 (13.4%) with score of 1.67 and Thika278 (6.6%) with score of 1.33. Four WAG, Kiboko271 recorded highest incidence (33.4%) with severity of 3.0, susceptible 990067 had incidence (26.6%) with severity score of 3, Thika278 and Thika280 had incidence of 20% and severity of 1.67, Kiboko295 and 990072 had incidence of 20% with severity of 2. At six WAG, Kiboko271 had CMD incidence (53.4%) with score of 3, 990067, Thika278 and Kiboko281 had 40% CMD incidence with severity of 3. Kiboko274 and 990072 was the mildest with incidence (20%) and severity score of 2 (Fig 4.5). Kiboko271 was observed to be the most susceptible genotype to CMD at the end of eight weeks observation with incidence of 60% and severity score of 3, followed by 990067 and Thika280 with 53.4% and score of 3. Thika278 had incidence of 40% with severity of 3, Kiboko276 and Kiboko295 had 40% incidence with severity score of 2. The susceptible genotype 990072 recoded least incidence of 33.4% with severity score of 3 (Fig 4.5)

Cassava mosaic geminiviruses- induced CMD leaf symptoms first appeared on top young leaves. The symptoms observed were yellow to pale green chlorotic mosaic, leaf curling and distortion (Plate 4.5). Generally, out of the fifteen genotypes inoculated, only five (Kiboko300, Thika272, Thika279, Kiboko297 and Kiboko275) did not show the CMD symptoms at the end of eight weeks period of observations. The remaining ten genotypes and two susceptible controls exhibited CMD symptoms.

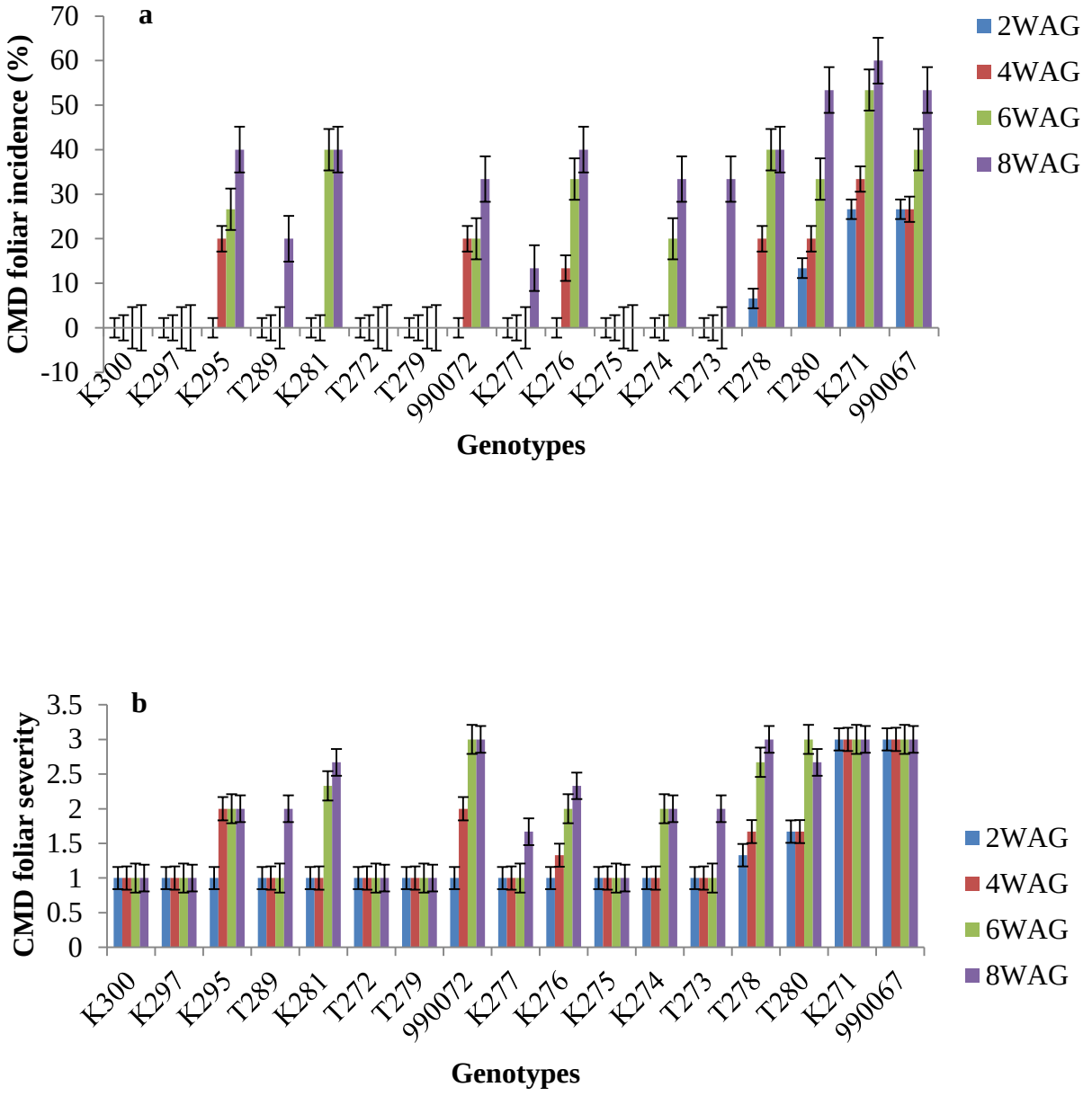

Figure 4.5: Means by weekly variations in CMD symptoms development on seventeen genotypes artificially infected with CMGs by chip bud grafting: (**a**) percentage disease incidence development at 2, 4, 6 and 8 weeks after grafting; (**b**) foliar symptom development severity scores at 2, 4, 6 and 8 weeks after grafting. WAG= week after grafting, CMD= cassava mosaic disease, CMGs= cassava mosaic geminiviruses, error bars= based on Duncan multiple range test.

CHAPTER FIVE

5.0: Discussion.

5.1: Cassava mosaic disease, CBSD and Associated Whitefly Vector in lower eastern Kenya

Foliar CBSD symptoms observed were yellow vein banding expressed mainly on the lower, older leaves and chlorosis which occurred along the lamina similar to those reported by Munga (2008), while CMD included yellow to pale green chlorotic mosaic, stunting of severely affected plants, leaf curling and distortion similar to those reported by Were *et al.,* (2004a).

The role of whitefly vectors in propagation of CMGs and CBSVs was corroborated through identification of the three species (*B. tabaci, B. afer,* and *A. disperses*) in the surveyed Counties. The spiraling whitefly *A. disperses,* have been reported in Machakos and Makueni Counties by Moffat *et al.,* (2016) who reported whiteflies species distribution and abundance on cassava crop in different agro-ecological zones of Kenya. *A. disperses* has not been reported in Kitui County in the previous studies and hence the present study presents the first information on *A. disperses* in Kitui County.

The wide range of CMD (20% to 100%) and CBSD (20% to 60%) prevalence at mean altitude of 1175.78m above sea level confirms report by Mware *et al.,* (2009) who reported that CBSD which was previously endemic in the coastal region of Kenya is spreading to high altitude areas This is contrary to previous findings by Hillocks *et al.,* (2002) that restricted high CBSD prevalence to altitude below 300m above sea level and less common between 300 to 700m.

The common local landrace grown by farmers showed high CMD and CBSD incidences in Makueni and Kitui County where high adult whitefly (*B. tabaci*) population was also recorded. Similarly, significantly positive correlations between CBSD and CMD incidence and the whitefly count indicated positive contribution of (*B. tabaci*) to the spread of CBSV and CMGs in surveyed region. In concurrence, Mware *et al.,* (2009) and Alicai *et al.,* (2007) reported that high abundance of *B. tabaci* seemed to enhance the spread of CBSD and CMD.

5.2: Cassava Response to CMD and CBSD Infections under Field and Greenhouse Conditions.

Negative correlation between the growth parameters (plant height, Branching and number of branches) and disease (CMD and CBSD) incidence and severity indicated that as plant height increases, the disease incidence and severity decreased, The negatively correlation between first branching height and disease severity and incidences suggested that CBSD and CMD decrease with the branching height this is due to the resistance to long distance movement of virus within the plant previously reported by Lecoq *et al.,* (2004). The positive correlation between the plant establishment and disease (CMD and CBSD) incidence and severity indicated that, higher plant establishment lead to high disease incidence and severity due to presence of more host plant for the disease vector (*B. tabaci*). Large cassava field with more vigorously growing plants attracts large number of whitefly for feeding; this enhances the transmission of CMGs and CBSV from one plant to the other and hence high viral disease incidences and severity already reported by Alicai *et al.,* (2007).

Unlike two susceptible local genotypes (990072 and 990067) that exhibited severe CMD and CBSD symptoms (foliar and root necrosis), all improved genotypes did not show symptoms for CBSD and CMD under field conditions in SEKU. The same improved genotypes however showed varied CMD and CBSD expression after disease inoculation through chip bud grafting under greenhouse conditions. This variation in genotypic response to CBSD and CMD between field and greenhouse conditions is probably due to differences in efficiency of virus transmission into the tested genotypes previously reported by Rwegasira *et al.,* (2015). In additional, moderate population of virus transmitting vectors (*B.tabaci*) of about 0-3 whitefly per plant was recorded in survey at Kitui County, where SEKU field experiment was done. This resulted to low virus transmission into the improved genotypes and hence no CMD and or CBSD symptoms was observed. This concurs with previous findings that whitefly population significantly and positively correlates with CBSD and CMD incidences (Maruthi *et al.,* 2005).

The tested genotypes showed significant variations in response to CBSD and CMD after virus inoculation through chip bud grafting in the greenhouse, this indicated efficiency of chip bud grafting in disease transmission and that not all the tested improved genotypes were immune of the cassava virus diseases. Also transmissions of CMGs and CBSVs through grafting have been

reported to be more efficient both in the field and greenhouse conditions compared to vector transmission method (Rwegasira *et al.*, 2015).

High severity of root necrosis was observed on the susceptible local genotypes (990072 and 990067) while the improved genotypes were free of necrosis. This is consistent with Hillocks *et al.*, (1996) who indicated that root symptoms usually develop after foliar symptoms and that in the most sensitive cultivar where the planting material has been derived from infected stock; root necrosis becomes apparent five months after planting.

Positive correlation between CBSD and CMD was recorded. These suggested that CBSV and CMGs benefits each other, perhaps through viral synergism where, one virus may assist a second co- infecting virus, leading to increased titres and severe infection. Similar findings have been reported on synergetic interaction between CBSD and CMD causing severe symptoms on a plant infected by both CBSVs and CMGs (Irungu, 2011).

The five improved genotypes (Kiboko300, Kiboko297, Thika272, Thika279 and Kiboko275) that did not show CBSD and CMD under both field and greenhouse trials indicated possible tolerance or resistance to the two diseases. This finding is consistent with previous report on definition of a resistant variety by Thresh *et al.*, (2008) who reported that truly resistant cultivars are not readily infected, even when exposed to large amounts of vector-borne inoculum.

Upon inoculation with CBSVs and CMGs through chip bud grafting technique under greenhouse conditions, four out of fifteen improved genotypes were free of CBSD compared to CMD while only five of the improved genotypes were CMD free. This indicated that eleven of newly bred genotypes were resistance of CBSD while ten are susceptible to CMD. Similar challenges have been reported in Tanzania where genotypes reported to be resistant to CBSD were found to be susceptible to CMD and those promoted for their resistance to CMD were severely infected by CBSD (Pheneas and Legg, 2007).

CHAPTER SIX

6.0: CONCLUSIONS and RECOMMENDATIONS

6.1: conclusion

The incidence and severity recorded in three counties of lower eastern Kenya, showed low CBSD and CMD incidence and prevalence in Machakos County and a moderate to high CMD incidence and prevalence in Kitui and Makueni Counties respectively due to wide distribution of whitefly vectors recorded in Kitui and Makueni. Eleven genotypes were CBSD-free while only five (Kiboko300, Thika272, Kiboko297, Thika279 and Kiboko275) of the newly bred genotypes were immune of both CMD and CBSD under field conditions in SEKU and after virus inoculation through chip bud grafting under greenhouse conditions. None of the improved genotypes developed symptoms under field conditions.

Although Kiboko271 showed no CMD and CBSD symptoms under field conditions, it appeared to be more affected by CMD after virus-inoculation under greenhouse conditions. Also, genotypes Thika289, Kiboko277, Thika273, Thika280 and Kiboko276 showed mild symptoms on either of the two diseases. On the hand, the susceptible controls 990072 and 990067 were reported to have dual infection of CMD and CBSD with 990072 being more affected by CBSD while 990067 most affected by CMD and hence different cassava genotypes response differently to both cassava viral diseases whereby, in most cases susceptible genotypes are severely affected compared to the improved genotypes.

6.2: Recommendations

1. Further molecular analysis of the five genotypes that showed tolerance to CMD and CBSD under both field and greenhouse assays.
2. Farmers should be sensitized on CMD and CBSD and be provided with improved genotypes as a way of managing cassava viral diseases.
3. Detailed study on distribution of *A. disperses* in Kitui county and their effectiveness in CMGs and CBSVs transmission.

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