THE STATUS AND FARMERS' KNOWLEDGE ON CASSAVA MOSAIC DISEASE AND THE RESPONSE OF LOCAL VARIETIES TO CASSAVA MOSAIC AND CASSAVA BROWN STREAK DISEASES IN COASTAL KENYA

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Thesis submitted in partial fulfillment of the requirements for the award of a Master of Science in Crop Protection of the University of Nairobi

2008

Declaration

This thesis is my original work and has not been presented for a degree in any other university

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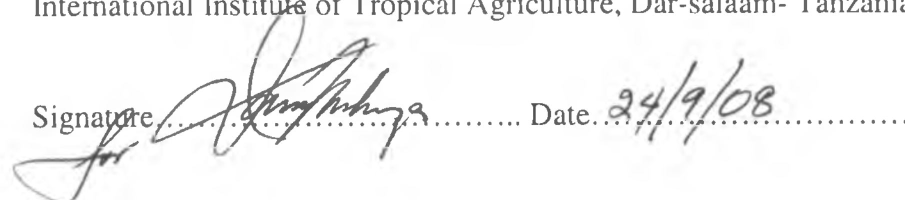
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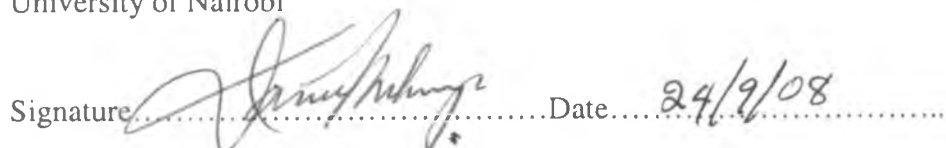
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List of Acronyms and abbreviations

ACMV	African Cassava Mosaic Virus
Anony.	Anonymous
ANOVA	Analysis of variance
bp	base pairs
CBSD	Cassava Brown Streak Disease
CBSV	Cassava brown streak virus
CMD	Cassava Mosaic Disease
CMGs	Cassava Mosaic Geminiviruses
CV	Coefficient of variation
DAS-ELISA	Double Antibody Sandwich Enzyme Linked Immunosorbent Assay
DNA	Deoxyribonucleic Acid
EACMV	East African Cassava Mosaic Virus
EACMZV	East African Cassava Mosaic Zanzibar Virus
EcoRV	Restriction enzyme isolated from *Escherichia coli*
EDTA	Ethylendiamine tetra-acetic acid
ELISA	Enzyme Linked Immunosorbent Assay
et al.	and others
FAO	Food and Agricultural Organization
Fig.	figure
ICMV	Indian Cassava Mosaic Virus
IITA	International Institute of Tropical Agriculture
KARI	Kenya Agricultural Research Institute
LSD	Least Significance Difference
MAP	Month After Planting
Mrna	Mitochondrial ribonucleic acid
NS	Not significant
Nt	Nucleotides
PCR	Polymerase Chain Reaction
RCBD	Random Complete Blocked Design

RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic acid
rpm	Revolutions per minute
SACMV	South African Cassava Mosaic Virus
ss	Single stranded
TAE	Tris-Acetate
TAS-ELISA	Triple Antibody Sandwich Enzyme Linked Immunosorbent Assay
TMS	Tropical Manihot Series

Abstract

Cassava Mosaic Disease (CMD) and Cassava Brown Streak Disease (CBSD) are the main biotic constraints to cassava production in coastal Kenya. A survey to document the incidence and severity of CMD and farmers' knowledge and perceptions on the disease was carried out in the region in October 2005. A total of 27 cassava fields were visited and the disease incidence and severity determined. A semi-structured questionnaire used to obtain information on farmers' knowledge on the disease. The incidence of CMD ranged between 73-100% among the surveyed fields. The dominant type of infection was cutting borne whereas the whitefly borne infection was lower and positively correlated to the *Bemisia tabaci* vector counts. Co-infection with both CMD and CBSD was common in all the districts but was highest (85%) in Lamu and lowest (10%) in Malindi. Kibandameno was the most popular variety in the region followed by Agriculture, Kahutele and Kaleso in a descending order. The results of a semi-structured questionnaire administered to the farmers showed that majority of the farmers (48%) obtained planting materials from the previous season's crop. Majority of the growers could recognize the disease but only 7% attributed it to viruses. In addition, 84% of the interviewees did not employ any management practices against CMD. Though 52% of the interviewees had observed varietal differences in disease susceptibility, they continued to grow the susceptible ones due to their superior culinary properties (34%) and lack of planting materials (7%).

An experiment to determine the role of selection of clean planting materials and roguing for the management of CMD was carried out in two growing seasons. *Bemisia tabaci* population was monitored on a net plot of 20 plants for the first five months of growth.

Roguing of diseased plants was carried out in the first month after planting. The monthly disease severity was monitored monthly for all the plants in each plot for eight months. The plant height was measured monthly for all the plants in the net plot for eight months. The crop was harvested at 10 months after planting and the number of roots, number of marketable roots and total root weight for each of the plants in the net plot were measured. The plant height and *B. tabaci* count were not significantly ($P \leq 0.05$) different between the treatments. The disease incidence and severity were higher in randomly selected materials compared to the clean ones. All the yield parameters determined were not significantly ($P \leq 0.05$) different between the treatments in the short rain season. During the long rain season only the number of marketable roots was significantly ($P \leq 0.05$) different among the treatments.

The response of local germplasm to co-infection with CMD and CBSD was determined in field experiments using three varieties popular in the region namely Kibandameno, Guzo and Kaleso. The control comprised of clean planting materials. Disease severity was determined for all the plants in the net plot. The plant height was also measured for all the plants in the net plot. Kibandameno was the most susceptible variety to both CMD and CBSD. Kaleso ranked second in susceptibility to CMD, on the other hand the variety did not show any CBSD symptoms during the vegetative growth period. In all the three varieties, the clean plants produced roots of highest quality and quantity compared to the virus infected ones. Yield losses due to CMD were higher when compared to those of CBSD. In Guzo, there was no significant ($P \leq 0.05$) difference in root yield parameter between the clean and CBSD infected plants. Qualitative and quantitative yield was highly reduced in Kibandameno. The yield loss ranged from 6-53%, 15-62%, 33-90%

and 41-61% for root length, total root counts, marketable number of roots and root weight respectively. Yield reduction was due to reduced root weight and number of roots in CMD infected plants. In CBSD infected plants, yield reduction was due to severe root constriction and pitting. Plants having mixed infections with CMD + CBSD had the highest yield reduction. Co-occurrence of cassava mosaic and cassava brown streak diseases threatens cassava production in coastal Kenya.

CHAPTER 1

1.0 GENERAL INTRODUCTION

Cassava *(Manihot esculenta* Crantz) is a member of the family Euphorbiaceae that comprises the latex-producing plants. Cassava is the only member of the genus *Manihot* that is cultivated for food (Purseglove, 1968). The crop originated in South America and was introduced to East Africa by Portuguese in the 19th century (Purseglove, 1968).

Cassava is grown for human consumption; the leaves are a source of minerals, vitamins and proteins, while the roots are a major source of starch (Dahniya, 1994). There is also potential for use of the crop as livestock feed where both leaves and roots can be used and be complemented with other nutrients (Hahn, 1988).

Cassava is one of the widely grown staple food crops in Africa, with a total annual production of about 600000 metric tones (Table 1). It is regarded as a food security crop as it adapts easily to diverse environmental conditions and farming systems. The yields are appreciable in adverse conditions and in soils that are poor to grow other crops (Ngeve, 1999). Cassava is tolerant to drought and can grow well even in areas with 600mm rainfall per annum (Hahn, 1992). The crop is therefore important in reducing famine by providing sustained food supplies where other crops fail (Dahniya, 1994).

Cassava takes 8-12 months to mature (Anon., 2000). The roots can be stored underground for 3-36 months after maturity and are always available for consumption when they mature at 9 months after planting (Hahn, 1992). Fresh roots are highly perishable and deteriorate if kept for more than 2–3 days (Dahniya, 1994)

Table 1: Cassava production in Africa

	Production (Mt)	Area harvested (ha)	Yield Kg ha^{-1}
World	195 574 112	17 870 626	10944
Africa	103 423 009	11 662 941	8868
Kenya	600 000	77 502	7643

Source FAO, 2005

Cassava productivity in Africa is low compared to the world average. The main reason being several pests and diseases, which seriously decrease the growth and yield of the crop (Osiru *et al.,* 1999).

The cassava mealy bug, *Phenococcus manihoti* Matile Ferroro., introduced to Africa from South America, has been the most devastating pest of cassava in the continent. Biological control has helped in the management of the pest (Neuenschwander, 1994) following the introduction of the exotic parasitoid wasp, *Anagyrus lopezi* De santis (IITA, 2004).

The cassava green mite, *Mononychellus tanajoa* Bondar, is an important exotic, arthropod pest on cassava in many regions of Africa. Yield losses due to the pest in Africa are estimated at 30 – 80% (Yaninek, 1994). This too was accidentally introduced from South America and is now being managed following importation and dissemination of the predatory mite, *Typhlodromalus aripo* DeLeon. The mite was introduced from Brazil and has established in many African countries including Kenya (Kariuki *et al.*, 2005).

Apart from the above pests, several diseases are also known to attack cassava. Cassava bacterial blight caused by *Xanthomonas axonopodis* pv. *manihotis* is present in almost all cassava-growing areas in Africa (Boher and Verdier, 1994). The disease can cause total yield loss where conditions are favourable for development and spread of the pathogen (Lozano, 1986). The two major viral diseases of cassava are cassava mosaic disease (CMD) and cassava brown streak disease (CBSD). Cassava mosaic disease is the biggest constraint to cassava production in Africa (Fauquet and Fargette, 1990) and it occurs in all the cassava-growing regions of sub-Saharan Africa (Thresh *et al.,* 1994a).

1.1 Problem statement and justification

The main cassava-growing regions in Kenya are the coastal and western regions. An epidemic of a severe form of CMD occurred in the western region in the early 1990s. Consequently, a lot of CMD related work was carried out in the region. CMD diagnostic surveys were conducted and the Cassava Mosaic Geminiviruses (CMGs) occurring in the region were determined. In addition, CMD resistant varieties bred at International Institute of Tropical Agriculture (IITA) were introduced in the region from Uganda. However, these varieties were not released in the coastal region due to quarantine considerations.

Phytosanitation decreases the availability of source of inoculum through crop hygiene, use of CMD-free cuttings and rouging of diseased plants. It is regarded as a feasible approach in the management of CMD. However, research to find out the benefits of this management strategy have not been carried out in coastal Kenya. There was a need to

determine the potential role of phytosanitation in the management of CMD in coastal Kenya.

The prevalence and distribution of Cassava Mosaic Geminiviruses (CMGs) in coastal Kenya is documented for Kilifi and Kwale districts in the region. It was therefore important to carry out an intensive survey and determine the distribution of CMGs in the region. In the coastal region, cassava production is further constrained by CBSD. There are reports of co-infection with CMD and CBSD from Tanzania (Legg and Raya, 1998) and a similar scenario exists in coastal Kenya. However, the rate of co-infection in coastal Kenya with the two diseases has not been determined. The pathological implications of this co-occurrence are also unknown. Therefore, the response of local germplasm to co-infection with CMD and CBSD was determined

1.2 Objectives of the study

The main objective of this study was to develop an integrated package for the management of cassava mosaic disease in coastal Kenya.

The specific objectives were;

1. To determine the incidence and severity of CMD and farmers' knowledge on CMD in coastal Kenya
2. To determine variability among the cassava mosaic geminiviruses in coastal Kenya
3. To evaluate the role of phytosanitation (selection of clean planting material and roguing of diseased plants) in the management of CMD in coastal Kenya
4. To determine the response of local cassava varieties to mixed infections with CMD and CBSD

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Cassava Mosaic Disease

Cassava mosaic disease (CMD) was first reported in coastal Tanzania in 1894 and occurs in all cassava-growing areas in Africa. It was later shown to be transmissible by grafting and by the whitefly, *Bemisia tabaci* Gennadius (Bock and Woods, 1983). However, the disease is mainly disseminated through vegetative propagation of infected cuttings.

The CMD symptoms appear on leaves as a characteristic mosaic pattern affecting discrete areas and are observable at an early stage of leaf development. There is unequal expansion of the leaf lamina causing malformation and distortion. The mosaic is of two different types, the green and the yellow mosaic. For the yellow mosaic, severely affected leaves are reduced in size, misshapen and twisted with yellow areas separated by areas of normal green colour (Storey and Nichols in 1938). The plants appear stunted and young leaves abscise (Hillocks and Thresh, 1999). In green mosaic, contrasting green and dark green areas occurs. This is not as conspicuous as the yellow mosaic and there is no apparent reduction in leaf growth, plant growth and tuberous root yield (Storey and Nichols, 1938).

2.2 Aetiology

2.2.1 Taxonomy and structure of cassava mosaic geminiviruses

The viral aetiology of the disease was first confirmed following isolation and electron microscopy of geminivirus particles and fulfillment of Koch's postulates (Bock and

woods, 1983). In Africa, CMD is caused by geminiviruses, family *Geminiviridae* genus *Begomovirus* (Bock and woods, 1983).

The geminviruses are geminate particles measuring 30 x 20nm with a protein coat of about 30 kDa. The protein coat encapsidates a circular single stranded (ss) DNA genome that is small (2.5-3.0 kb) in size and replicates in the host nucleus (Sequira and Harrison, 1982; Harrison *et al.*, 1977). Geminiviruses are persistently transmitted by insect vectors and have a tendency to infect the phloem cells. The family *Geminiviridae* consist of four genera (*Mastreviruses*, *Curtoviruses*, *Topocuviruses* and *Begomoviruses*) based on host range, genome organization and species of the transmitting vector (Fauquet *et al.*, 2000)

Members of the genus Begomovirus are the only geminiviruses transmitted by the whitefly vectors of the genus *Bemisia* (Harrison and Robinson, 1999). The circular ss DNA genomes of most begomoviruses are bipartite with a few being monopartite. The two DNA molecules namely DNA-A and DNA-B are approximately 2500 to 2800nt and carry a total of six genes (Stanley and Gay, 1983). The monopartite begomoviruses lack the DNA-B. There are four genes in DNA-A, one (AV1) in the virus strand and three (AC1, AC2 and AC3) in the complementary strand. The AV1 encodes for coat protein whereas AC1, AC2 and AC3 influence viral replication (Stanley and Gay, 1983). Two genes are present in DNA-B namely BV1 in the virus strand and BC1 in the complementary strand. The two genes are responsible for movement of the virus within the host (Stanley and Gay, 1983). However variation occurs in DNA-A of begomoviruses in the old and new worlds. The gene AV2 occurs among begomoviruses in the old world

(Zhou *et al.*, 1998) whereas AC4 is functional in begomoviruses that lack DNA-B (Harrison and Robinson, 1999).

2.2.2 Prevalence of cassava mosaic geminiviruses in Africa

The begomoviruses known to attack cassava in Africa include *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV) and a recently described strain, *East African cassava mosaic virus-Uganda* (EACMV-UG) (Otim-Nape and Thresh, 1997). EACMV-UG has properties of a recombinant between EACMV and ACMV (Zhou *et al.*, 1997). *South African cassava mosaic virus* has also been reported to occur in some southern African countries (Berry and Rey, 2001; Briddon *et al.*, 2004). The begomoviruses may infect cassava singly or in combination (Thresh *et al.* 1994a); Were, 2001). Most severe symptoms result from co-infection with both ACMV and EACMV-UG (Harrison *et al.,* 1997). The severe form of CMD has adverse effects on growth and yield. The cultivation of sensitive varieties has therefore been widely abandoned in epidemic areas leading to decline in cassava cultivation and production (Thresh *et al.,* 1994b).

2.2.3 Distribution of cassava mosaic geminiviruses

East African cassava mosaic virus and the African cassava mosaic virus are the most common in Africa. *Indian cassava mosaic virus* (ICMV) infects cassava in India and Sri-Lanka but has not been reported in Africa (Were, 2001; Legg and Fauquet, 2004). In Africa, ACMV occurs in all countries where cassava is grown. *South African cassava mosaic virus* (SACMV) has been reported in South Africa, Swaziland (Berry and Rey, 2001) and Zimbabwe (Briddon *et al.*, 2004).

Though EACMV occurs primarily in East Africa and Madagascar (Swanson and Harrison, 1994) it has been reported in Central Africa and West Africa including Cameroon (Fondong *et al*., 2000), Ghana (Offei *et al*., 1999) and Ivory Coast (Pita *et al*.,2001). However, recent evidence suggests that the EACMV-like virus occurring in West/Central Africa is a distinct species, *East African cassava mosaic Cameroon virus* (EACMCV) (Fondong *et al*., 2000).

EACMV-UG occurs in Kenya, Uganda, Tanzania, Democratic Republic of Congo, southern Sudan, Rwanda, Burundi and Gabon (Legg and Fauquet, 2004). In southern Africa the virus occurs in Mozambique, South Africa, Swaziland and Zimbabwe (Berry and Rey, 2001). Unlike in other parts of the continent the presence of EACMV-UG in southern Africa has not been associated with an epidemic (Legg and Fauquet, 2004). In Kenya EACMV occurs mainly in the coastal region, in isolated pockets in western region around Lake Victoria (Were, 2001) and in Eastern province. EACMV-UG occurs in western Kenya. There is a report on the presence of EACMZV in coastal Kenya (Bull *et al*., 2003).

2.3 Detection of cassava mosaic geminiviruses

The double-antibody sandwich version of enzyme-linked immunosorbent assay (DAS-ELISA) can detect the viral antigen in extracts of young symptomatic cassava leaves. The drawback is that infected plants of many cultivars produce symptom-bearing leaves interspersed with healthy leaves in which the virus is not detected by DAS-ELISA (Fargette *et al.,* 1987). The disease can also be detected by murine monoclonal antibodies (Mabs) raised against CMGs and used in triple antibody sandwich ELISA (TAS-ELISA).

TAS-ELISA is more sensitive than DAS-ELISA. The advantage of TAS-ELISA is that the background given by extracts of assymptomatic leaves is negligible (CABI, 2003).

The virus can be latent within infected plants and there is a period of approximately one month before plants infected with whitefly-borne inoculum develop symptoms hence sensitive methods of virus detection are employed (Fargette *et al.*, 1987). Polymerase Chain Reaction (PCR) based diagnostics are the most reliable for virus detection in cassava. They can be used to diagnose large numbers of samples for the presence of CMGs using specific primers for virus identification. Amplification of viral DNA sequences makes detection of CMGs largely independent of initial virus DNA concentration (Were, 2001). Furthermore, PCR can distinguish the different CMGs and CMGs mixtures (Sseruwagi *et al.*, 2004).

2.4 Transmission and spread of cassava mosaic geminiviruses

The cassava mosaic geminiviruses (CMGs) are transmitted by the whitefly, *Bemisia tabaci* Gennadius (*Aleyrodidae*, Homoptera) (Storey and Nichols, 1938; Seif, 1981). The adults require an acquisition-feeding period of about 3 – 5 hours, a latent period of at least 8 hours with an inoculation-feeding period of 10 minutes to transmit the virus, and the vector remains viruliferous for 9-10 days. Transtadial but not transovarial transmission has been reported and the virus is not lost through moulting. Optimal transmission is achieved using 10 adult whiteflies per plant (Dubern, 1994). Burban *et al.* (1992) reported that there is a specific cassava biotype of *B. tabaci* and there is a report on colonization of five non-cassava species by the cassava specific whitefly (Sseruwagi *et al.*, 2006).

The temporal and spatial spread of CMD is related to the movement of adult whiteflies. There is no uniform distribution of whiteflies within cassava fields; their numbers are highest on the upwind borders and lowest within fields irrespective of field shape and size. This is confirmed by the higher incidence CMD incidence along the downwind edges of newly infected fields (Fargette *et al.*, 1985). Wide plant spacing favours a higher disease incidence when compared to close spacing (Fargette *et al.*, 1990).

Temporal spread of the disease is also highly variable. It varies between months and is periodical and fluctuates seasonally (Fargette *et al.*, 1994). There is a decrease in susceptibility as the plant ages with little infection occurring after the third month after planting. The incidence correlates positively with fluctuations in vector populations but it also varies with changes in climatic factors especially temperature (Fargette *et al.*, 1994). Studies on regional spread reported that little spread occurs when susceptible cultivars are established at sites isolated from other cassava fields. This indicates that the inoculum pressure in a given site is related to the density of cassava in the locality (Bock, 1994a). CMD is mainly disseminated in infected cuttings given that the virus does not occur in true seed.

2.5 Economic importance of cassava mosaic disease

Cassava mosaic disease greatly reduces the growth and yield of cassava particularly of local unimproved varieties. Almost total loss of yields of storage roots can be realized though reductions mainly range from 24-75% (Seif, 1982). Thresh *et al.* 1997 estimated yield losses caused by CMD in Africa as 12-23 million tones based on an overall incidence of 50-60% and a 30-40% loss of the total yield of diseased plants. In Uganda,

the losses were estimated as 600000 tones of harvest worth US$ 60 million following the 1990s epidemic (Otim-Nape *et al.,* 2001). When the same epidemic spread to western Kenya losses estimated to be greater than US$ 10 million occurred. In addition, some farmers in the region lost their susceptible local germplasm to the disease. As a result of the CMD epidemic, most farmers in the affected areas abandoned cultivation of the crop leading to famine-related deaths (Legg, 1999).

2.6 Management of cassava mosaic disease

2.6.1 Use of resistant germplasm

The use of virus-resistant cultivars is the most effective and sustainable approach to controlling CMD (Storey and Nichols, 1938). Breeding for resistance to CMD is a complementary and sustainable method of CMD control. For instance, disease incidence can be decreased by combining two different attributes, resistance to CMD and resistance to the whitefly (Fauquet *et al*., 1988). In addition, the resistant varieties show recovery whereby infected plants become symptomless with time. In more resistant varieties, there is complete recovery to an extent that the viruses are not detectable by serological methods (Njock *et al*., 1996).

Cassava mosaic-resistant germplasm has been developed that confers resistance to all cassava mosaic viruses including the recently described EACMV-UG (Legg, 1999). These include the Tropical Manioc Series (TMS) selections namely TMS 30572, TMS 60142, TMS 30337, TMS 30786, TMS 30395 and TMS 60140 (Otim-Nape *et al.,* 1994). The varieties have not been widely adopted and in many countries, farmers continue to grow local varieties that have little or no resistance to CMD making the disease more

prevalent (Calvert and Thresh, 2002). CMD-resistant varieties are not widely adopted perhaps because of shortage of planting materials and lack of information on their performance and suitability. In addition, the flavour, cyanide content of the roots, branched growth habit, poor storage characteristics and lack of adaptation to local conditions may also influence the adoption of these varieties (Calvert and Thresh, 2002).

The popular local varieties in Kenya include Adhiambolela, Serere, Obwanaterani, Muwumba, Siprosa, Mwakamoja, and Habune in Western and Nyanza Provinces. In Coast Province Kibandameno, Guzo, Number 4 and Kibesho are the popular local varieties (Were, 2001). A socio-economic study in Kenya reported that though 42% of the farmers noted differences in response to CMD among varieties only 4% of them linked this to differences in resistance (Kamau *et al*., unpublished). Following the CMD epidemic in the 1990s, resistant varieties bred at IITA were introduced to western Kenya from Uganda (Legg *et al*., 1999). These varieties were however not introduced in coastal Kenya due to quarantine considerations. Movement of cassava germplasm from western to coastal Kenya is not allowed to prevent introduction of cassava bacterial blight which is confined to the region.

2.6.2 Phytosanitation

Phytosanitation decreases the availability of sources of inoculum through crop hygiene involving use of disease- free cuttings and roguing of diseased plants (Thresh and Cooter, 2001). This has not been widely adopted because of in some areas, CMD is highly prevalent and is regarded as a normal feature of cassava.

Due to unavailability of CMD-free stocks, farmers obtain planting materials from available plants regarded as suitable for providing cuttings. However, in areas where CMD is prevalent farmers rarely select cuttings from healthy plants, it may be difficult or impossible for them to distinguish clean plants when cuttings are required as the plants are leafless due to disease and pest attack (Calvert and Thresh, 2002). Field sanitation and use of resistant cultivars may be combined in the management of CMD since sanitation is possible and practical if the level of resistance in relation to infection pressure is adequate (Cours *et al.*, 1997). In Kenya, 38% of cassava farmers practice roguing while 32% practice selection for disease control (Kamau, unpublished). Roguing is not widely adopted as farmers tend to associate the practice with decline in overall yields due to reduced plant population being higher compared to the overall reduction in disease incidence (Legg and Fauquet, 2004).

2.6.3 Genetic Engineering

Cassava has been genetically engineered to introduce pathogen-derived resistance against cassava mosaic disease. Transgenic cassava plants with increased ACMV resistance have been developed using improved antisense RNA technology by targeting the viral mRNAs of the Viral DNA. The transgenic ACMV-resistant plants demonstrate reduced viral DNA accumulation in their infected leaves (Zhang *et al.*, 2005). However, the genetically engineered cassava is yet to be evaluated under field conditions (Thresh and Cooter 2005).

2.6.4 Cultural practices

Intercropping cassava with other crops may help in vector control and therefore reduce CMD spread. Cassava mosaic disease incidence has been shown to decrease when cassava is intercropped with maize or cowpea (Ahohuendo and Sarkar, 1995; Fargette and Fauquet, 1988; Fauquet and Fargette, 1990). Lower infestations of whiteflies have also been reported in cassava intercropped with maize when compared to monocropped cassava (Gold, 1994). A cassava-maize intercrop influences the behaviour of vectors within mixed stands such that they spend less time on cassava. Adult whiteflies tend to aggregate on the more vigorous plants in a stand hence cassava grown alone will harbour more whiteflies than cassava facing competition from intercrops.

Resistant varieties may not be widely adopted by farmers if they possess negative traits like unfavourable taste. The resistant varieties can be established in mixtures with the susceptible but highly preferred variety. This makes it possible to retain the susceptible variety especially in areas of lower infection pressure (Sserubombwe *et al.*, 2001).

It is advantageous to plant cassava in large compact blocks to avoid open spaces as vector population and disease incidence tend to be higher on field edges (Fargette *et al.*, 1985; Fargette *et al.*, 1990). Small plots should be oriented along the direction of wind to decrease the proportion of plants likely to be infected by whiteflies. Planting material should not be obtained from the outermost rows of plots used for multiplication of clean planting stocks (Fargette *et al.*, 1985).

CHAPTER THREE

3.0 INCIDENCE AND SEVERITY OF CASSAVA MOSAIC AND FARMER KNOWLEDGE ON THE DISEASE IN COASTAL KENYA

3.1 Introduction

Cassava ranks second to maize as the main staple food in coastal Kenya. It is an important food security crop and a source of income to many farmers. In coastal Kenya, cassava yields are 5-10t/ha against a potential of 32t/ha (Munga, 2000). This low yield is largely attributed to viral diseases. The two major viral diseases of cassava are cassava mosaic disease (CMD) and cassava brown streak disease (CBSD). Cassava mosaic disease is caused by whitefly borne viruses of genus *Begomovirus* (family *Geminiviridae*) and is the most important of the diseases affecting cassava in Africa (Calvert and Thresh, 2001). The disease occurs in all cassava-growing areas of the continent (Thresh *et al.*, 1994). It is spread through infected planting materials and the whitefly vector, *Bemisia tabaci* (Bock and Woods, 1983). Losses due to the disease in Africa are estimated at 30-40 % (Calvert and Thresh, 2002).

Several cassava mosaic geminiviruses are reported to infect cassava in Kenya. The ACMV occurs in western Kenya (Were, 2001), EACMV occurs mainly in the coastal region, in isolated pockets in western region around Lake Victoria (Were, 2001) and in Eastern province. EACMV-UG occurs in western Kenya. There is a report on the presence of EACMZV in coastal Kenya (Bull *et al.*, 2003).

The objectives of this study were to document farmers' knowledge on the identification and management of CMD and to determine the CMGs infecting cassava in coastal Kenya.

3.2 Materials and methods

3.2.1 Incidence and severity of cassava mosaic disease in coastal Kenya

A survey was conducted in four districts in coastal Kenya namely Lamu, Malindi, Kilifi and Kwale to document the incidence and severity of CMD as well as to determine farmers' knowledge on the disease. The survey was conducted in October 2005 when the crop was five months so as to facilitate the distinction between whitefly-borne infection and infection due to use of diseased cuttings. Presence of symptoms on first-formed leaves near the ground level denotes cutting-borne infection while current-season infection due to the whitefly (*Bemisia tabaci*) virus transmission occurs on the upper newly formed leaves (Legg and Raya, 1998).

Sampling was done from randomly selected farms approximately five kilometers apart along rural roads. About thirty cassava plants were randomly selected on a diagonal transect in each field. The *Bemisia tabaci* count was determined for the thirty plants. The upper five fully formed leaves of each plant were slowly turned upwards and the adult whitefly count obtained. The total population for a particular plant was recorded as the sum total for the five leaves. The mean for the farm was the mean vector count for the thirty plants.

The CMD severity and whitefly count was determined for the thirty plants within the diagonal transect. Presence of CBSD on these plants was noted but the CBSD severity was not determined. Whitefly population was also determined by counting the number of adults on the five upper fully expanded leaves of the thirty plants within the transect. Plants were observed for typical CMD symptoms while distinguishing whitefly-borne and cutting-borne infections. Disease incidence was determined as the percentage of plants showing CMD symptoms within the thirty plants sampled in the field. The disease severity was obtained from the thirty plants sampled using a scale of 1 – 5 adopted from Hahn *et al*. (1980) where; 1-Healthy and assymptomatic leaves, 2-Mild chlorotic patterns affecting most leaves or mild distortions at the base of most leaves while the remaining parts of the leaves and leaflets appear green and normal, 3-Moderate mosaic pattern throughout the leaf, narrowing and distortion of the lower one third of the leaflets, 4-Severe mosaic, distortion of two thirds of most leaves and general reduction in leaf size, some stunting of shoots and 5-Very severe mosaic symptoms on all leaves, distortions, twisting, misshaping and severe reduction of leaves of most plants accompanied by severe stunting of plants. The mean severity for thirty plants was then obtained to give the mean severity for the farm.

Cuttings from the varieties grown in coastal Kenya were collected from each of the sampled fields. Five cuttings were collected from each of the field; these included plants showing representative symptoms for the variety, those with symptoms contrasting with the representative type and healthy plants. From the plant from which a cutting was obtained, a record of the symptom severity and type of infection was taken. The cuttings

were established in an insect-proof screen house at KARI-Mtwapa for use in molecular diagnosis of the geminiviruses affecting cassava in the region.

3.2.2 Farmers' knowledge on cassava mosaic disease in coastal Kenya

Farmers' knowledge on CMD was gathered during the survey by administering a semi-structured questionnaire (Appendix 1). The information in the questionnaires was analyzed using the Statistical Package for Social Scientists (SPSS).

3.3 Variability among cassava mosaic geminiviruses in coastal Kenya

Fifty-four fresh samples were collected from the top-most tender leaves showing various CMD symptoms two months after establishment of the cuttings in an insect–proof screen house in KARI-Mtwapa. Leaf samples were also collected from the assymptomatic plants. The leaf samples were carried in appendorf tubes in an icebox to the University of Nairobi molecular biology laboratory.

The universal primers UNIF (5'RSGGGTCGACGTCATCAATGACGTTRTA-3') and UNIR (5'-AARGAATTCATKGGGGCCCARARRGACTGGC-3') were used for amplification of near full-length DNA fragments. PCR was performed at 90 volts for an hour. The first cycle was at 94°c for one minute followed by 35 cycles at 94°c 1 min, 55°c for 1.5 min, 72°c for 10 min and finally a cycle of 94°c for 1 min, 55°c for 1 min and *70°c* for 10 min. The amplified DNA was then digested using *Mlu*1 and *EcoR*V restriction enzymes. The restriction product was then subjected to 1.5% agarose gel electrophoresis

at 90V for one hour. The gel was then removed and the DNA fragments visualized under UV light.

3.3.1 DNA extraction

Total DNA was extracted using the Dellaporta method (Dellaporta *et al.*, 1983). Polymerase Chain Reaction (PCR) was then performed on these samples at the University of Nairobi laboratory using universal begomovirus primers.

The leaf samples were ground in a microfuge tube containing 500μl of Dellaporta buffer after which 33μl of 20% lauryl sulphate was added to each tube, mixed thoroughly and incubated in a water bath at 65°C ten minutes. To each tube, 160μl of 5M sodium acetate was added and mixed thoroughly. The tubes were then kept in a freezer for ten minutes and spun in a microfuge at 13000 rpm for ten minutes. 450μl of the supernatant was transferred to a clean microfuge tube into which 450μl of cold isopropanol was added and mixed thoroughly. Spinning was then done at 13000rpm for ten minutes to precipitate the DNA. The supernatant was then removed carefully to leave the DNA. This was followed by addition of 500μl of 70% ethanol to each tube and spinning at 13000rpm for 5 minutes. The supernatant was discarded and the DNA air–dried in the tube for about one hour. The DNA was the suspended in 500μl of sterile distilled water and stored at 4°C. The DNA template was then added to the PCR mix (Promega®) following the manufacturer's instructions. The samples were then loaded onto a thermocycler and the PCR process carried out. The amplified DNA was then subjected to gel-electrophoresis.

3.3.2 Agarose gel preparation and electrophoresis

A 1.2% agarose solution was prepared by dissolving 1.2g in 100ml of Tris-acetate (TAE) buffer. 5µl of stock ethidium bromide solution was then added to the molten agarose mix, cooled to about 38°C and then poured into a gel tray pre-fitted with combs. After solidification, the gel was immersed into an electrophoresis tank and TAE buffer was poured to cover the gel. The combs were then removed to expose the formed wells. 2µl of loading dye was put into omni wells corresponding to the number of samples prepared and one well for the molecular marker. 18µl of amplified DNA sample was added to the loading dye and the marker added to the well at the edge of the gel. The gel tray was then connected to a power supply and 96 volts applied across the gel for an hour. The gel was then removed from the tray and placed on a UV light source and the DNA bands observed. The sizes of amplified DNA segments were estimated through comparison with bands of the DNA marker.

3.3.3 Precipitation of the PCR product

The PCR product was transferred into a clean tube and an equal volume of cold isopropanol was added, mixed well and centrifuged at 13000rpm for 10 minutes. The supernatant was discarded and the pellets washed with 100µl of 70% alcohol. The tubes were then centrifuged at 13000rpm for ten minutes. The supernatant was then removed and the DNA air-dried. The DNA pellets were then re-suspended in 20µl of sterile distilled water and stored at 4°C.

Restriction Fragment Length Polymorphism (RFLP) was performed using the enzymes *EcoR*V and *Mlu*1. Firstly, 10µl of purified DNA was pipetted into a tube, 0.5 µl of the

enzyme was added followed by 2 µl of buffer. The samples were incubated at 37°c for one hour. One µl of loading dye was added to 20µl of the digestion product, mixed and loaded into the wells of 1.5% agarose gel stained with ethidium bromide. The samples were then run at 95volts for 45 minutes after which the DNA fragments visualized under UV light.

3.4 Results

3.4. 1 The Incidence, Severity and Farmers' Knowledge on Cassava Mosaic Disease in coastal Kenya

Cassava mosaic disease and CBSD were observed in all the fields visited (Plates 1and 2). The CMD incidence in the surveyed fields ranged from 73 to 100%. The average incidence for the districts was 76-97% (Table 3). The disease status varied across the varieties (Table 2).

Table 2. Incidence and severity of CMD across the varieties in four districts in coastal Kenya

	DISTRIBUTION			
VARIETY	KILIFI	MALINDI	LAMU	KWALE
Kibandameno	76 %(3)	97% (3)	97% (3)	83% (3)
Kahutele	89 % (3)	-	-	95% (3)
Agriculture	54% (2)	100% (3)	-	-
Msomali	100 % (3)	-	-	-
Muhogo wa chango	0% (1)	-	-	-
Mtsetsetsi	-	100 % (3)	-	-
Kaleso	-	30% (2)	-	-
Chokorokote	-	90 % (3)		-
Katsunga	-	100% (3)	-	-
Tingisha	-	-	-	100% (3)
Gushe	-	-	-	100 % (4)
Ambari	-	-	-	90% (3)
Mgiriama	-	-	-	100% (3)
Mzungu	-	-	-	100% (3)
Guzo	-	-	100% (3)	100 % (3)
Sagalato	-	-	-	100% (3)
Kibiriti mweusi	-	-	-	97% (2)
Kibiriti mwekundu	-	-	-	67 (2)

Disease incidence is in percent while values in parentheses show the disease severity while – indicates absence of the variety in the surveyed fields in the district.

The mean *Bemisia tabaci* was highest (9) in Kwale and lowest (2) in Lamu. The CMD infection was mainly the cutting-borne type, with a range of 79 - 97% among the districts. The whitefly-borne infection in the region was 2-6% (Table 3). Whitefly-borne infection was shown to be positively correlated to the *B. tabaci* population. Mixed infections with both CMD + CBSD was observed in all the districts at a rate of 79-97% (Fig. 1). Mixed infection rate was highly significant among the four districts at $P \leq 0.05$.

Table 3. Incidence of cassava mosaic disease in coastal Kenya

District	% Incidence	Mean *B. tabaci* Per plant	% Whitefly-borne infection	% Cutting-borne infection
Kilifi	76	6	5	95
Malindi	94	5	4	96
Lamu	97	2	2	98
Kwale	92	9	6	94

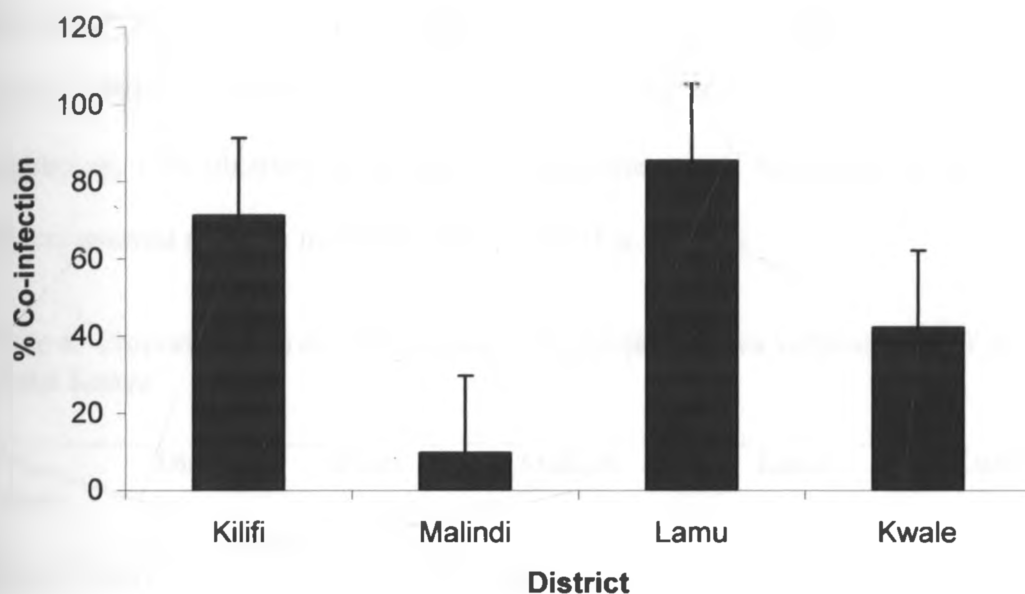

Fig. 1. Mixed infection with cassava mosaic and cassava brown streak viruses in coastal Kenya

Knowledge on varieties grown in the region varied among the farmers. Kibandameno was the most popular variety among the farmers (96%) interviewed in coastal Kenya. Other popular varieties were Agriculture (30%), Kahutele (20%) and Kaleso (7%). The distribution of the varieties varied across the region (Table 2). Cassava was mainly grown as a sole crop or intercrop (Plate 4). In most of the fields (63%), cassava was grown in an

intercrop system with maize (57%), maize and coconut (14%), maize and pumpkin (14%), cowpeas (7%) and citrus (4%). Varietal mixtures were also common (Plate 5). In Kwale district, 22% of the farmers who had more than one variety in their fields had established them in separate blocks. Some farmers (18.5%) had established a young crop next to an already mature one (Plate 3). Ratoon crops were observed in 15% of the cassava fields all within Malindi and Lamu districts and they were all of the Kibandameno variety (Plate 6).

Sources of planting materials varied among the farmers. Thirty–three percent of the farmers obtained cuttings from the previous season's crop, 48 % sourced from neighbours, 17% obtained from both previous season and neighbours while 2% of the farmers sourced planting materials from KARI (Fig. 2).

Table 4. Proportion (%) of farmers aware of popular cassava varieties in four districts in coastal Kenya

District / Variety	Kilifi	Malindi	Lamu	Kwale
Kibandameno	83	100	100	100
Agriculture	50	67	-	26
Guzo	20	20	17	33
Kahutele	67	0	-	15
Kaleso	-	20	-	15
Ambari	-	-	-	60

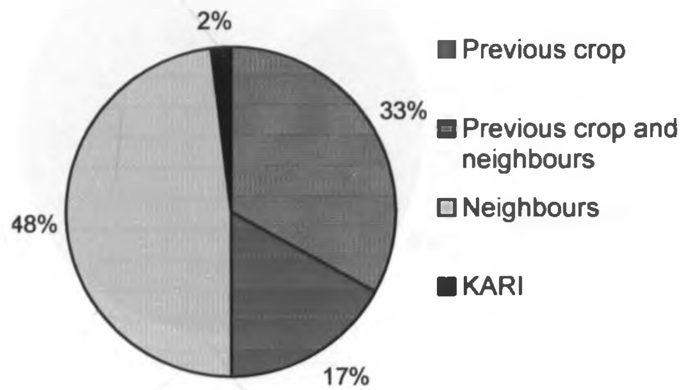

Fig. 2. Sources of planting materials among farmers in coastal Kenya.

Majority of the farmers (82%) could recognize the disease but attributed it to drought (12%), insects (12%) and low temperature (7%) whereas 65% had no idea as to what causes the disease (Fig. 3). Only 4% of the farmers associated the disease with viruses. Most of the farmers (70 %) regarded the disease as a problem in their fields with 63% reporting annual occurrence of the disease.

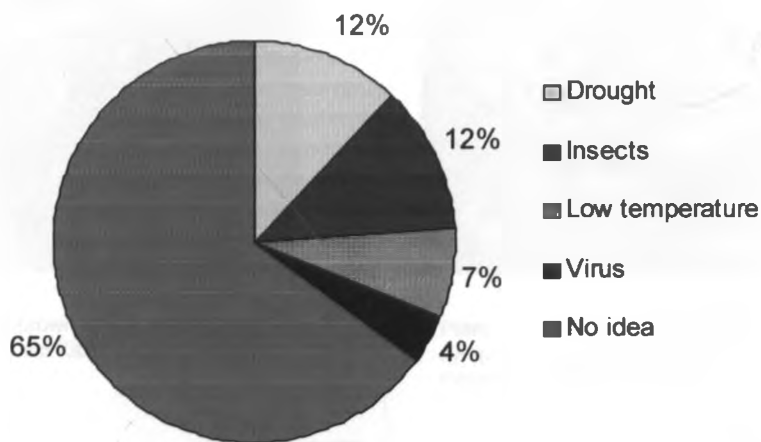

Fig 3. Perceived causes of cassava mosaic disease among farmers in coastal Kenya

Majority of the farmers (84%) had not adopted any management practices against CMD. Fifty-two percent of the farmers had observed difference in susceptibility among cultivars but continued to grow the susceptible ones due to good culinary properties (4%), non bitterness (30%) and lack of planting materials (7%). Among the reported resistant varieties were Guzo (15%), Ambari (11%) and Kaleso (7%).

Plate 1. Cassava brown streak foliar chlorosis on a three months old Kibandameno variety

Plate 2. Cassava mosaic disease symptoms on a Kibandameno plant three months after planting

Plate 3. A young cassava crop established next to a mature crop

Plate 4. A maize-cassava intercrop in Kwale district

Plate 5. Varieties of different cassava mosaic disease susceptibility grown in a mixture

Plate 6. A ratoon crop in Malindi district

3.4.2. Variability in cassava mosaic geminiviruses occurring in coastal Kenya

Most (89%) of the samples from the survey tested positive for CMD when PCR was carried out using the universal primers (Plate 7). When RFLP was done using the restriction enzyme *Mlu*1, three samples from a field in Kilifi District tested positive for EACMV-Ug (Plate 8, lanes 2, 5 and 9). A sample from the same field also had a dual infection of EACMV and EACMV-Ug (Plate 8, lane 7). The variety from which these samples were obtained was not established. The rest (85%) of the CMD positive samples were infected with EACMV.

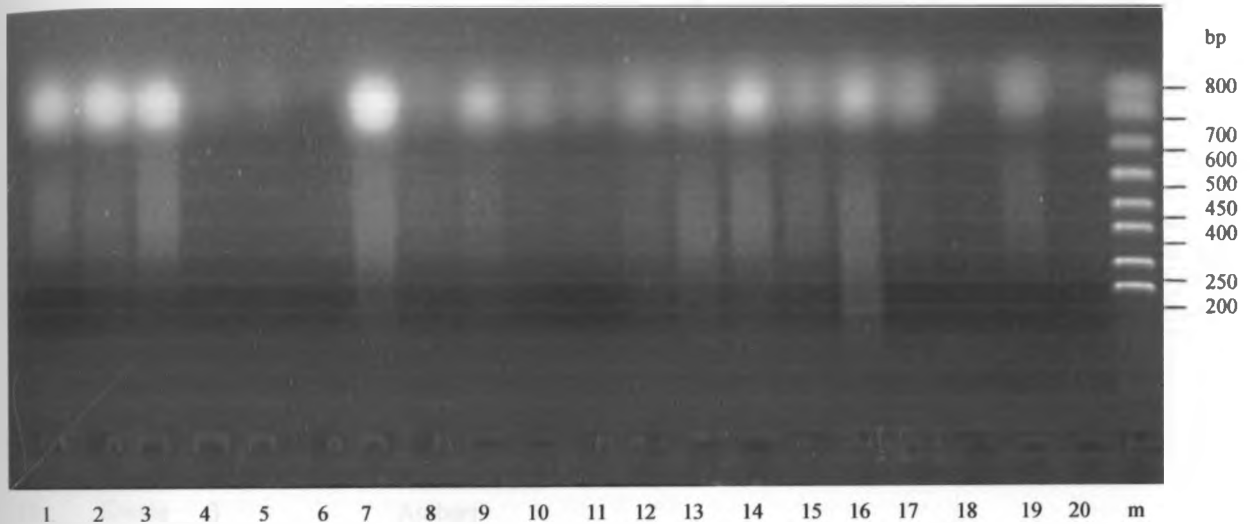

Plate 7. Gel electrophoresis of the PCR product amplified using Universal primer. Lanes 1-19comprised of the DNA samples where while lane 20 comprised of distilled water and lane m had the molecular marker. The DNA samples in the lanes 1-19 are as follows;

Lane	Source	CMD severity	variety
1	Kilifi	3	Unknown
2	Kilifi	3	Unknown
3	Kilifi	3	Unknown
4	Kwale	1	Agriculture
5	Kwale	2	Ambari
6	Malindi	2	Mgiriama
7	Kilifi	1	Kahutele
8	Malindi	5	Kibandameno
9	Lamu	1	Kibandameno
10	Kilifi	3	Msomali
11	Lamu	3	Kibandameno
12	Kwale	2	Agriculture
13	Malindi	2	Kibandameno
14	Kwale	3	Kibandameno
15	Malindi	4	Kazunga
16	Lamu	3	Kibandameno
17	Malindi	1	Kaleso
18	Lamu	3	Kibandameno
19	Kwale	1	Ambari

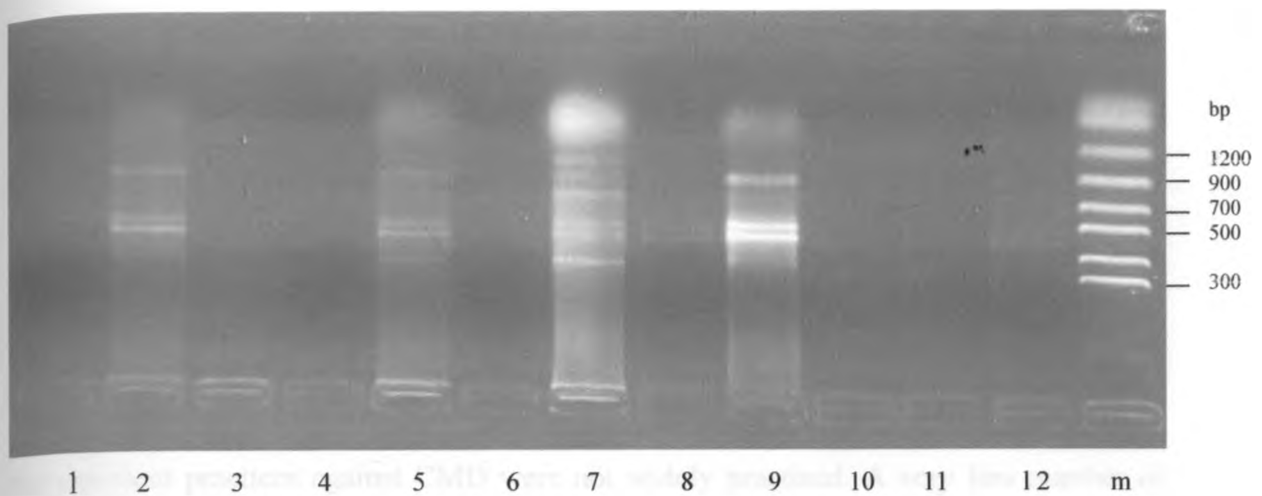

Plate 8. Gel electrophoresis of *Mlu*1 restriction of PCR product where lanes 2-12 comprised of samples lane1 comprised of distilled water while lane m comprised of the molecular marker. The DNA samples in lanes 2-12 were as follows;

Lane	Source	CMD severity	variety
2	Kilifi	3	Unknown
3	Kwale	1	Agriculture
4	Kwale	2	Ambari
5	Kilifi	3	Unknown
6	Lamu	2	Agriculture
7	Kilifi	3	Unknown
8	Kilifi	3	Unknown
9	Kilifi	3	Unknown
10	Kilifi	3	Msomali
11	Lamu	3	Kibandameno
12	Kwale	3	Ambari

The restriction product of *EcoRV* was too diffuse and could not be visualized upon gel-electrophoresis.

Discussion

The status and farmers' knowledge of CMD in coastal Kenya

The results from the survey showed that CMD is prevalent in coastal Kenya as supported by the high disease incidences. Based on previous surveys the incidence is on an increasing trend, it was 50% in 1998 (Kamau *et al.*, unpublished), 25-50% in 1999 (Were, 2004) and 58% in 2000 (Munga and Thresh, 2002). The present high disease

prevalence is mainly due to use of infected cuttings from previous season's crop and neighbours, which is consistent with the results of a survey undertaken by Bock (1994). Use of infected cuttings is a key factor in disease spread given that both CMD and CBSD can be transmitted through infected planting materials making dual infection with both diseases a common phenomenon.

Management practices against CMD were not widely practiced. A very low number of respondents sourced planting materials from research institutions. Most of the farmers did not select clean planting materials and roguing was equally unpopular. In addition, the ratoon crops observed in Lamu and Malindi Districts acted as disease foci as they had higher disease severity and in most instances were dually infected with CMD + CBSD. Disease symptoms were clear in weeded fields as compared to the unweeded ones. In weedy fields, the disease symptoms were accompanied by pest infestation especially mealy bugs. Most of the plants in such fields had also turned chlorotic. The *Bemisia tabaci* population was also higher in the weedy fields.

The *B. tabaci* population was lower on plants with a higher disease severity probably due to reduced leaf area (Fishpool and Burban, 1994) and reduced sap content of the brittle leaves. The population was also low in fields where cassava was intercropped with coconut. Possibly, the palm trees acted as wind breaks which impeded dispersion of the vectors. The highest *B. tabaci* population (over 80 adults per plant) was observed in a variety Sigalato growing in a farmers' field in Kwale district. The variety had reddish leaves that were very tender and was in a mature maize intercrop. The maize may have

influenced the dispersal of the vector while the young leaves were conducive for feeding by the whiteflies.

Majority of the farmers grew Kibandameno as the main variety in their fields because of its attributes such as high sugar content, high dry matter content, early maturity, good culinary properties and its marketability. Preference of more susceptible cultivars by farmers may have contributed to the wide spread nature of the disease as they are easily infected in the field. The variety Kibandameno is the most susceptible to the virus diseases while Kaleso; a hybrid is tolerant (Njeru and Munga, 2002). Most of the cassava varieties in the region are local landraces save for Kaleso, Guzo and Agriculture. Kaleso and Guzo are a result of the early breeding programme at Amani Tanzania that ran from 1920s-1940s (Hillocks and Jennings, 2003). Agriculture is a result of agricultural researchers to clean Kibandameno though it was not well received, as it did not match the culinary qualities of the latter. Apparently, none of the three varieties is resistant to CMD. Though varietal mixtures featured in most of the fields, the incidence was high. This contradicts a report from Uganda (Sserubombwe *et al.*, 2001) that growing varieties of different disease susceptibility reduces the disease status on the most susceptible variety. The failure of effectiveness of the mixtures in coastal Kenya is due to use of infected cuttings. Farmers in the region are also constrained by lack of cuttings. Most of them could not raise enough planting materials and had to supplement by borrowing from neighbours.

Conclusion

The results of this survey emphasize the need to enhance farmers' knowledge on cassava virus diseases in coastal Kenya. Emphasis should be on accurate disease diagnosis, enhancing the availability of planting materials and feasible management practices such as selection of clean planting materials, roguing and use of resistant varieties. There is also the need to avail new varieties as the ones currently available are all susceptible to CMD. This will be a feasible approach since most of the infection in the region is of the cutting-borne type. Clean planting materials need to be availed to the farmers. In addition, resistant cultivars should be promoted among the growers. Ensuring good agronomic and culinary characteristics of the resistant/tolerant varieties can increase their popularity. Furthermore, the approach worked for a decade following area wide release of CMD free materials to displace infected materials in farmers' fields in Uganda (Jameson 1964). Resistant cultivars also played a role in restoration of cassava production in Uganda following the CMD epidemic of 1990s (Otim-Nape *et al.*, 2001). There is need for researchers to pay attention to co-infection with CMD and CBSD. The rates reported in this survey are expected to increase as the crop matures since CBSD is reported to infect cassava late in the season.

The variation among strains and isolates of CMGs occurring in coastal Kenya

The results of this diagnostic study show that a wide range of CMGs infect cassava in coastal Kenya. The study by Were *et al.,* 2004 only identified EACMV. Further work on the same collection documented the presence of EACMZV from the same region (Bull *et al.*, 2003).

In this study, EACMV was found to be the dominant virus species in agreement with the findings of Were *et al.,* 2004. In addition, EACMV-UG was also isolated from a sample collected in Kilifi. This implies that the virus species is no longer confined to the western region of Kenya. However, it was not established how this virus may have spread to the coastal region. The virus is a recombinant between ACMV and EACMV (Zhou *et al.*, 1997) and it is unlikely that there was any recombination in coastal Kenya given that ACMV occurrence is not reported. The fact that it was isolated from an unknown variety indicated that it was probably introduced to the area through movement of materials from the Western region. It may also have been introduced through the open quarantine movement of cassava germplasm for breeding work.

Conclusions and recommendations

There is need to carry out an intensive study on the CMGs occurrence in coastal Kenya with special reference to Kilifi District so as to map out the extent distribution of EACMV-UG in the region. This should be accompanied by an effort to control its spread to avoid an epidemic usually associated with EACMV-UG. The case in coastal Kenya further threatens cassava production in the region given the co-occurrence of CMD and CBSD that is common across the region. This is complicated further by the lack of resistant varieties in the region. It is important to emphasize on the need to carry out thermotherapy and meristem tip culture whenever exchange of germplasm within regions is to take place.

CHAPTER FOUR

4.0 THE ROLE OF USING PHYTOSANITATION (SELECTION OF CLEAN PLANTING MATERIALS AND ROGUING) IN THE MANAGEMENT OF CASSAVA MOSAIC DISEASE

4.1 Introduction

Cassava is an important staple food crop in coastal Kenya. In addition, it serves an important role in food security given the erratic rains experienced in the region. Production in the region is largely constrained by prevalence of two viral diseases, CMD and CBSD. As a result, the yield of the crop is about 30% of the regions potential (Munga, 2000).

Phytosanitation decreases the availability of sources of inoculum through removal of diseased cassava plants and alternative CMD hosts in the vicinity of newly established fields, use of CMD free planting material and rouging of infected plant (Thresh and Cooter, 2001). It has been demonstrated that disease free planting material can be obtained through careful visual selection of cuttings from the available plants (Jameson, 1964, Bock, 1994b and Otim-Nape *et al.*, 1998). This observation was also noted by farmers in Rwanda (Njeru and Gashaka, 2007). Though phytosanitation is regarded as a feasible approach to management of CMD, scientific studies to establish the benefits of the practice have not been carried out in coastal Kenya.

Therefore, this study was conducted to find out the role of selecting clean planting material and roguing of diseased plants in the management of CMD in coastal Kenya.

4.2 Materials and methods

Four treatments namely; planting disease-free cuttings without roguing, disease-free cuttings where no roguing was done, randomly selected cuttings without roguing and randomly selected cuttings where roguing was done were evaluated for the management of CMD. The experiment was carried out for two seasons, the 2005 short rain season and the long rain season in 2006.

Trials were conducted at KARI-Mtwapa during the 2005/2006 short rain season and 2006/2007 long rain season. The research station is in the coastal lowland semi-humid coconut/cassava agroclimatic zone and at an altitude of 15m above sea level. The maximum temperature for the station is 32°C while the minimum temperature is 23 °C. The soil is sandy, poorly developed and is of low fertility. Cuttings of the variety Kibandameno, (CMD susceptible) was planted in a randomised complete block design (RCBD) with four replicates. Cuttings were planted in plots measuring 6 x 7m with spacing of 1 x 1m resulting in a population of 42 plants per plot.

Roguing was carried out one Month After Planting (MAP). Any plant showing CMD symptoms was uprooted and discarded from the experimental plots. Vector population was determined by counting the number of whitefly adults on the five top most leaves of selected shoots of five randomly selected plants. The leaves were carefully turned upwards so as not to disturb the vectors. The vector population count was done on a monthly basis up to the fifth MAP.

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The disease incidence and severity data was collected monthly on all the 42 plants in a plot up to the ninth month after planting. The disease severity determined using a scale of 1 – 5 adopted from Hahn *et al.* (1980) where; 1-Healthy and assymptomatic leaves, 2-Mild chlorotic patterns affecting most leaves or mild distortions at the base of most leaves while the remaining parts of the leaves and leaflets appear green and normal, 3-Moderate mosaic pattern throughout the leaf, narrowing and distortion of the lower one third of the leaflets, 4-Severe mosaic, distortion of two thirds of most leaves and general reduction in leaf size, some stunting of shoots and 5-Very severe mosaic symptoms on all leaves, distortions, twisting, misshaping and severe reduction of leaves of most plants accompanied by severe stunting of plants.

Plant height was determined on five randomly selected plants within the net plot. The perpendicular height of the tallest shoot of the plant was measured using a metre rule. On the tenth month, the roots were harvested. The number of roots of all the plants in each net plot of 20 plants was counted, the length measured ,the fresh roots weighed and the roots separated into marketable or unmarketable and the number of roots in each category recorded.

The data was subjected to analysis of variance and the means separated using the least significant difference. The software used in the analysis was Genstat 10th edition.

4.3 Results

During the short rain season, the monthly CMD incidence and severity were significantly ($P \leq 0.05$) different in the first 5 MAP. The disease incidence and severity were higher in

randomly selected materials compared to the clean ones. The plant height was not significantly (P≤0.05) different among the treatments (Table 5). The vector population was highest in the first MAP and decreased gradually in the subsequent counts, it was however not significant among the treatments (Fig. 4).

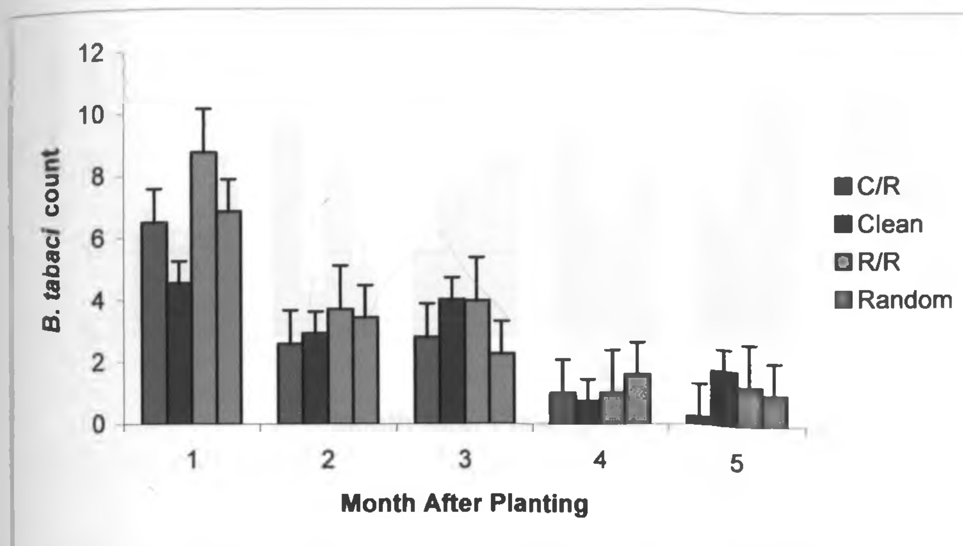

Fig 4. *Bemisia tabaci c*ounts for Kibandameno variety established from clean and randomly selected planting material in the short rain season

Key
C/R- Clean planting material with rouging, R/R- Randomly selected planting material with rouging
R- Randomly selected planting material without rouging, Clean- Clean planting material without roguing

During the long rain season the CMD incidence was only significantly (P≤0.05) different between the first two MAP but not significantly different between the treatments. The disease severity was not significant beyond the fifth MAP. The randomly selected materials had a higher severity than the clean ones. The Plant height was not significantly (P≤0.05) different among the treatments (Table 5). The *B. tabaci* population increased gradually over the evaluation period (Fig. 5). All the yield parameters measured were not significantly (P≤0.05) different between treatments in the short rains. During the long

rain season only the number of marketable roots was significantly ($P \leq 0.05$) different among the treatments (Table 6).

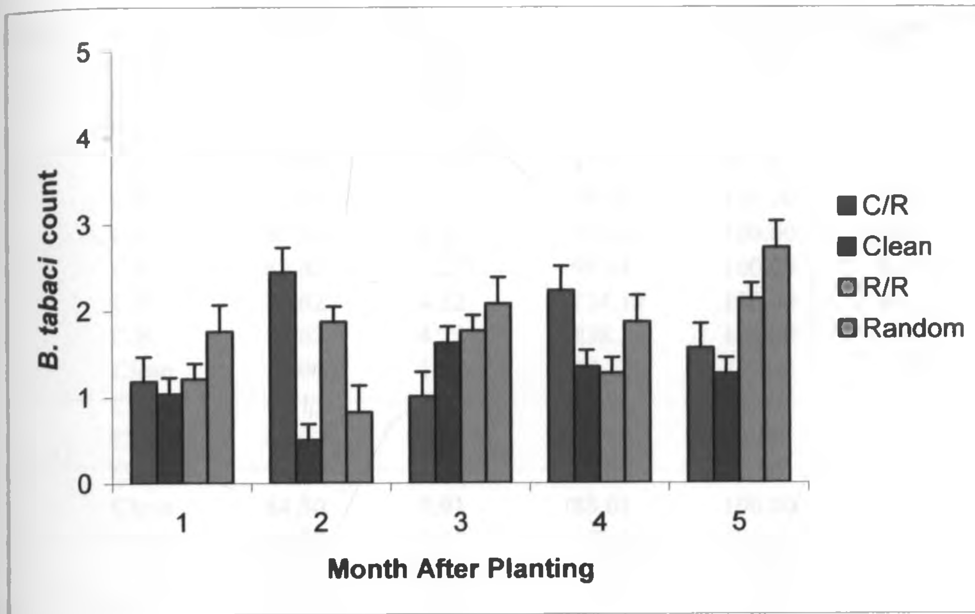

Fig 5. Bemisia tabaci counts for Kibandameno variety established from clean and randomly selected planting material in the long rain season

Key
C/R- Clean planting material with rouging, R/R- Randomly selected planting material with rouging, R- Randomly selected planting material without rouging, Clean- Clean planting material without roguing

Table 5. Cassava mosaic disease incidence and severity and plant height per plant of Kibandameno variety established from clean and randomly selected planting material in the long and short rain seasons at KARI-Mtwapa

		Short rain season			**Long rain season**		
MAP	Treatment	CMD incidence	CMD severity	Height	CMD incidence	CMD severity	Height
1	C/R	30.66	1.48	16.93	64.52	1.68	18.00
2	C/R	58.92	2.48	28.69	99.21	2.95	34.78
3	C/R	69.49	3.39	43.05	99.21	3.71	48.25
4	C/R	77.10	3.77	59.19	100.00	3.95	58.61
5	C/R	82.53	4.13	77.10	100.00	4.09	83.15
6	C/R	83.62	4.22	98.34	100.00	4.15	108.56
7	C/R	83.62	4.22	124.13	100.00	4.15	137.71
8	C/R	83.62	4.22	138.72	100.00	4.15	149.34
1	Clean	31.04	1.38	17.13	72.03	1.74	16.75
2	Clean	49.13	2.45	28.22	98.57	2.80	34.27
3	Clean	67.02	3.25	45.71	98.82	3.75	52.29
4	Clean	74.72	3.57	62.05	99.50	3.95	66.79
5	Clean	84.30	3.91	83.01	100.00	4.01	84.46
6	Clean	84.87	4.11	100.50	100.00	4.05	99.99
7	Clean	84.87	4.15	121.16	100.00	4.05	144.30
8	Clean	84.87	4.15	139.11	100.00	4.05	155.68
1	R/R	57.61	1.74	16.97	66.67	1.80	17.31
2	R/R	74.77	2.55	31.97	95.02	2.88	36.94
3	R/R	91.41	3.59	45.09	95.02	3.71	51.13
4	R/R	91.41	4.08	59.37	98.05	4.04	57.50
5	R/R	92.22	4.32	76.85	99.00	4.43	78.32
6	R/R	92.22	4.33	92.33	99.00	4.45	92.25
7	R/R	92.22	4.33	120.74	99.00	4.45	124.64
8	R/R	92.22	4.33	129.40	99.00	4.45	133.82
1	Random	62.81	1.81	17.43	82.00	1.92	18.51
2	Random	85.91	2.61	31.08	98.68	2.75	35.74
3	Random	90.30	3.49	44.47	98.68	3.56	51.31
4	Random	95.92	4.09	56.65	99.32	4.02	62.66
5	Random	95.92	4.23	74.37	99.32	4.23	79.02
6	Random	95.92	4.34	102.85	99.32	4.44	103.75
7	Random	95.92	4.34	121.55	99.32	4.44	124.32
8	Random	95.92	4.34	128.60	99.32	4.44	132.57
P<0.05		NS	NS	NS	NS	NS	NS
LSD		-	-	-	-	-	-
CV (%)		0.83	6.89	9.22	4.56	6.79	19.66

Key
C/R- Clean planting material with rouging, R/R- Randomly selected planting material with rouging, R-Randomly selected planting material without rouging, Clean- Clean planting material without roguing. NS-not significant

Table 6. The mean root length. number of roots. number of marketable roots and root length for the clean and randomly selected planting materials per plant of Kibandameno variety

	Short rains season				Long rains season			
Treatment	Root length (cm)	Number of roots	Number of marketable roots	Root weight (kg)	Root length (cm)	Number of roots	Number of marketable roots	Root weight (kg)
Clean	22.21	6.8	4.75	2.17	26.92	4.60	0.61	1.75
Clean/roguing	19.82	6.3	5.25	3.10	27.14	3.60	0.68	2.22
Random Selection	22.95	5.75	4.7	3.20	24.67	3.35	0.57	1.39
Random/Roguing	21.66	4.2	3.15	2.89	22.27	4.30	0.13	1.73
P<0.05	NS	NS	NS	NS	NS	NS	0.0001	NS
LSD	-	-	-	-	-	-	0.16	-
CV (%)	16.26	25.19	29.67	24.14	22.93	22.48	20.57	28.85

NS- not significant

Discussion

The population dynamics of *B. tabaci* observed in this study were influenced by the prevailing weather conditions. The short rains crop was planted during a dry period that persisted up to 6 MAP. The time of planting in the long rains crop coincided with the start of a rainy season in the region. The rainy period was then followed by a period of relatively high temperatures (Appendix 2).Temperature range of 20-30°C is known to favour increase in *B. tabaci* population as a result of increased fecundity, rapid development and increased lifespan (Legg, 1994). An abundance of *B. tabaci* has been reported at the onset of a rainy period preceded by a dry period (Legg and Ogwal, 1998) although rainfall above 280mm per month leads to a decline in the population density. This may explain the higher vector population in the long rains crop when compared with

the short rains crop. In addition, studies done in Uganda showed that the vector population increases during periods of rapid growth of the cassava crop and declined in the slow growth period (Legg, 1994). Incidentally, the period of rapid growth in cassava coincides with rainy weather conditions.

There was no significant ($P \leq 0.05$) difference in plant height and yield among the various treatments. This shows that roguing was not effective for CMD management. On the contrary, there are reported cases of success involving selection of clean planting materials and roguing in CMD management (Jameson, 1964; Otim-Nape *et al.*, 2001). In both cases, roguing was combined with varietal resistance in disease management. Jameson (1964) reported that roguing was only effective at centres with slight infections. After the 1990s pandemic of the severe CMD, roguing was affective after the disease had led to a change in the dominant varieties where the most susceptible variety was replaced by tolerant or resistant materials (Otim-Nape *et al.*, 2001).

There are reports where roguing for CMD management has proved futile, for instance two attempts in Ivory coast (Fauquet and Fargette, 1990) and earlier by Colon (1984) cited by Thresh *et al.*, (1998). The effectiveness of the approach is expected to vary among countries and regions given the differences in cropping systems, extent of damage in relation to virus strain and varietal susceptibility, and prevailing disease incidence. Disease incidence in the field is influenced mainly by the proportion of infected cuttings planted and the varietal sensitivity to vector transmission as a consequence of movement within or between fields (Hillocks, 1997).

It is suggested that roguing may be carried out in areas where the disease incidence is not greater than 20% (Hillocks, 1997; Hillocks and Jennings, 2003). An earlier report gives a success of the practice only in the event of slight infections (Jameson, 1964). The disease incidence at 1 MAP when roguing was carried out in this study was above 40 and 60% in the short and long rain season respectively. This high disease incidence was observed even in plots established from visually clean planting materials. This implies that the variety Kibandameno harbours latent infection and/or is associated with a high rate of disease spread within the plants. The former is more likely to be the case given the low vector population and the fact that most of the infections were not whitefly-borne. In addition, CMD incidence depends on the availability of inoculum and latent infections produce visible symptoms at the onset of the rains (Jameson, 1964).

Conclusions and recommendations

Roguing for CMD management should be employed in integration with resistant varieties and selection of clean planting materials so as to increase the success of the practice by ensuring a low level of disease incidence. The benefits of selection of clean planting material and roguing may not be realised in a single season attempt. There should be a working scheme of availability and sequential release of clean planting materials for the effect of phytosanitation to take effect. Susceptible varieties should also be subjected to thermotherapy and meristem tip culture to ensure that they are free of disease. Roguing and selection of clean planting material can then be carried out to get rid of resultant vector- borne infections.

It is important therefore to promote phytosanitation for disease management in coastal Kenya integrated with resistant varieties. The farmers should be educated on the long-term benefits of the practice in restoration of the crop productivity in the region and in line with suggestions of Thresh *et al.* (1994b) that the practice should be area wide. In addition, there is need for further studies on the effect of selection of clean planting materials for CMD management using a resistant variety. The susceptible local cultivar also needs to be cleaned through thermotherapy and tissue culture.

CHAPTER FIVE

5.0 RESPONSE OF LOCAL VARIETIES TO CO-INFECTION WITH CASSAVA MOSAIC AND CASSAVA BROWN STREAK DISEASES

5.1 Introduction

Cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) are the main diseases of cassava in coastal Kenya. The two diseases are spread through use of infected planting materials and the whitefly vector, *Bemisia tabaci* (Bock and Woods, 1983; Legg and Raya, 1998 and Maruthi *et al.*, 2005). Cassava mosaic disease is known to occur in all areas where cassava is grown in Africa while CBSD has been reported in Malawi and Tanzania (Nichols, 1950), Zanzibar (Thresh and Mbwana, 1998), Uganda (Thresh *et al.*, 1994b), Zambia and Mozambique (Hillocks *et al.*, 2002). In Kenya, the disease was formely confined to the coastal region (Bock, 1994b; Munga and Thresh, 2002). However, there are now reports of CBSD occurrence in the Western region (Ntwaruhunga, 2007)

Co-infection of plants by two viruses is common in nature. It has varying epidemiological implications. These may be in form of increased symptom severity and virus accumulation resulting in high yield losses. The level of one of the viruses may remain constant while that of the co-infecting virus increases (Anjos *et al.*, 1992; Calvert and Ghabrial, 1983; Goldberg and Brakke, 1987). Sometimes, the concentration of both viruses may increase (Fondong *et al.*, 2000). This increased level of the virus in co-infected plants is due to an increase in the number of virus particles per host cell (Goodman and Ross, 1974). In addition, there may be an increase in RNA due to a change in the regulation of virus replication (Pruss *et al.*, 1997). In event of dual

infections, one virus may cause an increase or decrease in efficiency of transmission of the other virus (Zhang *et al.*, 2000).

Co-infection with cassava mosaic and cassava brown streak diseases is known to occur in areas where the two diseases are prevalent. This phenomenon is reported to complicate the assessment of disease severity in diagnostic surveys due to masking of CBSD symptoms (Legg and Raya, 1998). Therefore, an experiment to determine the response of local germplasm to co-infection with the two diseases in coastal Kenya was carried out.

5.2 Materials and methods

A field experiment was conducted to determine the response of local germplasm to mixed infections with CMD and CBSD at KARI – Mtwapa. The experiment was carried out in 2005/2006 short rain and 2006/2007 long rain seasons. Cuttings from plants infected with a combination of CMGs and CBSV were planted in plots measuring 6 x 7m at a spacing of 1x1m using a RCBD. The control plots comprised of cuttings from plants that were visually disease-free. Three varieties popular with farmers in coastal Kenya, namely Kaleso, Guzo and Kibandameno were planted in three replicates. The treatments included; cuttings infected with CMD and CBSD, only CMD-infected cuttings, only CBSD-infected cuttings and visually virus-free cuttings.

To maintain the integrity of the treatments, vectors were controlled using Dimethoate`40% E.C and Confidor (Imidacloprid) at the rate of 5ml/litre for both pesticides. Spraying was done on a monthly basis and Confidor was applied as a soil drench around cassava plant bases at sprouting and at 6 MAP. Data on incidence and

severity of CMD and CBSD was collected on a monthly basis up to the eighth month after planting for all plants. The CMD severity determined using a scale of 1 – 5 adopted from Hahn *et al.* (1980) where; 1-Healthy and assymptomatic leaves, 2-Mild chlorotic patterns affecting most leaves or mild distortions at the base of most leaves while the remaining parts of the leaves and leaflets appear green and normal, 3-Moderate mosaic pattern throughout the leaf, narrowing and distortion of the lower one third of the leaflets, 4-Severe mosaic, distortion of two thirds of most leaves and general reduction in leaf size, some stunting of shoots and 5-Very severe mosaic symptoms on all leaves, distortions, twisting, misshaping and severe reduction of leaves of most plants accompanied by severe stunting of plants.

For the co-infected plants, the CMD and CBSD severity were recorded separately. Severity of CBSD shoot symptoms was determined using a scale of 1-5 where;1-No apparent symptoms, 2-Slight foliar mosaic, no stem lesions, 3-Foliar mosaic, mild stem lesions, no dieback, 4-Foliar mosaic and pronounced stem lesions, no dieback and 5-Defoliation with stem lesions and pronounced dieback. The CBSD root necrosis severity was determined on a scale of 1-5 where;1- No apparent symptoms, 2- Less than 5% of root necrosis, 3- 5-10% root necrosis, 4- 10-25% of root necrosis, mild root constriction and 5- 25% of the root necrotic and severe root constriction (Hillocks *et al.,* 1996).

The height of five plants in the plants in the net plot was determined monthly. The perpendicular height of the tallest plant shoot was measured. Harvesting was carried out at 10 MAP and data collected was number of roots, root length and fresh roots weight for each of the plant in the net plot. The roots for five randomly sampled plants within the net

plot were also scored for root necrosis. The results were subjected to analysis of variance and means separated by the least significant difference.

5.3 Results

5.3.1 Symptom development

Most of the CMD infected plants showed symptoms at sprouting during the two growing seasons. The CBSD symptoms were visible at sprouting on Guzo and Kibandameno varieties. Some Kibandameno plants showed mild symptoms of CMD whereby the mosaic had highly reduced chlorotic areas. Though the leaves felt brittle, they were not malformed. Such plants did not exhibit stunting. Some plants of variety Kaleso manifested CMD symptoms on some shoots whereas the rest of the plant remained healthy throughout the growth period. This was also observed in variety Guzo but at a lower magnitude.

Some co-infected Kibandameno plants developed a rosette appearance by the third month. The upper leaves were reduced in size due to mosaic while the lower leaves were CBSD infected and maintained their normal size (Plate 10). In co-infected plants, the CMD symptoms developed fast as compared to CBSD and the latter was masked (Plate 8). Some CBSD and co-infected Kibandameno plants showed die back from 8 MAP in the short rain season. The die back symptoms were however not observed in the long rain season.

At 4 MAP, the CBSD symptoms could be observed 16 cm from the shoot tip in Guzo but only on fully developed leaves. The variety was the first to show CBSD stem lesions at

4MAP. In some Guzo plants, it was possible to find only a single leaf with severe CBSD symptoms before the disease symptoms appeared on the other leaves.

In the short rain season, the root symptoms due to CBSD comprised of root constrictions and slight necrosis on Kibandameno. Guzo showed mild constrictions and necrosis and one plant was observed having roots with lesions. During the long rain, Kibandameno showed severe root constrictions, severe pitting and discoloration (Plate 12). The severely constricted roots became lignified and dry. Root necrosis was observed in a few plants though the root cortex showed signs of developing discolouration in majority of the plants. The root symptoms in Guzo were mild, during the two seasons, some plants showed mild constrictions and necrosis whereas majority of the plants produced healthy roots (Plate 13). Though Kaleso did not show any foliar symptoms due to CBSD, some plants produced constricted roots in the short rain season. This was however not observed in the long rain season.

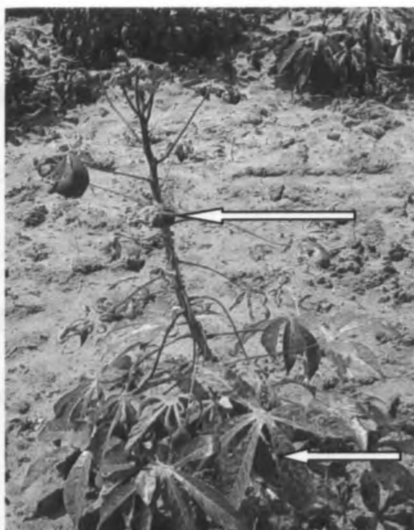

Plate 10. Co-infected Kibandameno with upper leaves reduced by CMD and normal sized CBSD -infected lower leaves

Plate 11. Masking of CBSD foliar symptoms by CMD on a four month old Kibandameno plant

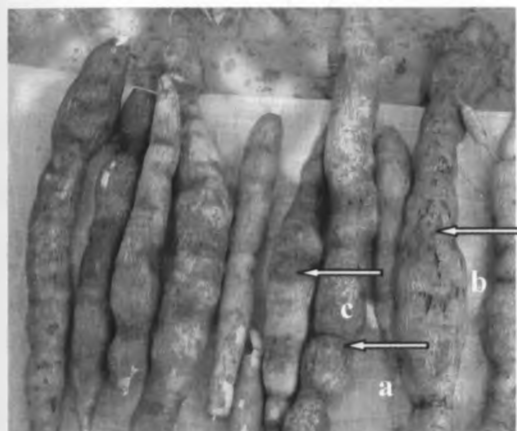

Plate 12. Root constrictions (a), pitting (b) and discolouration (c) on Kibandameno plants harvested 10 MAP

Plate 13. Root constrictions due to CBSD in Guzo variety

5.3.2 Incidence and severity of CMD

In the two seasons, the effect of time (months after planting), varieties and treatment on CMD incidence and severity were highly significant ($P \leq 0.05$). The CMD incidence and severity were highest in variety Kibandameno and lowest in Guzo.

In variety Guzo monthly CMD incidence was significantly ($P \leq 0.05$) higher up to the sixth MAP only in the short rain season. In the long rain season the CMD incidence and severity was significantly ($P \leq 0.05$) different among the first seven months of growth. The CMD incidence and severity were also significantly different ($P \leq 0.05$) among treatments. In the two seasons, CMD incidence was highest in plants established from co-infected materials and lowest in those established from CBSD infected materials.

In Variety Kaleso, CMD incidence was significantly ($P \leq 0.05$) different up to the sixth MAP in the short rain season as compared to fifth MAP in the long rain season. The monthly increase in CMD incidence was significant ($P \leq 0.05$) up to the seventh MAP in short rain season and sixth MAP in the long rain season. In the two seasons, cassava plants raised from clean planting materials had the lowest CMD incidence and severity and yielded the tallest plants. The variety did not show foliar symptoms of CBSD.

In Kibandameno variety, the monthly rise in CMD incidence was significantly ($P \leq 0.05$) different up to the sixth MAP in the short rain season whereas that for disease severity was not significant ($P \leq 0.05$) after the seventh MAP. In the long rain season, the rise in incidence was significant ($P \leq 0.05$) up to the fifth MAP and the severity did not increase significantly ($P \leq 0.05$) after the sixth MAP (Tables 7 and 8)

Table 7. Mean cassava mosaic disease incidence and severity per plant of Guzo, Kaleso and Kibandameno varieties established from visually clean, CMD, CBSD and CBSD+CMD infected materials in the short rain season at KARI-Mtwapa

	GUZO			KALESO		KIBANDAMENO	
MAP	Treatment	% CMD Incidence	CMD Severity	% CMD Incidence	CMD severity	% CMD Incidence	CMD Severity
1	CBSD	5.04	1.15	2.00	1.02	32.33	1.56
2	CBSD	7.46	1.36	21.00	1.26	40.00	2.73
3	CBSD	11.00	2.55	38.67	2.15	52.00	3.54
4		15.50	2.92	53.67	2.42	58.67	3.82
5	CBSD	22.54	3.33	56.67	2.69	70.00	3.85
7	CBSD	44.78	3.64	60.67	2.83	78.33	4.05
8		45.15	3.74	60.67	3.23	78.33	4.48
1	Clean	45.15	3.85	60.67	3.44	79.00	4.48
2		4.00	1.16	6.00	1.06	22.33	1.33
3	Clean	20.29	1.27	20.00	1.40	40.00	2.87
4		26.54	1.65	27.67	2.44	57.33	3.81
5	Clean	30.37	2.15	42.00	2.78	79.15	3.87
6		40.68	2.55	46.67	3.50	85.33	4.08
7	Clean	46.34	2.81	46.67	3.41	93.33	4.21
8		46.34	3.18	46.67	3.96	93.33	4.27
1	CMD	47.03	3.22	46.67	4.18	93.33	4.39
2		51.67	1.45	52.67	1.67	85.33	1.84
3	CMD	68.00	2.01	89.33	2.24	99.00	3.04
4		73.00	2.34	92.67	3.65	100.00	3.91
5	CMD	83.33	3.03	97.33	4.13	100.00	4.09
6		85.00	3.33	97.33	4.42	100.00	4.38
7	CMD	88.33	3.49	97.33	4.64	100.00	4.79
8		92.00	3.43	98.33	4.60	100.00	4.86
1	CMD +CBSD	94.67	3.58	98.33	4.68	100.00	4.86
2		24.00	1.47	37.33	1.39	41.33	1.44
3	CMD +CBSD	64.33	1.75	91.33	2.58	100.00	2.71
4		70.67	2.65	93.67	3.62	100.00	3.66
5	CMD +CBSD	93.33	2.95	93.67	3.77	100.00	3.93
6		95.33	3.33	96.62	4.07	100.00	3.98
7	CMD +CBSD	97.33	3.80	96.62	4.16	100.00	4.16
8		99.00	3.91	96.62	4.42	100.00	4.16
$P \leq 0.05$		>0.0001	>0.0001	<0.0001	<0.0001	<0.0001	<0.0001
LSD		4.30	0.19	2.29	0.11	1.77	0.09
CV (%)		13.72	12.31	6.15	6.21	3.80	4.21

Key

CBSD- cassava brown streak disease infected materials, Clean - Visually clean planting materials, CMD- cassava mosaic disease infected materials, MAP- Month after planting

Table 8. Mean cassava mosaic disease incidence and severity per plant in Guzo, Kaleso and Kibandameno varieties established from visually clean, CMD. CBSD and CBSD+CMD infected materials in the long rain season at KARI-Mtwapa

		GUZO		KALESO		KIBANDAMENO	
MAP	Treatment	% CMD Incidence	CMD Severity	% CMD Incidence	CMD severity	% CMD Incidence	CMD Severity
1	CBSD	6.56	1.20	6.33	1.07	32.33	1.59
2	CBSD	9.22	1.57	31.67	1.48	55.67	2.39
3	CBSD	15.26	2.30	40.67	2.03	60.33	3.30
4	CBSD	17.67	2.77	50.67	2.41	65.67	3.70
5	CBSD	38.97	3.15	54.67	2.59	77.00	3.93
6	CBSD	50.11	3.49	59.00	2.74	90.00	4.10
7	CBSD	50.11	3.64	59.00	2.91	95.00	4.25
8	CBSD	50.11	4.10	61.33	3.19	95.00	4.34
1	Clean	7.00	1.06	7.67	1.07	25.67	1.43
2	Clean	28.68	1.50	22.33	1.35	84.67	2.62
3	Clean	32.54	1.86	27.00	1.77	84.67	3.67
4	Clean	36.03	2.12	43.33	2.25	84.67	3.92
5	Clean	44.11	2.50	43.33	2.43	84.67	4.04
6	Clean	46.01	2.74	43.33	2.73	84.67	4.21
7	Clean	46.01	3.16	44.33	3.06	84.67	4.27
8	Clean	46.01	3.55	46.33	3.33	84.67	4.39
1	CMD	5.00	1.21	8.00	1.25	81.33	1.91
2	CMD	39.00	1.69	77.67	2.23	99.00	2.98
3	CMD	77.00	2.53	85.33	3.51	99.00	3.84
4	CMD	83.00	2.70	92.33	4.14	100.00	4.07
5	CMD	81.00	3.10	94.00	4.23	100.00	4.25
6	CMD	85.00	3.28	94.00	4.34	100.00	4.70
7	CMD	91.67	3.43	95.00	4.37	100.00	4.70
8	CMD	97.33	3.58	95.00	4.40	100.00	4.73
1	CMD +CBSD	17.33	1.27	55.00	1.69	46.00	1.43
2	CMD +CBSD	55.33	1.70	84.67	2.41	95.33	2.34
3	CMD +CBSD	63.67	2.60	84.67	2.95	97.33	3.45
4	CMD +CBSD	77.33	2.89	86.33	3.51	97.33	3.69
5	CMD +CBSD	93.67	3.29	88.67	3.74	97.33	3.89
6	CMD +CBSD	96.00	3.77	92.00	4.19	97.33	3.99
7	CMD +CBSD	99.00	3.90	92.00	4.27	97.33	4.06
8	CMD +CBSD	99.00	3.99	94.67	4.42	97.33	4.19
$P \leq 0.05$		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
LSD		4.42	0.25	5.59	0.29	3.70	0.14
CV (%)		14.5	16.07	15.81	17.43	7.62	6.92

Key

CBSD- cassava brown streak disease infected materials, Clean - Visually clean planting materials, CMD- cassava mosaic disease infected materials, MAP- Month after planting

5.3.3 Incidence and severity of Cassava brown streak disease

The CBSD incidence and severity was significantly ($P \leq 0.05$) different among diseased and clean planting materials in Guzo variety. The incidence was significantly different up to the sixth and seventh MAP during the short rain and long season respectively. The monthly CBSD severity was significant ($P \leq 0.05$) throughout the evaluation period. The plants established from co-infected cuttings had the highest severity in the two seasons (Tables 9 and 10).

In Kibandameno variety, the CBSD incidence did not increase significantly ($P \leq 0.05$) after the sixth MAP in the two seasons. The rise in severity was not significantly ($P \leq 0.05$) different after the fifth MAP in the short rain season whereas it was significant throughout the evaluation period in the long rain season. In the two seasons, CBSD incidence was highest in plants raised from co-infected cuttings and lowest in those from CMD infected materials. The CBSD severity was highest in plants established from co-infected materials and lowest in those raised from clean cuttings in the two seasons. However, in the short rain season there was no significant ($P \leq 0.05$) difference in severity among the treatments CMD+CBSD, CMD and CBSD whereas it was not significant among treatments in the long rain season (Tables 9 and 10).

Table 9. Mean cassava brown streak disease incidence and severity per plant in Guzo and Kibandameno varieties established from visually clean, CMD, CBSD and CBSD+CMD infected materials in the short rain season at KARI-Mtwapa

		GUZO		KIBANDAMENO	
MAP	Treatment	CBSD incidence	CBSD severity	CBSD Incidence	CBSD Severity
1	CBSD	7.33	1.06	39.33	1.32
2	CBSD	85.67	1.82	87.00	1.93
3	CBSD	93.00	2.65	90.00	2.97
4	CBSD	94.67	2.97	97.33	3.12
5	CBSD	94.67	3.31	97.33	3.75
6	CBSD	94.67	3.43	97.33	3.88
7	CBSD	94.67	3.59	97.33	3.89
8	CBSD	94.67	3.66	97.33	3.89
1	Clean	4.67	1.18	9.00	1.14
2	Clean	24.00	1.32	51.67	2.16
3	Clean	27.35	2.26	59.00	2.67
4	Clean	33.27	2.73	66.67	3.36
5	Clean	41.95	3.15	74.33	3.66
6	Clean	41.95	3.50	91.33	3.57
7	Clean	41.95	3.59	95.33	3.94
8	Clean	41.95	3.64	95.33	4.00
1	CMD	1.67	1.01	16.33	1.45
2	CMD	13.49	1.41	47.33	2.11
3	CMD	21.64	1.88	54.33	2.41
4	CMD	36.43	2.52	60.33	3.44
5	CMD	42.98	2.66	60.33	3.60
6	CMD	48.39	3.17	60.33	3.74
7	CMD	48.39	3.59	60.33	3.91
8	CMD	48.39	3.76	60.33	3.94
1	CMD +CBSD	57.00	1.66	46.33	1.48
2	CMD +CBSD	80.00	1.92	71.76	1.99
3	CMD +CBSD	95.00	2.70	100.00	2.92
4	CMD +CBSD	98.67	3.06	100.00	3.54
5	CMD +CBSD	98.67	3.52	100.00	3.66
6	CMD +CBSD	99.33	3.77	100.00	3.88
7	CMD +CBSD	100.00	3.81	100.00	3.92
8	CMD +CBSD	100.00	3.92	100.00	3.92
$P \leq 0.05$		<0.0001	<0.0001	<0.0001	NS
LSD		3.58	0.18	4.43	0.18
CV (%)		10.43	11.37	10.31	10.13

Key

CBSD- cassava brown streak disease infected materials, Clean - Visually clean planting materials, CMD- cassava mosaic disease infected materials, MAP- Month after planting

Table 10. Mean cassava brown streak disease incidence and severity per plant in Guzo and Kibandameno varieties established from visually clean, CMD, CBSD and CBSD+CMD infected materials in the long rain season at KARI-Mtwapa

		GUZO		KIBANDAMENO	
MAP	Treatment	CBSD incidence	CBSD severity	CBSD Incidence	CBSD Severity
1	CBSD	7.33	1.06	39.33	1.32
2	CBSD	85.67	1.82	87.00	1.93
3	CBSD	93.00	2.65	90.00	2.97
4	CBSD	94.67	2.97	97.33	3.12
5	CBSD	94.67	3.31	97.33	3.75
6	CBSD	94.67	3.43	97.33	3.88
7	CBSD	94.67	3.59	97.33	3.89
8	CBSD	94.67	3.66	97.33	3.89
1	Clean	4.67	1.18	9.00	1.14
2	Clean	24.00	1.32	51.67	2.16
3	Clean	27.35	2.26	59.00	2.67
4	Clean	33.27	2.73	66.67	3.36
5	Clean	41.95	3.15	74.33	3.66
6	Clean	41.95	3.50	91.33	3.57
7	Clean	41.95	3.59	95.33	3.94
8	Clean	41.95	3.64	95.33	4.00
1	CMD	1.67	1.01	16.33	1.45
2	CMD	13.49	1.41	47.33	2.11
3	CMD	21.64	1.88	54.33	2.41
4	CMD	36.43	2.52	60.33	3.44
5	CMD	42.98	2.66	60.33	3.60
6	CMD	48.39	3.17	60.33	3.74
7	CMD	48.39	3.59	60.33	3.91
8	CMD	48.39	3.76	60.33	3.94
1	CMD +CBSD	57.00	1.66	46.33	1.48
2	CMD +CBSD	80.00	1.92	71.76	1.99
3	CMD +CBSD	95.00	2.70	100.00	2.92
4	CMD +CBSD	98.67	3.06	100.00	3.54
5	CMD +CBSD	98.67	3.52	100.00	3.66
6	CMD +CBSD	99.33	3.77	100.00	3.88
7	CMD +CBSD	100.00	3.81	100.00	3.92
8	CMD +CBSD	100.00	3.92	100.00	3.92
P≤0.05		<0.0001	<0.0001	<0.0001	NS
LSD		4.42	0.25	4.43	-
CV (%)		14.5	16.07	10.31	10.13

Key

CBSD- cassava brown streak disease infected materials, Clean - Visually clean planting materials, CMD- cassava mosaic disease infected materials, MAP- Month after planting

4.3.4 Plant height

During the two seasons, plant height was highly significant ($P \leq 0.05$) among the treatments in all the three varieties. The clean planting materials attained the highest height than the infected ones in both seasons. Plants of variety Guzo established from clean planting materials were the tallest while those from the co-infected planting material were the shortest (Fig. 6). This was consistent in the two planting seasons.

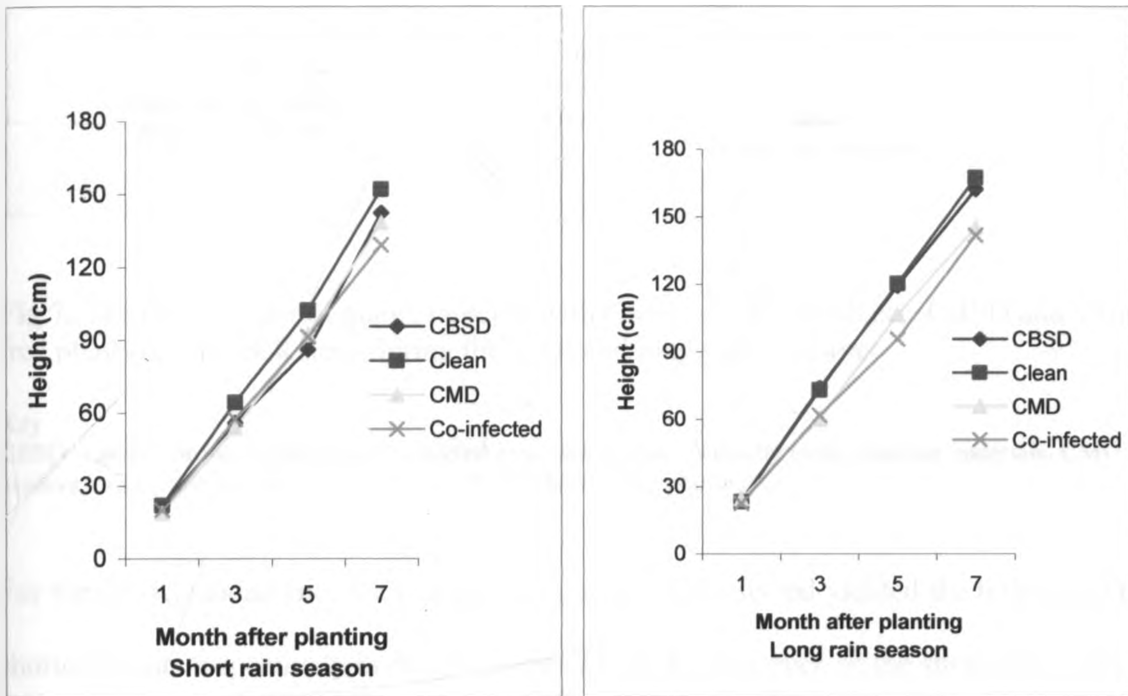

Fig 6. The mean height of plants infected with CMGs, CBSV, CMGs + CBSD and visually virus free plants of variety Guzo during the short and long rain seasons

Key
CBSD- cassava brown streak disease infected materials, Clean - Visually clean planting materials, CMD- cassava mosaic disease infected materials, MAP- Month after planting

In the short rain season, plants of variety Kaleso raised from clean planting material were the tallest whereas those from CMD infected cuttings were the shortest (Fig 7). In the long rains season the height was not significantly ($P \leq 0.05$) different among the treatments.

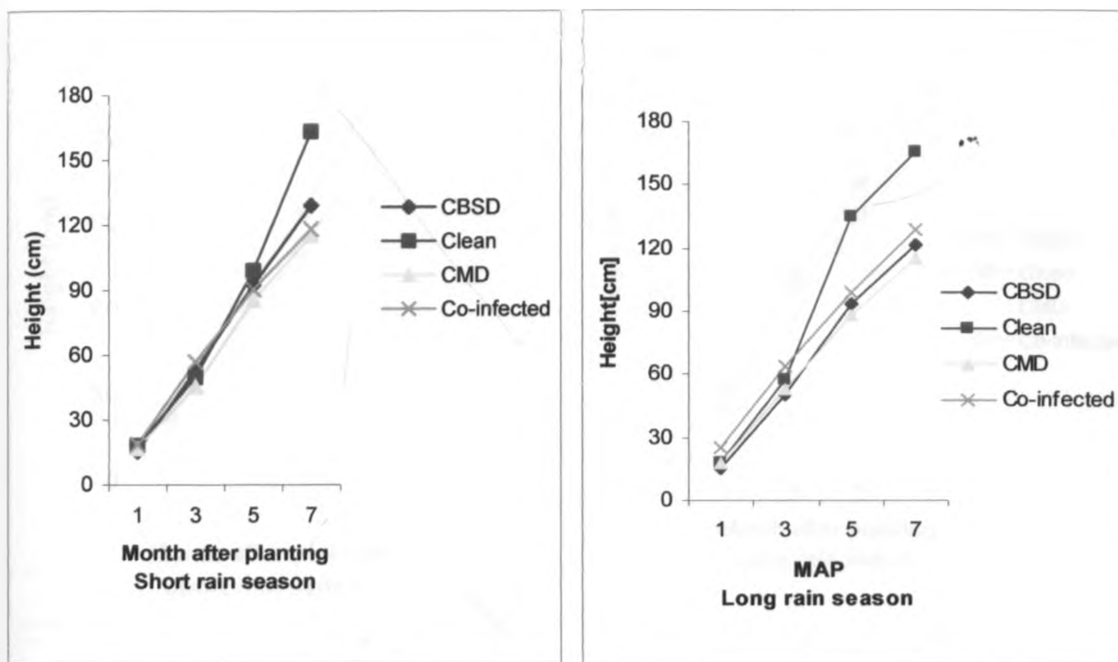

Fig 7. The mean height of plants infected with CMGs, CBSV, CMGs + CBSD and virus free plants of variety Kaleso during the short and long rainy seasons

Key
CBSD- cassava brown streak disease infected materials, Clean - Visually clean planting materials, CMD- cassava mosaic disease infected materials, MAP- Month after planting

For variety Kibandameno, the treatments clean and co-infected yielded the tallest and the shortest plants respectively in the two seasons (Fig 8). However, in the short rains season, the plant height was not significantly ($P \leq 0.05$) different among the plants raised from CMD and CBSD infected planting materials.

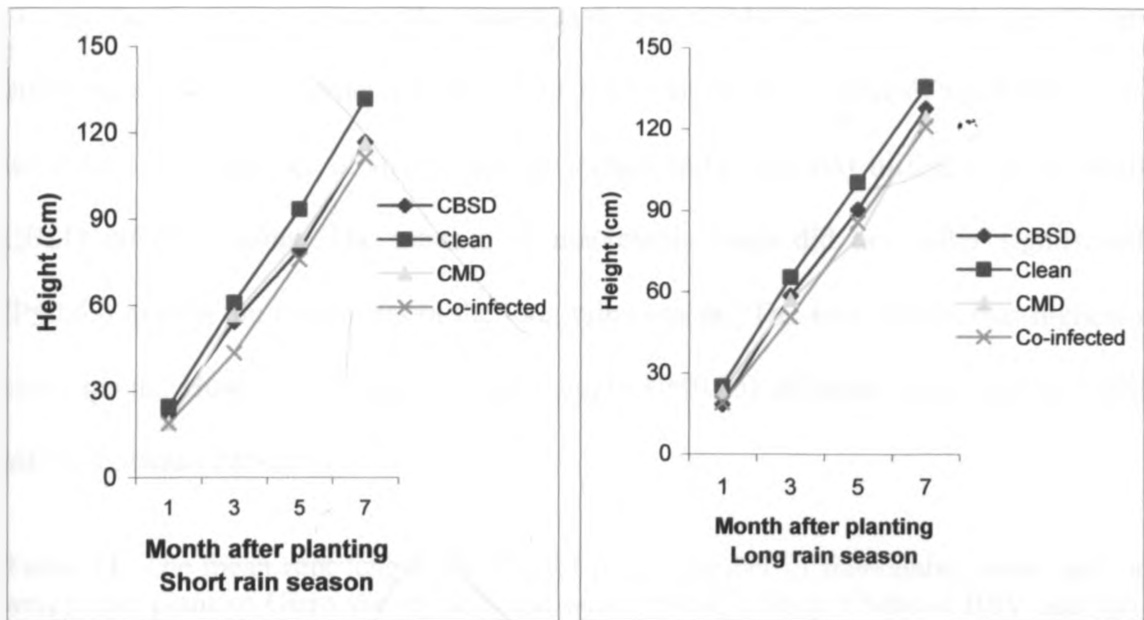

Fig 8. The mean height of plants infected with CMGs, CBSV, CMGs + CBSD) and virus free plants of variety Kibandameno during the short and long rainy seasons

Key

CBSD- cassava brown streak disease infected materials, Clean - Visually clean planting materials, CMD- cassava mosaic disease infected materials

4.3.4 Root yield

During the short rain season, the root yield parameters were different among some treatments in Guzo variety. The root length and number of roots were significantly different ($P \leq 0.05$) among treatments. The clean plants yielded the longest roots while co-infected plants produced the shortest roots. The numerical root yield and number of marketable roots were highest in clean plants but it did not differ significantly ($P \leq 0.05$) among the other treatments. The root weight was highest in clean plants; it was not significantly ($P \leq 0.05$) different between the plants singly infected with CMGs and CBSV. The co-infected plants produced the lowest root weight in the short rains season (Table 11).

During the long rain season, the root length and number of roots were significantly different among the treatments whereas the root weight and number of marketable roots were not. The number of roots was not significantly (P≤0.05) different in clean and CBSD infected plants. The number of marketable roots did not differ significantly (P≤0.05) among the treatments in the long rains season. The root weight was highest in clean plants. However, it was not significantly (P≤0.05) different from that of CBSD infected plants (Table 11).

Table 11. The mean root length, number of roots, number of marketable roots and root weight per plant of Guzo variety infected with CMGs, CBSD, CMBs+CBSV and virus free plants during the short and long rain seasons

	Short rain season				Long rain season			
Virus infecting plants	Root length (cm)	Number of roots	Number of marketable roots	Root weight (kg)	Root length (cm)	Number of roots	Number of marketable roots	Root weight (kg)
CBSV	28.93	6.27	4.0	3.56	31.89	7.2	5.13	3.52
CMGs	24.62	4.6	4.86	3.07	30.96	5.22	4.0	2.93
No virus	30.85	10.28	6.25	4.87	33.9	8.53	4.0	3.78
CMGs + CBSV	20.24	4.47	4.17	2.53	24.57	4.53	3.93	2.27
P≤0.05	0.004	0.0002	NS	NS	0.003	NS	NS	NS
LSD	4.38	1.45	-	-	3.52	-	-	-
CV (%)	8.4	11.4	19.9	26.4	5.81	14.85	28.89	19.28

Key
CBSV- cassava brown streak virus materials, CMGs – Cassava mosaic geminiviruses

In Kibandameno, all the root yield parameters taken in the short rain season were significantly (P≤0.05) different among treatments. The root length was not significantly (P≤0.05) different between clean and CBSD infected materials; though the CMD and co-

infected plants produced shorter roots they were not significantly (P≤0.05) different. The number of marketable roots and root weight were highest in clean and lowest in co-infected plants. The root weight was not significantly (P≤0.05) different between CBSD and co-infected plants. During the long rains, the co-infected plants yielded the shortest roots; the root length was not significantly (P≤0.05) different in the other treatments. The total root yield, number of marketable roots and root weight were all highest in healthy plants and lowest in co-infected plants (Table 12).

Table 12. The mean root length, number of roots, number of marketable roots and root weight per plant of Kibandameno variety infected with cassava mosaic geminiviruses, cassava brown streak virus and virus free plants during the short and long rain seasons

	Short rain season				Long rain season			
Virus infecting plants	Root length (cm)	Number of roots	Number of marketable roots	Root weight (Kg)	Root length (cm)	Number of roots	Number of marketable roots	Root weight (Kg)
CBSV	24.04	6.2	2.07	1.62	25.29	5	1.67	1.82
CMGs	14.11	3.64	3.07	1.87	18.02	3.87	1.8	2.26
No virus	25.42	7.27	5.23	2.83	31.19	5.9	3.6	3.1
CBSV + CMGs	11.95	2.73	0.5	1.23	15.77	3.47	0.73	1.22
P≤0.05	0.005	0.004	0.0009	0.05	0.0008	0.05	0.002	0.0014
LSD	6.45	1.92	1.39	1.07	4.8	1.77	0.99	0.94
CV (%)	17.06	19.32	25.48	28.36	10.65	19.48	25.41	22.48

Key
CBSV- cassava brown streak virus, CMGs – Cassava mosaic geminiviruses

All the yield parameters measured during the short rains were significantly (P<0.05) different between clean and infected materials in Kaleso variety. During the long rains,

all the parameters were not significantly (P<0.05) different among the treatments (Table 13).

... The mean root length, number of roots, number of marketable roots and root weight per plant of Kaleso variety infected with cassava mosaic geminiviruses, cassava brown streak virus and virus free plants during the short and long rain seasons

	Short rain season				Long rain season			
Virus infecting plants	Root length (cm)	Number of roots	Number of marketable roots	Root weight (kg)	Root length (cm)	Number of roots	Number of marketable roots	Root weight (kg)
CBSV	28.93	6.27	4	4.87	27.46	4.67	3.8	3.13
CMGs	24.62	7.57	4.86	3.57	31.13	3.33	2.67	1.81
No virus	30.85	9.28	6.25	3.07	39.72	7.40	5.60	4.75
CBSV +CMGs	20.34	5.93	4.17	2.53	27.79	3.53	2.13	2.59
$P \leq 0.05$	0.004	NS	NS	NS	NS	NS	0.05	0.04
LSD	4.38	-	-	-	-	-	2.41	1.89
CV (%)	8.39	18.02	19.89	26.44	20.60	21.40	33	30

Key
CBSV- cassava brown streak virus, CMGs – Cassava mosaic geminiviruses

Discussion

The emergence of Kibandameno as the most susceptible variety to both CMD and CBSD is in agreement with reports from previous authors, whose judgement was based on visual observations (Njeru and Munga, 2002; Bock, 1994b). Though, Guzo could get infected by both CMD and CBSD the effect of the diseases on yield was not highly significant ($P \leq 0.05$). This befits the description of the variety by Bock (1994b) as tolerant to CBSD with symptoms being confined to the lower leaves. The response of Kaleso is also parallel to reports that it is tolerant to CMD (Njeru and Munga 2002) Kaleso did not

develop CBSD symptoms during the vegetative growth, in agreement with reports that the variety is tolerant (Hillocks and Jennings, 2003).

The mild CMD symptoms observed on some of the Kibandameno plants may indicate the presence of EACMZV. There is a report on the presence of EACMZV in coastal Kenya and the symptoms observed conform to the description given by Maruthi *et al.* (2002). The plants of variety Kaleso and Guzo that only showed symptoms on some shoots while others remained healthy throughout the growth period could be attributed to cutting-borne infection (Thresh and Mbwana, 1998).

In all the three varieties, the clean plants produced superior roots as compared to the other treatments. In most of the Guzo plants, there was no significant difference in root yield parameters between the clean and the CBSD infected plants. This can be attributed to the tolerant nature of the variety to CBSD (Bock 1994b) perhaps due to restricted movement of the virus. Furthermore, there are reports that yield losses due to CBSD in tolerant varieties may not be realized in some varieties unless they are left in the ground for at least 12 months (Hillocks *et al.*, 2001).

Kibandameno showed a significant reduction in quantative and qualitative yield. This could be a result of the high disease severity observed on the variety. Yield reduction was mainly in form of reduced root length, weight, root fill and reduction in quality due to severe constriction and pitting. This concurs with the findings of Hillocks *et al.* (1999) who observed reduced root size in plants with severe symptoms. The reduced root weight is contrary to earlier reports that CBSD has no significant effect on root weight (Nichols,

1950; Bock 1994(b)). However, it conforms to more recent findings (Hillocks *et al*., 2001) where weight loss of up to 70% was reported in the most susceptible cultivars.

During the long rains, the yield parameters were not significantly different among the treatments in variety Kaleso. This observation is attributed to the ability of resistant varieties to restrict viral translocation and multiplication with negligible effects on yields (Sserubombwe *et al*., 2001). On the other hand, the yield parameters measured in the short rains were significantly different between clean and infected materials. The crop was exposed to dry conditions during which CMD symptoms are more apparent. This had an effect on the yield given that viral infection during the early stages of growth affects the physiological processes that determine the ultimate yield (Beck and Chant, 1958).

Yield losses due to CMD were generally higher when compared to those of CBSD save for number of marketable roots. This is due to the effects of CBSD on root quality that renders them unusable and unmarketable (Nichols, 1950). When yield was subjected to per variety analysis, the root weight in Kibandameno was lower in CBSD infected plants than in the CMD infected ones possibly due to the higher severity of the root symptoms. The marketability of the roots was reduced by the severe constrictions and pitting an observation that has also been reported in Malawi (Shaba *et al*., 2002).

Yield losses due to CMD reported in this study agrees with the earlier reports on reduced weight (Beck and Chant, 1958; Seif, 1982; Osiru *et al*., 1999 and Owor *et al*., 2004). Owor *et al*. (2004) also reported a reduction in the number of roots. The losses however vary depending on the variety (Terry and Hahn, 1980), age of the crop at infection

(Fargette *et al.*, 1988), the distribution of infected plants within the field (Osiru *et al.*, 1999) and the strain of the virus (Owor *et al.*, 2003).

Both CMD and CBSD induce chlorotic symptoms on cassava foliage. Co-occurrence of the two diseases may lead to masking of CBSD. The CMD can however be visually detected as it causes leaf distortion in addition to the leaf chlorosis. In such plants, the presence of CBSD can only be detected in plants that develop stem lesions. This masking effect has also been reported in Tanzania (Legg and Raya, 1998). Plants co-infected with both CMD and CBSD had the highest yield reduction indicating some form of interaction between the two viruses. Mixed virus infections have been known to occur and may have biological and epidemiological effects. For the case of CMD and CBSD, it is a co-infection with a geminivirus and an ipomovirus. In most of the documented cases of viral synergism, the titre of the co-infecting virus is known to increase while that of the other virus remains constant or declines altogether (Goldberg and Brakke, 1987; Vance *et al.*, 1995; Pruss *et al.*, 1997; Shceets, 1998). The masking of CBSD symptoms observed in the vegetative stage of this study may be indicative of an increased titre of the CMGs. This could account for the high yield loss in co-infected plants.

The dual infection could be one of the explanations behind the increased disease incidence and declining cassava productivity in coastal Kenya in the recent past. The diseased plants are the main reservoirs of the disease as the region is not associated with a high vector population. Alternatively, the dual infection could be increasing the efficiency of the vector in disease transmission.

Conclusions and Recommendations

Co-infection with both CMD and CBSD poses a challenge to the management of the two diseases in. The most appropriate approach is the management of the disease complex rather than a single disease as suggested by Hillocks and Jennings (2003). This is further confirmed by an attempt made in Tanzania where a CBSD resistant variety was introduced but turned out to be highly susceptible to mosaic thus the productivity was not restored (Kanju *et al.*, 2002). The most effective method will be to breed for resistance against the two diseases either by convectional breeding or genetic engineering approaches.

The observation made in Guzo whereby only one lower leaf remained infected in the early stages of growth could be as a result of vector transmission coupled with the ability of the virus to remain localised before eventual translocation to other leaves. It is evident from the results that there is some form of interaction between the two diseases. The co-infection comprises of a geminivirus and an ipomovirus, which are incidentally transmitted by a common vector, the *Bemisia tabaci*. Though there are many reports on dual infections involving a potyvirus there is no other reported case involving a geminivirus and an ipomovirus. Therefore, there is need for further investigations into this phenomenon with regard to the effect of co-infection on the efficiency of the vector to transmit either one or all of the pathogens involved and the titre of the viruses involved.

6. 0 GENERAL DISCUSSION AND CONCLUSION

The coastal Kenya is characterized by erratic rainfall and is prone to food shortages and poverty levels of about 60% are reported. The region is suitable for cassava cultivation given the ability of the crop to adapt to ecological conditions that can hardly support most of the common food crops. Cassava is therefore an important food security crop and a source of income to many small-scale farmers in coastal Kenya. However the yields realized are only 30% of the region's potential (Munga, 2002).

Cassava mosaic and cassava brown streak diseases have a negative effect on cassava production in coastal Kenya. They cause direct yield reduction and a decline in crop acreage. Farmers lack knowledge on management of these diseases. In addition, some of the varieties popular with farmers in the region are susceptible to the two diseases. Development of these varieties dates back to 1920s-1940s in Amani Tanzania (Hillocks and Jennings, 2003). Therefore, it is important for researchers to educate farmers on disease identification and management. Resistant varieties should also be developed and participatory evaluation adopted. This will ensure that the released varieties meet the farmer preferred attributes. These strategies will restore cassava productivity thus surplus yields may be realized which can be utilized in cassava based industries. In coastal Kenya, cassava is processed into chips, crisps and flour for human consumption by small-scale processors (Kiura *et al.*, unpublished).

Increased cassava productivity coupled with sound disease management and high yielding varieties will lead to increased yields in coastal Kenya. The surplus yield can be used for income generation through direct sales and value added products.

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APPENDICES

Appendix i

1.0 Cassava Mosaic Disease Survey-Questionnaire for producers

i. Field Number: ----------------District----------------Altitude-------

ii. Name of the farmer---------------Age-------

iii. Gender; male-----female----

1. What variety of cassava do you plant on this farm (Greatest to the least)?

Major characteristics of the variety Var. 1 ------------------------
----

Var. 2 --

Var. 3 --

Var. 4 --

3. Where do you usually obtain planting material? --------------------------------

----

----

4. Interviewer shows cassava plant with virus symptoms, and asks them what causes the disease. If there are no virus-diseased plants present, show them a picture.

(a) Can the producer recognize the disease? Yes-------------- (01), No------------- (02)

(b) What do you call the disease (local name------------------------meaning

(c) What causes/spreads it? --?

----

5. Is the disease a problem in your farm? Yes-------- (01) NO -------- (02) --------Don't know------ (03)

6. Does the problem appear every year? Yes---------------- (01) No--------------- (02)

7. What months is this disease severe? --

8. List methods you use to control the disease problem in order of importance (1=the most important): and give scores for their effectiveness in controlling the problem:

(1) Very effective; (2) Partly effective; (3) Not effective (4) Damaging

Control method	effectiveness
1. ----------------------------	------------------------------------
2. ----------------------------	------------------------------------
3. ---------------------------	------------------------------------
4. ---------------------------	------------------------------------

9. Do you ever plant these (diseased) plants? Yes--------- (01), No------------- (02)

10. Do you ever pull these (diseased) plants out? Yes------ (01) No------------ (02)

If yes, when-

11. Do you rotate cassava with other crops? Yes----No-----, if yes which ones? ------------

12. Are some varieties more severely damaged than others? Yes------- (01) No-------- (02)

13.a) If you have noticed differences, why do you grow the ones that are more diseased? ----------------------

----

----

b) Why do you think some varieties are more diseased than others? ---

----

----

14. Do you have any good resistant varieties? Yes------------ (01), No----------- (02)

If yes, give names--

----

15. Which other good traits does the variety have? ---

----

16. Would you be interested in receiving a new variety? Yes---------- (01), No--------- (02)

If yes, what characteristics would want in the new variety? (List them in order of importance)

(a)---

(b)---

(c)---

(d)---

(e)---

(f)---

16. Cropping system; Monoculture--------- (01),

Mixed cropping--------------- (02), with; -------------

COMMENTS OR OBSERVATIONS

Thank you

Appendix 2

2.0 Weather data for KARI-Mtwapa during the two seasons

Mean monthly temperatures (°c)

MAP / Season	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Short rain	28.0	92.0	4.6	28.0	28.7	28.5	27.6	26.5	25.3	25.0
Long rain	28.5	27.6	26.5	25.3	25.0	25.0	25.5	25.9	26.8	27.8

Total monthly rainfall (mm)

MAP / Season	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Short rain	28.0	92.0	4.6	1.0	1.6	49.7	321.2	305.9	158.6	103.0
Long rain	49.7	321.2	305.9	158.6	103.0	88.3	143.1	282.6	343.1	49.7

Appendix 3

3.0 ANALYSIS OF VARIANCES

3.1 RESPONSE OF LOCAL GERMPLASM TO CO-INFECTION WITH CASSAVA MOSAIC AND CASSAVA BROWN STREAK DISEASES

i) SHORT RAINS SEASON

Dependent valuable: CMD incidence

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Month After Planting (MAP)	7	74181.15	10597.31	383.45	<.0001
Block	2	394.73	197.37	7.14	0.0010
Variety	2	33747.51	16873.76	610.55	<.0001
Treatment	3	136103.30	45367.77	1641.56	<.0001
MAP*Variety*Treatment	83	27984.60	337.16	12.20	<.0001

Dependent Variable: CMD severity

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Month After Planting (MAP)	7	239.78	34.25	570.24	<.0001
Block	2	0.21	0.10	1.72	0.1824
Variety	2	43.29	21.64	360.33	<.0001
Treatment	3	21.11	7.04	117.15	<.0001
MAP*Variety*Treatment	83	32.22	0.39	6.46	<.0001

Dependent Variable: CBSD Incidence

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Month After Planting (MAP)	7	42637.54	6091.08	313.26	<.0001
Block	2	308.65	154.32	7.94	0.0005
Variety	2	320178.67	160089.33	8233.24	<.0001
Treatment	3	56364.27	18788.09	966.25	<.0001
MAP*Variety*Treatment	83	69365.61	835.73	42.98	<.0001

Dependent Variable: CBSD severity

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
MAP	7	101.15	14.45	435.51	<.0001
Block	2	0.76	0.38	11.51	<.0001
Variety	2	262.83	131.42	3960.76	<.0001
Treatment	3	1.15	0.38	11.52	<.0001
MAP*Variety*Treatment	83	61.89	0.75	22.47	<.0001

Dependent Variable: Height

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Month After Planting (MAP)	7	492943.26	70420.47	1473.26	<.0001
Block	2	748.22	374.11	7.83	0.0005
Variety	2	6346.89	3173.44	66.39	<.0001
Treatment	3	9250.46	3083.49	64.51	<.0001
MAP*Variety*Treatment	83	16039.14	193.24	4.04	<.0001

Dependent Variable: Root length

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Block	2	34.41	17.20	1.05	0.3665
Variety	2	344.09	172.05	10.51	0.0006
Treatment	3	581.72	193.91	11.85	<.0001
Variety*Treatment	6	230.88	38.48	2.35	0.0661

Dependent Variable: Number of roots

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Block	2	0.13	0.07	0.08	0.9269
Variety	2	18.43	9.22	10.76	0.0005
Treatment	3	102.22	34.07	39.79	<.0001
Variety*Treatment	6	23.75	3.96	4.62	0.0035

Dependent Variable: Number of marketable roots

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Block	2	0.29	0.14	0.21	0.8151
Variety	2	34.84	17.42	25.07	<.0001
Treatment	3	50.60	16.87	24.28	<.0001
Variety*Treatment	6	15.20	2.53	3.65	0.0115

Dependent Variable: root weight

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Block	2	0.46	0.23	0.28	0.7602
Variety	2	15.75	7.87	9.53	0.0010
Treatment	3	17.45	5.82	7.04	0.0017
Variety*Treatment	6	4.68	0.77993241	0.94	0.4840

ii) LONG RAINS SEASON

Dependent Variable: CMD Incidence

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Month After Planting (MAP)	7	74181.15	10597.31	383.45	<.0001
Block	2	394.73	197.37	7.14	0.0010
Variety	2	33747.51	16873.76	610.55	<.0001
Treatment	3	136103.30	45367.77	1641.56	<.0001
MAP*Variety*Treatment	83	27984.60	337.16	12.20	<.0001

Dependent Variable: CMD severity

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Month After Planting (MAP)	7	239.78	34.25	570.24	<.0001
Block	2	0.21	0.10	1.72	0.1824
Variety	2	43.29	21.64	360.33	<.0001
Treatment	3	21.11	7.04	117.15	<.0001
MAP*Variety*Treatment	83	32.22	0.39	6.46	<.0001

Dependent Variable: CBSD Incidence

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Month After Planting (MAP)	7	42637.54	6091.08	313.26	<.0001
Block	2	308.65	154.32	7.94	0.0005
Variety	2	320178.66	160089.33	8233.24	<.0001
Treatment	3	56364.27	18788.09	966.25	<.0001
MAP*Variety*Treatment	83	69365.61	835.73	42.98	<.0001

Dependent Variable: CBSD severity

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Month After Planting (MAP)	7	101.15	14.45	435.51	<.0001
Block	2	0.76	0.38	11.51	<.0001
Variety	2	262.83	131.42	3980.76	<.0001
Treatment	3	1.15	0.38	11.52	<.0001
MAP*Variety*Treatment	83	61.89	0.75	22.47	<.0001

Dependent Variable: Height

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Month After Planting (MAP)	7	492943.26	70420.47	1473.26	<.0001
Block	2	748.22	374.11	7.83	0.0005
Variety	2	6346.89	3173.44	66.39	<.0001
Treatment	3	9250.46	3083.49	64.51	<.0001
MAP*Variety*Treatment	83	16039.14	193.24	4.04	<.0001

Dependent Variable: Root length

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Block	2	26.54	13.27	0.60	0.5593
Variety	2	568.16	284.08	12.77	0.0002
Treatment	3	699.96	233.32	10.49	0.0002
Variety*Treatment	6	185.58	30.93	1.39	0.2624

Dependent Variable: Number of roots

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Block	2	2.58	1.29	1.20	0.3210
Variety	2	24.01	12.01	11.12	0.0005
Treatment	3	67.09	22.36	20.72	<.0001
Variety*Treatment	6	5.64	0.94	0.87	0.5313

Dependent Variable: Number of marketable roots

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Block	2	3.97	1.99	1.68	0.2089
Variety	2	50.77	25.38	21.51	<.0001
Treatment	3	46.24	15.41	13.06	<.0001
Variety*Treatment	6	3.64	0.61	0.51	0.7907

Dependent Variable: root weight

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Block	2	1.65	0.83	1.21	0.3172
Variety	2	8.05	4.02	5.89	0.0089
Treatment	3	17.60	5.87	8.59	0.0006
Variety*Treatment	6	5.60	0.93	1.37	0.2715

3.2 THE ROLE OF PHYTOSANITATION(SELECTION OF PLANTING MATERIAL AND ROGUING IN THE MANAGEMENT OF CASSAVA MOSAIC DISEASE

i) SHORT RAIN SEASON

Dependent Variable: CMD Incidence

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Month After Planting (MAP)	7	27170.56	3881.51	44.08	<.0001
Block	3	1432.28	477.43	5.42	0.0018
Treatment	3	9588.80	3196.27	36.30	<.0001
MAP*Treatment	21	2233.94	106.38	1.21	0.2633

Dependent Variable: CMD severity

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Month After Planting (MAP)	7	108.99	15.57	261.01	<.0001
Block	3	1.41	0.47	7.88	<.0001
Treatment	3	1.86	0.62	10.37	<.0001
MAP*Treatment	21	0.42	0.02	0.34	0.9970

Dependent Variable: plant height

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Month After Planting (MAP)	7	203973.87	29139.12	645.67	<.0001
Block	3	55.05	18.35	0.41	0.7486
Treatment	3	172.09	57.36	1.27	0.2889
MAP*Treatment	21	771.16	36.72	0.81	0.6967

Dependent Variable: Number of roots

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Block	3	6.39	2.13	1.01	0.4321
Treatment	3	15.22	5.08	2.41	0.1344

Dependent Variable: Number of marketable roots

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Block	3	2.35	0.78	0.45	0.7259
Treatment	3	9.93	3.31	1.89	0.2022

Dependent Variable: root weight

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Block	3	0.82	0.27	0.22	0.8819
Treatment	3	2.80	0.93	0.74	0.5521

ii) LONG RAIN SEASON

Dependent Variable: CMD Incidence

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Month After Planting (MAP)	7	10838.51	1548.36	80.82	<.0001
Block	3	36.33	12.11	0.63	0.5961
Treatment	3	171.15	57.05	2.98	0.0355
MAP*Treatment	21	669.12	31.86	1.66	0.0515

Dependent Variable: CMD severity

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Month After Planting (MAP)	7	91.22	13.03	210.29	<.0001
Block	3	0.82	0.27	4.38	0.0062
Treatment	3	1.06	0.35	5.71	0.0012
MAP*Treatment	21	1.18	0.06	0.90	0.5865

Dependent Variable: Height

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Month After Planting (MAP)	7	227558.82	32508.40	138.68	<.0001
Block	3	5341.85	1780.62	7.60	0.0001
Treatment	3	1212.95	404.32	1.72	0.1673
MAP*Treatment	21	2495.00	118.81	0.51	0.9612

Dependent Variable: Root length

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Block	3	73.02	24.34	0.73	0.5616
Treatment	3	62.33	20.78	0.62	0.6196

Dependent Variable: Number of roots

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Block	3	6.85	2.28	2.88	0.0956
Treatment	3	4.11	1.37	1.73	0.2311

Dependent Variable: Number of marketable roots

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Block	3	0.10	0.03	3.24	0.0744
Treatment	3	0.76	0.25	24.38	0.0001

Dependent Variable: root weight

Source	DF	ANOVA SS	Mean Square	F Value	Pr > F
Block	3	0.76	0.25	0.97	0.4468
Treatment	3	1.41	0.47	1.81	0.2163

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