ASSESSMENT OF CASSAVA GENOTYPES FOR AGRONOMIC PERFORMANCE AND RESISTANCE TO CASSAVA MOSAIC AND CASSAVA BROWN STREAK DISEASES IN WESTERN KENYA

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(Plant Breeding)

JOMO KENYATTA UNIVERSITY OF

AGRICULTURE AND TECHNOLOGY

2021

Assessment of Cassava Genotypes for Agronomic Performance and Resistance to Cassava Mosaic and Cassava Brown Streak Diseases in Western Kenya

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A thesis submitted in partial fulfilmentof the Requirements for the degree of Master of Science in Plant Breeding of the Jomo Kenyatta University of Agriculture and Technology

2021

DECLARATION

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

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DEDICATION

This work is dedicated firstly, to my Lord Almighty God in Christ Jesus, for strength, favour and perfect health during the entire course of this study. Secondly, to my Late father, Mwalimu Johann Nyongesa Navangi and my mother Ruth Nasimiyu Navangi for inspiration. Thirdly, to my children, Silas, Paul, Raphael and Esther and my brother Edwin for their immense encouragement and support. My husband, Dr. Patrick Ongadi Mudavadi whose immense love and support made this journey bearable. Finally, to my dear grandmother, Bilha Nasimiyu for consistently praying for me and encouragement. I attribute my current success individually and collectively to them.

ACKNOWLEDGEMENTS

First, I wholeheartedly thank the Almighty God in the name of Jesus Christ for granting me good health, strength and perseverance to undertake this research to completion. I am immensely thankful to the International Institute of Tropical Agriculture (IITA) and the Bill and Melinda Gates Foundation under the "The New Cassava Varieties and Clean Seed to Combat CBSD and CMD (5CP) Project, for the MSc scholarship and funding of the research.

I also sincerely thank the Director General, KALRO for permission to undertake the studies, and the staff of KALRO-Kakamega, KALRO-Embu, KALRO-Kibos and KALRO-Alupe for providing technical and logistical assistance during the study. I am greatly thankful to the Department of Horticulture, Jomo Kenyatta University of Agriculture and Technology (JKUAT) for admission to study MSC in Plant Breeding and for providing excellent institutional and professional support. During this research. Special thanks goes to my university supervisor, Prof. Githiri Stephen Mwangi for excellent support and guidance throughout this research.

Special gratitude goes out to my other supervisors Dr. Edward Eneah Kanju (IITA, Tanzania) and Dr. Theresia L. Munga (KALRO ICRC, Mtwapa); and mentors Prof. Elijah Miinda Ateka and Dr. Silver Tumwegamire (IITA) for their support, advice and efforts in supervision. Dr. Edward was a true inspiration in unwavering commitment to support my studies. I am also indeed thankful to Dr. Philip Onyimbo Kwena (KALRO-Kakamega) for assistance in data analysis. Finally, I extend my gratitude to my dear husband, Dr. Patrick Ongadi Mudavadi, colleagues at the Department of Horticulture JKUAT, Researchers and staff at Embu, Kibos, Kakamega and Alupe KALRO Centres for their invaluable support during the entire period of studies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND ACRONYMS

ACMV	African Cassava Mosaic Virus
ANOVA	Analysis of Variance
AMMI	Additive Main effect and Multiplicative Interaction
ASV	AMMI Stability Value
BMGF	Bill and Melinda Gates Foundation
5CP	New Cassava varieties and Clean Seed to Combat CBSD and CMD (5CP) Project
CBSD	Cassava Brown Streak Disease
CBSVs	Cassava Brown Streak Viruses
CI	Confidence Interval
CMD	Cassava Mosaic Disease
CMVs	Cassava Mosaic Viruses
ECA	East and Central Africa
EACMV	East African Cassava Mosaic Virus
ESA	Eastern and Southern Africa
FAO	Food and Agriculture Organization of the United Nations
GEI	Genetic and Environment Interaction

GTIL	Genetic Technologies International Laboratories
IITA	International Institute for Tropical Agriculture
KALRO	Kenya Agricultural and Livestock Research Organization
MAP	Months after Planting
NARS	National Agricultural Research Systems
RCBD	Randomized Complete Block Design
UCBSV	Ugandan Cassava Brown Streak Virus

ABSTRACT

Cassava is an important food crop in sub-Saharan Africa. More than a third of the region's potential cassava harvest is continuously lost to pest and diseases. The most important of these are the cassava viruses: cassava mosaic geminiviruses (CMGs) and cassava brown streak viruses (CBSVs). The objectives of the study were to: (1) evaluate 23 elite cassava genotypes for variation in CMD and CBSD resistance parameters at Alupe, Kakamega and Kibos in Western Kenya; and (2) assess the 23 elite cassava genotypes for variation in agronomic traits and correlate this to CMD and CBSD resistance parameters. The study used twenty-three (23) elite cassava genotypes that had shown promise in terms of their resistance to both CMD and CBSD, and had been officially released or were in the final stages of official release in the five cassava project (5CP) countries, namely Kenya, Malawi, Mozambique, Tanzania and Uganda. The genotypes were evaluated using balanced Alpha Lattice design, with three replicates at Alupe, Kakamega and Kibos for an extended cropping cycle between 2015/16 and 2016/17. Data was collected at 1 month after planting (1 MAP), 3 MAP, 6 MAP, 9 MAP and 12 MAP, and analyzed using generalized linear models using Genstat Version 15 software. Mean separation was carried out using least significant difference (LSD) at $P \leq 0.05$ significance level. Whiteflies (*Bemisia tabaci Genn.*) abundance was significantly ($P \leq 0.05$) influenced by genotype, environment (location), month after planting (MAP) time and interaction. Agronomic traits were highly influenced ($P \leq 0.05$) by the location, and less ($P \geq 0.05$) by genotype and interaction. All genotypes were considered sweet as cyanide content score was between 3.00 and 6.00 across the three locations. Mean DM yield (t ha^{-1}) was 5.49, but 3.69, 4.65 and 8.14 at Alupe, Kakamega and Kibos respectively. Mean CMD and CBSD incidence was 0.60 and 0.84, respectively across the three locations. Similarly, mean CMD and CBSD severity was 1.09 and 1.13, respectively across locations. Mean CMD and CBSD incidence and severity was higher in Alupe, compared to Kakamega and Kibos. There was significant ($P \leq 0.05$) negative association between CMD, CBSD incidence and severity with all agronomic parameters evaluated. Additive main effect and multiplicative interaction (AMMI) model detected highly significant ($P \leq 0.001$) environmental effects in response to CMD and CBSD severity and incidence, biomass and fresh root yield. Highly significant variation ($P \leq 0.001$) was detected by the AMMI model against genotype, environment and GEI in response to CMD incidence and severity. The AMMI analysis for all traits studied showed that more than 50% of the variation in GEI sum of squares (SS) was accounted for by integrated principal component analysis (IPCA1). IPCA2 were non-significant, indicating that they largely captured random error. AMMI stability values (ASV) were used to determine stable genotypes for dual disease resistance and agronomic performance at Alupe, Kakamega and Kibos. Combined (dual) resistance was, therefore confirmed for CMD and CBSD incidence and not severity. Therefore, best stable genotypes based on combined ASV and ranking in response for dual resistance to both CMD and CBSD incidence across the three locations were Colicanana, F10-30-R5, Orera, Tajirika and Kizimbani. Best stable genotypes in response to agronomic performance across the three locations were Nase-18, F10-30-R5, Nase-3, Eyope and

Tajirika. Finally, based on combined ASVs and ranking, best stable genotypes in response to dual resistance for both CMD and CBSD incidence and agronomic performance across the three locations were KBH/2002/066, Kizimbani, Nase-18, F10-30-R5, Tajirika, CH05-203, Nase-3, Eyope and Orera. It is recommended that these stable elite cassava genotypes be further screened for wider adaptability and dual resistance to CBSD and CMD, including agronomic performance under farmer conditions in diverse farming systems, vector and disease pressures, for the possibility of future varietal release.

Key Words: Agronomic; AMMI; CBSD; CMD; Environment; Evaluation; Genotypes; Incidence; Location; Performance; Severity; Stability Value

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Cassava (*Manihot esculenta* Crantz) is an important food crop in Africa (Tumwegamire *et al*. 2018), ranking number one among the root crops with up to 185 MT of annual root production (Avijala *et al*., 2015). Africa alone produces more than 55% of the world's cassava (Legg *et al.,* 2014). Cassava is a major source of carbohydrates in the diet of millions of people and is grown as a famine reserve crop owing to its tolerance to harsh environmental conditions (Ribaut, *et al.*, 2010; Zhang *et al*. 2015). However, according to Legg *et al*., 2014, this production is direly threatened by Cassava Brown Steak Disease (CBSD) and Cassava Mosaic Disease (CMD). The two diseases, both or singularly are the current principal biotic factors affecting cassava production in East and Southern Africa, with high risks of spreading to the western Africa sub-region if not contained (Legg *et al.,* 2014). Cassava mosaic disease (CMD) is present in all cassava-growing countries in Africa and causes losses of at least 145-180 MT each year (Legg & Thresh, 2003; Tembo, *et al*., 2017). The disease is triggered by the emergence and spread of at least eight species of geminiviruses transmitted by whiteflies (*Bemisia tabaci Genn.*) and disseminated through infected cuttings (Kuria *et al*. 2017; Thresh and Cooter 2005). It first appeared in 1894 in Tanzania, and since then several CMD epidemics in Africa have been reported (Jeremiah *et al.*, 2015; Pita *et al*., 2001). The most recent outbreak and by far the most economically important began in Uganda in the late 1980s (Legg and Thresh, 2003; Uzokwe *et al.,* 2016). The disease has subsequently invaded most of East and Central Africa (ECA). The pandemic of severe CMD has been reported in 12 African countries, including Cameroon in West Africa, and continues to spread (Bart and Taylor, 2017; Legg *et al*., 2014; Legg and Thresh, 2003) and has also been reported in south India and Sri Lanka (Legg *et al.,* 2014; Mignouna and Dixon, 1997; Stansly *et al.*, 2010).

A new cassava virus disease namely, cassava brown streak disease (CBSD), currently presents the greatest threat to cassava production in ECA (Legg *et al.*, 2014). CBSD is caused by two species of ipomoviruses (Legg et al., 2014). First reported in coastal East Africa , the disease began to spread in the Great Lakes region of East Africa in 2003 (Jeremiah *et al.*, 2015; Legg and Thresh, 2003). CBSD has a limited effect on the growth and appearance of plants, but can be catastrophic for production as the dry rot that it produces in tuberous roots can render entire crops unusable (Legg *et al.,* 2014). Both cassava mosaic geminiviruses (CMGs) and cassava brown streak viruses (CBSVs) are transmitted by the whitefly vector *Bemisia tabacii,* and perpetuated through infected cuttings (Hillocks *et al*. 2000; Jeremiah *et al*. 2015; Njoroge *et al.* 2016). The increasing spread of super-abundant whiteflies raises justifiable fears that CMD and CBSD will spread further on the African continent and worldwide (Stansly *et al.,* 2010). This would have obvious major and unanticipated consequences for food security, economic development, and social stability in many countries, as much of the world's cassava germplasm is highly susceptible to these viruses (Legg *et al*., 2014; Legg and Thresh, 2003). Moreover, global warming is likely to exacerbate the situation because higher temperatures will favor the whitefly vector (Njoroge *et al*. 2016). This potential additional impact from pest and disease is more significant as cassava is one of the very few crops that may otherwise be relatively unscathed by future patterns of climate (Legg *et al*., 2014). Although high levels of genetic improvement have been achieved for CMD, the ravaging scourge of CBSD in ECA threatens to erode genetic gains made in the fight against CMD (Kizito, 2006). The most applicable control strategy is a combination of tactics that include cultural practices and use of virus-resistant (Tumwegamire *et al.* 2018). Breeding for dual resistance is currently being pursued as the most sustainable way to tame devastating effects of the two diseases (Tumuhimbise, 2013).

1.2 Statement of the Problem

Cassava production faces several constraints among which include socio-economic factors, market conditions, biotic and abiotic factors. Cassava mosaic and brown streak diseases, singularly or both are the current principal biotic factors affecting cassava production in eastern and southern Africa (Legg *et al.*, 2014). Yield losses of up to 70% associated with CBSD have been reported from Africa. Further, losses from CMD in Africa have been estimated to be 12-23 million tons of harvestable roots annually, equivalent to US$ 1.2-2.3 million (Uzokwe *et al.*, 2016). Generally, the two viral diseases, CMD and CBSD, have a major impact on cassava production in sub- Saharan Africa, together causing estimated losses of US$1 billion per year (Bart and Taylor, 2017). A recent report from Kenya estimates US$1,300/hectare losses (Bart and Taylor, 2017; Masinde *et al*., 2016). Both viruses are transmitted by the whitefly vector *Bemisia tabacii*. Therefore, dual infection of CBSD and CMD diseases could potentially devastate cassava production with up to 100% yield loss. There is another concern that CBSD is spreading beyond its relatively limited distribution in south eastern Africa and, the Great Lakes region, with high risks of spreading to the western Africa sub-region if not contained. This study, therefore intends to contribute to knowledge that will further development of more productive and resistant genotypes and, ultimately effective management of two of Africa's most pernicious threats to food security.

1.3 Justification

This study was conducted as part of on-going research on cassava under the "New Cassava Varieties and Clean Seed to Combat CBSD and CMD (5CP) Project funded by the Bill and Melinda Gates Foundation. The project aimed to improve cassava productivity and food security in Eastern and Southern Africa (ESA) region by deploying new virus-free cassava varieties that have dual resistance to cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) and by developing novel systems for producing clean breeders' seed (Tumwegamire *et al*., 2018). Breeding for

dual resistance could, therefore, be defined as the control of resistance to CMD and CBSD, that is genetically linked or random occurrence, whenever, CMD and CBSD is present in a genotype. The Bill and Melinda Gates Foundation (BMGF) has already played a key role in supporting a diverse set of cassava virus disease control initiatives, ranging from upstream biotechnology research to more development-oriented field programs. However, new opportunities exist, and must be exploited maximally if the regional impact of CBSD is to be mitigated as rapidly as possible (Legg et al., 2014; Legg et al., 2006). The desired goal of breeding efforts is to produce stable resistant genotypes, and breeding for dual resistance is currently being pursued as a sustainable way to tame the devastating effects of both CMD and CBSD (Tumwegamire *et al.*, 2018). Twenty-three (23) clones (varieties) that had shown great promise in terms of their resistance to both CMD and CBSD had been officially released or were in the final stages of official release in Kenya, Malawi, Mozambique, Tanzania and Uganda. A large number of highly promising new clones are in the final stages of official release (Tumwegamire *et al*., 2018). Much of the final stage varietal development work remains poorly resourced (Legg et al., 2014). The entire CBSD-affected region stands to benefit greatly by sharing these elite varieties amongst the National Agricultural Research Systems (NARS). Systems for providing clean, virus-tested cassava planting material (hereafter referred to as seed) could therefore play a critically important role in supplementing varietal resistance in the control of cassava viruses, thereby boosting production in cassava-producing countries. However, the performance of most cassava varieties/genotypes in disease hotspot areas is influenced by environmental factors (rainfall, temperature regimes and soil types), a phenomenon called genotype by environment interaction (GEI). Therefore, there is need to test varieties in varying agro-climatic conditions in order to identify those with specific and those with wide adaptation and hence this study is proposed.

1.4 Objectives of the Study

1.4.1 Overall Objective

To contribute towards production of high yielding cassava clones with resistance to CMD and/or CBSD at Alupe, Kakamega and Kibos in Western Kenya region.

1.4.2 Specific Objectives

1) To evaluate twenty-three (23) elite cassava genotypes for CMD and CBSD resistance traits and agronomic performance at Alupe, Kakamega and Kibos
2) To determine the relationship between agronomic traits and CMD and CBSD resistance traits for the twenty-three (23) elite cassava genotypes at Alupe, Kakamega and Kibos.
3) To evaluate the stability of elite cassava genotypes across Alupe, Kakamega and Kibos.

1.5 Null Hypotheses of the Study

1) There were no differences amongst the 23 elite cassava genotypes for CMD and CBSD resistance traits and agronomic performance at Alupe, Kakamega and Kibos.
2) There were no differences in the relationship between agronomic traits with CMD and CBSD resistance traits for the elite cassava genotypes at Alupe, Kakamega and Kibos.
3) There were no differences in the stability of elite cassava genotypes across Alupe, Kakamega and Kibos.

CHAPTER TWO

LITERATURE REVIEW

2.1 Cassava: Origin and Uses

Cassava (*Manihot esculenta* Crantz), belongs to the family *Euphorbaiceae*, synonyms: yucca, manioc, and mandioca). It is native to South America, and is believed to have been introduced into sub-Saharan Africa by the Portuguese traders during the 16th century (Mignouna and Dixon, 1997). According to the Food and Agriculture Organization of the United Nations (FAO), cassava is currently the third most important source of calories in the tropics, after rice and maize, and more than 800 million people use it as a source of food and income generation in Africa, Asia, and Latin America (Liu, 2017). Among the cassava-growing regions of the world, Africa accounts for more than 50% of the global cassava root production of 233.8 million metric tons (Tumwegamire *et al*., 2018). The resilience of cassava enables it to grow successfully under a wide range of agro-ecological zones where cereals and other crops cannot thrive, making it a suitable crop for resource poor farmers to cultivate under marginal environments in Africa (Chikoti, *et al.*, 2019; Patil and Fauquet, 2009; Tumwegamire *et al.*, 2018). Cassava is cultivated as a tuberous root crop and its roots are a major source of dietary starch (Legg *et al.*, 2014; Mignouna and Dixon, 1997). The tubers are eaten fresh and in various forms of processed food (Mignouna and Dixon, 1997). Cassava is grown in sub-Saharan Africa by resource-poor farmers, many of them women, as an intercrop with vegetables, plantation crops (coconut, oil palm and coffee), yams, melon, sweet potato, maize, sorghum, millet, cowpea, groundnut (peanut), and other legumes for food security and assures household income (Isabella, *et al.*, 2017; Slakie, *et al.*, 2013; Uzokwe *et al*., 2016). Cassava leaves are also consumed as a green vegetable, especially in East Africa, to provide an important source of proteins, minerals, and vitamins (Kizito, 2006). Several African countries are gradually replacing wheat flour with cassava flour in the production of staples like bread and noodles (Noorfarahzilah, *et*

al., 2014; Onyenwoke and Simonyan, 2014). However, there is an increased awareness that excessive reliance on cassava could lead to malnutrition in these countries, since the tubers are a poor source of protein, vitamins A and E, iron, and zinc (Kizito, 2006). In recent years, cassava has been increasingly used as raw material in the manufacture of various industrial products such as starch and flour (Masumba *et al.*, 2017; Tumwegamire et al., 2018). With increased prospects of starch from cassava as a source of ethanol for bio-fuels, its cultivation is transforming from subsistence to a more commercially-oriented farming enterprise (Mabasa, 2007). Consequently, this has resulted in continuous increase in cassava acreage has been increasing throughout Africa (Titus, *et al*., 2011).

2.2 Production and Economic Importance of Cassava

Cassava plays a key role in food security and in income generation for the small scale farmers of the eastern Africa region (Gedil & Sartie, 2010). It is a hardy crop that gives a decent harvest amidst erratic rainfall and infertile soils. Therefore, improvement of the cassava production systems can be a pathway to food security and adaptation to climate change (Legg *et al*., 2014). In the East African region, cassava productivity is 10 t/ha (that is 9.8 t/ha in Tanzania, 10.6 t/ha in Kenya and 12 t/ha in Uganda (Alene, *et al.*, 2013; Kintché *et al*., 2017). According to Tesfaye *et al*., 2017, these yields are about half of those obtained in some South Asian countries, such as China (16.3 t/ha), Indonesia (16.2 t/ha), Thailand (22.9 t/ha) and India (31.4 t/ha). The low yields in East Africa are caused by an array of factors including susceptibility of commonly grown varieties to major diseases and pests, and variability in climate patterns (Kintché *et al*., 2017; Munyahali, *et al*., 2017). Among the major diseases, viral diseases are the most important in the region (Bart and Taylor 2017; Legg *et al*. 2014; Nduwumuremyi *et al.*, 2017; Sserubombwe *et al.*, 2008; Tumwegamire *et al.*, 2018; Uzokwe *et al*., 2016).

2.3 Viral Diseases Affecting Cassava

Viral diseases, especially cassava mosaic disease and cassava brown streak disease are important biotic constraints to cassava production in East Africa (Hillocks *et al.*, 2000). It has been estimated that CMD causes an estimated yield loss of over US$ 14 million per annum (Bull *et al.* 2006; Tembo *et al.* 2017; Thresh and Cooter 2005), while CBSD causes an estimated yield loss of up to 70% (Abaca *et al.*, 2012; Katono *et al.*, 2015; Ndyetabula *et al.*, 2016). CMD is associated with three virus species, African cassava mosaic virus (ACMV), East African cassava mosaic virus (EACMV) and East African cassava mosaic virus-Uganda variant (UgV), which are widely distributed wherever cassava is grown (Hillocks *et al.*, 2000; Slakie *et al.*, 2013), and endemic more across tropical Africa (Legg *et al.*, 2006). CBSD has been endemic in western and coastal regions of Kenya and other parts of East Africa (Abaca *et al.*, 2012; Jeremiah *et al.*, 2015; Kawuki *et al.*, 2016; Legg and Thresh, 2003; Tumwegamire *et al.*, 2018).

Generally, losses from CMD in Africa have been estimated to be 12-23 million tons of harvestable roots annually, equivalent to US$ 1.2-2.3 million (Legg and Thresh, 2003). CBSD has two typical effects, reduction of root yield and quality. This in turn affects marketability of the roots (Hillocks *et al.*, 2000). Indeed, yield losses of up to 70% associated with CBSD have been reported (Mware, *et al.*, 2009). The two viral diseases, CMD and CBSD, have a major impact on cassava production in sub- Saharan Africa, together causing estimated losses ofUS$1 billion per year (Tumwegamire *et al.*, 2018). A recent report from Kenya estimates US$1,300/hectare losses (Masinde *et al.*, 2016). Both viruses are transmitted by the whitefly vector *Bemisia tabacii Genn.*

2.4 Aetiology and Effects of Cassava Mosaic Disease on Cassava

Cassava mosaic disease (CMD) is caused by either the African cassava mosaic virus (ACMV), East African cassava mosaic virus (EACMV) or South Africa mosaic virus (SAMV) and is transmitted by whiteflies (Katono *et al.* 2015; Kuria *et al.* 2017; Rwegasira & Rey, 2012). Of these three virus strains, EACMV and ACMV are the most common and important in Africa (Bull *et al.*, 2006; Kuria *et al.*, 2017). In addition to

these viruses, sub-viral catalysts known as satellites cause undesirable effects in cassava plants through virus accumulation and increase the severity of the expression of the symptoms of their helper virus (Chikot, 2011). The satellites associated with CMD were recently discovered and have been reported to enhance disease symptoms in CMD infected cassava plants (Chikot, 2011). Cassava infected plants with either ACMV or EACMV show severe symptoms depending on the cultivar. With the presence of satellites in plants with CMD, symptoms are more severe depending on the virus/combination and host plant (Anjanappa *et al.,* 2016; Chikot, 2011; Hillocks *et al.*, 2000; Thresh & Cooter, 2005). Cassava mosaic disease occurs as a mixed or single infection (Hillocks *et al.*, 2000). Dual infections with two different cassava mosaic germini-viruses (CMGs) cause more severe symptoms than neither virus alone (Legg *et al*., 2011). In Africa, yield losses have been estimated to be between 15 and 40% (Masinde *et al*., 2016; Stansly *et al*., 2010; Thresh and Cooter, 2005). In Zambia, CMD is a major threat to cassava production and is found in all major cassava producing areas (Chikoti *et al*., 2019). It causes yield losses of 50-70% per year (Tumwegamire *et al*., 2018).The yield loss is a result of viruses interfering with photosynthetic processes in the leaves thereby leading to stunted plants and reduced storage root size and quality (Anthony *et al*., 2015; Dubey, 1999; Kizito, 2006; Slakie *et al.,* 2013; Soyode & Oyetunji, 2010) .

2.5 Aetiology and Effects of Cassava Brown Streak Disease on Cassava

Cassava brown streak disease has for a long time been known to be caused by *Cassava brown streak virus* (CBSV) (Tumwegamire *et al*., 2018). Recent findings have indicated that CBSD may sometimes be caused by mixed infection of two viruses, CBSV cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV), with both viruses belonging to the genus *Ipomovirus* in the family *Potyviridae* (Jeremiah *et al.,* 2015; Kawuki *et al*., 2016; Masumba *et al.,* 2017; Rwegasira and Rey, 2012; Tumwegamire *et al*., 2018). The disease was reported for the first time in the East African coast in 1936 (Thresh & Cooter, 2005), as one of the most damaging viral

diseases of cassava (Kawuki *et al.* 2016; Ndyetabula *et al.* 2016; Tumwegamire *et al.* 2018). Although the disease is primarily known to spread through infected planting materials, other researchers have attributed its spread to the whitefly vector, *Bemisia tabacii. Genn* (Jeremiah *et al.*, 2015; Njoroge *et al.*, 2016; Pita *et al*., 2001; Thresh & Cooter, 2005).

The tuberous root yield loss caused by CBSD has been estimated to be more than 70% (Legg, 2009; Stansly *et al.*, 2010). The disease is known to occur in various countries including Kenya, Malawi, Mozambique, Tanzania, Uganda and more recently in Burundi, Democratic Republic of Congo and Rwanda (Legg, 2009; Tumwegamire *et al.*, 2018). Initial description of the disease symptoms were given before 1950s, but more comprehensive descriptions were subsequently provided by (Hillocks *et al*., 2000). There is, however, reported variability in the magnitude of symptom expressions among CBSD-affected cultivars when grown in different environments (Jeremiah *et al*. 2015; Legg *et al.* 2011). Variation among cassava cultivars in expressing root and foliar symptoms of CBSD has been reported, with most susceptible cultivars exhibit pronounced foliar and root symptoms soon after sprouting in the cutting-derived infection (Kawuki *et a*l., 2016; Masumba *et al.*, 2017; Ndyetabula et al. 2016; Tumwegamire *et al.* 2018). Some cultivars develop mild root symptoms without foliar symptoms, and in most instances stem symptoms start as minor necrotic spots which fuse into bigger necrotic lesions culminating into shoot die-back as most of tender portion of stem becomes necrotic (Katono *et al.* 2015; Kawuki *et al*. 2016; Legg *et al.* 2011; Masumba *et al.* 2017; Ndyetabula *et al.* 2016).

2.6 Combined Effects of Cassava Mosaic and Brown Streak Diseases

The effects of a combination of CMD and CBSD diseases on cassava are very complex and can lead to serious yield losses depending on environment, genotypes and virus strains, with the two diseases severely affecting establishment and overall yield (Legg *et al*., 2014; Legg & Thresh, 2003; Tumwegamire *et al*., 2018). The overall effect of

CBSD when combined with cassava mosaic disease can cause up to 90-100% yield loss, due to profound reduction in photosynthetic leaf area (Abaca *et al*. 2012; Anthony *et al*. 2015; Kawuki *et al.* 2016). However, the susceptibility of varieties is genetically governed and some resistant/tolerant clones have been obtained through hybridization (Brumlop & Finckh, 2011; Pita *et al.*, 2001). Both the cassava mosaic geminiviruses (CMGs) and cassava brown streak viruses (CBSVs) are transmitted by the whitefly vector *Bemisia tabacii,* and since the 1990s, more than 100-fold increases in abundance of this insect have been recorded on cassava in parts of East Africa (Avijala *et al.* 2015; Kawuki *et al.* 2016) Although not definitively proven, this increase is believed to be the result of genetic changes in whiteflies that have enabled them to become better adapted to cassava and the broader cassava-farming environment (Nduwumuremyi *et al.*, 2017; Pita *et al*., 2001; Sserubombwe *et al.,* 2008). The so-called super-abundant whiteflies are so numerous that they can cause direct physical damage to cassava plants that leads to losses of up to 50 %, and when this is combined with the transmission of CMGs and CBSVs, almost complete crop loss is common (Njoroge *et al.*, 2016; Tumwegamire *et al.*, 2018). Whiteflies reproduce rapidly and have strong flight capabilities, and so have been driving the virus pandemics of CMD and CBSD throughout ECA, leading to the current direct threats to West Africa (Legg *et al.,* 2014). The initial component of the war on cassava viruses' strategy aimed to attack this problem at its core by dramatically reducing the numbers of whiteflies (Slakie *et al*., 2013). It was felt that doing so will eliminate the problem of physical damage and greatly reduce the spread of existing and future cassava viruses. However, this requires the natural balance of cassava plants supporting low numbers of whiteflies effectively and sustainably be re-established, managed by natural enemies (Jeremiah *et al.,* 2015; Slakie *et al.*, 2013). Immediate actions and interventions in threatened countries on the potential future impacts of whiteflies and cassava viruses provides opportunity on how to counter these threats.

2.7 Control of Cassava Mosaic and Cassava Brown Streak Diseases

The CMD epidemic in East Africa in the 1990s prompted strong breeding initiatives that led to the discovery and deployment of stronger sources of resistance, with single-gene resistance from CMD2 applied successfully in combating the disease in Nigeria and elsewhere (Katono *et al*. 2015; Legg *et al.* 2014; Tumwegamire *et al.* 2018). More recently, another resistance gene, CMD3, has been hypothesized to explain higher levels of resistance to CMD in some progenies (Bart & Taylor, 2017; Kuria *et al*., 2017; Rabbi *et al*., 2013). While extensive deployment of CMD-resistant varieties has been pursued throughout the cassava belt of Africa, it is based on a limited number of varieties (Rabbi *et al*., 2013; Stansly *et al.*, 2010). Other key challenges affecting breeding capacity include the limited understanding of mechanisms of resistance, as well the limited knowledge of resistance genes for CMD (and even less for CBSV; none for whiteflies) (Katono *et al.*, 2015; Legg *et al*., 2014; Tumwegamire *et al*., 2018). Therefore, focused genetic improvement and breeding strategies based on efficient molecular tools and simplified protocols are central to the war on cassava viruses. High levels of genetic improvement have been achieved for CMD, but the ravaging scourge of CBSD in ECA threatens to erode the genetic gains made in the fight against CMD (Gedil & Sartie, 2010; Kawuki *et al*., 2011; Kizito, 2006; Tumwegamire *et al.*, 2018).

Breeding for CBSD resistance is still relatively recent, because though an old disease, it became a major threat only in the last decade compared to CMD (Anthony *et al*. 2015; Kaweesi *et al.* 2016; Kawuki *et al.* 2016; Tumuhimbise *et al.* 2014a). The most economically viable method for CMD and CBSD management is the use of host-plant resistance and development of cassava varieties that are resistant to both CMD and CBSD (Katono *et al.* 2015; Kawuki *et al.* 2016). However, the most applicable control strategy for CMD and CBSD is a combination of tactics that include cultural practices and use of virus-resistant cultivars. Resistant cultivars are considered as the most important tool for management of both viruses. Continuous deployment of new resistant cultivars is necessary as CMGs are known to evolve producing virulent strains while different strains of CBSD are being reported (Sing'ombe Ombiro, 2016; Thresh and Cooter, 2005; Tumwegamire *et al*., 2018). When two or more viruses co-infect a plant

they may influence each other where one virus may assist a second, co-infecting virus, leading to increased titres and more severe symptoms (Bull *et al.* 2006; Legg *et al*. 2014; Legg *et al.* 2006; Monger *et al*. 2001). Therefore, designing and deploying effective control strategies for control of CMD and CBSD requires thorough knowledge on the development of disease symptoms and interactions between viruses and their host plants. Selection for cultivars that possess morphological traits that reduces the severity of these two diseases will have tremendous effect on cassava production in Africa.

2.8 Agro-Morphological Evaluation of Cassava Genotypes

Since the Mendel period, breeders and geneticists have used morphological characteristics such as leaf and flower colour attributes to follow segregation of genes in hybrid selections of different crops (Gedil & Sartie, 2010). However, most morphological traits are not associated with easily observed phenotypic markers (Bart & Taylor 2017; Mezette, *et al*., 2013; Ribaut, *et al*., 2010). Therefore, morphological characterization through genetic and phenotypic identification and classification of plants is commonly based on the morphological traits assessed and recorded in the field (Mezette, *et al*., 2013; Upadhyaya, *et al*., 2008; Vicente *et al*., 2005). Agro-morphological characterization has also been used for purposes like the identification of duplicates, studies of genetic variation patterns, and correlation with characteristics of agronomic potential (Fukuda, *et al*., 2010; Mezette *et al*., 2013; Temegne, *et al.*, 2016).

Plant characterizations are grouped according to either their variable or constant characteristics (Avijala *et al*., 2015; Fukuda *et al*., 2010). Variable characteristics are those associated largely with genotype by environment interaction. While, constant characteristics typify the species or cultivar, for instance, the branching types in cassava cultivars (Fukuda *et al.* 2010; Gedil & Sartie 2010; Upadhyaya, *et al*., 2008). Because cassava grows in several different ecological environments, it is difficult to describe the morphological characteristics, and therefore, the influence of the environment in the genotype is always important (Masumba *et al*. 2017; Nduwumuremyi *et al*. 2017;

Ribaut, *et al*., 2010). Traditionally, the characterization and classification of cassava germplasm has been accomplished by the use of morphological descriptors, which is a defined set of relatively stable morphological traits useful for cassava genotype characterization (Fukuda *et al.,* 2010). They include shoot and root parts characterization of cassava with quantitative and qualitative measurements. This important taxonomic method has been extended by molecular approaches, where cassava cultivars are generally distinguished based on morphological traits. Generally, cassava has large numbers of cultivars, sometimes with lack of definitive identification by the influence of changing environmental conditions (Kizito 2006; Mezette, *et al.,* 2013). Therefore, many studies have used agro-morphological characterization to determine the genetic diversity and responses (resistance/tolerance) to CMD and CBSD among cassava genotypes (Anthony *et al*. 2015; Sharifi *et al*. 2017; Zhang *et al.* 2015).

2.9 Association between CMD and CBSD Resistance with Agronomic Performance

Breeding for resistance to cassava mosaic disease (CMD) and CBSD was initiated in 1937 in Amani, Tanzania. Due to insufficient levels of resistance in cultivated cassava (*M. esculenta Crantz*), a strategy to incorporate resistance from wild species, particularly from *M. glaziovii and M. melanobasis* (now regarded as *M. esculenta subsp. Flabellifolia*) through inter-specific hybridization and backcrossing was adopted (Esuma *et al.,* 2016; Kawuki *et al.,* 2016). The hybrids form an important genepool for CMD and CBSD resistance, and comprise some of the genotypes adopted for this study. Breeding for dual CMD and CBSD resistance is being pursued as the most cost-effective and sustainable way to manage the devastating effects of the viral diseases in ESA region (Tumwegamire *et al.*, 2018). Although high resistance for CMD has been found, only limited success has been documented for CBSD (Legg *et al.,* 2014; Tumwegamire *et al.,* 2018).

Yield in plants refers to the mass of produce harvested from a single plant or the quantity of produce harvested per unit of land area (Tumuhimbise, 2013). In cassava, it

is often defined in terms of marketable storage root yield, although leaves, stems or even seeds could potentially be additional economic products (Tumuhimbise, 2013). Therefore, some of **t**he desired goals of the breeding efforts is stable genotypes with resistance to both viral diseases and high yielding. Incidence and severity of CMD and CBSD symptom expression varies considerably among cassava varieties and with the environment (Esuma *et al.*, 2016; Jeremiah *et al*., 2015; Katono *et al*., 2015; Tumuhimbise *et al*., 2014). Some varieties show severe shoot and root symptoms while others show either marked leaf symptoms and mild root necrosis or *vice versa*, as well as combinations of milder versions of both leaf and root symptoms (Hillocks *et al*., 2000).

A recent study by Kaweesi *et al*., 2016, using a graft-inoculated cassava glasshouse study showed that 'resistant' and 'tolerant' varieties, with mild symptoms, restrict virus accumulation in the plant and support lower virus titres than susceptible genotypes. This was supported by the findings of Kaweesi *et al.*, 2016 and Tumwegamire *et al.*, 2018, suggesting that 'resistant' and/or 'tolerant' varieties possess molecular resistance mechanisms that impair the replication of CMG's and CBSVs. Although different levels of resistance/tolerance to CMD and CBSD are recognized, dual resistance and complete immunity has not been observed. Therefore, in this present study, cassava genotypes with resistance/tolerance to CMD and/or CBSD were systematically evaluated under on-station research conditions. The study aimed to quantify their response to virus infection in known hotspot locations in Western Kenya and determine the relationship between relative incidence, severity and agronomic performance.

2.10 Assessment of Cassava Genotypes' Stability and Adaptability across Environments

Genotype by environment interaction (GEI) is an important issue for plant breeders and agronomists in particular (Mtunguja, *et al.*, 2016), in the face of a wide range of agro-ecologies and variable climate. Breeders generally strive to develop genotypes that are superior in a number of agronomic, quality and disease resistance traits for a wide range

of environmental conditions. Therefore, GEI is of great interest when evaluating the stability of cassava genotypes under different environmental conditions (Jalata, 2011). The measured yield of each genotype (cultivar) in each test environment is a result of genotype main effect (G), an environment main effect (E) and genotype x environment interaction (GEI) (Gurmu, 2017). Though the environment (E) accounts for about 80% of the total yield variation; it is only G and GEI that are relevant to cultivar evaluation and mega environment classification (Yan, *et al.*, 2000). GEI is related to component of yield variation across environments for a genotype that cannot be explained either by G or E alone (Yan & Kang, 2003; Yan & Tinker, 2006).

GEI reduces the genetic progress in plant breeding programs through minimizing the association between phenotype and genotype (Hongyu, *et al*., 2014). Hence, GEI must be either exploited by selecting superior genotype for each a specific target environment or avoided by selecting widely adapted and stable genotype across wide range of environments (Jeberson *et al*., 2018; Nduwumuremyi *et al*., 2017). GEI is used to determine if a genotype is widely adapted for a wide range of environmental conditions or selected for different sub environments (Scapim and Gonçalves-vidigal, 2017). In their investigation of genotype x environment interaction (GEI), researchers proposed and used different procedures to analyze GEI (Jalata, 2011) and proposed various statistics to measure the stability of the genotypes across environments (Farshadfar, 2012).

However, as reported by Jalata, 2011, no single method can adequately explain cultivar performance across environments. Therefore, statistical methods for measuring genotypic stability should partition the information from a Genotype-Environment data matrix into simpler components representing real responses vs. random variation (Gauch & Zobel, 1988; Ly *et al*., 2013). These statistical methods can be classified into two groups, namely univariate and multivariate (Farshadfar, *et al*., 2012). Univariate models comprise: regression coefficient (Finlay & Wilkinson, 1963), sum of squared deviations from regression – joint regression analysis (JLR) (Eberhart & Russell, 1966), stability

variance (Shukla, 1972), coefficient of determination (Pinthus, 1973), and coefficient of variability (Francia & Kannenberg 1978). Multivariate models includes a wide range of methods such as principal component analysis (PCA) (Mishra *et al.*, 1964), cluster analysis (Gower, 1967; Souza, 2014), genotype main effect plus genotype by environment interaction (GGEI) bi-plot analysis (Yan *et al*., 2000; Yan & Kang, 2003; Yan & Tinker, 2006), and additive main effects and multiplicative interaction models (AMMI) (Gauch and Zobel, 1988). AMMI and GGE bi-plot analyses are currently the two methods that are widely used to overcome the difficulties in multi-environment trial data analysis (Anthony *et al.*, 2015; Gurmu, 2017; Hongyu *et al*., 2014; Jeberson *et al*., 2018; Rad *et al*., 2013; Yan & Kang, 2003).

AMMI analysis is the most reliable for identifying specific adaptations of cassava genotypes to favorable and unfavorable environments (Gauch & Zobel, 1988). AMMI model estimates the magnitude and significance of GEI effects on each genotype's response by using a single model, combining analysis of variance for main effects of genotypes and environments and principal component analysis (PCA) of GEI (Esuma *et al.*, 2016; Nduwumuremyi *et al.*, 2017; Tumuhimbise, 2013). GGE bi-plot provides more information about environments and genotype performance than the AMMI bi-plot analysis (Nduwumuremyi *et al.*, 2017; Yan & Kang, 2003). However, GGE bi-plot method is unable to separate genotype from GEI effects, in contrast to AMMI (Jeberson *et al*., 2018; Nduwumuremyi *et al*., 2017; Sharifi *et al*., 2017). Since analysis of variance (ANOVA), principal component analysis (PCA), and linear regression (LR) are sub-cases of the more complete AMMI model, then the AMMI offers a more appropriate fast statistical analysis of disease infection (incidence and severity) and yield trials that may have GEI (Akcura *et al*., 2006; Farshadfar *et al.*, 2012; Hongyu *et al*., 2014; Rad *et al*., 2013; Tadesse, 2019; Tumuhimbise *et al*., 2014).

The combination of analysis of variance and principal components analysis in the AMMI model, along with prediction assessment, is a valuable approach for understanding GEI and obtaining better yield estimates. The interaction is explained in

the form of a bi-plot display where, PCA scores are plotted against each other and it provides visual inspection and interpretation of the GEI components. Integrating bi-plot display and genotypic stability statistics enable genotypes to be grouped based on similarity of performance across diverse environments. In studies by Nduwumuremyi *et al.*, 2017, evaluating 30 cassava genotypes in five (5) different environments (locations) across Rwanda in 2014/15, GGE bi-plot analysis was found better than Eberhart and Russell joint linear regression analysis in identifying stable and high yielding genotypes. This finding was similar to studies elsewhere (Esuma *et al*., 2016; Farshadfar *et al*., 2012; Jeberson *et al*., 2018; Rad *et al*., 2013; Tadesse, 2019). Although AMMI and GGE are equivalent in achieving predictive accuracy, the AMMI method is considered superior to GGE for evaluating yield trial data (Hongyu *et al*., 2014; Yan and Kang, 2003), because it shows genotype main effects, environment main effects and interaction effects, whilst the GGE bi-plot only displays G and *G x E* effects (Gauch & Zobel, 1988; Ly *et al*., 2013; Steyn *et al*., 1993).

The AMMI model combines the analysis of variance for the genotype and environment main effects with principal components analysis of the genotype environment interaction (Gauch & Zobel, 1988). The combination of analysis of variance and principal components analysis in the AMMI model, along with prediction assessment, is a valuable approach for understanding GEI and obtaining better yield estimates (Hongyu *et al*., 2014). The interaction is explained in the form of a bi-plot display where, PCA scores are plotted against each other and it provides visual inspection and interpretation of the GEI components (Akcura *et al.,* 2006; Farshadfar, *et al.*, 2012) Integrating bi-plot display and genotypic stability statistics enable genotypes to be grouped based on similarity of performance across diverse environments (Hongyu *et al*., 2014; Tadesse, 2019). AMMI model, however, does not make provision for a quantitative stability measure. Such a measure is essential in order to quantify and rank genotypes according their yield stability.

The AMMI stability value (ASV) was proposed by Purchase, *et al.*, 2013, as a measure to quantify the stability coefficient, important in ranking the genotypes based on stability and adaptability.

$$AMMI\ Stability\ Value\ (ASV) = \sqrt{\{\frac{IPCA\ 1\ Sum\ of\ squares}{IPCA\ 2\ Sum\ of\ Squares}\}(\text{IPCA1 Score})^2 + (\text{IPCA2 Score})^2}$$

In effect the ASV is the distance from zero in a two dimensional scatter gram of IPCA 1 (Interaction Principal Component Analysis axis 1) scores against IPCA 2 scores. Since the IPCA 1 score contributes more to G x E sum of squares, it has to be weighted by the proportional difference between IPCA 1 and IPCA 2 scores to compensate for the relative contribution of IPCA 1 and IPCA 2 total G x E sum of squares. The ASV as described by Purchase, *et al*., 2013, is comparable with the other stability parameters of AMMI model and other methods of stability such as joint regression by Eberhart and Russell (1966) and Shukla (1972) stability methods in the study of GxE interaction. This statistical method can be used to evaluate stability after reduction of noise from the GEI effects. Since the IPCA1 score contributes more to GEI sum of square, it has to be weighted by the proportional difference between IPCA1 and IPCA2 scores to compensate for the relative contribution of IPCA1 and IPCA2 total GE sum of squares (Amiri, *et al.*, 2013; Purchase, *et al.*, 2013).

CHAPTER THREE

EVALUATION OF CASSAVA GENOTYPES FOR RESISTANCE TO CASSAVA MOSAIC AND CASSAVA BROWN STREAK DISEASES IN WESTERN KENYA

3.1 Abstract

More than a third of Sub Saharan Africa's (SSA) potential cassava harvest is continuously lost to pest and disease constraints. The most important of these are the cassava viruses: cassava mosaic geminiviruses (CMGs) and cassava brown streak viruses (CBSVs). Twenty-three (23) cassava genotypes that had shown great promise in terms of their resistance to both CMD and CBSD were used in this study. The elite cassava genotypes were evaluated for resistance to cassava mosaic and brown streak diseases at Alupe, Kakamega and Kibos in Western Kenya. The trial was conducted using an alpha lattice balanced design using 23 genotypes with three replicates during an extended cropping cycle between 2016 and 2017. Results for combined analysis of variance showed that genotype, location, month after planting (MAP) and their interactions significantly influenced ($P \leq 0.05$) incidence and severity of CMD and CBSD. Within location analysis for CMD and CBSD incidence and severity among the elite cassava genotypes from 1 MAP up to 12 MAP gave varying results. High CMD incidence and severity was recorded for genotypes at Alupe (0.730; 1.256) as opposed to Kakamega (0.000; 1.000) and Kibos (0.031; 1.006) which recorded. Low values. Similarly, CBSD root incidence and severity were high in Alupe (0.848; 1.310), as opposed to Kakamega (0.020; 1.006) and Kibos (0.188; 1.078). Genotype Kibandameno, a local standard check, had the highest CMD and CBSD incidence and severity in all three locations. Additive Main effect and Multiplicative Interaction (AMMI) model was used to confirm dual resistance and to determine the best stable genotypes for disease resistance based on AMMI Stability Value (ASV). Based on combined ASVs for disease resistance traits, genotypes that were stable for dual resistance to CMD and CBSD incidence across Alupe, Kakamega and Kibos comprised Colicanana, F10-30-R5, Orera,

Tajirika and Kizimbani, while, genotypes that were unstable for dual resistance to CMD and CBSD incidence, with specific adaptability across Alupe, Kakamega and Kibos were KBH/2006/026, F19-NL, Nase-1, Kalawe and Kibandameno. Dual resistance was thus confirmed for CMD and CBSD incidence and not severity. Whiteflies abundance was significantly influenced ($P \leq 0.05$) by genotype, location, MAP time and the interaction between genotype and location. In conclusion, significant positive correlation ($P \leq 0.05$) between all disease resistance traits further confirmed dual resistance amongst some of the 24 cassava genotypes, however this was location specific and not generalized.

3.2 Introduction

Cassava (*Manihot esculenta* Crantz) production is increasingly constrained by the two principal biotic constraints (Liu, 2017), namely cassava mosaic disease (CMD), caused by cassava mosaic geminiviruses (CMGs); and cassava brown streak disease (CBSD), caused by cassava brown streak viruses (CBSVs) (Bull *et al*., 2006; Legg *et al.*, 2014). CMD was first described in 1894 in what is now Tanzania, and is currently known to occur in all the cassava-growing countries of Africa and the adjacent islands and also, in India and Sri Lanka (Hillocks *et al*., 2000). The spread of an unusually severe form of CMD, the so-called 'CMD Pandemic' was first recorded from Uganda in the late 1980s and has subsequently spread to affect an area greater than 4 million square kilometres across 11 countries of East and Central Africa (Tumwegamire *et al*., 2018). On the other hand, CBSD is caused by two distinct viruses: cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV), both of which belong to the genus Ipomovirus in the family Potyviridae, and generally produce similar symptoms in infected plants (Kawuki *et al*., 2016; Legg and Thresh, 2003). CBSD symptoms are usually variable and irregular, and depend on many factors including plant age, cultivar (genotype), environmental conditions (that is, altitude, temperature and rainfall quantity) and virus species (Kawuki *et al*., 2016; Legg and Thresh, 2003; Ndyetabula *et al.*, 2016). Control strategies for CBSD have mainly focused on host plant resistance (Bart and

Taylor, 2017; Katono *et al*., 2015). However, recent studies revealed that resistance to CBSD is inherited quantitatively, and therefore is likely to be influenced by environment, making multi-environmental evaluation necessary (Legg *et al.*, 2014; Tumwegamire *et al.*, 2018). The dual (mixed) effects of CMD and CBSD diseases on cassava are very complex and can lead to tremendous yield loss depending on severity and cassava genotypes (Irungu, 2001; Tumuhimbise, 2013). When combined, 100% yield loss can result (Katono *et al.*, 2015; Kawuki *et al.*, 2016; Tumuhimbise et al., 2014). The two diseases are responsible for production losses worth more than US$1 billion every year and are a threat to food and income security for over 30 million farmers growing cassava in East and Central Africa (Legg *et al.*, 2014). Both the cassava mosaic *geminiviruses* (CMGs) and cassava brown streak viruses (CBSVs) are transmitted by the whitefly vector *Bemisia tabacii Genn* (Legg *et al.*, 2014; Njoroge *et al.*, 2016) . Whiteflies reproduce rapidly and have strong flight capabilities, and so have been driving the virus pandemics of CMD and CBSD throughout the region leading to the current direct threats to the entire SSA (Jeremiah *et al*., 2015; Rwegasira and Rey, 2012). CMD and CBSD management has mostly relied on the identification of existing sources of virus resistance, the introgression of virus resistance traits into farmer-preferred cultivars, and the deployment of virus resistant varieties in the field (Thresh and Cooter, 2005). These strategies have been particularly important for mitigating the impact of CMD in the CMD pandemic regions of Africa (Legg *et al., 2*014). However, the CMD-resistant cultivars and landraces deployed in CMD-affected regions were not tested for resistance to CBSD (Tumwegamire *et al.*, 2018). They later appeared to be susceptible to CBSD, which may have facilitated the spread of CBSD in East and Central Africa during the last two decades (Legg *et al.*, 2014; Tumwegamire *et al.*, 2018). Moreover, genotypes reported to be resistant to a given disease in one location could turn out to be susceptible to the same disease when grown in a different location (Kuria *et al*., 2017; Rwegasira & Rey, 2012). Thus, renewed measures to identify, characterize, and preserve dual CMD and CBSD resistance in cassava germplasm are required for sustainable disease management strategies in the region. Multi-location

selection of genotypes with dual resistance to CMD and CBSD that possess agro-morphological traits that reduces the severity of these two diseases will have tremendous effect on cassava productivity in Sub Saharan Africa. The objectives of this study were to: (1) Identify elite cassava genotypes resistant to CMD and CBSD at Alupe, Kakamega and Kibos; (2) Determine stability amongst the elite genotypes resistant to CMD and CBSD using AMMI Stability Value (ASV) at Alupe, Kakamega and Kibos.

3.3 Materials and Methods

3.3.1 Experimental Material

Twenty three (23) elite cassava genotypes that had shown great promise in terms of their resistance to CMD and/or CBSD and were officially released or were in the final stages of official release in the New Cassava Varieties and Clean seed to Combat CMD and CBSD (5CP) project countries namely, Kenya, Malawi, Mozambique, Tanzania and Uganda (Tumwegamire et al., 2018), were evaluated in this study (Mkumba was used twice to balance the experimental design, hence Mkumba-2) and are presented in Table 3.1. Kibandameno from Kenya, is a local landrace, not yet officially released, but with high susceptibility to both CMD and CBSD and was included as a standard local check and infector plot.

3.3.2 Experimental locations

3.3.2.1 Pre-Trial: Multiplication of test materials

Twenty-four (24) elite cassava genotypes (Table 3.2) from the 5CP cassava project countries were taken to Genetic Technologies International Laboratories (GTIL) where they were regenerated through tissue culture to ensure that they were virus-free. The tissue culture plantlets were then multiplied at the KALRO Embu Centre during the 2014/15 cropping season. KALRO-Embu is known to have low (zero) incidences of both CMD and CBSD (Mware, *et al.*, 2009). KALRO Embu is located in the central

highlands of Kenya at latitude 0° 30' S, longitude 37°30' E and an altitude of 1480 metres above sea level (m a.s.l) (Gachimbi, 2002). The experiment was laid out in a randomized complete block design (RCBD), with twenty-three treatments (genotypes) replicated three times. The purpose of the pre-trial experiment was to multiply and bulk enough planting materials for the subsequent evaluations.

3.3.2.2 Main Trial Locations

Main evaluation trials were conducted on-station, for an extended cropping season between 2016-2017, in three locations namely KALRO Kakamega, KALRO Kibos and KALRO Alupe, representing three agro-ecological zones: Upper Midlands zone 1 (UM1); Lower Midlands zone 2 (LM2) and Lower Midlands zone 1 (LM1) respectively (Jaetzold, et al., 2005) as described in Table 3.2. These sites are known CMD and CBSD hot spot areas (Legg & Bouwmeester, 2010).

Table 3.1: Origin and characteristics of cassava genotypes used in this study

Country of origin	Genotype name	Id Code	Potential fresh root yield (t/ha)	DM content (%)	Reaction to diseases		Release status as at 2014
					CMD resistance	CBSD resistance	
Tanzania	KBH 2002/066	TZ01	34.1	28.0	Moderate	Moderate	In pipeline
	Pwani	TZ02	50.8	29.2	Moderate	Moderate	Released
	Mkumba	TZ03	23.3	27	Weak	Moderate	In pipeline
	KBH 2006/026	TZ04	30.0	29.0	Moderate	Moderate	In pipeline
	Kizimbani	TZ05	28.6	28.0	Moderate	Moderate	Released
	Mkumba 2*	TZ06	20.0	32.0	Weak	Moderate	In pipeline
Malawi	Sangoja	MW01	35	33	Moderate	Moderate	Released
	Sauti	MW02	30	34	Moderate	Moderate	Released
	Yizaso	MW03	35	33	Moderate	Moderate	Released
	Kalawe	MW04	28	36	Moderate	Moderate	Released
	CH05-203	MW05	33	34	Moderate	Moderate	In pipeline
Mozambique	Colicanana	MZ123	20.0	33.0	Weak	Moderate	Released
	Orera	MZ126	23.0	32.0	Weak	Moderate	Released
	Eyope	MZ127	25.0	32.0	Moderate	Moderate	Released
Kenya	LM 08/363	KE01	69	27	Moderate	Moderate	In pipeline
	F19-NL	KE02	39.4	25	Moderate	Moderate	In pipeline
	Tajirika	KE03	61	25.7	Moderate	Moderate	Released
	F10-30-R2	KE04	58	40	Moderate	Moderate	Adv. yld trial
	Kibandameno**	KE05	26.1	40	Susceptible	Susceptible	Not released
Uganda	TZ 130	UG01	31.2	35.0	Strong	Moderate	In pipeline
	Nase-14	UG02	26.9	31.5	Strong	Moderate	Released
	Nase-18	UG03	38.6	35.5	Strong	Moderate	Released
	Nase-1	UG04	14.9	32.5	Strong	Moderate	Released
	Nase-3	UG05	<10	30.0	Moderate	Moderate	Released

*DM = Dry matter; Adv. yld = Advanced Yield; *=Mkumba 2, similar to Mkumba, adopted as 24th variety to balance the ALPHA Lattice design; **=standard susceptible local check; MW Malawi; MZ Mozambique; KE Kenya; TZ Tanzania; UG Uganda. Adopted from* (Tumwegamire et al., 2018)

Table 3.2: Description of the experimental locations

Experimental Trial	Region/ Location	Location Description	CBSD pressure	CMD pressure	CMG diversity
Pre-Trial Experiment	Eastern – Embu	Latitude 0° 30' S, longitude 37°30' E and an altitude of 1480m.a.s.l. Rainfall 1200-1500 mm/year. soils are Humic Nitisols from basic volcanic rocks	Absent	Low	Low
Main Experiment	Nyanza – Kibos	Latitude $0^0$37'S and longitude $37^0$20'E; Mid Altitude (1173m.a.s.l); Rainfall (1200-1300mm/year; Temperatures (20^0C - 35^0C.); Soils (black cotton)	Moderate	Moderate	Moderate
	Western – Alupe	Latitude 0°30' 0 N; longitude 34°7' 50 E; Altitude (1170m.a.s.l); Rainfall (680.5–860 mm/year); Temperature (16-34°C); Soils (Ferralo - orthic Acrisol with pH of 5.0 as determined by water)	High	Moderate	High
	Western – Kakamega	Latitude 0^0 28'N and longitude 34'E; Mid-High altitude (1240-2000m.a.s.l); Rainfall (1240.1-2214mm/year); Temperature (18-29˙C); Soils (clay loams and sandy loams)	Low	Low	Moderate

Source: (Jaetzold et al., 2005)*; (KALRO-Kakamega/Kibos/Embu/Alupe Meteorological weather stations, 2015);* (Legg and Bouwmeester, 2010)*; m.a.s.l – metres above sea level.*

3.3.3 Experimental Design and Planting Details

The trial at each location was laid out in a Balanced Alpha Lattice Design with twenty-four (24) treatments (genotypes), with three replications of six (6) blocks and four (4) plots each. Healthy stem cuttings each 25 cm in length were horizontally planted in a flat seedbed at a spacing of 1m×1m within rows and 1m x 1m between rows giving a population density of 10,000 plants ha^{1}. Each plot measured 6m×7m ($42m^{2}$), comprising 6 rows of 7 plants each to give a total of 42 plants in each plot. The first and last rows and the first and last plant within the middle row of each plot were considered as border plants. The plots and blocks were separated by 2.0 m and 2.0 m alleys, to reduce inter-plot and inter-block plant competition, respectively. The trials were conducted without supplemental irrigation and weeded regularly.

3.3.4 Data Collection

Disease resistance traits recorded in this study are presented in Table 3.3 To obtain primary infection, foliar incidence and severity of both CBSV and CMV symptoms were recorded at three (3) MAP and thereafter at 6, 9, and 12 MAP. Counts of adult *B. tabacii* whiteflies were recorded from the underside of 10 fully expanded leaves on the tallest shoot of each of the 20 randomly selected plants per genotype in a plot starting at 1 MAP, and then at 3, 4, and 6 MAP; and the mean numbers per genotype were computed. Severity and incidence for CMD and CBSD on leaves (foliar) were evaluated quarterly through foliar symptom scoring, while on the roots it was done at harvest time (12 MAP) through root symptom scoring. Severity (Figure 3.1) of CBSD and CMD infection was assessed using a scale of 1-5 as described by Fukuda *et al.*, 2010, where: 1 means no apparent symptoms, 2 means slight leaf chlorosis, 3 means severe leaf chlorosis and mild stem lesions, 4 means severe leaf chlorosis and severe stem lesions while 5 means defoliation, severe stem lesions and dieback. The mean CBSD and CMD incidence was determined by expressing the number of plants showing CBSD foliar symptoms as a percentage of the total number of plants in a plot. CBSD root damage

severity (Figure 3.2) was assessed by slicing each root five times transversely for all the 20 plants using a 1–5 point scale as described by Fukuda *et al.*, 2010, where 1= no symptoms i.e. no apparent necrosis, 2= less than 5% of root necrosis, 3= 5–10% of root necrosis, 4= 10–25% of root necrosis, mild root constriction, and 5= >25% of root necrosis with severe lesions and high root constriction. The guides for CMD and CBSD incidence and severity scoring are shown in Appendices 1, 2, 3, and 4.

Table 3.3: Description of CMD and CBSD resistance traits recorded in this study

Abbreviation	Disease trait	Description
cmd_inc	CMD incidence (%)	cassava mosaic disease incidence is the proportion of plants showing CMD symptoms, scored at 3, 6, 9 and 12 months after planting (MAP
cbsv_inc	CBSD incidence (%)	cassava brown streak virus incidence is the proportion of plants showing CBSV symptoms, scored at 3, 6 and 9 MAP
cbsv_serv	CBSD severity	cassava brown streak virus (CBSV) severity rated on a scale from 1 (no symptoms) to 5 (extremely severe), scored at 3, 6 and 9 MAP
cmd_serv	CMD severity	cassava mosaic disease (CMD) severity rated on a scale from 1 (no symptoms) to 5 (extremely severe), scored at 3, 6, 9, 12 MAP
cbsd_rts_serv	CBSD Roots severity	cassava brown streak disease (CBSD) roots severity rated on a scale from 1 (no symptoms) to 5 (extremely severe), scored at 12 MAP
cbsd_rts_inc	CBSD Roots incidence	cassava brown streak disease roots incidence is the proportion of plants showing CBSD roots symptoms, scored at 12 MAP
wf_plot_av	Average whitefly count per plot	counts the average number of the whiteflies per plot at the time, scored at 1, 3, 4, and 6 MAP

Figure 3.1: A and B. Severity of CMD and CBSD foliar (leaf) infection on cassava genotypes Mkumba and Kalawe through leaf lesions, chlorosis and dieback

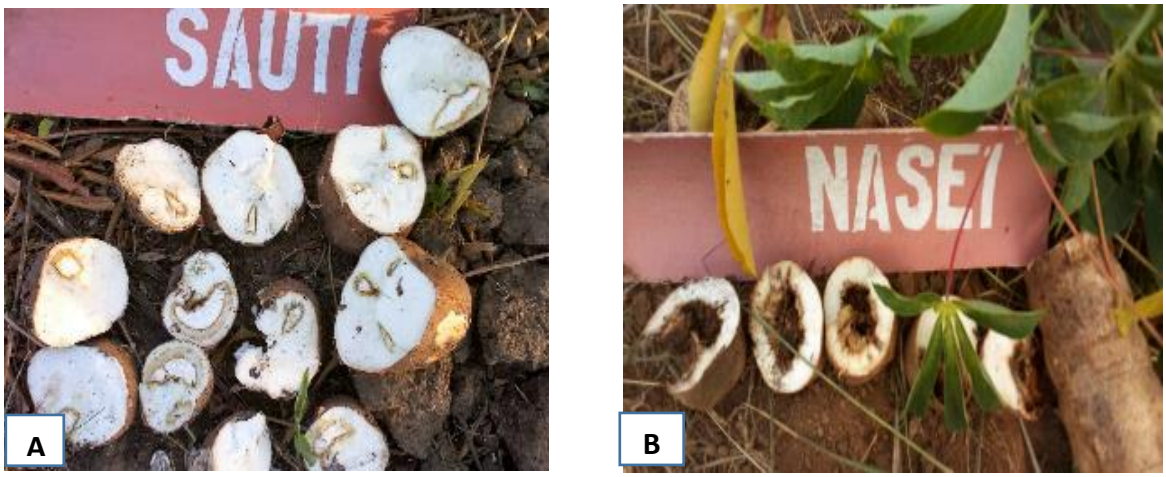

Figure 3.2:.A and B. Severity of CBSD roots infection on cassava genotypes Sauti and Nase-1 through root lesions, necrosis and severe constriction

3.3.5 Data Analysis

Square root transformation was carried out on CMD and CBSD incidence, severity and whitefly population data before further analysis. Data on transformed CMD, CBSD and whitefly values were subjected to analysis of variance (ANOVA) to establish whether or not significant differences existed among the cassava genotypes and where significance was obtained. Means were separated using least significance differences (LSD) at 5% significance level ($P < 0.05$). Variance components $\sigma^2_{G,}$ $\sigma^2_{E,}$ σ^2_{GxExT} and σ^2_e were estimated based on the generalized mixed effect model, with genotype declared as fixed effects and location/environment (Alupe, Kakamega and Kibos), plus Months after Planting (MAP) time (Plant Age) as random effects using the following model: Yi = G + E + TE + GE + TGE (where Yi = observation (response variable); E = environment; T = Months after Planting (MAP) time (plant age); and G = Genotype. Further, Pearson's correlations (correlation coefficient, R^2 and significance, $P < 0.05$), were carried out on the square root transformed data amongst root and foliar incidence and severity scores at 3, 6, 9 and 12 MAP.

CMD and CBSD resistance stability analyses were performed by the Additive Main effect and Multiplicative Interaction (AMMI) method as described in (Gauch and Zobel, 1988) using the following statistical model:

$$Y_{ij} = \mu + g_i + e_j + \sum_{K=n}^{n} \lambda_k \alpha_{ik} y_{jk} + r_{ij} + \varepsilon_{ij'}$$

Where Y_{ij} is the mean response of genotype i in the environment j; μ is the overall mean; g_i is the fixed effect of genotype i (i = 1, 2, ... g); e_j is the random effect of environment j (j = 1, 2, ... e); ε_{ij} is the average experimental error; the GEI is represented by the factors; λ_k is a unique value of the k^{th} interaction principal component analysis (IPCA), (k = 1, 2, ... p, where p is the maximum number of estimable main components), α_{ik} is a singular value for the i^{th} genotype in the k^{th} IPCA, y_{jk} is a unique value of the j^{th} environment in the k^{th} IPCA; r_{ij} is the error for the G × E interaction or AMMI residue (noise present in

the data); and k is the characteristic non-zero roots, k = [1, 2, ... min (G - 1, E - 1)]. The sum of squares for the GEI ($SS_{G\times E}$) was divided into *n* singular axes or main components of interaction (IPCA), which was described the standard portion, each axis corresponding to an AMMI model (Tables 6 and 8 in Appendices).

Since the AMMI model does not make provision for a quantitative stability measure, and as such a measure is essential in order to quantify and rank genotypes in terms of disease resistance stability, the following measure as proposed by (Purchase *et al*., 2013) was adopted for this study:

$$AMMI\ Stability\ Value\ (ASV) = \sqrt{\{\frac{IPCA\ 1\ Sum\ of\ squares}{IPCA\ 2\ Sum\ of\ Squares}\}(\text{IPCA1 Score})^2 + (\text{IPCA2 Score})^2}$$

In effect the ASV is the distance from zero in a two dimensional scatter gram of IPCA I (Interaction Principal Component Analysis axis I) scores against IPCA2 scores. Since the IPCA I score contributes more to G x E sum of squares, it has to be weighted by the proportional difference between IPCA 1 and IPCA2 scores in order to compensate for the relative contribution of IPCA 1 and IPCA2 scores to total G x E sum of squares. The genotypes with the highest ASV values are considered the most unstable in the test environments (specifically adapted to certain environments), while genotype with lowest ASV values close to zero (0) and one (1) are the most stable across environments (Appendix 5). Similarly, test environments with highest ASV values were considered most unstable, while those with low ASV values stable (Farshadfar, *et al*., 2012; Gauch and Zobel 1988; Hongyu *et al*. 2014; Purchase, *et al.*, 2013)

3.4 Results

3.4.1 Cassava Mosaic Disease (CMD) Incidence and Severity

3.4.1.1 Effect of Location, Genotypes and their Interaction on CMD Severity and Incidence

Combined analysis of variance (Table 3.4) for CMD incidence at Alupe, Kakamega and Kibos, revealed that the effect of genotype, location and interaction of genotype by location was highly significant ($P \leq 0.001$). Further, combined analysis of variance for CMD severity at Alupe, Kakamega and Kibos, revealed that the influence of genotype and location was highly significant ($P \leq 0.001$), while the interaction between genotype by location was not significant ($P \geq 0.05$).

3.4.1.2 Effect of Location on CMD Severity and Incidence

Location as a factor had very high significant influence ($P \leq 0.01$) on CMD incidence and severity at Alupe, Kakamega and Kibos. Generally, low CMD, incidence was recorded at Kakamega (0%) and Kibos (0.07%) 12 MAP (Table 3.5), as opposed to Alupe (1.74%), with an overall mean of 0.60%. Highest CMD severity and incidence was recorded on Kibandameno and Kalawe genotypes at Alupe with mean of 2.24 and 7.53%; and 1.58 and 4.04%, respectively (Tables 3.5 and 3.6). Mean CMD severity for Alupe, Kakamega and Kibos was 1.26, 1.00 and 1.01 respectively (Table 3.6). On the contrary, as shown in Figures 3.3 and 3.5, after assessment of the genotypes from planting, CMD incidence and severity progressively increased at Alupe from 6 MAP to 12 MAP, as opposed to Kakamega and Kibos, where it decreased from 6 MAP to 12 MAP.

Table 3.4: Combined ANOVA of square root transformed data for disease resistance traits and whiteflies population recorded on elite cassava genotypes at Alupe, Kakamega and Kibos in Western Kenya

Source of Variation	DF	Mean Squares (MS) – 12 MAP				MS – 6 MAP
		CMD Severity	CMD Incidence	CBSD Severity	CBSD Incidence	Whitefly Abundance
Genotype (G)	23	4.33***	1867.10***	1.43	230.17***	11.95**
Location (L)	2	13.76***	9800.30***	15.41***	1557.56***	1003.52***
Genotype (G) x Location (L)	46	1.17	796.10***	1.06	107.80***	5.73*
Residual	144	0.31	128.9	0.63	39.85	2.54
Overall Mean		1.01	6.60	1.13	0.84	6.28
$LSD_{0.05}$ (L) x (G)		0.35	1.07	0.55	1.25	2.21
SE		0.12	0.38	0.20	0.45	0.72
CV $(\%)_{Rep}$		19.90	24.20	30.00	20.8	6.4

*DF=Degrees of Freedom; SE= Standard Error of Mean; CV=Coefficient of Variation, expressed as a percentage; Level of significance test *=P<0.05, **=P<0.01, ***=P<0.001;* $LSD_{0.05}$*=least significant difference at 5%; MAP Time=Month After Planting time (3, 6, 9 and 12)*

3.4.1.3 Effect of MAP Time (Plant Age) on CMD Incidence and Severity

CMD incidence at Kakamega increased slightly from 3MAP to 6 MAP before decreasing to 0% by 12 MAP (Figure 3.5). CMD severity increased progressively with MAP time at Alupe as opposed to Kakamega and Kibos (Figure 3.3), where severity increased up to 6 MAP before reducing progressively towards 9 MAP and 12 MAP. Generally, Kakamega had lowest mean CMD severity score (Figure 3.3). CMD

incidence across the 24 cassava genotypes, increased in Kibos from 3 MAP and was highest at 6 MAP (18.75%) before reducing significantly towards 12 MAP (Figure 3.5). This was in contrast to Alupe, where CMD incidence rose steadily from 3 MAP to 6 MAP, before decreasing at 9 MAP and increasing sharply again towards 12 MAP (Figure 3.5).

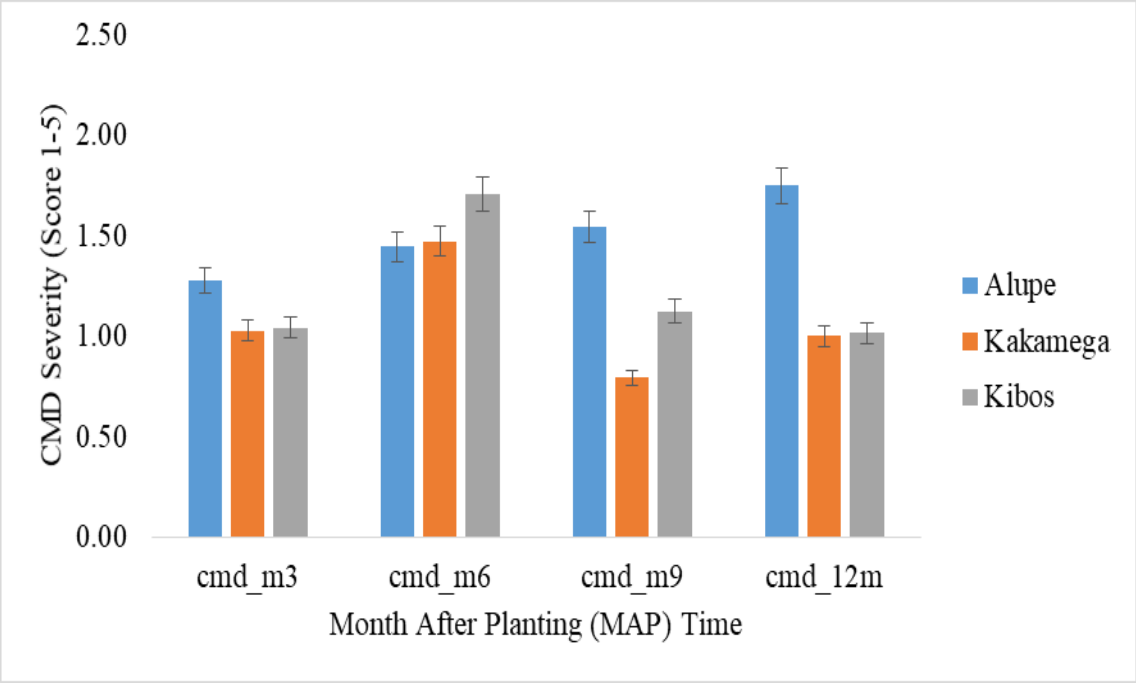

Figure 3.3: Mean CMD Severity variation with MAP time (95% CI) at Alupe, Kakamega and Kibos. cbsv = cassava brown streak visrus; m stands for month; CBSD = Cassava Brown Streak Disease

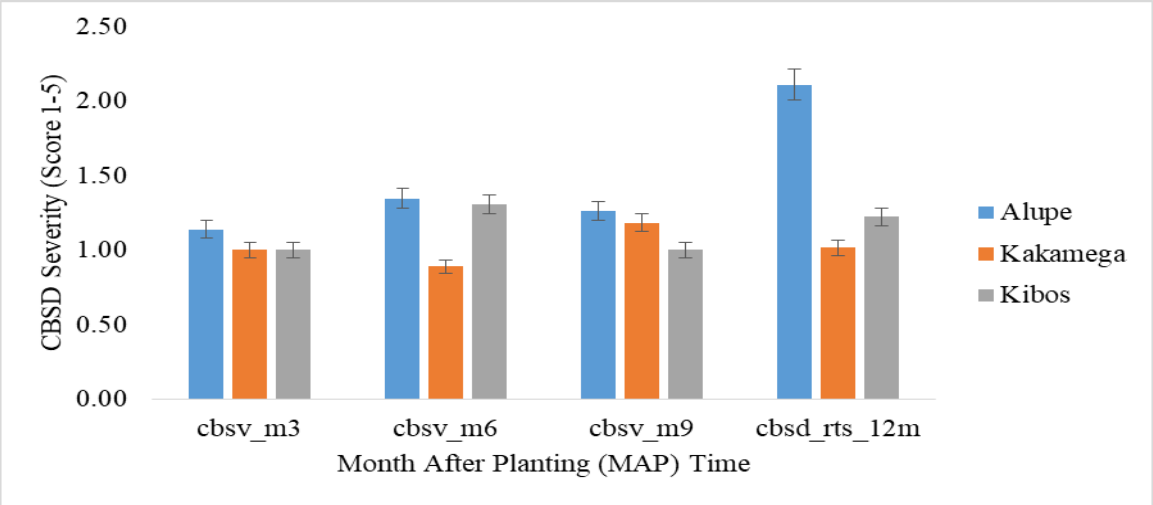

Figure 3.4: Mean CBSD Severity variation with months after planting time (95% confidence interval) at Alupe, Kakamega and Kibos. cbsv = cassava brown streak visrus; m stands for month; CBSD = Cassava Brown Streak Disease

3.4.1.4 Effect of Genotypes on CMD Severity and Incidence

Results at 12 MAP in Tables 3.5 and 3.6 showed that Kibandameno, a local standard check, recorded the highest severity and incidence at Alupe (mean 2.24 and 7.53%) and Kibos (1.14 and 0.75%), as opposed to Kakamega (1.00 and 0.00%). Similarly, Kalawe had relatively high severity and incidence at Alupe (1.58 and 4.04%), as opposed to Kakamega (1.00 and 0.00%) and Kibos (1.00 and 0.00%). As recorded in Tables 3.5 and 3.6, other cassava genotypes with relatively high CMD severity and incidence at Alupe and not Kakamega and kibos were Nase-1 (1.35 and 3.88%), Sangoja (1.38 and 3.32%) and TZ-130 (1.47 and 3.47%). Tables 3.5 and 3.6, further showed that very low CMD severity (1.00) and incidence (0.00%) at Alupe, Kakamega and Kibos were recorded on cassava genotypes Eyope, KBH/2002/066, Mkumba, Mkumba-2, Nase-14, Nase-18, Nase-3, Pwani and Yizaso.

Table 3.5: Variation in means of CBSD and CMD Incidence 12 MAP, AMMI Stability Value (ASV) and rank for elite cassava genotypes within and combined across Alupe, Kakamega and Kibos

Cassava Genotypes	CBSD Incidence (%)						CMD Incidence (%)					
	Alupe	Kakamega	Kibos	Mean	ASV	Rank	Alupe	Kakamega	Kibos	Mean	ASV	Rank
CH05-203	2.38	0.00	0.00	0.79	0.05	3	2.25	0.00	0.00	0.75	0.08	5
Colicanana	1.77	0.00	0.00	0.59	0.01	1	1.94	0.00	0.00	0.65	0.02	2
Eyope	0.00	0.00	0.00	0.00	0.31	8	0.00	0.00	0.00	0.00	0.81	19
F10-30-R5	2.38	0.00	0.00	0.79	0.05	4	2.10	0.00	0.00	0.70	0.05	4
F19-NL	3.75	0.00	0.00	1.25	0.39	16	3.83	0.00	0.00	1.28	1.27	21
Kalawe	4.76	0.00	2.51	2.42	1.21	23	4.04	0.00	0.00	1.35	1.54	23
KBH/2002/066	4.76	0.00	0.00	0.00	0.31	9	2.75	0.00	0.00	0.92	0.31	7
KBH/2006/026	0.00	0.00	1.12	1.96	0.61	20	0.00	0.00	0.00	0.00	0.81	10
Kibandameno	6.80	0.00	0.00	2.26	2.45	24	7.53	0.00	0.75	3.10	7.44	24
Kizimbani	2.25	0.00	1.12	1.12	0.04	2	1.12	0.00	0.00	0.37	0.09	6
LM/2008/363	2.50	0.00	1.59	1.36	0.10	7	2.50	0.00	0.00	0.83	0.18	8
Mkumba	0.00	0.00	0.00	0.00	0.31	10	0.00	0.00	0.00	0.00	0.81	11
Mkumba-2	0.00	0.00	2.51	0.84	0.61	21	0.00	0.00	0.00	0.00	0.81	12
Nase-1	3.63	0.00	0.00	1.21	0.35	15	3.88	0.00	0.00	1.30	1.34	22
Nase-14	0.00	0.00	0.00	0.00	0.31	11	0.00	0.00	0.00	0.00	0.81	13
Nase-18	0.00	0.00	0.00	0.00	0.31	12	0.00	0.00	0.00	0.00	0.81	14
Nase-3	0.00	0.00	0.00	0.00	0.31	13	0.00	0.00	0.00	0.00	0.81	15
Orera	2.38	0.00	0.00	0.79	0.05	5	1.37	0.00	0.00	0.46	0.03	3
Pwani	0.00	0.00	0.00	0.00	0.31	14	0.00	0.00	0.00	0.00	0.81	16
Sangoja	4.63	0.00	1.12	1.92	0.55	19	3.32	0.00	0.00	1.11	0.73	9
Sauti	0.00	0.00	0.79	0.26	0.47	17	0.00	0.00	0.00	0.00	0.81	17
Tajirika	2.51	0.00	0.00	0.84	0.06	6	1.59	0.00	0.00	0.53	0.00	1
TZ-130	3.97	0.00	0.00	1.32	0.48	18	3.47	0.00	0.00	1.16	0.88	20
Yizaso	0.00	1.12	0.00	0.37	0.69	22	0.00	0.00	0.00	0.00	0.81	18
Location Mean	2.02	0.05	0.45	0.84			1.74	0.00	0.07	0.60		

ASV=AMMI Stability Value ((Nduwumuremyi *et al*., 2017; Purchase *et al*., 2013)*; LSD=Least Significance Difference (CBSD incidence, $LSD_{0.05}$=0.25;; CMD incidence, $LSD_{0.05}$=0.22); SE=Standard Error (CBSD incidence, SE=0.09; CMD incidence, SE=0.08); CV=Coefficient of Variation (CBSD incidence, CV% $_{Rep}$=20.80; CMD incidence, CV% $_{Rep}$=24.10); CMD incidence scored on a scale of 1-5, and expressed as a percentage of total plants per plot.*

Table 3.6: Variation in Means of CBSD and CMD severity 12 MAP for elite cassava genotypes within and combined across Alupe, Kakamega and Kibos

Cassava Genotypes	CBSD Severity			CMD Severity		
	Alupe	Kakamega	Kibos	Alupe	Kakamega	Kibos
CH05-203	1.41	1.00	1.00	1.41	1.00	1.00
Colicanana	1.41	1.00	1.00	1.24	1.00	1.00
Eyope	1.00	1.00	1.00	1.00	1.00	1.00
F10-30-R5	1.41	1.00	1.00	1.33	1.00	1.00
F19-NL	1.41	1.00	1.00	1.55	1.00	1.00
Kalawe	1.66	1.00	1.41	1.58	1.00	1.00
KBH/2002/066	1.82	1.00	1.00	1.28	1.00	1.00
KBH/2006/026	1.00	1.00	1.14	1.00	1.00	1.00
Kibandameno	2.07	1.00	1.00	2.24	1.00	1.14
Kizimbani	1.28	1.00	1.14	1.14	1.00	1.00
LM/2008/363	1.24	1.00	1.24	1.28	1.00	1.00
Mkumba	1.00	1.00	1.00	1.00	1.00	1.00
Mkumba-2	1.00	1.00	1.41	1.00	1.00	1.00
Nase-1	1.41	1.00	1.00	1.55	1.00	1.00
Nase-14	1.00	1.00	1.00	1.00	1.00	1.00
Nase-18	1.00	1.00	1.00	1.00	1.00	1.00
Nase-3	1.00	1.00	1.00	1.00	1.00	1.00
Orera	1.41	1.00	1.00	1.14	1.00	1.00
Pwani	1.00	1.00	1.00	1.00	1.00	1.00
Sangoja	1.82	1.00	1.28	1.38	1.00	1.00
Sauti	1.00	1.00	1.24	1.41	1.00	1.00
Tajirika	1.41	1.00	1.00	1.14	1.00	1.00
TZ-130	1.67	1.00	1.00	1.47	1.00	1.00
Yizaso	1.00	1.14	1.00	1.00	1.00	1.00
Location Mean	1.31	1.01	1.08	1.26	1.00	1.01

LSD=Least Significance Difference: CMD severity, $LSD_{0.05}$=0.12; CBSD severity, $LSD_{0.05}$=0.11; SE=Standard Error (CMD severity, SE=0.03; CBSD severity, SE=0.04); CV=Coefficient of Variation (CMD severity, CV% $_{Rep}$=19.90; CBSD severity, CV% $_{Rep}$=30.00;); CBSD severity scored on a scale of 1-5, (Fukuda et al., 2010)

3.4.2 Cassava Brown Streak Disease (CBSD) Incidence and Severity

3.4.2.1 Effect of Location, Genotypes and their Interaction on CBSD severity and Incidence

Combined analysis of variance as shown in Table 3.4 for Alupe, Kakamega and Kibos revealed that genotype; location and interaction of genotype by location had highly significant influence ($P \leq 0.01$) on CBSD incidence. The analysis further showed that genotype alone had a highly significant influence ($P \leq 0.01$) on CBSD severity.

3.4.2.2 Effect of the Location on CBSD Incidence and Severity

Results in Table 3.6 revealed that the highest CBSD severity at 12 MAP across the 24 cassava genotypes was recorded at Alupe (mean 1.31) followed by Kibos (mean 2.08) and Kakamega (mean 1.01) with an overall mean of 1.13. Similarly, the highest CBSD incidence was recorded at Alupe (mean 2.02), followed by Kibos (0.45) and Kakamega (mean 0.05) with an overall mean of 0.84 (Table 3.5). As shown in Figure 3.6, there was an increase of CBSD incidence at Kakamega and Kibos from 3 MAP to 6 MAP before marked reduction from 9 MAP to 12 MAP. However, the trend of CBSD incidence at Alupe was almost the same from 3 MAP to 12 MAP (Figure 3.6). Similarly, the trend of CBSD severity at Alupe, Kakamega and Kibos was variable from 3 MAP to 12 MAP (Figure 3.4). CBSD severity increased at Alupe steadily from 3 MAP to 9 MAP, followed by a sharp increase to 12 MAP (Figure 3.4). However, the trends of CBSD severity at Kakamega and Kibos were almost the same from 3 MAP to 12 MAP (Figure 3.4).

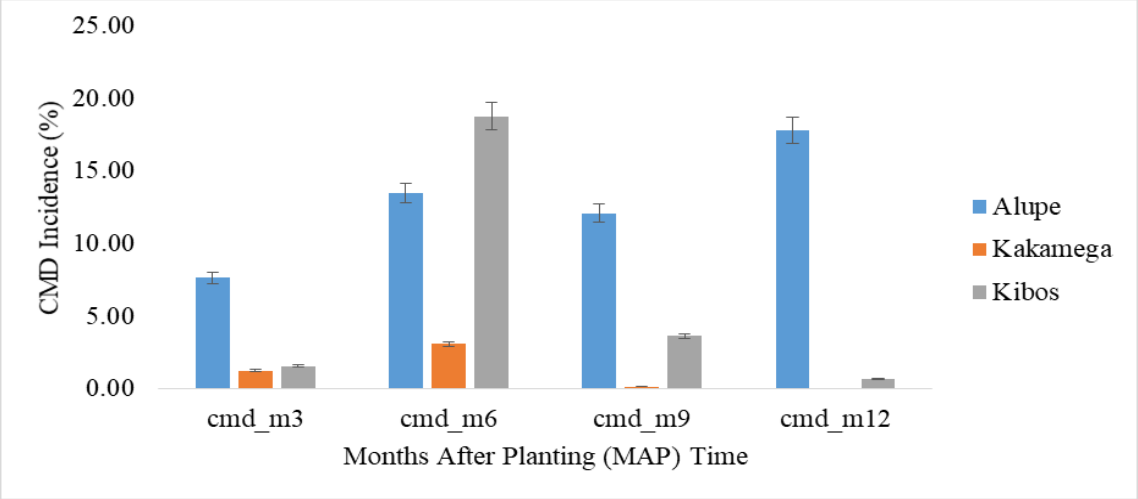

Figure 3.5: Mean percentage (%) variation of CMD Incidence with MAP time 95% confidence interval) at Alupe, Kakamega and Kibos. m stands for month; CMD = Cassava Mosaic Disease

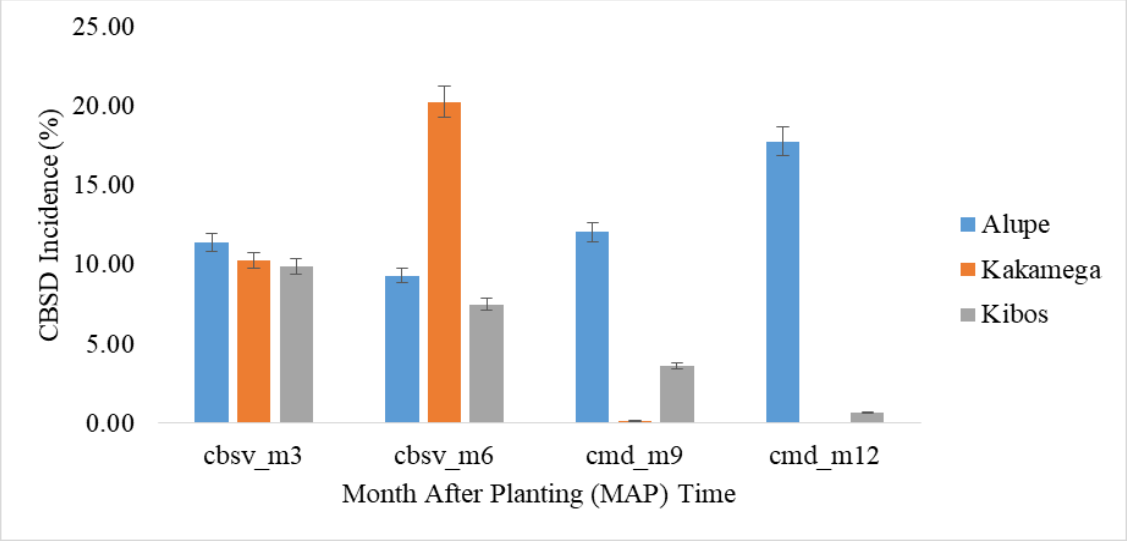

Figure 3.6: Mean percentage (%) variation of Cassava Brown Streak Disease Incidence with months after planting (95% confidence interval)) at Alupe, Kakamega and Kibos. cbsv = cassava brown streak visrus; m stands for month; CBSD = Cassava Brown Streak Disease

3.4.2.3 Effect of MAP Time on CBSD Incidence and Severity

CBSD severity remained stable at Alupe from 3 MAP to 9 MAP, before increasing progressively towards 12 MAP (Figure 3.4). CBSD severity decreased at Kakamega from 3 MAP to 6 MAP, before increasing slightly towards 9 MAP and progressively decreasing again towards 12 MAP. Whereas, CBSD severity at Kibos increased from 3 MAP to 6 MAP, before decreasing towards 9 MAP and progressively increasing again towards 12 MAP (Figure 3.4). As shown in Figure 3.6, CBSD incidence at Alupe decreased slightly from 3 MAP to 6 MAP, before sharply increasing again towards and 9 MAP and 12 MAP. On the other hand, CBSD incidence at Kakamega increased steadily from 3 MAP to 6 MAP, before decreasing again sharply towards 9 MAP and 12 MAP. Similarly, CBSD incidence at Kibos decreased progressively from 3 MAP to 12 MAP.

3.4.2.4 Effect of the Genotypes on CBSD Severity and Incidence

Results in Tables 3.5 and 3.6 revealed that the highest mean CBSD severity and incidence was recorded on Kibandameno, a local standard check (2.07 and 6.80%) at Alupe. In contrast, Kibandameno was relatively free from CBSD severity and incidence at Kakamega (1.00 and 0.00%) and Kibos (1.00 and 0.00%). Other cassava genotypes that recorded high CBSD severity and incidence at Alupe and not Kakamega and Kibos were Nase-1 (1.41 and 3.63%), TZ-130 (1.67 and 3.97%) and F19-NL (1.41 and 3.75%). Similarly, high CBSD severity and incidence was also recorded on Kalawe at Alupe (mean 1.66 and 4.76%) and Kibos (mean 1.41 and 2.51); KBH/2002/066 at Alupe (1.82 and 4.76%); and Sangoja at Alupe (1.82 and 4.63%) and Kibos (1.28% and 2.12%). Results, further, revealed that cassava genotypes that were clean and had very low severity (1.00) and incidence (0.00%) at Alupe, Kakamega and Kibos were Eyope, KBH/2006/026, Mkumba, Nase-14, Nase-18, Nase-3 and Pwani. Interestingly, Mkumba-2, that is same as Mkumba was clean with very low infection severity and incidence at Alupe (1.00 and 0.00%) and Kakamega (1.00 and 0.00%), as opposed to Kibos (1.41 and 2.51%).

3.4.3 Adult Whiteflies (Bemisia tabaciu Genn.) Abundance

The combined analysis of variance (Table 3.4) for adult whiteflies (*B. tabacii*) abundance in Alupe, Kakamega and Kibos, showed that genotype, location and interactions between genotype by location had significant influence ($P < 0.05$). Mean whiteflies abundance per genotype in Alupe, Kakamega and Kibos were 63.85, 56.28 and 15.84 respectively, with an overall mean of 45.32. The highest mean whiteflies numbers were recorded at Kakamega and Alupe on Kalawe (137.46) and Kibandameno (106.41) respectively (Table 3.7). The lowest mean numbers for adult whiteflies were recorded in Kibos across the 24 genotypes (Table 3.7). Nase-1 had the lowest mean whiteflies abundance of 7.77 at Kibos. Other genotypes with low mean whiteflies numbers (Table 3.6) at Kibos were Tajirika (11.08), Kizimbani (12.34), F19-NL (12.37), LM/2008/363 (12.58), Eyope (12.79) and Mkumba-2 (12.93). The mean whitefly abundancies recorded (‘0) at the different MAP times (plant age) are presented in Figure 3.7. The lowest mean whiteflies abundance (< 20.00) were recorded at Kibos, where the number decreased from 19.30 at 1 MAP to 8.30 at 6 MAP (Figure 3.7). Mean whiteflies abundance per genotype reduced from 1 MAP. 3 MAP and 6 MAP and were 48.99, 46.40 and 40.59 respectively. The highest mean whiteflies numbers were recorded 1 MAP (78.24) at Alupe, but these also decreased to 50.68 by 6 MAP. The scenario was quite different at Kakamega, where the mean whiteflies abundance increased from 49.42 at 1 MAP up to 62.79 at 6 MAP (Figure 3.7).

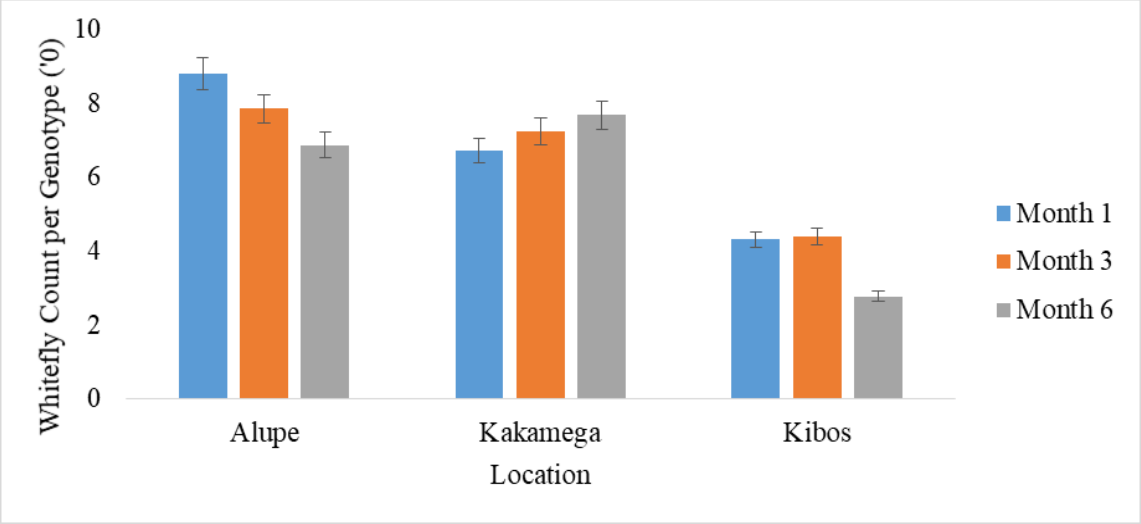

Figure 3.7: Mean number of Adult Whiteflies count per genotype (‘0) with MAP time (plant age) at Alupe, Kakamega and Kibos

Table 3.7: Mean number of adult whiteflies per genotype at Alupe, Kakamega and Kibos in western Kenya 6 MAP

Cassava Genotypes	Alupe	Kakamega	Kibos	Genotype Mean	ASV	Rank
CH05-203	68.51	50.54	15.29	44.78	1.40	9
Colicanana	59.33	39.83	16.54	38.57	4.01	18
Eyope	70.43	79.98	12.79	59.40	9.53	20
F10-30-R5	55.56	64.33	17.88	45.92	3.55	16
F19-NL	68.36	56.27	12.37	45.66	0.83	7
Kalawe	85.24	137.46	21.67	81.46	82.12	24
KBH/2002/066	65.41	60.26	14.69	46.79	0.34	2
KBH/2006/026	59.40	59.56	19.11	46.02	1.03	8
Kibandameno	106.41	68.32	14.22	60.99	26.56	23
Kizimbani	52.08	60.59	12.34	41.67	3.36	15
LM/2008/363	61.26	38.68	12.58	37.50	3.73	17
Mkumba	59.66	26.16	15.24	33.69	13.47	22
Mkumba-2	50.29	27.31	12.93	30.18	8.91	19
Nase-1	56.49	54.94	7.77	39.73	0.71	5
Nase-14	67.43	57.07	17.77	47.42	0.10	1
Nase-18	62.52	58.63	21.34	47.50	0.61	4
Nase-3	57.48	55.34	10.60	41.14	0.43	3
Orera	65.16	51.68	22.81	46.55	1.74	10
Pwani	50.70	29.76	18.62	33.03	11.17	21
Sangoja	60.66	53.43	19.69	44.59	0.82	6
Sauti	62.92	67.94	23.39	51.42	2.14	12
Tajirika	67.20	65.54	11.18	47.97	2.54	14
TZ-130	61.61	42.11	13.07	38.93	2.36	13
Yizaso	58.29	45.01	16.32	39.87	1.79	11
Location Mean	63.65	56.28	15.84	45.32		

ASV=AMMI Stability Value ((Nduwumuremyi *et al*., 2017; Purchase *et al*., 2013); $LSD_{0.05}$ =Least Significance Difference (2.22); SE=Standard Error (0.40); $CV\%_{Rep}$ = Coefficient of Variation (6.40)

3.4.4 Pearson's Correlations amongst Disease Resistance Traits for Elite Cassava Genotypes

There were highly significant positive correlations ($P < 0.05$) between CMD and CBSD Severity across Alupe, Kakamega and Kibos at most sampling dates (Table 3.8), with the exception ($P > 0.05$) of CMD 6 MAP vs CBSD 6 MAP, CMD 9 MAP vs CMD 6 MAP, CMD 12 MAP vs CBSD 6 MAP and CBSD roots severity 12 MAP vs CMD 6 MAP. However, the relationship between CMD severity 12 MAP vs CMD severity 6 MAP and CMD severity 12 MAP vs CBSD severity 9 MAP was not significant ($P > 0.05$), but inverse. Similarly, results (Table 3.9), revealed that there were also highly significant positive correlations ($P < 0.05$) between CMD and CBSD Incidence across Alupe, Kakamega and Kibos at most sampling dates, with the exception ($P > 0.05$) of CMD 6 MAP vs CBSD 6 MAP, CBSD 3 MAP vs CBSD 9 MAP, CMD 9 MAP vs CBSD 6 MAP, CMD 6 MAP vs CBSD 9 MAP and CMD 12 MAP vs CBSD roots incidence 12 MAP. The relationship between CBSD incidence 9 MAP vs CBSD incidence 6 MAP and CBSD roots incidence 12 MAP vs CBSD incidence 6 MAP was not significant ($P > 0.05$), but inverse (Table 3.9). In both cases (CMD and CBSD incidence and severity), the correlation at 6 MAP, though not significant ($P > 0.05$), was positive. This could have been attributed to normal host plant resistance, and not sampling error. Results also revealed that there was a highly significant positive correlation ($P = 0.001$) between CMD and CBSD severity with incidence and whiteflies abundance from 1 MAP to 6 MAP (Figure 3.7). Further, there was a non-significant ($P \geq 0.05$) but positive correlation between incidence with severity and whiteflies abundance 6 MAP towards 9 MAP, an indication that whiteflies abundance had little or no influence on CMD and CBSD severity and incidence beyond 6 MAP.

Table 3.8: Pearson's correlation among disease severity traits recorded on elite cassava genotypes at Alupe, Kakamega and Kibos in Western Kenya

Disease Severity Parameter	cmd_sev_ 3MAP	cbsv_seve _3MAP	cbsv_sev _6MAP	cmd_sev_ 6MAP	cmd_sev_ 9MAP	cbsv_sev_ 9MAP	cmd_serv_ 12MAP
cbsv_seve _3MAP	0.1786**						
cbsv_sev_ 6MAP	0.4868***	0.2763***					
cmd_sev_6 MAP	0.1292*	0.2441***	0.0653				
cmd_sev_9 MAP	0.5773***	0.2623***	0.3839***	0.1244			
cbsv_sev_ 9MAP	0.5070***	0.2103***	0.4835***	0.1580*	0.4706***		
cmd_serv_ 12MAP	0.1579*	0.2869***	0.1146	-0.1214	0.1930**	-0.0456	
cbsd_rts_s erv_12 MAP	0.2668***	0.1695*	0.7473***	0.0366	0.2187***	0.2831***	0.1502*

*Correlation Coefficient, R^2; and Level of significance test *=P < 0.05, **=P < 0.01, ***=P < 0.001*

Table 3.9: Pearson's correlation among disease incidence traits recorded on elite cassava genotypes at Alupe, Kakamega and Kibos in Western Kenya

Disease Incidence Parameter	cbsv_inc_ 3MAP	cmd_inc_ 3MAP	cbsv_inc_ 6MAP	cmd_inc_ 6MAP	cmd_inc_ 9MAP	cbsv_inc_ 9MAP	cmd_inc_12 MAP
cmd_inc_3 MAP	0.1482*						
cbsv_inc_ 6MAP	0.3045***	0.0168					
cmd_inc_6 MAP	0.2774***	0.3221***	0.0373				
cmd_inc_9 MAP	0.2403***	0.5598***	0.0002	0.3818***			
cbsv_inc_ 9MAP	0.0898	0.3279***	-0.0249	0.0384	0.2565***		
cmd_inc_12 MAP	0.2488***	0.4921***	0.0071	0.1956**	0.4428***	0.4805***	
cbsv_rts_incd_12 MAP	0.2013***	0.3392***	-0.0120	0.1555*	0.2751***	0.3055***	0.8196***

*Correlation Coefficient, R^2; and Level of significance test *=P < 0.05, **=P < 0.01, ***=P < 0.001*

3.4.5 Confirmation of Dual CMD and CBSD Resistance amongst Elite Cassava Genotypes

As reported in Tables 3.5, confirmation of dual resistance to CMD and CBSD in elite cassava genotypes at Alupe, Kakamega and Kibos was achieved through AMMI analysis based on AMMI Stability Value (ASV). The results showed significant ($P \leq 0.01$) genotype by location interaction for CMD and CBSD severity. Therefore, dual (combined) resistance was confirmed for only CMD and CBSD incidence amongst cassava genotypes across Alupe, Kakamega and Kibos. Hence, top five genotypes with CMD and CBSD combined (dual) incidence resistance stability across the three locations, due to their very low ASV values were Colicanana (0.02), Tajirika (0.04), Orera,(0.04) F10-30-R5 (0.05) and Kizimbani (0.07) as reported in Table 3.10. On the basis of individual disease resistance stability, only genotype F10-30-R5 had dual resistance against CMD incidence (0.05), CBSD roots incidence (0.06) and combined (0.05) across Alupe, Kakamega and Kibos. Cassava genotypes with marked stability for whiteflies abundance (population per genotype) due to their low ASVs across Alupe, Kakamega and Kibos were Nase-14 (0.10), KBH/2002/066 (0.34), Nase-3 (0.43), Nase-18 (0.61) and Nase-1 (0.71) as shown in Table 3.10. Results also showed that genotypes Kalawe and Kibandameno (due to their very high ASVs) were unstable for dual resistance to both CMD and CBSD severity and incidence, including whiteflies abundance across Alupe, Kakamega Kibos (Table 3.10).

Table 3.10: Confirmation of stable and unstable genotypes amongst elite cassava genotypes based on ASV and rank for dual resistance to CMD and CBSD Incidence across Alupe, Kakamega and Kibos

Parameter	Rank 1	Rank 2	Rank 3	Rank 4	Rank 5
			Stable genotypes across environments		
CMD incidence	Tajirika	Colicanana	Orera	F10-30-R5	CH05-203
		(0.02)		(0.05)	(0.08)
	(0.00)		(0.03)		
CBSD Roots	Colicanana	Kizimbani	CH05-203	F10-30-R5	Tajirika
incidence	(0.01)	(0.04)		(0.06)	
			(0.05)		(0.06)
Combined CMD,	Colicanana	Tajirika	Orera	F10-30-R5	Kizimbani
CBSD incidence	(0.02)			(0.05)	(0.07)
		(0.04)	(0.04)		
Whiteflies	Nase-14	KBH/2002/066	Nase-3	Nase-18	Nase-1
Abundance		(0.34)		(0.61)	
	(0.10)		(0.43)		(0.71)
			Unstable genotypes adapted more to specific environments		
	Rank 20	Rank 21	Rank 22	Rank 23	Rank 24
CMD incidence	TZ-130	F19-NL	Nase-1	Kalawe	Kibandameno
					(7.44)
	(0.88)	(1.27)	(1.34)	(1.54)	
CBSD Roots	KBH/2006/026	Mkumba-2	Yizaso	Kalawe	Kibandameno
incidence					
	(0.61)	(0.61)	(0.69)	(1.21)	(2.45)
Combined CMD,	Yizaso	F19-NL	Nase-1	Kalawe	Kibandameno
CBSD incidence					(4.95)
	(0.75)	(0.83)	(0.85)	(1.38)	
Whiteflies	Eyope	Pwani	Mkumba	Kibandameno	Kalawe
abundance					
	(9.53)	(11.17)	(13.47)	(20.50)	(82.12)

3.5 Discussion

This study was intended to: (1) evaluate elite cassava genotypes for resistance to cassava mosaic and brown streak diseases at Alupe, Kakamega and Kibos in western Kenya. (2) Identify stable genotypes with dual resistance to cassava mosaic and brown streak diseases. The study, therefore, presents the most comprehensive evaluation of a large number of cassava genotypes (23) for both CMD and CBSD at Alupe, Kakamega and Kibos in Western Kenya reported to date. It is important to note that Mkumba cassava genotype, was used twice to balance the Alpha Lattice design, hence making 24 genotypes used in the analysis. There was marked variation in CMD and CBSD incidence and severity from 3 MAP to 12 MAP across Alupe, Kakamega and Kibos, similar to findings by other researchers (Abaca *et al*., 2012; Jeremiah *et al*., 2015; Rwegasira and Rey, 2012; Tembo *et al*., 2017). A number of factors were observed likely to amplify the genotype × environment interaction for CMD and CBSD incidence and severity in hotspot locations in this study. These included genotype susceptibility levels, predominant viruses in locality and/or season, and climatic factors that either influenced the abundance of whitefly vectors and/or the growth rate of the crop, as similarly reported by Kawuki *et al*., 2016. CMD and CBSD incidence and severity increased up to 6 MAP, before declining towards 12 MAP across the 24 cassava genotypes and locations. This could have been attributed to setting in of host plant resistance mechanism in this study, and not sampling error.

The results from this study showed significant differences among genotypes and location for all the CMD and CBSD resistance traits evaluated, namely incidence and severity 1 MAP, 3 MAP, 6 MAP, 9 MAP and 12 MAP. Significant interactions were also detected for all the disease traits studied and whiteflies abundance. This implies that the 24 cassava genotypes responded differently at Alupe, Kakamega and Kibos with regards to CMD and CBSD incidence and severity including whiteflies abundance. This phenomenon was similar to (Anthony *et al*., 2015; Nduwumuremyi *et al*., 2017; Rwegasira and Rey, 2012; Tembo *et al*., 2017; Tumuhimbise *et al*., 2014), who reported

significant variation among genotypes, harvest times, locations and their interactions on most agro-morphological traits evaluated. Whiteflies played a major role in the spread of CMD and CBSD epidemic through their vectoring of CMGs and CBSVs, especially in the first six (6) months after planting in this study, similar to findings by (Jeremiah *et al*., 2015; Njoroge *et al*., 2016; Rwegasira and Rey, 2012). Thereafter, there was no direct relationship between whitefly population and cassava mosaic and brown streak diseases incidence and severity from 6 MAP to 12 MAP. This finding was similar to that by Jeremiah *et al*., 2015 and Njoroge *et al*., 2016, who observed that the spread of CMD and CBSD was not directly related to whitefly population for plants beyond 6 MAP. Kalawe and Kibandameno genotypes with recorded high whiteflies abundance in Alupe, Kakamega and Kibos had also the highest CMD and CBSD incidence and severity.

Pearson Correlation analysis (correlation coefficient, R^2 and significance, $P < 0.05$) was carried out amongst the two diseases incidence and severity of infection and symptoms expression in leaves, stems and roots. This suggests that the occurrence of CMD and CBSD symptoms in any of the plant parts was interdependent and incidence was positively related to severity in the respective plant part. Similar observations have been reported by (Abaca *et al*., 2012; Kawuki *et al*., 2016; Masumba *et al*., 2017). The significant influence ($P \leq 0.05$) of whitefly on both CBSD and CMD disease indices concur with previous reports (Jeremiah *et al*., 2015; Tumwegamire *et al*., 2018), that the whitefly, *B. tabacii* is responsible for unlimited spread of the two diseases throughout the Central, East and Southern parts of Africa. Occurrences of CBSD and CMD at Alupe, Kakamega and Kibos were not dependent of each other, due to negative correlation and non-significant interaction ($P \geq 0.05$) between genotypes and location. Therefore, infection with either CMD or CBSD seemed to affect the incidence and severity of the elite cassava genotyoes within location and not across locations. Similar observations on responses of the different varieties to the two diseases were reported previously (Jeremiah *et al.*, 2015; Katono *et al*., 2015; Nduwumuremyi *et al.*, 2017; Ndyetabula *et al*., 2016; Rwegasira & Rey, 2012; Tumwegamire *et al.*, 2018).

Confirmation of dual resistance to CMD and CBSD amongst the 24 cassava genotypes at Alupe, Kakamega and Kibos was achieved using AMMI Stability Value (ASV). ASV is the distance from the coordinate point to the origin in a two-dimensional scatter gram of IPCA 1 scores against IPCA 2 scores (Appendix 11 and 12). With this method, the genotypes with larger IPCA scores, either negative or positive, are the more specifically adapted to certain environments and those with smaller IPCA scores indicates a more stable genotype across environments. Hence, genotypes with lower ASV values were considered more stable and genotypes with higher ASV were unstable. Accordingly, genotypes Colicanana, Tajirika, Orera, F10-30-R5 and Kizimbani with very low ASVs exhibited remarkable stability for dual resistance against CMD and CBSD incidence across Alupe, Kakamega and Kibos. While, genotypes Kalawe and Kibandameno mainly, but also Yizaso, F19-NL and Nase-1 with relatively high ASVs were the most unstable for dual resistance against CMD and CBSD incidence across the three locations. Genotypes Nase-1, NASE-3, Nase-14, Nase-18 and KBH/2002/066 genotypes were more stable against whiteflies abundance across Alupe, Kakamega and Kibos due to their low ASVs. While, Eyope, Pwani, Mkumba, Kalawe and Kibandameno were unstable against whiteflies abundance across the three locations due to their high ASVs.

3.6 Conclusion

Both CMD and CBSD incidence and severity at Alupe, Kakamega and Kibos was genotype and location specific and not generalized. Therefore, through AMMI stability analysis based on AMMI Stability Value (ASV), the study confirmed combined (dual) resistance amongst some of the elite cassava genotypes to CMD and CBSD incidence and not severity. Confirmed stable genotypes for dual resistance to CMD and CBSD incidence and wider adaptability based on ASV across Alupe, Kakamega and Kibos were Colicanana, Tajirika, Orera, F10-30-R5 and Kizimbani. While, confirmed unstable genotypes for dual resistance to CMD and CBSD incidence, but with more specific adaptability at Alupe, Kakamega and Kibos were Kalawe, Kibandameno, F19-NL, Nase-1 and Yizaso. Dual resistance by the elite cassava genotypes was selective

(genotype and location specific, and not both) in response to both CMD and CBSD incidence and severity. This finding should be of value to cassava breeding and development efforts throughout Kenya, and other parts of sub Saharan Africa (SSA) affected or threatened by CMD and CBSD. This will hopefully contribute to the development of much improved and/or resistant genotypes and, ultimately more effective management of two of Africa's most pernicious threats to food security.

CHAPTER FOUR

EVALUATION OF CASSAVA GENOTYPES FOR AGRONOMIC PERFORMANCE, CORRELATION WITH CMD AND CBSD TRAITS AND STABILITY IN WESTERN KENYA

4.1 Abstract

Cassava production in sub-Saharan Africa (SSA) is constrained by the two biotic constraints namely, cassava mosaic disease (CMD) and cassava brown streak disease (CBSD). The aim of this study was to evaluate elite cassava genotypes for variation in agronomical traits, correlate them to CMD and CBSD parameters and identify stable genotypes across Alupe, Kakamega and Kibos in Western Kenya. Twenty-three (23) elite cassava genotypes that had shown resistance to either one or both of CMD and CBSD in Eastern Africa were evaluated. The trial was conducted using an alpha lattice balanced design with twenty-three (23) genotypes, replicated three times at Alupe, Kakamega and Kibos in Western Kenya for an extended cropping cycle between 2016 and 2017. Results showed significant differences ($P < 0.05$) between genotypes and location (or agro-ecology), but not interaction ($P < 0.05$), for all the agronomic traits evaluated in this study, namely; fresh root yield, t ha^{-1}, biomass yield, tha^{-1}, dry matter yield, t ha^{-1}, harvest index (%), dry matter content (%) and cyanogenic potential. All the 23 cassava genotypes evaluated across the three locations had mean cyanide potential levels ranging from of 3.00–6.00, and were therefore, sweet and not bitter. The significant but negative relationship between CMD and CBSD incidence and severity with agronomic performance implied that their relationship was inverse. Confirmation of stability for agronomic performance was achieved through AMMI analysis, using AMMI stability value (ASV). Stable genotypes based on AMMI stability values (ASV) for fresh root yield across Alupe, Kakamega and Kibos were KBH/2002/066, Kibandameno (a local standard check), Nase-18, Kizimbani and Nase-3. The study

recommends these genotypes to be further evaluated in more environments to assess their wider adaptability and stability.

4.2 Introduction

Cassava (*Manihot esculenta* Crantz) is a key food security crop in sub-Saharan Africa (SSA) and increasingly offers opportunities for income generation from the sale of fresh roots and diverse processed products (Bart and Taylor, 2017; Legg *et al*., 2014; Tumwegamire *et al*., 2018). World-wide, cassava is a staple food for more than 800 million people (Muengula-Manyi *et al*., 2012). However, agronomic performance of cassava is increasingly constrained by the two principal biotic constraints, cassava mosaic disease (CMD), caused by cassava mosaic geminiviruses (CMGs); and cassava brown streak disease (CBSD), caused by cassava brown streak viruses (CBSVs) (Nduwumuremyi *et al*., 2017; Tesfaye *et al.*, 2017; Tumuhimbise *et al*., 2014; Tumwegamire *et al*., 2018). In Africa, yields are only 8-10 tonnes per hectare, on average approximately half of those achieved in Asia and Latin America (Kintché *et al.*, 2017).

Even though cassava is one of the most widely grown staple crops in Nyanza, Western and Coast regions of Kenya, cassava production losses in Kenya are estimated at over US$ 14 million per annum by CMD, and weight loss of produced roots of up to 70% by CBSD (Masinde *et al.*, 2016; Mware *et al*., 2009). Whereas CMD is widely distributed wherever cassava is grown, CBSD has been endemic in the coastal region of Kenya and currently emergence has been reported in the Western region of Kenya (Mware *et al.*, 2009; Njoroge *et al.*, 2016). Breeding for dual resistance is currently being pursued as the most cost-effective and sustainable way to manage the devastating effects of the viral diseases in ESA (Tumwegamire *et al*., 2018). Continuous deployment of new resistant cultivars is necessary as CMGs are known to evolve producing virulent strains while different strains of CBSD are being reported (Anjanappa *et al.*, 2016). Although high resistance for CMD has been found, only limited success has been documented for CBSD (Legg *et al.*, 2014; Tumwegamire *et al.*, 2018). The desired goal of the breeding

efforts is to identify stable genotypes that are high yielding and resistant to both viral diseases.

Identification of sources of virus resistance was achieved by screening germplasm by grafting or sap inoculation with the virus under greenhouse conditions or in the field under natural whitefly-mediated infection (Anjanappa *et al*., 2016; Irungu, 2001; Slakie *et al*., 2013). The work was undertaken in the early stages of the project "New Cassava varieties and Clean Seed to Combat CMD and CBSD (5CP)", and aimed at exchanging elite germ plasm among countries most affected by CMD and CBSD for adaptability breeding (Tumwegamire *et al*., 2018). These collaborative efforts with different national cassava breeding programs have identified germplasm resistant or tolerant to CBSD/CMD (Tumwegamire *et al*., 2018). However, these have been evaluated so far under a narrow range of conditions of environment, virus strains, and vector abundance (Legg *et al*., 2014). These genotypes also need to be distinguished by their agronomic traits such as plant height, height to first branching, time of maturity, biomass yield, fresh root yield, dry matter (DM), dry matter content (% DMC), Harvest Index (HI) and cyanogenic glycosides content in the roots.

However, most of these important cassava agronomic traits have high genotype by environment interaction (Bart and Taylor, 2017; Katono *et al.*, 2015; Kaweesi *et al.*, 2016; Mignouna and Dixon, 1997; Tumwegamire *et al.*, 2018). Suitable genotypes are those adapted to the target environment, and requires breeding for both specific and broad/wider adaptation. Farmers, on the other hand, grow cassava under diverse cropping systems, and therefore prefer genotypes that suit their cropping systems, are resistant to pests and diseases, especially CMD and CBSD, with resultant high yields. Therefore, multi-location variety trials are conducted to identify disease tolerant/resistance and high yielding genotypes but also to identify sites that best represent the target environment for specific and wide adaptability (Gedil and Sartie, 2010; Kvitschal *et al*., 2009; Mignouna and Dixon, 1997; Tumwegamire *et al.*, 2018).

Stable genotypes within and across environments can be determined using various methods, that range from parametric; such as environmental stability variance (Shukla, 1972), regression slope (Finlay and Wilkinson, 1963), deviation from regression (Eberhart and Russell, 1966) and coefficient of determination (Pinthus, 1973); and multivariate methods such as Analysis of Mixed effect and Multiplicative Interaction (AMMI) (Purchase *et al.*, 2013). Therefore, the Eberhart and Russell (1966) model and AMMI stability analysis could be the preferable tools to identify stable, high yielding and adaptable genotype (s) for wider or specific environments. However, since analysis of variance (ANOVA), principal component analysis (PCA), and linear regression (LR) are sub-cases of the more complete AMMI model, then AMMI offers a more appropriate statistical analysis of agronomic performance trials that may have GEI (Jeberson *et al. 2018;* Purchase*., et al*., 2013; Sharifi *et al*. 2017). AMMI clarifies the GEI and summarizes patterns and relationships of genotypes and environments, to improve the accuracy of agronomic performance, including yield estimates (Farshadfar, *et al*., 2012; Rad *et al.,* 2013; Steyn *et al*., 1993; Tadesse 2019; Tumuhimbise et al. 2014). The objectives of this study were to: (1) Assess variation in agronomic performance of elite cassava genotypes at Alupe, Kakamega and Kibos in Western Kenya; (2) correlate agronomic performance traits with CMD and CBSD traits at Alupe, Kakamega and Kibos; and (3) identify stable genotypes at Alupe, Kakamega and Kibos in Western Kenya.

4.3 Materials and Methods

4.3.1. Experimental Material

Refer to section 3.3.1.

4.3.2 Experimental Locations

Refer to section 3.3.2.

4.3.3 Experimental Design and Planting Details

Refer to section 3.3.3.

Table 4.1: Description of agronomic parameters of interest for elite cassava genotypes recorded in this study

Name of Parameter	Description and Estimation Formula
Biomass Yield (t ha^{-1})	Total fresh weight of harvested foliage and stems in tonnes per hectare = Dry Matter Content (% DMC)|100*Fresh Biomass Weight in kgs
Harvest Index (% HI)	Ratio of fresh root weight divided by total plant weight (biomass and fresh roots), expressed as percentage (%) = Fresh Root Weight, kg|100*Total Plant Weight in kgs (Fresh Root Weight in kgs + Fresh Biomass Weight in kgs)
Dry Matter Root Yield (t ha^{-1})	Dry weight of harvested roots derived by multiplying fresh storage root yield by dry matter content expressed in tonnes per hectares = 250g Fresh Root Weight - Dried Root Weight)|(250g Fresh Root Weight *100
Root dry matter content (% DMC)	Percentage dry matter content of storage roots. It is the ratio of dry root weight to the weight of 100 g fresh weight expressed in percentage = Dry Matter (DM)|250*100
Cyanogenic potential (CNP)	Cyanogenic potential of the fresh storage roots, determined by Picrate Concentration (PC) score method on a scale of 1-9
Fresh Root Yield (t ha^{-1})	Total fresh yield of storage roots harvested per plot measured in tonnes per hectare = Dry Matter Content (% DMC)|100*Fresh Root Weight in kg

Reference: (Fukuda et al., 2010).

4.3.4 Data Collection

Agronomic performance parameters of interest in this study are described in Table 4.1. At harvest time, 12 MAP, data on three agronomic traits, namely harvest index (HI), root dry matter content (DMC) and Cyanide content were computed. For estimating harvest index all harvested plants per genotype were partitioned into roots and biomass (stumps, stems and foliage). Thereafter, separate weights of roots and above-ground biomass were made and HI computed as the ratio of roots to the total biomass, expressed as a percentage (%). However, it's important to point out that the trials were carried out under open field conditions, hence leaf fall and all the roots were not accounted for in estimating HI. DMC was determined using the oven dry method, where fresh root samples of each variety (250 g) were taken in triplicate and dried to constant weight in an oven maintained at 72°C for 48h. The difference between fresh root weight and oven dried root weight (dry matter, DM) was then used to compute the dry matter content (% DMC) for each genotype. The Cyanogenic Potential (CNP) was carried out according to the procedure described by Fukuda et al., (2010). Cyanide content of fresh storage roots was determined by Picrate score (PC) method, characterized by colour change of the picrate on a 125 mm Whatman® filter paper strip as described by Fukuda et al., (2010). Colour change from pale green to dark brown was scored on a scale of 1 to 14 corresponding to a cyanide content of between <10ppm to>450ppm. Root sampling was standardized (using standard paper and a blank provided with the kit) to account for known root variation in cyanide concentration and analysis was done within an hour after harvesting.

4.3.5 Data Analysis

Data was entered into MS Excel spreadsheet and analysis was carried out using Genstat statistical software Release 15.2 (Genstat procedure library release PL23.2, VSN International, 2015). Agronomic performance was subjected to analysis of variance (ANOVA) to establish differences among cassava genotypes with dual resistance to cassava mosaic and brown streak diseases. Pearson's correlation analysis (correlation

coefficient R2 and significance level, $P < 0.05$) was carried out between: root and foliar incidence and severity; and root incidence and severity with DMC, HI, other agronomic traits, CMD and CBSD disease traits 12 MAP for associations. Combined analysis of variance across the environments (Alupe, Kakamega and Kibos) and partitioning the variance into components σ^2_G, σ^2_E, σ^2_{GxE} and σ^2_e were estimated based on the generalized mixed effect model, with genotype declared as fixed effects and location/environment as random effects using the following model:

$$\boldsymbol{Y_{ijkl} = \mu + G_i + E_j + GE_{ij} + R_{k(j)} + B_{l(k)} + \varepsilon_{ijkl}}$$

Where: Y_{ijkl} is observed value of genotype *i* in block *l* and replication *k* of environment *j*, μ is grand mean, G_i is effect of genotype *i*, E_j is environment or location effect, GE_{ij} is the interaction effect of genotype *i* with environment *j*, $R_{k(j)}$ is the effect of replication *k* in environment *j*, $B_{l(k)}$ is the effect of block *l* in replication *k*, ϵ_{ijkl} is error (residual) effect of genotype *i* in block *l* and replication *k* of environment *j*.

4.3.6 AMMI Stability Analysis of Agronomic Performance Traits

Similarities among test environments based on environmental main and GEI effects were evaluated using additive main effect and multiplicative interaction analyses. The method uses a combination of ANOVA and principal components analysis (PCA) (Jeberson et al., 2018; Purchase et al., 2013; Sharifi et al., 2017). Therefore, while ANOVA partitioned the variance into three components: genotype, environment and G× E deviations from the grand mean, the PCA partitioned the G × E deviations into different interaction principal component axes (IPCA). These were tested for statistical significance using AMMI ANOVA. Since the AMMI model does not make provision for a quantitative stability measure, and as such a measure is essential in order to quantify and rank genotypes in terms of agronomic performance i.e. yield, biomass, etc., stability, the following measure as proposed by (Purchase et al., 2013) was adopted for this study:

$$AMMI\ Stability\ Value\ (ASV) = \sqrt{\{\frac{IPCA\ 1\ Sum\ of\ squares}{IPCA\ 2\ Sum\ of\ Squares}\}(\text{IPCA1 Score})^2 + (\text{IPCA2 Score})^2}$$

Where, SSIPC1/SSIPC2 is the weight given to the IPC1 value by dividing the IPC1 sum of square on the IPC2 sum of square. The larger the IPCA (interaction principal component analysis) scores, either negative or positive, the more specifically adapted a genotype is to certain environments, smaller IPCA scores indicate a more stable genotype across environments. Therefore, genotypes with the highest ASV values are considered the most unstable in the test environments (specifically adapted to certain environments), while genotype with lowest ASV values close to zero (0) and one (1) are the most stable across environments (Gauch and Zobel 1988; Hongyu *et al*., 2014; Purchase, *et al*., 2013; Tadesse 2019) as shown in Appendix 5, 7 and 9.

4.4 Results

4.4.1 Analysis of Variance (ANOVA) for Agronomic Performance Traits of Elite Cassava Genotypes across Alupe, Kakamega and Kibos

Analysis of variance (Table 4.2) on agronomic performance traits of elite cassava genotypes with resistance to CMD and CBSD, evaluated across Alupe, Kakamega and Kibos, revealed that location alone had a highly significant influence ($P \leq 0.001$) on biomass yield (t ha^{-1}), fresh root yield (t ha^{-1}), dry matter (DM) yield (t ha^{-1}), harvest index (HI %) and dry matter content (% DMC). Analysis of variance (Table 4.2) further revealed that genotype alone had significant influence ($P \leq 0.001$) on cyanogenic potential (CNP). The interaction between genotype and location did not have any significant influence ($P \geq 0.05$) on fresh root yield, t ha^{-1}, biomass yield, tha^{-1}, dry matter yield, t ha^{-1}, harvest index (%), dry matter content (%) and cyanogenic potential at Alupe, Kakamega and Kibos (Table 4.2).

4.4.2 Fresh Root Yield (t ha-1)

The mean fresh root yield (t ha-1) is shown in Table 4.3, and was 9.21 t ha^{-1}, 11.62 t ha^{-1} and 20.35 t ha^{-1} at Alupe, Kakamega and Kibos respectively and 13.73 t ha^{-1}, across the three locations. Kibos recorded the highest fresh root yield on genotypes TZ-130 (32.85 t ha^{-1}) and LM/2008/363 (28.66 t ha^{-1}), and least on Orera (13.71 t ha^{-1}) and Colicanana (15.22 t ha^{-1}). Kakamega recorded the highest fresh root yield (Table 4.4) on genotypes Mkumba-2 (24.48 t ha^{-1}) and Mkumba (18.57 t ha^{-1}), and least on Kalawe (2.13 t ha^{-1}) and KBH/2006/026 (2.82 t ha^{-1}). Alupe recorded the highest fresh root yield on genotypes Eyope (14.32 t ha^{-1}), KBH/2006/026 (13.58 t ha^{-1}) and Nase-14 (13.65 t ha^{-1}), and least on Kalawe (2.43 t ha^{-1}) and Sauti (4.80 t ha^{-1}). Cassava genotypes with the highest mean fresh root yield across the three locations (Table 4.3) were TZ-130 (19.13 t ha^{-1}) and Mkumba-2 (17.92 t ha^{-1}), while the least was Kalawe (7.70 t ha^{-1}).

4.4.3 Dry Matter (DM) Yield (t ha-1)

The mean DM yield is shown in Table 4.3, and was 3.69 t ha^{-1}, 4.65 t ha^{-1} and 8.14 t ha^{-1} at Alupe, Kakamega and Kibos respectively, and 5.49 t ha^{-1} across the three locations. Alupe recorded the highest DM yield on genotypes KBH/2006/026 (7.43 t ha^{-1}), Eyope (5.73 t ha^{-1}) and TZ-130 (5.26 t ha^{-1}), and least on genotypes Kalawe (0.97 t ha^{-1}) and Sauti (1.92 t ha^{-1}). Kakamega recorded the lowest mean DM yield on genotypes Kalawe (0.85 t ha^{-1}) and KBH/2006/026 (1.13 t ha^{-1}), and the highest on genotypes Mkumba-2 (9.79 t ha^{-1}) and Mkumba (7.42 t ha^{-1}). Kibos, on the other hand, recorded the highest mean DM yield on genotypes TZ-130 (13.14 t ha^{-1}) and LM/2008/363 (11.46 t ha^{-1}), and the least on genotypes Orera (5.48 t ha^{-1}) and Yizaso (5.87 t ha^{-1}). Across the three locations (Table 4.3), the highest DM yield was recorded TZ-130 (7.65 t ha^{-1}) and the least on Kalawe (3.09 t ha^{-1}).

4.4.4 Biomass Yield (t ha-1)

The mean biomass yield (t ha-1) is shown in Table 4.4, and was 3.72 t ha^{-1}, 7.98 t ha^{-1} and 9.50 t ha^{-1} at Alupe, Kakamega and Kibos respectively, and 7.07 t ha^{-1} across the three locations (). Cassava genotype TZ-130 recorded the highest biomass yield at Kakamega (12.03 t ha^{-1}) and Kibos (12.37 t ha^{-1}), but not at Alupe (3.04 t ha^{-1}). Alupe recorded the highest biomass yield on genotypes CH05-295 (6.63 t ha^{-1}) and Mkumba-2 (6.24 t ha^{-1}), and the least on Sauti (2.31 t ha^{-1}). Kakamega recorded the highest biomass yield on genotype Yizaso (12.22 t ha^{-1}), and the least on KBH/2002/066 (4.08 t ha^{-1}). While Kibos recorded the highest biomass yield on genotypes Kizimbani (13.94 t ha^{-1}) and Kibandameno (13.56 t ha^{-1}), and the least on Colicanana (4.82 t ha^{-1}). Cassava genotypes with the highest mean biomass yield across the three locations (Table 4.4), were Kizimbani (9.55 t ha^{-1}), CH05-203 (9.40 t ha^{-1}) and Sangoja (9.31 t ha^{-1}). While, those genotypes with the least biomass yield were Nase-1 (5.42 t ha^{-1}), Colicanana (5.48 t ha^{-1}) and Kalawe (5.55 t ha^{-1}).

Table 4.2: ANOVA for agronomic performance of cassava genotypes 12 MAP at Alupe, Kakamega and Kibos

	Location (L)			Genotype (G)			Interaction (G*L)		
Agronomic Traits	**DF**	**Mean Square**	**F-value**	**DF**	**Mean Square**	**F-value**	**DF**	**Mean Square**	**F-value**
Fresh Root Yield, t ha^{-1}	2	2471.17	39.51***	23	47.85	0.77	46	62.71	1.00
Biomass Yield, tha^{-1}	2	640.61	49.56***	23	13.15	1.02	46	14.98	1.16
Dry Matter Yield, t ha^{-1}	2	395.39	39.51***	23	7.66	0.77	46	10.03	1.00
Harvest Index (%)	2	3752.35	15.39***	23	205.10	0.84	46	191.28	0.78
Dry Matter Content (%)	2	1243.56	35.07***	23	42.98	1.21	46	43.54	1.23
Cyanogenic Potential	2	0.12	0.88	23	11.12	79.51***	46	0.20	1.41

*DF=Degrees of Freedom; ***=Significance (P≤0.001)*

Table 4.3: Means and AMMI Stability Values (ASV) with ranks of fresh root and dry matter (DM) yield for elite cassava genotypes 12 MAP at Alupe, Kakamega and Kibos

Cassava Genotypes	Fresh Root Yield, t ha^{-1}						Dry Matter (DM) Yield, t ha^{-1}					
	Alupe	**Kakamega**	**Kibos**	**Mean**	**ASV**	**Rank**	**Alupe**	**Kakamega**	**Kibos**	**Mean**	**ASV**	**Rank**
CH05-203	11.98	9.52	23.49	15.00^{ab}	1.05	6	4.79	3.81	9.40	6.00^{ab}	0.42	6
Colicanana	9.22	16.67	15.22	13.70^{ab}	3.16	17	3.69	6.67	6.09	5.48^{ab}	1.27	17
Eyope	14.32	14.16	19.04	15.84^{ab}	1.28	7	5.73	5.66	7.61	6.33^{ab}	0.51	7
F10-30-R5	7.07	16.74	23.98	15.92^{ab}	1.80	10	2.83	6.70	9.59	6.37^{ab}	0.72	9
F19-NL	5.51	7.76	25.16	12.81^{ab}	3.01	15	2.20	3.11	10.06	5.12^{ab}	1.21	15
Kalawe	2.43	2.13	18.59	7.70^{a}	1.89	11	0.97	0.85	7.44	3.09^{a}	0.75	11
KBH/2002/066	9.34	9.52	18.18	12.35^{ab}	0.21	1	3.74	3.81	7.27	4.94^{ab}	0.08	1
KBH/2006/026	13.58	2.82	17.82	12.35^{ab}	5.31	23	5.43	1.13	7.13	4.94^{ab}	2.12	23
Kibandameno	7.15	11.22	21.93	13.43^{ab}	0.41	2	2.86	4.49	8.77	5.37^{ab}	0.16	2
Kizimbani	9.18	11.45	15.88	12.17^{ab}	0.77	4	3.67	4.58	6.35	4.87^{ab}	0.31	4
LM/2008/363	9.68	10.38	28.66	16.24^{ab}	3.17	18	3.87	4.15	11.46	6.49^{ab}	1.27	18
Mkumba	7.26	18.57	16.30	14.05^{ab}	4.16	21	2.91	7.43	6.52	5.62^{ab}	1.67	21
Mkumba-2	10.72	24.48	18.57	17.92^{b}	7.20	24	4.29	9.79	7.43	7.17^{b}	2.88	24
Nase-1	5.89	6.81	25.46	12.72^{ab}	3.50	20	2.36	2.73	10.18	5.09^{ab}	1.40	20
Nase-14	13.65	6.01	21.04	13.57^{ab}	3.16	16	5.46	2.40	8.42	5.43^{ab}	1.26	16
Nase-18	9.67	8.97	22.19	13.61^{ab}	0.65	3	3.87	3.59	8.88	5.44^{cd}	0.26	3
Nase-3	12.89	11.32	19.31	14.50^{ab}	0.79	5	5.15	4.53	7.72	5.80^{ab}	0.31	5
Orera	10.00	11.45	13.71	11.72^{ab}	1.99	13	4.00	4.58	5.48	4.69^{ab}	0.80	13
Pwani	10.11	16.35	17.13	14.53^{ab}	1.93	12	4.04	6.54	6.85	5.81^{ab}	0.77	12
Sangoja	5.42	15.22	20.49	13.71^{ab}	1.67	8	2.17	6.09	8.20	5.49^{ab}	0.67	8
Sauti	4.80	14.63	17.93	12.45^{ab}	1.80	9	1.92	5.85	7.17	4.98^{ab}	0.72	10
Tajirika	10.25	5.18	20.75	12.06^{ab}	2.11	14	4.10	2.07	8.30	4.82^{ab}	0.84	14
TZ-130	13.15	11.40	32.85	19.13^{ab}	5.14	22	5.26	4.56	13.14	7.65^{b}	2.06	22
Yizaso	7.83	16.23	14.67	12.91^{ab}	3.26	19	3.13	6.49	5.87	5.16^{ab}	1.30	19
Location Mean	9.21	11.62	20.35	13.70			3.69	4.65	8.14	5.49		

ASV=AMMI Stability Value (Nduwumuremyi et al. 2017; Purchase, Hatting, and Deventer 2013)*; $LSD_{0.05}$=least significance difference at 5% (Fresh root yield, $LSD_{0.05}$ location=7.34, $LSD_{0.05}$ variety=2.61, $LSD_{0.05}$ loc*var=12.77; Dry Mater (DM) yield - $LSD_{0.05}$ location= 2.95, $LSD_{0.05}$ variety=1.04, $LSD_{0.05}$ loc*var=5.11); CV_{rep}=% Coefficient of Variation (; Fresh root yield, CV% $_{rep}$=4.70; DM yield, CV% $_{rep}$=4.78); SE-Standard Error (Biomass yield, SE=1.20; Fresh root yield, SE=1.05); Means with different superscript letters were significantly different (P<0.05)*

Table 4.4: Means of biomass yield and harvest index for elite cassava genotypes 12 MAP at Alupe, Kakamega and Kibos

Cassava Genotypes	Biomass Yield, t ha^{-1}				Harvest Index (%)			
	Alupe	Kakamega	Kibos	Mean	Alupe	Kakamega	Kibos	Mean
CH05-203	6.63	10.06	8.51	9.40^{a}	40.63	33.85	52.45	42.31^{ab}
Colicanana	3.12	8.49	4.82	5.48^{a}	49.01	43.64	56.13	49.59^{b}
Eyope	4.13	9.41	10.41	7.98^{a}	56.49	37.38	42.85	45.57^{ab}
F10-30-R5	2.92	10.63	11.38	8.31^{a}	50.62	37.57	47.23	45.14^{ab}
F19-NL	3.72	5.30	10.77	6.59^{a}	41.75	35.20	51.89	42.94^{ab}
Kalawe	2.68	4.56	9.41	5.55^{a}	22.58	15.87	47.51	28.65^{a}
KBH/2002/066	2.76	4.08	11.28	6.75^{a}	59.97	41.06	38.55	46.55^{ab}
KBH/2006/026	4.10	5.27	11.47	6.04^{a}	57.83	26.39	38.01	43.91^{ab}
Kibandameno	4.20	8.08	13.56	8.62^{a}	53.88	35.95	39.07	43.97^{ab}
Kizimbani	2.94	8.77	13.94	9.55^{a}	55.48	37.65	32.32	41.82^{ab}
LM/2008/363	4.16	6.34	8.14	6.21^{a}	47.97	37.51	58.47	47.98^{b}
Mkumba	3.07	9.75	7.40	6.74^{a}	40.74	43.89	46.83	43.82^{ab}
Mkumba-2	6.24	9.95	6.23	7.47^{a}	43.08	40.36	54.97	46.14^{ab}
Nase-1	2.45	5.49	8.31	5.42^{a}	49.59	31.62	55.60	45.60^{ab}
Nase-14	3.73	8.50	5.69	5.97^{a}	57.23	26.98	60.48	48.28^{b}
Nase-18	3.51	4.71	8.71	5.64^{a}	52.63	40.39	50.19	47.7^{4b}
Nase-3	3.27	5.48	8.74	5.83^{a}	60.67	37.41	47.29	48.46^{b}
Orera	4.03	5.35	11.87	7.08^{a}	49.77	39.15	32.85	40.59^{ab}
Pwani	4.88	11.30	6.69	7.62^{a}	45.66	36.73	50.11	44.17^{ab}
Sangoja	4.68	11.24	12.01	9.31^{a}	30.00	29.93	39.94	33.29^{ab}
Sauti	2.31	7.06	10.53	6.63^{a}	37.08	43.47	38.92	39.83^{ab}
Tajirika	4.17	7.36	9.12	6.68^{a}	46.39	25.31	45.10	38.93^{ab}
TZ-130	3.04	12.03	12.37	9.15^{a}	55.70	23.62	50.33	43.22ab
Yizaso	2.62	12.22	6.67	7.17^{a}	54.17	33.94	45.74	44.62^{ab}
Location Mean	3.72	7.98	9.50	7.07	48.29	34.79	46.79	43.40

*$LSD_{0.05}$=least significance difference at 5% (Biomass yield - $LSD_{0.05}$ locations=2.95, $LSD_{0.05}$ varieties=1.04, $LSD_{0.05}$ loc*var=5.11; Harvest Index - $LSD_{0.05}$ locations=14.23, $LSD_{0.05}$ varieties=5.03, $LSD_{0.05}$ loc*var=9.58); CVrep=% Coefficient of Variation (Biomass yield, CV% $_{rep}$=4.70; Harvest Index - CV% $_{rep}$=8.50); SE-Standard Error (Biomass yield, SE=2.63; Harvest Index, SE=3.20); Means with different superscript letters were significantly different (P<0.05)*

Table 4.5: Means of dry matter content (% DMC) and cyanogenic potential (CNP) for elite cassava genotypes 12 MAP in Alupe, Kakamega and Kibos

Genotype	Dry Matter Content (% DMC)				Cyanogenic Potential (CNP)			
	Alupe	Kakamega	Kibos	Mean	Alupe	Kakamega	Kibos	Mean
CH05-203	39.30	53.30	41.50	44.69^{c}	6.00	6.00	6.00	6.00^{e}
Colicanana	34.30	50.50	40.60	41.82^{abc}	4.00	4.00	4.00	4.00^{b}
Eyope	37.40	44.50	42.50	41.47^{abc}	6.00	5.33	6.00	5.78^{e}
F10-30-R5	38.80	45.50	43.80	42.71^{abc}	4.00	3.67	4.00	3.89^{b}
F19-NL	35.90	44.10	43.70	41.24^{abc}	4.00	4.00	4.00	4.00^{b}
Kalawe	32.70	42.50	42.00	39.07^{abc}	5.00	5.00	5.00	5.00^{d}
KBH/2002/066	38.30	38.30	46.10	40.91^{abc}	3.00	3.00	3.00	3.00^{a}
KBH/2006/026	39.30	29.70	40.60	36.27^{a}	6.00	6.00	6.00	6.00^{e}
Kibandameno	28.90	45.00	43.70	39.22^{abc}	4.00	4.00	4.00	4.00^{b}
Kizimbani	39.80	45.70	45.60	43.71^{bc}	5.00	4.67	5.00	4.89^{cd}
LM/2008/363	36.50	41.40	40.10	39.31^{abc}	6.00	6.00	6.00	6.00^{e}
Mkumba	38.30	47.60	43.80	43.24^{bc}	3.00	4.00	3.00	3.33^{a}
Mkumba-2	37.90	47.10	44.40	43.10^{1abc}	3.00	3.00	3.00	3.00^{a}
Nase-1	26.40	45.90	42.90	38.40^{abc}	3.00	3.00	3.00	3.00^{a}
Nase-14	42.60	45.00	39.40	42.33^{abc}	5.00	4.00	5.00	4.67^{cd}
Nase-18	33.20	41.10	45.90	40.04^{abc}	3.00	3.00	3.00	3.00^{a}
Nase-3	41.20	42.90	44.90	43.00^{abc}	6.00	5.00	6.00	5.67^{e}
Orera	36.00	42.10	43.30	40.44^{abc}	4.00	4.00	4.00	4.00^{b}
Pwani	41.80	47.10	41.90	43.58^{bc}	6.00	6.00	6.00	6.00^{e}
Sangoja	38.90	45.70	41.70	42.11^{abc}	4.00	4.33	4.00	4.11^{b}
Sauti	30.50	43.90	45.20	39.87^{abc}	3.00	3.33	3.00	3.11^{a}
Tajirika	36.50	44.30	42.30	41.04^{abc}	5.00	5.00	5.00	5.00^{d}
TZ-130	30.30	42.10	39.90	37.47^{ab}	5.00	4.67	4.00	4.56^{c}
Yizaso	35.60	45.30	41.70	40.84^{abc}	6.00	6.00	6.00	6.00^{e}
Location Mean	36.30	44.20	42.80	41.10	4.54	4.46	4.50	4.50
Std. Error (SE)	2.00	2.00	2.00	2.00	0.22	0.22	0.22	0.22
$LSD_{0.05}$ Locat	5.53	5.53	5.53	5.53	0.12	0.12	0.12	0.12
$LSD_{0.05}$ Variety	1.96	1.96	1.96	1.96	0.35	0.35	0.35	0.35
$LSD_{0.05}$ L*V	9.58	9.58	9.58	9.58	0.61	0.61	0.61	0.61
CV% rep	2.80	2.80	2.80	2.80	0.30	0.30	0.30	0.30

$LSD_{0.05}$=least significance difference at 5%; CV=% Coefficient of Variation; SE-Standard Error; *Means with different superscript letters were significantly different (P<0.05)*

4.4.5 Harvest Index (HI %)

The harvest index (%) is shown in Table 4.5, and was 48.29%, 34.79% and 46.79% at Alupe, Kakamega and Kibos respectively, and 43.40% across the three locations. Alupe recorded the highest HI on genotypes Nase-3 (60.67%) and KBH/2002/066 (59.97%), and the least HI on genotypes Kalawe (22.58%) and Sangoja (30.00%). Kakamega recorded the least HI on genotypes Kalawe (15.87%), TZ-130 (23.62) and Tajirika (25.31%), and the highest HI on genotypes Mkumba (43.89%) and Colicanana (43.64). Kibos recorded the least HI on genotypes Kizimbani (32.32%) and Orera (32.85%), and the highest HI on genotypes Nase-14 (60.48%) and LM/2008/363 (58.47%). Across the three locations, (Table 4.5), HI for all cassava genotypes ranged from 2865 – 49.59%, and was highest on genotypes Colicanana (49.59%) and Nase-3 (48.46%), and least on genotypes Sangoja (33.29%) and Kalawe (28.65%).

4.4.6 Dry Matter Content (% DMC)

The % DMC is shown in Table 4.5, and was 36.30%, 44.20% and 42.80% at Alupe, Kakamega and Kibos, respectively, and 41.10% across the three locations. Alupe recorded the highest DMC on genotypes Nase-14 (42.60%), Nase-3 (41.20%) and Pwani (41.80%), and the least DMC on genotypes Kibandameno (28.90%) and Nase-1 (26.40%). Kakamega recorded the highest DMC on genotypes CH05-203 (53.30%) and Colicanana (50.50%), and the least DMC on genotypes KBH/2006/026 (29.70%) and KBH/2002/066 (38.30%). Kibos recorded the highest DMC on genotype KBH/2002/066 (46.10%) and NASE-18 (45.90%), and the least DMC on genotypes Nase-14 (39.40%) and TZ-130 (39.90%). Across the three locations (Table 4.5), genotypes with the highest DMC were CH05-203 (44.69%), Kizimbani (43.71%) and Pwani (43.58%), and genotypes with least DMC were TZ-130 (37.47%) and KBH/2006/026 (36.27%).

4.4.7 Cyanogenic Potential (CNP)

Results of cyanogenic potential in Table 4.5. revealed that all the 24 cassava genotypes across the three locations had mean CNP levels ranging from of 3.00–6.00, categorized as very low, low, moderately low and moderate CNP levels. As shown in Table 4.6, the analysis and results also revealed distinct genotypes for each picrate concentration (PC) category across the three locations. Hence, very low CNP (PC Score 3.00) genotypes comprised of KBH/2002/066 (3.00), Mkumba (3.33), Mkumba-2 (3.00), NASE-1 (3.00), Nase-18 (3.00) and Sauti (3.11). Low CNP (PC score 4.00) genotypes comprised Colicanana (4.00), F10-30-R5 (3.89), Kibandameno (4.00), Nase-14 (4.00), Orera (4.00), Sangoja (4.11) and F19-N (4.00). Moderately low CNP (PC score 5.00) genotypes comprised Kalawe (5.00), Kizimbani (4.89), Nase-14 (4.67), Tajirika (5.00) and TZ-130 (4.46). Moderate CNP (PC score 6.00) genotypes comprised CH05-203 (6.00), Eyope (5.78), KBH/2006/026 (6.00), LM/2008/363 (6.00), NASE-3 (5.67), Pwani (6.00) and Yizaso (6.00).

Table 4.6: Cyanogenic Potential (CNP) for elite cassava genotypes at 12 MAP at Alupe, Kakamega and Kibos

Very Low CNP (PC Score 3.00)	**Low CNP (PC Score 4.00)**	**Moderately Low CNP (PC Score 5.00)**	**Moderate CNP (PC Score 6.00)**
KBH/2002/066 (3.00^{a})	Colicanana (4.00^{b})	Kalawe (5.00^{d})	CH05-203 (6.00^{e})
Mkumba (3.33^{a})	F10-30-R5 (3.89^{b})	Kizimbani (4.89^{cd})	Eyope (5.78^{e})
Mkumba-2 (3.00^{a})	Kibandameno (4.00^{b})	Nase-14 (4.67^{cd})	KBH/2006/026 (6.00^{e})
Nase-1 (3.00^{a})	Orera (4.00^{b})	Tajirika (5.00^{d})	LM/2008/363 (6.00^{e})
Nase-18 (3.00^{a})	Sangoja (4.11^{b})	TZ-130 (4.56^{c})	Nase-3 (5.67^{e})
Sauti (3.11^{a})	F19-NL (4.00^{b})		Pwani (6.00^{e})
			Yizaso (6.00^{e})

Values in brackets represents the actual mean cyanide levels for each genotype; *Means with different superscript letters were significantly different (P<0.05)*

4.4.8 Correlation amongst CMD and CBSD Resistance Traits with Agronomic Traits

As shown in Tables 4.3 and 4.5, dry matter **(**DM) yield was used to calculate the % dry matter content (DMC). Hence the two agronomic traits had highly positive significant relationship (*P = 0.001*), with 1.000 as coefficient of correlation (Table 4.7). Therefore, the correlation coefficients and *P - values* of DM yield and % DMC with other agronomic and disease traits are the same (Table 4.7). The relationship of cyanogenic potential (CNP) with other agronomic traits was positive and weak, but not significant (*P > 0.05*) as shown in Table 4.7. DM yield was significant (*P < 0.05*) and positively correlated with biomass yield, harvest index (HI) and fresh root yield. The relationship between HI and fresh root yield was positive and highly significant (*P = 0.001*). However, HI was highly significant, but negatively (*P = 0.001*) associated with biomass yield. Further, HI was negative but significantly associated with DM yield (*P = 0.01*) and % DMC (*P = 0.05*). The relationship between biomass yield, DM yield, % DMC and fresh root yield was positive and highly significant (*P = 0.001*). There was also high significant (*P = 0.001*) and positive relationship between fresh root yield and HI for the 24 cassava genotypes across the three locations (Table 4.7). However, the relationship between fresh root yield, DM yield and % DMC was positive, weak but significant (*P < 0.05*). The association between CMD and CBSD incidence and severity 12 MAP across the three locations was positive and significant (*P < 0.05*) as shown in Table 4.7. The relationship between biomass yield and disease resistance parameters was negative (inverse), but significant (*P < 0.05*). While, the relationship between CNP and CMD and CBSD incidence and severity was inverse, but not significant (*P > 0.05*). The relationship between DM yield, % DMC and disease traits was inverse, but highly significant (*P ≤ 0.001*). The association between HI with CBSD incidence, CBSD severity and CMD incidence was positive, but not significant (*P > 0.05*), while CMD severity was inverse, but not significant (*P > 0.05*). The relationship between fresh root yield and CBSD incidence was inverse, but not significant (*P > 0.05*). However, as shown in Table 4.7., the relationship between fresh root yield with CBSD severity, CMD incidence and CMD severity was negative, but significant (*P < 0.05*).

Table 4.7: Correlations among agronomic performance and disease resistance traits for elite cassava genotypes 12 MAP at Alupe, Kakamega and Kibos

Agronomic and Disease Parameters	Biomass Yield	CBSD Incidence	CBSD Severity	CMD Incidence	CMD Severity	CNP	DM Yield	DM Content	Harvest Index
CBSD Incidence	-0.196*								
CBSD Severerity	-0.133*	0.916***							
CMD Incidence	-0.184**	0.820***	0.779***						
CMD Severity	-0.202**	0.758***	0.747***	0.931***					
CNP	0.102	-0.063	-0.047	-0.061	-0.073				
DM Yield	0.332***	-0.384***	-0.363***	-0.468***	-0.537***	0.012			
DM Content	0.332***	-0.384***	-0.363***	-0.468***	-0.537***	0.012	1.000***		
Harvest Index (%)	-0.353***	0.027	0.005	0.013	-0.027	0.019	-0.153**	-0.153*	
Fresh Root Yield	0.410***	-0.168	-0.140*	-0.183***	-0.208**	0.068	0.134*	0.134*	0.587***

Note: Cassava Mosaic and Brown Streak Disease scores are from Chapter 3; Correlation Coefficients and level of significance test *=P<0.05, **=P<0.01, ***=P<0.001

4.4.9 Confirmation of Stability for Root Yield amongst the Elite Cassava Genotypes

Confirmation of stability for root yield production (fresh root yield, dry matter yield and combined fresh root and dry matter yield) amongst the 24 elite cassava genotypes at Alupe, Kakamega and Kibos was achieved using AMMI analysis and AMMI Stability Value (Table 4.4). AMMI Stability Values (ASV) and hence genotype ranks, showed that yield performance amongst cassava genotypes was variable across Alupe, Kakamega and Kibos. However, based on ASV, the top five genotypes with best possible root yield performance due to their very low ASV values (towards zero) with high stability and wider adaptability across environments (location), are listed in Table 4.8. Dry matter (DM) yield was derived from root yield, hence genotype ASV between the two were the same (Table 4.4.and 4.8). Therefore, most stable genotypes for fresh root yield and DM yield, respectively, across Alupe, Kakamega and Kibos were KBH/2002/066 (0.21, 0.08), Kibandameno (0.41, 0.16), Nase-18 (0.65, 0.26), Kizimbani (0.77, 0.31) and Nase-3 (0.79, 0.31). AMMI analysis based on AMMI Stability Value (ASV) and genotype ranks identified unstable genotypes, with ASV towards one (1), and hence more specific adaptability, to either Alupe, Kakamega or Kibos are shown in Table 4.4 and 4.9. Most unstable genotypes for fresh root yield and DM yield, respectively, based on ASV were Nase-1 (3.50, 1.40), Mkumba (4.16, 1.67), TZ-130 (5.14, 2.06), KBH/2006/026 (5.31, 2.12) and Mkumba-2 (7.20, 2.88). Based on combined ASVs and ranking (Table 4.8 and Appendix 5), elite cassava genotypes that were stable for root yield performance across Alupe, Kakamega and Kibos comprised Nase-18, F10-30-R5, Nase-3, Tajirika and Eyope. While, genotypes that were unstable, with specific adaptability to either Alupe, Kakamega or Kibos comprised KBH/2006/026, TZ-130, Nase-14, Kalawe and Mkumba-2. It should be noted that Mkumba-2 was adopted from the remaining planting materials (left overs) of Mkumba to balance the Alpha lattice design. Hence, the quality of the planting materials could have been low, leading to poor performance compared to the original Mkumba.

Table 4.8: Confirmation of stable and unstable amongst elite cassava genotypes based on ASV and ranking for root yield performance across Alupe, Kakamega and Kibos

Parameter	Rank 1	Rank 2	Rank 3	Rank 4	Rank 5
	Stable genotypes across environments				
Fresh Root Yield	KBH/2002/066	Kibandameno	Nase-18	Kizimbani	Nase-3
	(0.21)	(0.41)	(0.65)	(0.77)	(0.79)
Dry Matter Yield	KBH/2002/066	Kibandameno	Nase-18	Kizimbani	Nase-3
	(0.08)	(0.16)	(0.26)	(0.31)	(0.31)
Combined Yield	KBH/2002/066	Kibandameno	Nase-18	Kizimbani	Nase-3
	(0.15)	(0.29)	(0.46)	(0.54)	(0.55)
	Unstable genotypes adapted more to specific environments				
	Rank 20	Rank 21	Rank 22	Rank 23	Rank 24
Fresh Root Yield	Nase-1	Mkumba	TZ-130	KBH/2006/026	Mkumba-2
	(3.50)	(4.16)	(5.14)	(5.31)	(7.20)
Dry Matter Yield	Nase-1	Mkumba	TZ-130	KBH/2006/026	Mkumba-2
	(1.40)	(1.67)	(2.06)	(2.12)	(2.88)
Combined Yield	Nase-1	Mkumba	TZ-130	KBH/2006/026	Mkumba-2
	(2.45)	(2.92)	(3.60)	(3.72)	(5.04)

4.5 Discussion

The results from this study showed significant differences ($P \leq 0.05$) between genotypes and location or agro-ecology, but not the interaction between genotype and location ($P \geq 0.05$), for all the agronomic traits evaluated. This implied that the 24 elite cassava genotypes responded differently to agronomic performance at Alupe, Kakamega and Kibos. These findings were similar to what was reported by (Tembo *et al*., 2017; Tumuhimbise, 2013), who found out significant variation in agronomic traits among cassava genotypes evaluated in diverse locations and at different harvesting times and interaction between genotypes and location. The same experiences on genotype by environment interaction were variously reported by (Anthony *et al*., 2015; Esuma *et al*., 2016; Mtunguja *et al*., 2016; Nduwumuremyi *et al*., 2017; Tesfaye *et al*., 2017; Tumuhimbise *et al.*, 2014; Tumwegamire *et al*., 2018).

Previous studies have shown cyanide potential (CNP) varies considerably with genotypes and across environment (Mtunguja *et al*., 2016). However, this study found significant difference ($P \leq 0.05$) for CNP between cassava genotypes, but not location and genotype by location interaction, contrary to what has been reported in previous studies. Cyanide content of fresh storage roots, in this study, was determined by Picrate Acid Concentration score (PC) method, characterized by colour change of the picrate on a 125 mm Whatman® filter paper strip as described by (Fukuda *et al*., 2010). Colour change from pale green to dark brown was scored on a scale of 1 to 14 corresponding to a cyanide content of between < 10ppm to > 450ppm. According to Brito, *et al*., 2009; Mbah, Nwankwo, *et al.*, 2019; and Mtunguja *et al.*, 2016, different varieties of cassava also have variations in their root's cyanogenic content, ranging from 10 to 450 mg HCN-.kg-1 fresh weight. Among the two main cassava groups, bitter cassava is characterized by its high contents of Cyanogenic Glycosides (15–450 mg HCN per kilogram of fresh weight of roots) while sweet cassava with low cyanide contents will typically contain approximately 10–150 mg HCN per kilogram of fresh weight of roots. All the 24

cassava genotypes evaluated across the three locations in this study had mean CNP levels ranging from of 3.00–6.00 (specifically, very low, low, moderately low and moderate CNP levels based on Picrate Acid Concentration, PC scale), and were therefore, sweet and not bitter.

Harvest index (HI) is used to determine the efficiency by which cassava converts the dry matter into the economic tuberous roots yield (Tumuhimbise, 2013). The shorter the variety the higher the index value. The more the value of the HI of a crop or a variety the more is the efficiency of the crop to convert the dry matter into the economic part, which is the tuberous root for cassava crop. Studies have shown that HI negatively correlates with plant biomass yield and positively correlates with tuberous roots yield. It is expected that an increase in plant biomass yield consequently reduces HI since it represents the ratio between tuberous root yields and total plant weight. On the other hand, increasing tuberous roots yield induces higher harvest indexes (Avijala *et al.*, 2015; Esuma *et al.*, 2016) This finding was consistent with the present study, where harvest index was average (mean 43.29%) for the 24 genotypes. Further, harvest index was negatively correlated to biomass yield, hence an increase in biomass yield would result into a decrease in HI and vice versa; but positively and significantly correlated to fresh root yield. As previously reported by Avijala *et al.*, 2015, and similar to findings of this study, there was great variability among the data for agronomic performance of the elite cassava genotypes, which was verified by the range of the results of evaluated traits: mean tuberous fresh roots yield = 7.70 - 19.13 t ha^{-1}; mean dry matter (DM) yield = 3.09 -7.65 t ha^{-1}; mean shoot biomass yield = 5.42 -o 9.55 t ha^{-1}; mean harvest index = 28.65 - 49.59%; mean dry matter content = 36.27 - 44.69, and cyanogenic potential (CNP) = 3.00 - 6.00, with the respective means of 13.70 t ha^{-1}; 5.49 t ha^{-1}; 7.07 t ha^{-1}; 43.10%, 41.10% and 4.50.

Although CMD and CBSD incidence and severity correlated negatively in many cases with biomass yield, fresh root yield and harvest index, some of the 24 elite cassava genotypes had significant low fresh root yield, even with mild or no symptoms,

indicating lack of a general correlation between symptom severity and yield loss. The presence of significant differences ($P < 0.05$) between the test environments for fresh root yield revealed that the 24 elite cassava genotypes performed differently across the three locations. The significance of environmental effects in evaluating cassava genotypes for agronomic performance was also manifested by the significant G x E interaction effects. The current result was supported by previous similar findings (Esuma et al., 2016; Tadesse, 2019; Tumuhimbise et al., 2014). Correlation results, further, show that location level occurrences of CBSD and CMD were dependent of each other, due to positive correlation between them. Hence, infection with either disease seemed to affect the incidence and severity of the other. Similar observations on responses of the different varieties to the two diseases were reported previously (Katono *et al*., 2015; Rwegasira and Rey, 2012; Tumwegamire *et al*., 2018).

The significant but negative relationship between CMD and CBSD incidence and severity with agronomic performance implied that their relationship was inverse. This was consistent with findings reported by several authors (Abaca *et al.*, 2012; Kuria *et al.*, 2017; Nduwumuremyi *et al*., 2017; Rwegasira and Rey, 2012; Tembo *et al*., 2017; Tumuhimbise *et al*., 2014). Confirmation of stability for agronomic performance was achieved through AMMI analysis. The AMMI model combines the analysis of variance for the genotype and environment main effects with principal components analysis of the GEI interaction effect. Stability (genotype-environment productivity and performance) was confirmed by the AMMI stability value (ASV), developed by (Purchase, *et al*., 2013), based on the AMMI model's IPCA1 and IPCA2 (interaction principal components axes 1 and 2, respectively) scores for each genotype. The ASV is comparable with the joint regression methods of (Eberhart and Russell, 1966) and (Shukla, 1972) to determine stability. Hence, genotypes with lower ASV values are considered more stable and genotypes with higher ASV are unstable. Based on ASV, this study was able to identify stable and unstable genotypes for yield performance across Alupe, Kibos and Kakamega. Stable genotypes for fresh root yield and DM yield,

across Alupe, Kakamega and Kibos were KBH/2002/066, Kibandameno, Nase-18, Kizimbani and Nase-3. Unstable genotypes for fresh root yield and DM yield, based on ASV and ranks were Nase-1, Mkumba, TZ-130, KBH/2006/026 and Mkumba-2.

4.6 Conclusion

The relationship between CMD and CBSD incidence and severity and their combined influence on agronomic traits for the elite cassava genotypes was not similar (variable) across Alupe, Kakamega and Kibos. Stability for high yield is the ultimate objective of cassava breeding programmes, as cassava is mainly grown for its storage roots. The study, using AMMI analysis, based on AMMI Stability Value (ASV) identified stable genotypes for yield performance (tuberous fresh root yield and dry matter (DM) yield), across Alupe, Kakamega and Kibos. These were KBH/2002/066, Kibandameno (a local standard check), Nase-18, Kizimbani and Nase-3. All these genotypes were sweet, with cyanogenic potential between 3.00 to 6.00. These superior genotypes needs to be further evaluated in more environments to assess their specific and wider adaptability and stability, including possible recommendation for release to farmers for cultivation.

CHAPTER FIVE

GENERAL DISCUSSION

5.1 Intoduction General Discussion

As previously reported by Tumwegamire *et al*., 2018, breeding for dual resistance (defined as the control of resistance to CMD and CBSD is genetically linked or random occurrence, whenever, CMD and CBSD is present in a genotype), is currently being pursued as the most cost-effective and sustainable way to manage the devastating effects of the viral diseases in ESA. Although high resistance for CMD has been found, only limited success has been documented for CBSD (Legg *et al.*, 2014). The desired goal of the breeding efforts is stable genotypes, that are high yielding, but with resistance to both viral diseases. Further, collaborative efforts with different national cassava breeding programs have identified germplasm which is resistant or tolerant to CBSD/CMD. However, these have been evaluated so far under a narrow range of conditions of environment, virus species/strains, and vector abundance (Legg *et al.*, 2014). The exchange of germplasm between countries, affected by viral diseases, enhances the diversity of germplasm available to partner countries. It will also provide breeders with fresh opportunities to evaluate and release new varieties as well as to use them as parents in efforts to breed new genotypes with dual resistance to CBSD and CMD (Tumwegamire *et al.*, 2018).

Therefore, the main purpose of this study was to contribute towards production of high yielding, clean cassava planting materials through assessment of elite cassava clones for resistance to CMD and CBSD at Alupe, Kakamega and Kibos in Kenya. Further, to contribute to progress in breeding for host plant resistance, which is still slow currently, due to limited knowledge on variation in resistance to the two diseases. The specific objectives were to: (1) Evaluate elite cassava genotypes for CMD and CBSD disease resistance parameters at Alupe, Kakamega and Kibos, which are known CMD and

CBSD hot spot locations in Western Kenya; and (2) Evaluate elite cassava genotypes for variation in agronomic traits and determine their correlation with CMD and CBSD resistance traits at Alupe, Kakamega and Kibos in Western Kenya. The study presented here, therefore, describes comprehensive evaluation of dual resistance to CMD and CBSD and agronomic performance reported to date, involving twenty-three (23) elite cassava genotypes under three environments, with diverse agro-ecological conditions in Western Kenya.

5.2 Evaluation of Elite Cassava Genotypes for CMD and CBSD Resistance Traits

The results from this study showed significant differences between genotypes, locations, and month after planting (MAP) time (plant age), CMD and CBSD resistance traits and agronomic traits evaluated. However, significant one and two way interactions were detected for CMD and CBSD resistance and some agronomic traits. This implies that the genotypes responded differently to the locations and MAP time. This phenomenon was also reported by Tumuhimbise *et al.*, 2014, who observed similar results for cassava fresh root yield. Our findings also agreed with those of Tembo *et al.*, 2017, who reported that disease resistance and agronomic performance are genotype, environment (location) and MAP time (plant age) dependent. There were variations in CMD and CBSD incidence and severity across the three study locations, which was in agreement with other studies (Legg, 2009). Highest CMD and CBSD incidence and severity scores in this study were recorded at Alupe, as opposed to Kakamega and Kibos. Similar findings were reported from surveys by Legg and Bouwmeester, 2010, in CMD and CBSD epidemic areas, where severity scores of up to 3.0 have been recorded. Further, in agreement with this study, according to Legg *et al.*, 2011, CMD and CBSD incidence and severity has been found to be influenced by soil quality, cultivars, virus strains and amount of rainfall. Higher disease severity in the three study locations could also be linked to co-infection of ACMV and EACMV and possible recombination as reported by Legg and Bouwmeester, 2010 from cassava disease surveys in Western Kenya and Lake Victoria region. Similar studies by Tumwegamire *et al.*, 2018, also linked

incidence and severity of CMD and CBSD to different locations (eco zones) growing different cassava varieties.

The elite cassava genotypes also differed in whitefly abundance as similarly observed in previous studies (Legg, 2009). Hence, from this study, there was no association between whitefly abundance on the evaluated genotypes with CMD, CBSD incidence and severity observed. For example, Kalawe with high ASV, hence considered unstable genotype for resistance to CMD and CBSD incidence and severity across the environments, had more than average number of adult whiteflies. Similarly, Kibandameno (a local standard check) had highest CMD and CBSD incidence and severity, with high ASV value and considered unstable across environments, but with fewer than average adult whitefly numbers. These findings equally indicated some genotype preferences by the whiteflies, as similarly reported previously Stansly *et al.*, 2010. Other studies have also reported the lack of association between whitefly abundance and CMD, CBSD incidence and severity infection (Jeremiah *et al.*, 2015).

5.3 Evaluate Elite Cassava Genotypes for Variation in Agronomic Traits and determine their Correlation with CMD and CBSD Resistance Traits

Findings from this study, and as observed previously by Tumuhimbise et al., 2014, there was a significant but negative (inverse) relationship between CBSD and CMD incidence and severity traits with agronomic performance traits. With regards to agronomic performance, the elite cassava genotypes evaluated gave fresh root yield between 7.7 – 19.1 t/ha^{-1}, biomass yield between 4.42 – 9.15 t/ha^{-1} and harvest index (HI) between 28.7% - 49.6%. A similar study by Tembo *et al.*, 2017, revealed that the fresh root yield ability for cassava depends on several factors including the yield potential of the genotype, the genotype/temperature interaction, soil moisture and soil fertility. As would be expected, there was a significant but negative (inverse) relationship between CMD and CBSD incidence and severity 12 MAP with agronomic performance, similar to findings by Anthony *et al*., 2015; Esuma *et al*., 2016; Nduwumuremyi *et al.*, 2017; and

Tumuhimbise, 2013. However, there was a significant positive relationship between CMD incidence and severity with CBSD incidence and severity amongst the elite cassava genotypes across the three locations. Although CMD and CBSD incidence and severity correlated negatively in many cases with biomass yield, fresh root yield and harvest index, some of the 23 elite cassava genotypes had significantly low fresh root yield, even with mild or no symptoms, indicating lack of a general correlation between symptom severity and yield reduction. The analyses of variances (ANOVA) for disease resistance and agronomic performance traits showed significant differences for genotype and environment main effects as well as the GEI, except for cyanide content (HCN) where only the variation among genotypes was significant.

Cyanide is produced by plants as a by-product of ethylene metabolism, or as reduced form of nitrogen storage and defense against attack by herbivorous (Guédé, 2014; Mtunguja *et al*., 2016). Cassava plants produce high quantities of cyanogenic compounds compared to other crops, and it is mainly concentrated in leaves and roots. Variations in cyanide content from this study were highly due to genotype and not across environments or genotype by environment interaction. Previous studies showed that cyanide content varied with genotypes and across environment (Ubwa, *et al*., 2015). This study also found significant difference between genotypes ($P \leq 0.001$) for cyanogenic potential (CNP) levels and not GEI $P \geq 0.001$), therefore un-confirming previous studies. Our findings on cyanogenic potential also contrasted (Mtunguja *et al*., 2016), who reported that variations in cyanide content were mainly due to genotype and environment by genotype interaction. Further, the results contrasted Burns *et al*., 2012, who reported that cyanogenic potential varies with the genotype and within the same genotype and is further affected by planting season and soil type, hence same genotype can taste sweet in one locality and bitter in another. However, all the elite cassava genotypes evaluated across the three locations in this study had mean CNP levels ranging from of 3.00 – 6.00 (specifically, very low, low, moderately low and moderate

CNP levels based on Picrate Acid Concentration, PC scale), and were therefore, considered sweet and not bitter.

5.4 Confirmation of Stability of Resistance to CMD, CBSD and Agronomic Performance

Additive main effects and multiplicative interaction (AMMI) analysis, based on AMMI stability value (ASV), was used to determine the stability of elite cassava genotypes for CMD and CBSD resistance and agronomic performance in Alupe, Kibos and Kakamega environments. The cultivar superiority measure encompasses calculations (across environments) of the mean square difference between the performance of a variety and the best variety. ASV, therefore, is the distance from the coordinate point to the origin in a two- dimensional plot of IPCA1 scores against IPCA2 scores in the AMMI model. Because the IPCA1 score contributes more to the GEI sum of squares, a weighted value is needed. This weighted value was calculated for each genotype and each environment according to the relative contribution of IPCA1 to IPCA2 to the interaction sum of squares. Smaller ASV values indicate more stable genotypes across environments and vice versa. A genotype is thus considered to be stable if its environmental variance is small, referred to as static or biological stability (Farshadfar *et al.*, 2012). Hence, the stable genotype possesses an unchanged or least changed performance regardless of any variation of the environmental conditions. Biological stability is useful for quality traits, disease resistance, stress characters and agronomic performance. Stability analysis provides a general solution for the response of the genotypes to environmental change. In this way, linear regression analysis, has been widely used to assess stability (Farshadfar, *et al*., 2012; Kvitschal *et al*. 2009; Purchase, *et al.*, 2013). However, due to limitations with linear regression methods, multivariate methods i.e. AMMI analysis are now widely used with three main purposes: (i) to eliminate "noise" in the data set (for example, to distinguish systematic and non-systematic variation); (ii) to summarize the information and (iii) to reveal a structure in the data (Gauch and Zobel 1988; Hongyu *et al.* 2014; Purchase, *et al*., 2013). Based on AMMI IPCA1 and IPCA 2 scores, and

associated ASV, the study came up with first five stable and unstable genotypes for each disease resistance and agronomic performance traits, which were the most responsive or unresponsive to location (environment) effects (Appendix 11, 12 and 13). They represented either the best or the poorest performers in locations, corresponding to their ASV, hence placement nearer to or farther from the IPCA1 and IPCA2 origins (Esuma *et al.,* 2016).

Through AMMI analysis, dual resistance was, therefore, confirmed for CMD and CBSD incidence and not severity. Accordingly, genotypes Colicanana, Tajirika, Orera, F10-30-R5 and Kizimbani, LM/2008/363 and KBH/2002/066 with very low ASVs exhibited remarkable stability for dual resistance against both CMD and CBSD incidence across Alupe, Kakamega and Kibos. While, genotypes Kalawe and Kibandameno mainly, but also F19-NL, Nase-1 and Yizaso with relatively very high ASVs were the most unstable (more specifically adapted to certain environments) for dual resistance against both CMD and CBSD incidence across Alupe, Kakamega and Kibos. Cassava genotypes Nase-14, KBH/2002/066, Nase-3, Nase-18, Nase-1 and KBH/2002/066 were more stable against whiteflies abundance across Alupe, Kakamega and Kibos due to low ASVs. While, Eyope, Pwani, Mkumba, Kalawe and Kibandameno were unstable against whiteflies abundance across Alupe, Kibos and Kakamega due to high ASVs. Cassava genotypes that were more for fresh root yield and DM yield, across Alupe, Kakamega and Kibos were KBH/2002/066, Kibandameno, Nase-18, Kizimbani and Nase-3. Similarly, the study identified unstable genotypes (more specifically adapted to certain environments) for agronomic performance. Identified unstable genotypes for biomass yield (due to their high ASV) across Alupe, Kakamega and Kibos were Colicanana, KBH/2002/066, Pwani, Mkumba-2 and Yizaso. Identified unstable genotypes for fresh root yield and DM yield, based on ASV and ranks were Nase-1, Mkumba, TZ-130, KBH/2006/026 and Mkumba-2.

From this study, I observed that different genotypes exhibit varying measures or frequencies of disease resistance and agronomic performance traits within and across

locations (environments), a situation that complicates genotype categorization and/or comparison including confirmation for dual resistance, as similarly reported by Kawuki *et al*., 2016. For example, cassava genotypes F19-NL (0.03) and Nase-1 were stable for resistance to CBSD incidence, but they were unstable for resistance to CMD incidence. Similarly, genotypes Kibandameno (a local standard check) and Kalawe were highly unstable for dual resistance to CMD and CBSD incidence, however they (especially Kibandameno) were stable for agronomic performance (fresh root yield and DM yield) across the three locations. This study through AMMI analysis and based on combined ranking of AMMI stability value (ASV) identified stable and unstable genotypes in response to dual resistance to CBSD and CMD incidence and agronomic performance across Alupe, Kakamega and Kibos, as shown in Appendix 14. Stable genotypes based on combined ASV and ranking in response to dual resistance to CMD and CBSD incidence were Colicanana, F10-30-R5, Orera, Tajirika and Kizimbani. Stable genotypes in response to agronomic performance across the three locations were KBH/2002/066, Kibandameno, Nase-18, Nase-3 and Kizimbani.

Finally, based on combined ASVs and ranking, stable genotypes in response to dual resistances for both CMD and CBSD incidence and agronomic performance were KBH/2002/066, Kizimbani, Nase-18, CH05-203 and Nase-3. These stable genotypes could be of immediate importance for further evaluation and/or use in breeding. It suffices to note that the improved (elite) cassava genotypes evaluated in this study were a set drawn from an advanced breeding population, which means they could have already attained stability for important agronomic traits including biomass and fresh root yield. The significant differences observed between genotypes in reaction to CMD and CBSD indicate wide genetic diversity among the elite cassava genotypes for dual resistance to the two diseases. However, in spite of the foregoing discussion, the study confirmed genotypes that expressed profound immune responses to both diseases (hence dual resistance), with good agronomic performance in the three environments considered (Appendix XIV). Furthermore, the significant genotypes and environment (location)

interaction in response to CMD and CBSD incidence and severity and agronomic performance is indicative of the behavior of these quantitative traits, as similarly observed by Anthony *et al*., 2015.

CHAPTER SIX

GENERAL CONCLUSION AND RECOMMENDATION

6.1 General Conclusion

There was variation among the elite cassava genotypes, due to location differences and MAP time (plant age) in relation to CMD and CBSD incidence and severity, and agronomic performance traits studied. However, GEI was non-significant for biomass and fresh root yield and other agronomic performance traits, indicating that the genotypes had non-significantly different patterns of response to change in location and could be evaluated in terms of their mean response over locations. With regards to agronomic performance, the elite cassava genotypes did not significantly interact with locations, though there were apparent changes in AMMI stability value (ASV) and rank of the genotypes at each environment/location. There was a highly significant influence of genotypes, locations and GEI for CMD and CBSD incidence and severity. Hence, the study using AMMI analysis and based on AMMI stability value (ASV) confirmed stable genotype in response to dual resistance to CMD and CBSD, including agronomic performance. Stable genotypes based on combined ASV and ranking in response to dual resistance to CMD and CBSD incidence were Colicanana, F10-30-R5, Orera, Tajirika and Kizimbani. Stable genotypes in response to agronomic performance across the three locations were KBH/2002/066, Kibandameno, Nase-18, Nase-3 and Kizimbani. Finally, based on combined ASVs and ranking, stable genotypes in response to dual resistance for both CMD and CBSD incidence and and agronomic performance were KBH/2002/066, Kizimbani, Nase-18, CH05-203 and Nase-3. It is, however, important to point out that both the AMMI analysis and general ANOVA indicated that, a high proportion of the variation was explained by genotypic variances for both CMD and CBSD incidence and severity across the three environments. This suggests that resistance to CMD and CBSD was environment/location specific and not generalized. Further, the highly significant genotype by environment interaction implies that the

confirmed stable elite cassava genotypes have to be further evaluated in multiple environments to achieve reliable dual resistance to CMD and CBSD.

6.2 Recommendations

- The best performing stable genotypes identified for each of the disease resistance and agronomic performance traits studied across the three environments (locations) be further screened for resistance to CMD and CBSD, including other biotic stresses, for the possibility of advancing best clones for on-farm production.
- Additional multi-locational studies across different agro-ecological zones are needed further to determine the overall dual response of these elite cassava genotypes to CMD and CBSD. This will be important to link CMD and CBSD incidence and severity including agronomic performance to climatic and geographical considerations.
- The significant differences observed between genotypes for reaction to CMD and CBSD indicate wide genetic diversity among the genotypes for resistance to the two diseases. Hence, only one genotype (F10-30-R5) out of 24 genotypes evaluated expressed immune responses to incidence of both diseases in all the environments considered. This is an opportunity for further research.
- Genotype and location (environment) had a profound effect on all traits analysed. These variations indicate significant genetic diversity present in farmer fields that can be utilized to increase yield potential at different locations. Genotypes should be selected for specific adaptation to environments. Furthermore, dual CMD and CBSD resistance should also be based on specific variety and location for all the traits we have investigated in this study.

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APPENDICES

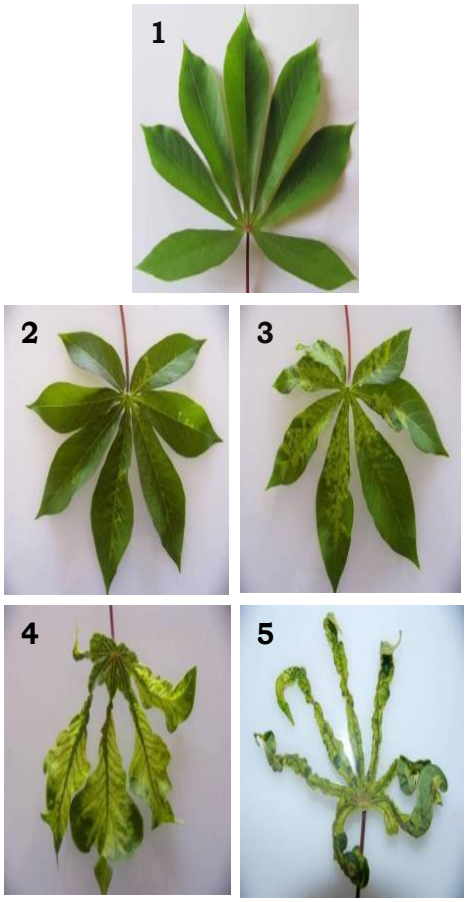
Appendix I: Standard scale for CMD and CBSD foliar and root severity symptom scoring

Score	CMD Foliar Symptom Description	CBSD Foliar Symptom Description	CBSD Root Symptom Description
1	No symptoms	No symptoms on leaves or stems	No symptoms on storage roots
2	Up to 25% leaf area chlorotic, mild leaf distortion, no stunting	Mild/slight vein yellowing or chlorotic blotches on leaves, no brown streaks/lesions on green stem portions	Less than 5% of storage root tissue is necrotic
3	25 - 50% leaf area chlorotic, moderate leaf distortion, no stunting	Mild/slight vein yellowing or chlorotic blotches on leaves mild brown streaks/lesions on green stem portions	5-10% of storage root tissue is necrotic
4	50 - 75% leaf area chlorotic, severe leaf distortion, moderate stunting	Severe/extensive vein yellowing or chlorotic blotches on leaves severe brown streaks/lesions on green stem portions no defoliation, stem dieback or stunting	10-50% of storage root tissue is necrotic
5	75 - 100% leaf area chlorotic, severe leaf distortion, small leaflets (almost no lamina), severe stunting	Severe/extensive vein yellowing or chlorotic blotches on leaves severe brown streaks/lesions on green stem portions defoliation, stem dieback or stunting	More than 75 % of storage root tissue is necrotic

Appendix II: Cassava Mosaic Disease foliar symptom scoring scale

CMD symptom scoring scale

Score	Description
1	no symptoms
2	Up to 25% leaf area chlorotic, mild leaf distortion, no stunting
3	25 - 50% leaf area chlorotic, moderate leaf distortion, no stunting
4	50 - 75% leaf area chlorotic, severe leaf distortion, moderate stunting
5	75 - 100% leaf area chlorotic, severe leaf distortion, small leaflets (almost no lamina), severe stunting



Appendix III: Cassava Brown Streak Disease foliar symptom scoring scale

Appendix IV: Cassava Brown Streak Disease root severity symptom scoring scale

CBSD Root Severity Scoring Scale

Score	Root Symptom Description	Pictorial
1	No symptoms on storage roots	
2	less than 5% of storage root tissue is necrotic	
3	5-10% of storage root tissue is necrotic	
4	10-50% of storage root tissue is necrotic	
5	More than 75% of storage root tissue is necrotic	

Appendix V: Combined AMMI Stability Values and ranking for CMD and CBSD resistance and agronomic performance across Alupe, Kakamega and Kibos in Western Kenya

Cassava Genotype	Disease Resistance AMMI Stability Values				Agronomic Performance ASVs				Combined ASVs	
	CMD Incidence	CBSD Incidence	Dual Disease ASV	Disease Rank	Fresh Root Yield	DM Yield	Agronomic ASV	Agronomic Rank	ASV Combined	Combined Rank
CH05-203	0.08	0.05	0.07	6	1.05	0.42	0.74	6	0.40	3
Colicanana	0.02	0.01	0.02	1	3.16	1.27	2.22	17	1.12	13
Eyope	0.81	0.31	0.56	9	1.28	0.51	0.90	7	0.73	8
F10-30-R5	0.05	0.05	0.05	4	1.80	0.72	1.26	9	0.66	6
F19-NL	1.27	0.39	0.83	21	3.01	1.21	2.11	15	1.47	17
Kalawe	1.54	1.21	1.38	23	1.89	0.75	1.32	11	1.35	15
KBH/2002/066	0.31	0.31	0.31	8	0.21	0.08	0.15	1	0.23	1
KBH/2006/026	0.81	0.61	0.71	18	5.31	2.12	3.72	23	2.21	22
Kibandameno	7.44	2.45	4.95	24	0.41	0.16	0.29	2	2.62	23
Kizimbani	0.09	0.04	0.07	5	0.77	0.31	0.54	4	0.30	2
LM/2008/363	0.18	0.10	0.14	7	3.17	1.27	2.22	18	1.18	14
Mkumba	0.81	0.31	0.56	10	4.16	1.67	2.92	21	1.74	21
Mkumba-2	0.81	0.61	0.71	19	7.20	2.88	5.04	24	2.88	24
Nase-1	1.34	0.35	0.85	22	3.50	1.40	2.45	20	1.65	19
Nase-14	0.81	0.31	0.56	11	3.16	1.26	2.21	16	1.39	16

Nase-18	0.81	0.31	0.56	12	0.65	0.26	0.46	3	0.51	4
Nase-3	0.81	0.31	0.56	13	0.79	0.31	0.55	5	0.56	5
Orera	0.03	0.05	0.04	3	1.99	0.80	1.40	13	0.72	7
Pwani	0.81	0.31	0.56	14	1.93	0.77	1.35	12	0.96	12
Sangoja	0.73	0.55	0.64	15	1.67	0.67	1.17	8	0.91	10
Sauti	0.81	0.47	0.64	16	1.80	0.72	1.26	10	0.95	11
Tajirika	0.00	0.06	0.03	2	2.11	0.84	1.48	14	0.75	9
TZ-130	0.88	0.48	0.68	17	5.14	2.06	3.60	22	2.14	20
Yizaso	0.81	0.69	0.75	20	3.26	1.30	2.28	19	1.52	18

Appendix VI: AMMI integrated principal component analysis (IPCA1 and IPCA2) for whiteflies abundance and disease resistance traits of elite cassava genotypes across Alupe, Kakamega and Kibos in Western Kenya

	CBSD Incidence		CBSD Severity		CMD Incidence		CMD severity		Whiteflies	
Genotype	**IPCA[1]**	**IPCA[2]**	**IPCAg[1]**	**IPCA[2]**	**IPCA[1]**	**IPCA[2]**	**IPCA[1]**	**IPCA[2]**	**IPCA[1]**	**IPCA[2]**
CH05-203	-0.11	0.11	-0.10	0.07	-0.11	0.06	-0.12	-0.04	-0.09	-0.20
Colicanana	0.00	0.11	-0.10	0.07	-0.05	0.05	0.01	-0.01	-0.35	0.08
Eyope	0.33	0.09	0.19	0.08	0.34	-0.06	0.20	0.03	0.60	-0.19
F10-30-R5	-0.11	0.11	-0.10	0.07	-0.08	0.06	-0.06	-0.03	0.27	0.45
F19-NL	-0.37	0.13	-0.10	0.07	-0.42	0.15	-0.23	-0.07	0.11	-0.28
Kalawe	-0.34	-0.55	-0.13	-0.35	-0.46	0.16	-0.25	-0.07	1.28	0.10
KBH/2002/066	-0.46	-0.17	-0.35	-0.07	-0.20	0.09	-0.02	-0.02	0.16	-0.09
KBH/2006/026	0.33	0.09	0.19	0.08	0.34	-0.06	0.20	0.03	0.07	0.33
Kibandameno	-0.94	0.15	-0.58	0.06	-1.00	-0.51	-0.71	0.19	0.07	-1.02
Kizimbani	0.01	-0.19	0.05	-0.07	0.11	0.00	0.09	0.01	0.38	0.29
LM/2008/363	0.01	-0.32	0.11	-0.17	-0.16	0.08	-0.02	-0.02	-0.71	-0.34
Mkumba	0.33	0.09	0.19	0.08	0.34	-0.06	0.20	0.03	-0.77	-0.18
Mkumba-2	0.56	-0.59	0.35	-0.34	0.34	-0.06	0.20	0.03	-0.59	0.09
Nase-1	-0.35	0.12	-0.10	0.07	-0.43	0.15	-0.23	-0.07	0.26	-0.26
Nase-14	0.33	0.09	0.19	0.08	0.34	-0.06	0.20	0.03	0.00	0.00
Nase-18	0.33	0.09	0.19	0.08	0.34	-0.06	0.20	0.03	-0.05	0.28
Nase-3	0.33	0.09	0.19	0.08	0.34	-0.06	0.20	0.03	0.23	-0.07
Orera	-0.11	0.11	-0.10	0.07	0.07	0.02	0.09	0.01	-0.30	0.23
Pwani	0.33	0.09	0.19	0.08	0.34	-0.06	0.20	0.03	-0.70	0.31
Sangoja	-0.43	-0.17	-0.30	-0.21	-0.32	0.12	-0.10	-0.04	-0.07	0.29
Sauti	0.41	-0.12	0.28	-0.17	0.34	-0.06	-0.12	-0.04	0.14	0.44
Tajirika	-0.14	0.11	-0.10	0.07	0.02	0.03	0.09	0.01	0.41	-0.28
TZ-130	-0.41	0.13	-0.29	0.07	-0.35	0.13	-0.17	-0.06	-0.20	-0.12
Yizaso	0.45	0.39	0.24	0.22	0.34	-0.06	0.20	0.03	-0.17	0.13

Appendix VII: AMMI integrated principal component analysis (IPCA) 1 and 2 for agronomic performance of elite cassava genotypes across Alupe, Kakamega and Kibos in Western Kenya

	Biomass Yield		Fresh Root Yield		Harvest Index		DM Yield	
Genotype	**IPCA[1]**	**IPCA[2]**	**IPCA[1]**	**IPCA[2]**	**IPCAg1]**	**IPCA[2]**	**IPCA[1]**	**IPCA[2]**
CH05-203	-0.66	0.62	0.76	0.32	1.43	0.19	0.48	0.20
Colicanana	-1.05	0.29	-1.38	0.27	0.87	0.65	-0.87	0.17
Eyope	-0.08	-0.24	-0.42	1.00	-1.31	-0.10	-0.26	0.63
F10-30-R5	-0.07	-0.94	-0.35	-1.26	-0.22	0.17	-0.22	-0.80
F19-NL	0.75	0.32	1.08	-1.05	1.23	0.32	0.68	-0.67
Kalawe	0.63	0.31	1.03	-0.43	2.92	-0.54	0.65	-0.27
KBH/2002/066	1.10	0.20	0.04	0.45	-2.20	0.36	0.03	0.29
KBH/2006/026	0.89	0.36	1.08	1.85	-1.84	-1.48	0.68	1.17
Kibandameno	0.81	-0.36	0.21	-0.58	-1.46	0.13	0.13	-0.37
Kizimbani	0.80	-0.91	-0.53	0.56	-2.41	0.61	-0.34	0.36
LM/2008/363	0.00	0.59	1.23	-0.85	1.33	-0.25	0.78	-0.54
Mkumba	-0.74	-0.26	-1.57	-0.40	0.63	1.91	-0.99	-0.25
Mkumba-2	-1.10	0.79	-2.08	-0.44	1.38	0.76	-1.32	-0.28
Nase-1	0.24	0.20	1.27	-0.95	0.92	-1.05	0.80	-0.60
Nase-14	-0.89	0.37	1.02	1.22	0.78	-2.61	0.64	0.77
Nase-18	0.44	0.62	0.63	0.06	-0.12	0.23	0.40	0.04
Nase-3	0.31	0.40	0.00	0.89	-1.22	-0.70	0.00	0.56
Orera	0.96	0.28	-0.78	1.00	-1.80	1.27	-0.49	0.63
Pwani	-1.22	0.09	-1.08	0.23	0.61	0.31	-0.68	0.15
Sangoja	-0.10	-0.60	-0.60	-1.04	1.12	1.24	-0.38	-0.66
Sauti	0.42	-0.38	-0.84	-0.80	0.10	2.60	-0.53	-0.51
Tajirika	0.02	0.29	1.02	0.64	0.13	-1.07	0.65	0.40
TZ-130	-0.13	-1.28	1.68	-0.76	-0.18	-2.39	1.06	-0.48
Yizaso	-1.33	-0.75	-1.42	0.09	-0.70	-0.55	-0.90	0.06

Appendix VIII: AMMI Analysis of variance (ANOVA) for CMD and CBSD incidence and severity across Alupe, Kakamega and Kibos in Western Kenya

Source	DF	CBSD Incidence			CBSD Severity			CMD Incidence			CMD Severity		
		SS	MS	F	SS	MS	F	SS	MS	F	SS	MS	F
Treatments	71	86.72	1.22	2.04***	12.15	0.17	1.46*	71.70	1.01	3.01***	9.08	0.13	2.68***
Genotypes	23	21.28	0.93	1.54	2.99	0.13	1.11	19.35	0.84	2.51***	2.28	0.10	2.07**
Environments	2	27.61	13.80	30.84***	3.64	1.82	39.08***	24.52	12.26	66.38***	3.07	1.53	206.44
Block	6	2.69	0.45	0.75	0.28	0.05	0.40	1.11	0.19	0.55	0.05	0.01	0.16
Interactions	46	37.84	0.8	1.37**	5.52	0.12	1.02	27.83	0.61	1.80**	3.74	0.08	0.70
IPCA	24	33.39	1.39	2.32**	4.82	0.20	1.71*	27.30	1.14	3.39***	3.72	0.16	3.25***
IPCA	22	4.45	0.20	0.34	0.70	0.03	0.27	0.53	0.02	0.07	0.01	0.00	0.01
Error	138	82.82	0.60		16.18	0.12		46.30	0.34		6.60	0.05	

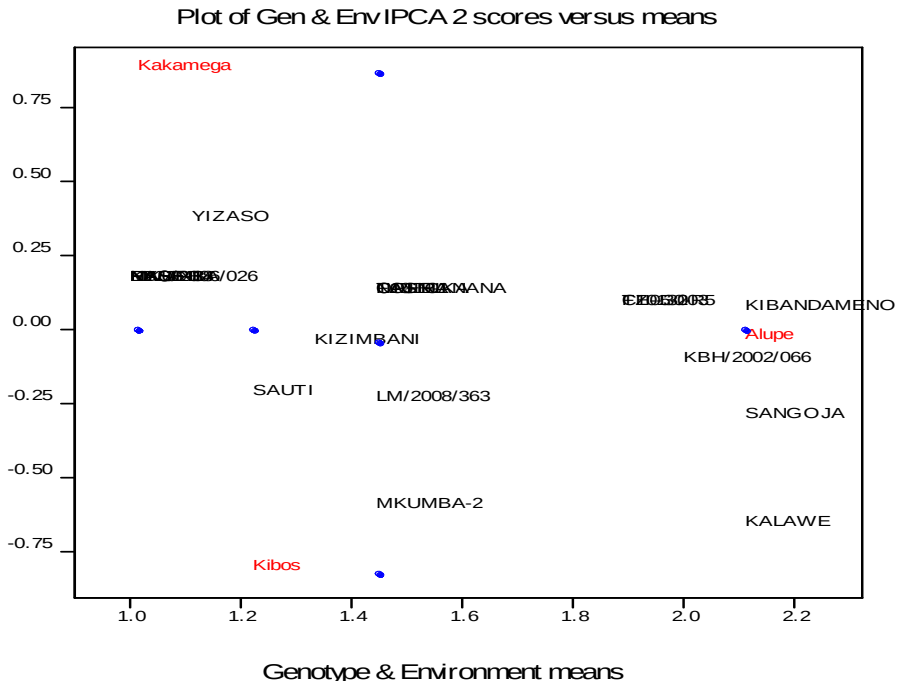
Appendix IX: AMMI Analysis of variance (ANOVA) for agronomic performance of elite cassava genotypes across Alupe, Kakamega and Kibos in Western Kenya

Source	DF	Biomass Yield			Fresh Root Yield			Harvest Index			Dry Matter Yield		
		SS	MS	F	SS	MS	F	SS	MS	F	SS	MS	F
Treatments	71	2264.00	31.88	3.06***	8960.00	126.20	2.34***	21600.00	304.20	1.46***	1433.50	20.19	2.34***
Genotypes	23	299.00	13.01	1.25	1101.00	47.90	0.89	4773.00	207.50	0.99	176.20	7.66	0.89
Environments	2	1291.00	645.50	9.11***	4942.00	2471.10	9.8***	7886.00	3943.00	3.78*	790.80	395.38	9.8***
Block	6	425.00	70.83	6.79***	1513.00	252.10	4.68***	6266.00	1044.30	5.01***	242.00	40.34	4.68***
Interactions	46	673.00	14.64	1.40	2916.00	63.40	1.18	8940.00	194.40	0.93	466.60	10.14	1.18
IPCA	24	511.00	21.29	2.04***	2114.00	88.10	1.64*	5571.00	232.10	1.11	338.20	14.09	1.64*
IPCA	22	163.00	7.39	0.71	802.00	36.50	0.68	3369.00	153.10	0.73	128.40	5.84	0.68
Error	138	1440.00	10.43		7433.00	53.90		28791.00	208.60		1189.30	8.62	

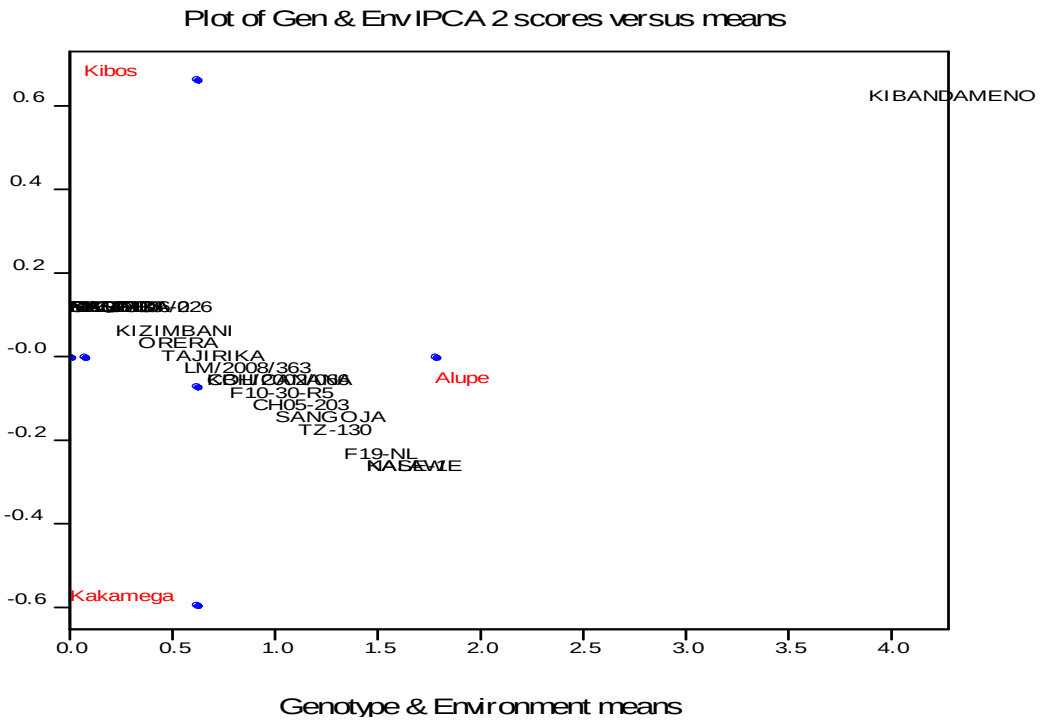
Appendix X; AMMI Anova for whiteflies (*Bemisia tabaci*) abundance on elite cassava genotypes across Alupe, Kakamega and Kibos in Western Kenya

Source	df	Whiteflies Abundance		
		SS	MS	F-Value
Treatments	71	848.5	11.95	16.44***
Genotypes	23	91.6	3.98	5.48***
Environments	2	669	334.51	46.59***
Block	6	43.1	7.18	9.88***
Interactions	46	87.9	1.91	2.63***
IPCA	24	69.7	2.9	3.99***
IPCA	22	18.2	0.83	1.14
Error	138	100.3	0.73	

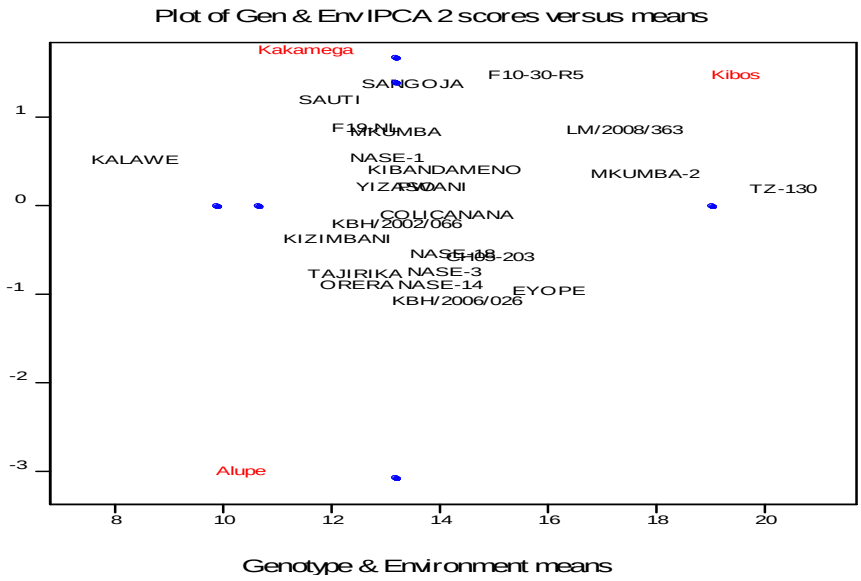
Appendix XI: GGE bi-plot of G x E IPCA1 scores against IPCA2 scores on genotype and environment means for CBSD severity at harvest



Appendix XII: GGE bi-plot of G x E IPCA1 scores against IPCA2 scores on genotype and environment means for CMD severity at harvest



Appendix XIII: GGE bi-plot of G x E IPCA1 scores against IPCA2 scores on genotype and environment means for Fresh root yield at harvest



Appendix XIV: Confirmation of stable and unstable genotypes for resistance to CMD and CBSD incidence and agronomic performance based on combined ASVs and ranking across Alupe, Kakamega and Kibos in Western Kenya

Parameter	Rank 1	Rank 2	Rank 3	Rank 4	Rank 5
Stable genotypes across environments					
Disease incidence	Colicanana (0.02)	Tajirika (0.04)	Orera (0.04)	F10-30-R5 (0.05)	Kizimbani (0.07)
Agronomic	KBH/2002/066 (0.15)	Kibandameno (0.29)	Nase-18 (0.46)	Kizimbani (0.54)	Nase-3 (0.55)
Combined	KBH/2002/066 (0.23)	Kizimbani (0.30)	CH05-203 (0.40)	NASE-18 (0.51)	Nase-3 (0.56)
Unstable genotype adapted to specific environments					
	Rank 20	**Rank 21**	**Rank 22**	**Rank 23**	**Rank 24**
Disease incidence	Yizaso (0.75)	F19-NL (0.83)	Nase-1 (0.85)	Kalawe (1.38)	Kibandameno (4.95)
Agronomic	Nase-1 (2.45)	Mkumba (2.92)	TZ-130 (3.60)	KBH/2006/026 (3.72)	Mkumba-2 (5.04)
Combined	Mkumba (1.74)	TZ-130 (2.14)	KBH/2006/026 (2.21)	Kibandameno (2.62)	Mkumba-2 (2.88)