GENETIC DIVERSITY OF CASSAVA (*MANIHOT ESCULENTA* CRANTZ) GERMPLASM AND EFFECT OF ENVIRONMENT ON RESISTANCE TO CASSAVA BROWN STREAK AND CASSAVA MOSAIC DISEASES IN BURUNDI

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN PLANT BREEDING AND BIOTECHNOLOGY

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DECLARATION

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

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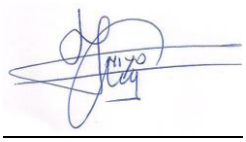
Signature:  Date: 11th November 2021

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DEDICATION

I dedicate this thesis to my wife, Jeanne Ntirenganya and my children, Uriel Kefasi, Karren Pierrot and Pierra Déborah who endured solitude during my absence from home but showed patience and love. I would like also to dedicate this thesis to my mother Mrs. Adèle Nahimana and in memory of my late father Mr. Pascal Mutunge as well as all members of my family, for their advice and support to my education.

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ABBREVIATIONS AND ACRONYMS

ACMV	: African Cassava Mosaic Virus
AFLP	: Amplified Fragment Length Polymorphisms
AHC	: Ascending Hierarchical Clustering
AMMI	: Additive Main effect and Multiplicative Interaction
ASV	: Analysis of the main effects and multiplicative interaction Stability Values
AUDPC	: Area Under the Disease Progress Curve
CBB	: Cassava Bacterial Blight
CBSD	: Cassava Brown Streak Disease
CEC	: Cation Exchange Capacity
CMD	: Cassava Mosaic Disease
CV	: Coefficient of Variation
DArT	: Diversity Arrays Technology
DArTseq	: Diversity Arrays Technology Sequencing
Df	: Degree of freedom
DM	: Dry Matter
DNA	: Deoxyribo Nucleic Acid
DR Congo	: Democratic Republic of the Congo
EACMV	: East African Cassava Mosaic Virus
EDTA	: Ethylenediaminetetraacetic Acid
Exch	: Exchangeable
FAO	: Food And Agriculture Organization
FAOSTAT	: Food And Agriculture Organization Statistics
FSRY	: Fresh Storage Root Yield
Fst	: Fixation index
Gm	: Genotype mean
GSI	: Genotype Stability Index
GWAS	: Genome -Wide Association Studies
GxE	: Genotype By Environment
He	: Expected Heterozygosity

HI : Harvest Index
Ho : Observed Heterozygosity
ICMV : Indian Cassava Mosaic Virus
Inc : Incidence
ISABU : Institut des Sciences Agronomiques du Burundi
LSD : Least Significant Difference
m.a.s.l : Meters above sea level
MAP : Months After Planting
MAS : Marker Assisted Selection
meq : Milliequivalents
NE : North East
ns : no significant
PCA : Principal component analysis
PE : Plant Establishment
pH : Potential of Hydrogen
QTL : Quantitative Traits Loci
RAPD : Random Amplified Polymorphic DNAs
RASV : Rank of the genotypes based on the AMMI Stability Value
RFLP : Restriction Fragment Length Polymorphisms
RNA : Ribonucleic Acid
RT-PCR : Reverse Transcription Polymerase Chain Reaction
RY : Rank of the genotypes based on performance for a given trait
SACMV : South African Cassava Mosaic Virus
Sev : Severity
SLCMV : Sri Lankan Cassava Mosaic Virus
SNP's : Single-Nucleotide Polymorphisms
SS IPCA :Sum of Squares of the Interaction Principal Component Analysis
SSR : Single Sequence Repeat
TE : Tris-Ethylenediamine tetra-acetic acid
WFN : White Fly Number
WFP : White Fly Population

General abstract

Cassava is an important cash crop for many small scale farmers in Burundi. Most small scale farmers use local landraces and though they have farmer preferred traits, their genetic diversity is unknown and marred by phenotypic susceptibility to Cassava Brown Streak Disease (CBSD) and Cassava Mosaic Disease (CMD). This limits future breeding programs to improve cassava production and resistance to diseases in Burundi. Due to this, disease tolerant genotypes from other countries were introduced to Burundi to help improve on their germplasm and then determine the relationships between local landraces and improved germplasm. Objectives of this study were to (1) assess the genetic diversity among cassava landraces and introduced cassava genotypes using morphological and molecular markers, (2) determine effects of genotype by environment (GxE) interaction on resistance to CBSD and CMD diseases in varied agro-ecological zones of Burundi. Genotype characterization was done using 17 qualitative agro morphological traits while molelcular analysis was conducted using SNP genotyping data from DaRTseq using KDCompute on 118 genotypes. For objective 2, the effect of GxE interaction on resistance to cassava viral diseases was determined using 18 accessions arranged in alpha lattice design on 9 blocks per site. Data was taken on sprouted cuttings, whiteflies population, foliar diseases, root necrosis, growth parameters and yield. Results for objective 1, on 118 accessions revealed more than 18,000 SNPs but there with low genetic distance (Fst<0.15) between local landraces and resistant genotypes. Phenotypic classification showed three main clusters based on Ward's Method with cluster III containing all introduced genotypes; genotypic classification showed six main clusters with cluster II and IV having 5 and 11 introduced genotypes, respectively. Overall, 73 accessions had unique genotypes while 16 accessions were genetic clones indicating that 73 could be used in hybridization programs. Morphological markers showed five paired accessions using Ward's method. According to the field experimental study, 3 genotypes had dual tolerance to CBSD and CMD on leaves; 5 genotypes were tolerant to CMD; 7 genotypes were tolerant to CBSD on leaves and stems while 2 genotypes were resistant. Overall, 8 clones showed high yield while 3 were tolerant to CMD and CBSD in all locations indicating that they could be used in breeding programs for germplasm improvement. In conclusion, results on molecular characterization will contribute to optimizing the conservation of genetic resources, together with understanding diversity and its use in crop improvement. Identification of resistant/ tolerant genotypes will be incorporated in cassava breeding program for transferring the genes to

farmer-preferred varieties. Dually resistant genotypes like Mkumba and Pwani were identified as putative duplicates clones, might be used as genetic stock that could combine resistance to CBSD and CMD a single genotype. From this study, it is recommended that these cassava genotypes could be included in Burundi genetic improvement programs for higher yield to realize genetic gains with time.

CHAPTER ONE

INTRODUCTION

1.1. Background information

Cassava (*Manihot esculenta* Crantz) is mainly cultivated in tropical countries, particularly in sub-Saharan Africa, South America and Asia as an important staple food (Perez and Villamayor, 1984). The cassava production is estimated to be 277 million tons on approximately 24.5 million hectares worldwide (FAOSTAT, 2020) and provides food for more than 800 million people (Thresh, 2006, FAO, 2007). According to Rojas et al. (2007), cassava crop rank fourth in terms of carbohydrate food source in the tropics after rice, sugar cane and maize and provides more than 60% of the daily calorific needs of the populations in tropical Africa and Central America. Plucknett et al. (1998) reported cassava as a crop with a major role in food security. It is an important staple crop where both the foliage and root are considered as a food source. In Africa, cassava production is estimated at 160 million tons on 18 million hectares (FAOSTAT, 2020), Nigeria being the largest producer country of cassava. According to FAOSTAT (2020), Eastern Africa production is estimated at 30 million tons on 3 million hectares, Tanzania leading the production with 5 million tons, followed by Uganda, Burundi, Rwanda, Kenya and South Sudan.

In Burundi, cassava is the most important staple crop with a production of 2.39 million tons in 2018, followed by fruits, bananas, sweet potatoes, vegetables and cereals (FAOSTAT, 2020). It is grown by farmers throughout the low, medium and high altitude areas and is particularly important to small-scale farmers in these zones. All cassava varieties grown in Burundi are used exclusively for human consumption as ugali. According to Aloys and Hui Ming (2006), Burundians eat the cassava roots in the form of “imikembe”, “ubuswage” and are processed into flour to be eaten as paste (Ugali). Moreover, the leaves of cassava are always eaten either as vegetables or as sauce. Despite its importance, cassava production has been declining from year 2003 to year 2011 and from 2015 to 2018 (FAOSTAT, 2020) due to biotic and abiotic stresses. The biotic stresses include viral, bacterial, and fungal diseases as well as insect pests. From 2010 to 2012, cassava production doubled, from 598,409 tons in 2010 to 1,244,607 tons in 2012, and decreased subsequently to reach 2,242,352 tons in 2014 (FAOSTAT, 2020). Cassava crop production is declining due to two important diseases, namely cassava brown streak disease (CBSD) with losses of 74% (Kawuki et al., 2016) and cassava mosaic disease (CMD) causing

losses of 25-100% (Brian et al., 2015). Other diseases such as cassava bacterial blight and anthracnose are also the most important diseases which limit cassava production in major producing countries such as Nigeria, Thailand, Brazil, Democratic Republic of the Congo and Indonesia (FAOSTAT, 2020). Bigirimana et al. (2011) reported the presence of CBSD in Burundi and recommended the need for urgent measures to mitigate the outbreak. In general, strategies used in plant virus disease management (Naidu and Hughes, 2001) are: (i) to prevent the virus from reaching the crop by eliminating the source of infection, (ii) to minimize the spread of the disease by controlling its vector, (iii) to utilize virus-free planting material and (iv) to incorporate the host-plant resistance to the virus by breeding. The aims of the proposed research are (1) to determine genetic diversity for local landraces grown in Burundi and (2) to identify cassava clones with specific and wide adaptability for CBSD and CMD dual resistance.

1.2. Statement of the problem

At the dawn of the third millennium, agriculture should meet the growing demand for food. It is becoming evident that increasing productivity of root crops is vital for the next green revolution (Villordon et al., 2014, Den Herder et al., 2010, Lynch, 2007). Among the root crops, cassava is an important staple food crop in the world (Thresh, 2006; FAO, 2013). However, cassava is susceptible to several diseases, among them CMD and CBSD, occasioning economic losses of more than one billion US dollars each year (IITA, 2014). CBSD is the most devastating disease to cassava and causes losses to cassava root production and quality. According to Masinde et al. (2018), the global annual economic losses are estimated to be in excess of US$ 726 million while Abaca et al. (2012) reported losses of 100% when CBSD is combined with CMD.

Recently, outbreaks of CBSD have been reported at mid altitude agro-ecologies (1200-1500 meters above sea level) in many countries in Eastern, Central and Southern Africa (Ndunguru et al., 2015 in Tanzania, Bigirimana et al., 2011 in Burundi, Mbewe et al., 2015, in Malawi, Mulimbi et al., 2012 in DR Congo and Zacarias et al., 2010 in Mozambique). Almost all varieties earlier selected for CMD resistance have succumbed to CBSD in countries such as Burundi, Uganda, Tanzania and Western Kenya (Bigirimana et al., 2011).

In Burundi, 5 resistant varieties to CMD were released and disseminated widely in 2006 by the Institut des Sciences Agronomiques du Burundi (ISABU) in collaboration with non-governmental organizations and the private sector. However, all the released CMD resistant

varieties succumbed to CBSD (Bigirimana et al., 2011). CBSD, has also been reported in the lowlands of Burundi resulting to low productivity and root yields, which probably accounts for the reported decrease in production from 2,757,583 tons in 2015 to 2,386,709 tons in 2018 (FAOSTAT, 2020). Due to this, the need to improve on cassava germplasm can be overstated both to improve production and enhance future breeding programs. Several methods are used for genetic improvement, such as introduction of resistant varieties, determination of the genetic diversity of germplasm and development of new genotypes (Ceballos et al., 2016, Acquaah, 2012). To enhance Burundian cassava breeding program, resistant germplasm were introduced and evaluated in different agro-ecological zones to determine the best varieties for farmers in Burundi. Secondly, the genetic diversity and similarity of cassava landraces and introduced germplasm in Burundi was unknown. Therefore, a component of this study focused on collecting and assessing genetic diversity of local germplasm. Furthermore, the identification of probable resistant genotypes was done for the local landraces against CBSD by comparing them with the introduced resistant genotypes for possible sites of homology within their genomes using morphological and molecular analyses.

1.3. Justification

One of the goals of plant breeders is to generate genetically diverse individuals through selection, hybridization or introduction for use in breeding programs. In Burundi, knowledge on genetic diversity of cassava genotypes is limited which limit genetic improvement programs. Knowledge of genetic diversity is a requirement to breeding programs to develop new cultivars in Burundi. Secondly, although there are many landraces in Burundi, their resistance to CBSD is presumed to be low but there is no documentation. Different morphological and molecular characterization techniques are used jointly to determine genetic diversity and similarity of genotypes to enhance breeding programs for different crops (Singh et al., 2017) including cassava. In cassava, these techniques have been used to determine the level of genetic and morphological variation (Karim et al., 2019, Montero-Rojas et al., 2011, de Souza, 2007, Kizito et al., 2005) but not much has been done on the composition of the local landraces compared to improved genotypes.

Many landraces are grown by farmers, but limited information to their resistance to CBSD is available. Therefore, a number of landraces were collected by ISABU for screening against CBSD. Preliminary screening against CBSD done in ISABU for 100 local landraces showed

phenotypic susceptibility but there was no available data to verify this. Secondly, since the genetic diversity of the landraces was unknown, there was a need to characterize them using molecular markers to identify a core collection for future breeding efforts in Burundi. Thirdly, since there is no reported resistant variety in Burundi, there was a need to introduce CBSD tolerant elite genotypes for adaptive evaluation across several agro-ecological zones to identify the best performing CBSD resistant varieties. This study seeks to develop core collection, which will be validated, improved and premier cassava clones used for future hybridization programs to improve resistance to CBSD and CMD. It also seeks to identify cassava genotypes with specific and wide adaptability for dual resistance to infection by CBSD and CMD.

1.5. Objectives

1.5.1. Overall objective

To contribute to improving cassava production in Burundi by establishing the genetic diversity of cassava genotypes and selection of genotypes with resistance to cassava diseases.

1.5.2. Specific objectives

1. To assess the genetic diversity among cassava landraces and introduced genotypes using morphological and molecular markers.
2. To determine the effects of genotype x environment interaction on resistance to CBSD and CMD diseases in varied agroecological zones of Burundi.

1.6. Hypotheses

1. The local germplasm are genetically diverse and distinct from the introduced germplasm.
2. There is dual resistance to CBSD and CMD among the introduced cassava genotypesacross environments in Burundi.

CHAPTER TWO

LITERATURE REVIEW

2.1. Origin of cassava

Paleobiolinguistics method was used to establish the origin and time of domestication of *Manihot* spp (Brown et al., 2013). The diversity of cassava (*Manihot* spp) is sourced from two probable centers of origin. Approximately, 98 species of *Manihot* originated from the New World (Nassar, 1978). Current evidence indicates that Mesoamerica is the place of origin of the genus, with approximately 17 species; and the other being Brazil with approximately 80 species (Duputié et al., 2011). It is reported that the only domesticated species *in Manihot esculenta* ssp. *esculenta* and is derived from ssp. flabellifolia (Brown et al., 2013). The species *Manihot esculenta* Crantz is native to Latin America (Nassar, 2003) and domesticated between 5000 - 7000 BC in Brazil (Leotard et al., 2009). In the 16th century, Portuguese brought cassava from South America to West Africa and spread it across the sub-Saharan countries (Hillocks, 2002; Okogbenin et al., 2007; Aloys and Hui Ming, 2006). Cassava was brought in East Africa by the Portuguese by the 18th century through ocean routes (Hillocks, 2002). The introduction of cassava to Uganda was facilitated by Arab traders between 1862 and 1875 through Tanzania and then to Great Lake Regions by numerous travelers by the 19th century (Langlands, 1966; Alloys and Hui Ming, 2006).

2.2. Botany and genetics of cassava

Cassava, *Manihot esculenta* Crantz, is the domesticated species. The cultivated strain *Manihot esculenta ssp. esculenta* and two wild forms *M. esculenta ssp. flabellifolia* and *M. esculenta ssp. peruviana* are the known three subspecies (Allem, 2002). There are 98 species belonging to the genus *Manihot* (Rogers and Appan, 1973) and are diploid with a chromosome number 2n = 36 (Nassar, 2009 and Soto et al., 2015). The high number of chromosomes suggests that *Manihot* species behave meiotically like diploids and are therefore allopolyploids species with basic chromosome number x = 9 (Carvalho et al., 1999 and OECD, 2014). Natural mating bas been observed between species in Manihot and those of distant relatives generating natural and artificial hybrids of cassava and *M. glaziovii* (OECD, 2014, Sécond et al., 1997). However, the genetics of cassava is less understood compared to other important staple crops, due to its natural

heterozygosity, long growing cycle and low level of seed production. Cassava is generally outcrossing species, but natural self-pollination may occur, and therefore suffers from inbreeding depression, making it difficult to express more genetic variation (Soto et al., 2015).

2.3. Constraints in cassava production in Africa

Cassava losses are mainly due to biotic stresses as opposed to abiotic stresses. The biotic stresses include viral, bacterial, fungal diseases and insect pests. Among viral diseases, cassava brown streak disease with losses of 74% (Kawuki et al., 2016) and cassava mosaic virus disease with estimated losses between 25% and 100% (Brian et al., 2015) are the most important threats of cassava production. CBSD and CMD are both transmitted by the whitefly vector *Bemisia tabaci* and infected stem cuttings. Diseases such as bacterial and anthracnose are also the most important diseases that limit cassava production in major producing countries (Nigeria, Thailand, Brazil, Democratic Republic of the Congo and Indonesia).

2.3.1. Cassava bacterial blight (CBB)

It is the most important bacterial disease in the cassava belt worldwide (Fanou et al., 2018) and in Africa (Hillocks and Wydra, 2002). According to Lozano (1986), an estimated yield losses of about 30% were reported when cuttings from an infected field were planted and losses of up to 80% can be reached after three cycles only if no control measures are adopted (Lozano, 1986). The causal agent of CBB, *Xanthomonas axonopodis pv. manihotis*, has several modes of survival and transimission (spread) that play a significant role as sources of inoculum for new infections. CBB infects newly planted cassava fields, the old fields, as well as cassava fields planted earlier before the beginning of rainy season (Fanou et al., 2018). Other bacterial diseases like bacterial angular leaf spot (or bacteial necrosis) caused by *Xanthomonas campestris pv. cassavae* and soft rot of stems and roots caused by *Erwinia carotovora ssp. carotovora* have low impact on cassava yield losses if well managed (Hillocks and Wydra, 2002).

2.3.2. Brown leaf spot and anthracnose

The fungal diseases in cassava are caused by various pathogens. Brown leaf spot is caused by *Cercospora manihotis* (Hillocks and Wydra, 2002). The disease was reported also to cause leaf chlorosis and extensive defoliation, and yield losses of 20% were observed on individual plants in the areas with a lot of rainfall (Hillocks and Wydra, 2002). Cassava anthracnose disease caused

by *Colletotrichum gloeosporioides*, is prevalent in most of the cassava producing countries of Africa (Moses et al., 2008). The disease affects both cassava leaves and stems and severe anthracnose attacks can cause death of stems which can affect the availability of planting materials (Msikita et al., 2000). It was estimated that the disease causes yield losses of 30% or more in susceptible cultivars (Moses et al., 2008).

2.3.3. Insect pests in cassava production

Insect pests include cassava mealybug, African root and tuber scale, cassava green mites (*Mononychellus tanojoa*) that cause losses of 25-50% (Brian et al, 2015), whitefly (*Bemisia tabaci*) that devastated cassava in cassava producing countries (Hillock and Thresh, 2000) and finally nematodes, particularly *Meloidogyne* and *Pratylenchus ssp*. Yield losses caused by nematodes are not quantified and the disease is not considered as important threat of cassava. However, when the nematode populations build up, the crop damage increases also (Hillocks and Wydra, 2002).

2.3.4. Cassava brown streak disease (CBSD)

It is a disease that infects leaves as well as cassava tuber, and causes loss of tuber production and quality. CBSD causes substantial root yield loss of up to 100% particularly in the worst affected areas. The disease can render susceptible varieties not usable if cassava roots are not harvested before nine months. CBSD and CMD together can cause losses of one billion of US dollars per year (IITA, 2014). The CBSD research history can be highlighted in a timeline. Between 1930 and 1940, CBSD was reported from Tanzania (Storey, 1936) and a breeding program for resistance to CMD/CBSD was initiated in the same country in 1937 (Jennings, 1957). In 1950, CBSD was reported as an important disease in east Africa, from Kenya, Tanzania to Mozambique and lowland of Malawi (Nichols, 1950). Later in 1990, CBSD was confirmed in single site in Uganda (Thresh et al., 1994) and high CBSD incidences were reported in coastal Tanzania (Legg and Raya, 1998). RT-PCR of CP sequence confirmed that causal agent belongs to genus Ipomovirus and family potyviridae in 2000 (Monger et al., 2001). Between 2000 and 2010, high CBSD incidence in northern Mozambique and many lakeshore fields in Malawi (Hillocks et al., 2002; Hillocks and Jennings, 2003), in western Kenya (Mware et al., 2009) and its re-emergence in Uganda at altitude >1000 masl (Alcai et al., 2007) were reported. Maruthi et al. (2005) confirmed *Bemisia tabaci* as vector of CBSD. Between 2010 and 2016, the presence of CBSD was reported in Burundi (Bigirimana et al., 2011), in DRC (Mulimbi et al., 2012) and in

Mayotte Island (Roux-Cuvelier et al., 2014). Nduguru et al. (2015) reported high diversity of CBSD viruses.

2.3.5. Cassava mosaic disease (CMD)

Several species with single-stranded DNA viruses that infect cassava plants have been reported from Africa. They include the African cassava mosaic virus (ACMV), the East African cassava mosaic virus (EACMV), and the South African cassava mosaic virus (SACMV) (Varshney and Tuberosa, 2013). However, the Indian cassava mosaic virus (ICMV) in India and the Sri Lankan cassava mosaic virus (SLCMV) in neighbouring islands are found as related species of viruses. Genomic sequencing and phylogenetic analysis revealed others nine species between Africa and India (Patil and Fauquet, 2009). CMD was first reported in Tanzania in 1894 (Fauquet and Fargette, 1990; Legg and Fauquet, 2004) and in Madagascar, Uganda, and Tanzania in the 1930s–1940s and affects all cassava growing area in sub-Saharan Africa. The distribution of the epidemic throughout the African continent resulted in cassava crop damage, high losses on yield, great economic loss and devastating famine (Legg et al, 2005; Legg and Fauquet, 2004).

2.3.6. Abiotic stress affecting the production of cassava

Cassava can be grown in infertile soil and where climatic conditions are difficult with little rainfall. In general, drought and low nitrogen stresses do not constitute major problem of the cassava crop (Xu et al., 2013). However, several mechanisms give the cassava crop a tolerance to drought, and once they are established, the crop tolerates the water stress. These mechanisms are divided into three groups: the reduction of water use by the crop and the efficient utilization of the limited amount consumed water to produce biomass (Howeler, 2012). Furthermore, cassava can grow in saline soil, mostly dominated by sodium chloride, and known as a very important constraint to food crop production. Cheng et al. (2018) highlighted the role of low salt in the growth of cassava, during the accumulation of starch in fibrous rootlets and increase the total protein content in new shoots.

2.4. Cassava improvement methods

2.4.1. Conventional methods of cassava improvement

The principle of breeding clonally propagated crops is to introduce a crossing step to produce sexual seed with genetic variation (Grüneberg et al., 2009). The population genotypes developed from seeds are highly heterozygous and different due to genetic recombination. Every seed is grown and potentially considered as new variety (Grüneberg et al., 2009). After the genetic recombination, all other propagation steps for cassava are asexual propagation. Breeding approaches include variety introduction, germplasm assembly and maintenance, clonal selection and hybridization are breeding procedures for clonally propagated crops (Acquaah, 2012). Breeding methods of cassava are defined by its genetic variability available, the mode of reproduction and the breeding objectives. Cassava is highly heterozygous species and presents a lot of segregation in the first generation progenies, that are then evaluated through phenotypic mass selection (Ceballos et al., 2015). The methods developed for self-pollinating crops are applicable to cassava with some modifications because of its specific characteristics.

According to Fukuda et al. (2002), there is no classic genetic improvement methods initiated for vegetative propagated crops. The main genetic improvement methods used in cassava are the assembly of the germplasm and selection followed by hybridization among selected elite clones (Acquaah, 2012 and Ceballos et al., 2004). The introduction of varieties and selection are the most important breeding methods used in Africa (Ceballos et al., 2016). However, crossing followed by selection of superior genotypes in the segregating population is the most universal method employed in cassava genetic improvement. Crossing requires selection of parental genotypes, which is generally based on their combining abilities, expected by the performance of the particular genotype (Fasahat et al., 2016).

The introduction and assembly of germplasm constitute the first step in breeding cassava. Just like seed, vegetative material may be introduced, evaluated and adapted to the new environment. Plantlets, seedlings and cuttings are introduced in sterile conditions to avoid contamination (Acquaah, 2012). Clonal selection on the other hand has the following objectives: maintenance of genetically pure and disease free clones, and the development of new varieties (Acquaah, 2012). Disease-free cassava is obtained by purifying an infected cultivar through screening for disease-free material by visually inspections for the presence of pathogens. When indexing reveals that a

pathogen is present, it may be eliminated by tissue culture, heat treatment, chemical treatment and use of apomictic seed methods (Acquaah, 2012).

2.4.2. Molecular methods of cassava improvement

Molecular methods for genetic improvement involve the use of DNA test results to facilitate the selection of parents for future generation of the genetic breeding program. Molecular markers are reported as strong tools in plant improvement with more effectiveness, reliability and less costly-than traditional breeding (http://bioscience.iita.org/index.php/en/research/molecular-breeding, Sraphet et al., 2011). Molecular methods are applied so that valuable genes and traits can be introgressed to germplasm for breeding programs and decision-making in conservation programs (Barcaccia, 2010). Gedil and Menkir (2019) emphasised the use of molecular methods in recurrent selection and composite population characterization. Various types of molecular marker methods have been developed and used to study genetic improvement of germplasm (Fregene et al., 2003, Kizito et al., 2005, Xia et al., 2005). They include restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNAs (RAPD), amplified fragment length polymorphisms (AFLP) and, most recently, single sequence repeat (SSR), single-nucleotide polymorphisms (SNP's) and Diversity Arrays Technology (DArT) markers.

2.4.2.1. Marker assisted selection techniques

Marker assisted selection (MAS) is an indirect selection method where a trait of interest is selected based on a marker (morphological, biochemical, DNA or RNA variation) associated to a trait of interest such as yield, disease resistance, abiotic stress tolerance, and quality. The technique has been successfully applied in cassava selection for resistance to CMD (Oliveira et al., 2018). The authors also reported the efficiency of MAS in identifying individuals with high level of inbreeding, providing a selection of about 25% of individuals in cassava self-pollinated progenies. Simple sequence repeat markers were developed and utilized to construct the genetic linkage map of cassava (Fregene et al., 1997) and to evaluate the genetic diversity of cassava (de Souza, 2007, Kizito et al. 2005 and Fregene et al. 2003). They are nucleotides tandem repeat units and provide excellent targets and a means to assess genetic variation within species (Ferguson et al., 2011). With codominant markers, like SSR and RFLP, heterozygotes can be differentiated from homozygotes (Kosman and Leonard, 2005). The first genetic linkage map of cassava was constructed from F1 intra-specific cross using SSR, RFLP and RAPD (Fregene et

al., 1997). The frequency and number of alleles per SSR marker in the Puerto Rican cassava collection were determined (Montero-Rojas et al., 2011). Restriction fragment length polymorphism is a kind of polymorphism that results from variation in the DNA sequence recognized by restriction enzymes that is used to cut DNA molecules at known positions. RFLP, AFLP and RADP markers were used to analyse the genetic diversity of cassava (Elias et al., 2000, Fregene et al., 2000 and Marmey et al., 1993). Furthermore, study on the genetic diversity and relationships within cassava germplasm using SNPs markers, was done by Karim et al. (2019). The utilization of SNPs speeded up the rhythm of assessment the genetic diversity and selection gains rather than using only traditional technique and also others molecular methods. Later, Diversity Arrays Technology (DArT) markers based-SilicoDArT and SNP markers for cassava were developed and reported as a tool for genotyping large germplasm collections (Xia et al., 2005) but this has not been used on Burundian cassava genotypes.

2.4.2.2. Linking specific traits to markers within cassava genomes

High-density single markers were used to perform genetic selection and reveal cassava performance, thereby reducing the breeding cycle from six years to one year (Sraphet et al., 2011). According to Sraphet et al. (2011), the marker associated to the quantitative traits loci (QTL) can be also applied to MAS to select cassava variety with desired phenotype. Molecular marker associated with agronomic traits contributed significantly in marker assisted cassava breeding programs (de Souza, 2007). The author also reported the identification of SSR and AFLP markers linked to the CMD-resistance gene in cassava landraces and RAPD markers linked to resistance to anthracnose. Quantitative traits loci for resistance to cassava bacterial blight were identified by Soto Sedano et al. (2017) and are the most useful for cassava improvement.

2.4.2.3. Latest molecular techniques used in molecular biology, genome wide association and DArT for genome profiling

Two research techniques have been developed in recent years with many implications: genome - wide association studies (GWAS) and Diversity Arrays Technology (DArT). Genome-wide association seeks to identify single nucleotide polymorphisms in the genome and to determine how polymorphisms are distributed across different populations. A genome-wide association approach involves rapidly scanning markers throughout the whole sets of genomes of many

samples to find interested trait linked to specific disease (Norrgard, 2008), and also detect genotype (common SNPs) linked to common phenotypes in a group of individuals (Tian et al., 2020 and Bush and Moore, 2012). According to Tian et al. (2020), a number of high-quality genotype-phenotype associations are detected in plants and animals such as rice, sorghum, maize cotton, soybean, sheep and pork.

On the other hand, DArT performs well in polyploid species and does not require any existing DNA-sequence information and can be used with little resources required for SNP platforms (Wenzl et al., 2008). The principle of DArT is based on revelations of DNA polymorphism that the technology reveals by investigating representations of genomic DNA samples for presence or absence of individual fragments (Kilian et al., 2012 and Wenzl et al., 2008). It is a sequence-independent genotyping method designed to detect genetic variation at several hundred genomic loci in parallel without relying on sequence information (Wenzl et al., 2008 and Wenzl et al., 2004).

2.5. Applications of molecular markers to cassava research and breeding

Ferguson et al. (2011) highlighted the advantages of molecular breeding in cassava. They include more precise genetic selection, the genetic improvement in quantitative traits, the reduction of the breeding population size, shorter the maturation time and preemptive breeding in environments where stresses like CBSD or CMD, are absent, but pose a significant problem. Molecular markers were reported by Acquaah (2012) as tool to assist breeders to select parents used to create new breeding population and to predict the performance of hybrids to be developed from different intergroup crosses. Thus, many molecular markers have been used in cassava. RAPDs, RFLPs (Beeching et al., 1993) and AFLPs (Fregene et al., 1997) were first genetically used to study the diversity of cassava.

Later, these markers have been replaced by SSR markers reported as highly reproducible, co dominant with many alleles, and distributed everywhere in the genome (Ferguson et al., 2011). Long time before SNP marker development, the study of the diversity of cassava was limited by the density of the markers and the cost. High density of SNPs was identified in cassava and facilitated the progress in cassava improvement (Ferguson et al., 2011). In 2000, microarray-based markers such as DArT was developed and utilized to characterize efficiently a big number of polymorphisms in a timely and at low cost (Mogga et al., 2018). It provides also rapid, high quality and affordable genome profiling, even from the most complex polyploid genomes.

In general, several molecular breeding techniques have been used in plant breeding. They include marker assisted backcrossing that reduce the number of backcrosses by three to four generations and conducted to transfer genes into a popular cultivar (Varshney et al., 2012) and backcross inbred lines to introgress wild genes (Jeuken et al., 2008). Others molecular breeding techniques used in the past are marker assisted "forward selection" that allow the recombination of alleles throughout the genome and the marker assisted gene pyramiding used to develop cultivars with durable resistance and the marker assisted early generation selection (Acquaah, 2012).

CHAPTER THREE

CHARACTERIZATION OF LOCAL AND INTRODUCED CASSAVA GERMPLASM USING QUALITATIVE MORPHOLOGICAL TRAITS MARKERS AND DART SEQ MARKERS ANALYSES

3.1. Abstract

Cassava is an important food crop and a source of income to small scale farmers in Burundi. In the long tradition of growing cassava, many small scale farmers mainly use local landraces that despite good local adaptability and highly preferred consumer attributes are susceptible to devastating cassava viral diseases. It is possible therefore to tap into good attributes of these varieties and eliminate their weak attributes in a breeding program. However, there is limited information on genetic diversity of local landraces, yet this information is critical in exploiting their genetic background. This limited information presents redundancy for cassava improvement in Burundi. Due to susceptibility of local landraces in Burundi, introduction of disease resistant germplasm developed in neighboring countries was done to improve local germplasm. Since the genetic composition of the introduced genotypes had not been compared to the local germplasm, this study aimed at determining genetic relationships and diversity for the local landraces and introduced germplasm using morphological and DArTSeq-based SNPs markers. A total of 118 accessions were evaluated with more than 18,000 SNPs identified. Molecular characterization revealed low genetic distance (Fst<0.15) between landraces and introduced resistant germplasm. Phenotypic traits distribution based on leaf, stems and root were diverse among the genotypes. Phenotypic characterization revealed three main clusters based on Ward's Method, with cluster III containing all introduced elite germplasm, while molecular characterization revealed six main clusters. A total of 73 accessions were distinct genotypes while 16 accessions were duplicates among local germplasm. Molecular characterization revealed three pairs of duplicates, which should be pooled as a single cultivar to avoid redundancy. Accessions that shared similar morphological traits were divergent at molecular level indicating that clustering was inconsistent for the former. The results revealed existing identical and differential relationships among clones. It is concluded that these results contribute to optimizing the conservation of genetic resources of Burundi and germplasm used for developing new clones. Despite variabilities found within the collection, it was observed that cassava germplasm in Burundi have a narrow genetic base hence a recommendation of further introductions should be made to broaden it.

3.2. Introduction

Cassava is an important staple crop and both the foliage and root are used as food sources. It is grown by farmers and is particularly important to small-scale farmers in the zones where it is cultivated. However, cassava cultivation has long history in Burundi and the knowledge of genetic diversity and relationship within the cultivated cultivars is poor which limits their use for genetic improvement. According to Afuape et al. (2011), understanding of the genetic diversity present in germplasm can assist in determining whether morphologically based taxonomic classifications reveal patterns of genomic variation. This can also provide information on the population structure, allelic richness, and diversity parameters of germplasm to help breeders the use of genetic resources for crop improvement. Such information is lacking for the cultivated cassava accessions in Burundi hence the reason why this study was conducted.

Genetic diversity and duplicates were assessed using SNP markers that are abundant, ubiquitous, and polymorphic and can be automated, resulting in a high analytical yield (Mammadov et al., 2012). Along with the phenotypic and passport data, the SNP markers contribute considerably to an efficient differentiation of germplasm. On the other hand, the cost of identifying the duplicates of cassava using molecular characterization is far low compared to the cost of conserving germplasm having duplicates. However, identified duplicates based on SNP markers must be characterized morphologically to verify whether they have similar enough traits to be considered synonyms in the germplasm. The results will then lead to understanding of genetic diversity among cassava landraces, identify and avoid duplicates. Generated results will also be used in breeding program for future improvement approach to increase cassava production in Burundi. The study aimed to characterize cassava genotypes using morphological and molecular markers as well as determine the relationship between the local landraces and the introduced resistant genotypes.

3.3. Materials and methods

3.3.1. Germplasm collection and establishment

One hundred local landraces of cassava were collected from 4 agro ecological zones in Burundi, including Imbo plain, Mumirwa slopes, East and north depressions and Central plateau (Table 3.1). These were established and multiplied at ISABU Bukemba research station (hot spot for CMD and CBSD infection) and Murongwe station (low spot for CMD and CBSD infection) for

morphological and molecular characterization. The study sites varied in altitude and amounts of rainfall (Table 4.1). Bukemba is located at 03°59′ 54″ S and 30°4′49″ E, 1180.9 m.a.s.l. in Rutana province in southeastern of Burundi while Murongwe located at 03°11′36″S and 29°53′47″E, 1523 m.a.s.l. in Gitega province in central of Burundi. Eighteen elite introduced resistant cassava genotypes were also characterized to assess their genetic relationship with the local landraces (Table 3.2).

Table 3. 1. Cassava landraces and their region of origin

Name of accession	Agro ecological zone	Name of accession	Agro ecological zone	Name of accession	Agro ecological zone
Nakarasi ya congo	1	Gatarina	3	Mpamba	4
Nakarasi y'ikirundi	1	Serereka	3	Mabare	4
Gitamisi_1	1	Bugiga annonciate_1	3	Imiduga_1	4
Muzinda	1	Yongwe_2	3	Tabika	4
Kwezikumwe	1	Gitikatika	3	Yongwe ederi	4
Rumonge	1	Gifunzo caritsa_1	3	Umukurajoro	4
Mbubute	1	Gifunzo caritsa_2	3	Rukokora	4
Yagata	1	Fyiroko	3	Kinazi dorothee1	4
Niga	1	Munebwe	3	Gasu	4
Ibigororoka	1	Ndoha	3	Inagitembe	4
Maguruyankware_1	1	Maguruyinkware_2	3	Umutuburano	4
Mwarabu	1	Rumarampunu	3	Gitamisi_2	4
Rushishwa	1	Imikabika	3	Rubona_2	4
Sosomasi	1	Hanyesi	3	Nakarasi_1	4
Myezisita	1	Rubona_1	3	Surupiya	4
Zegura	1	Bwome devote1	3	Sogota	4
Igipila	1	Umuyobera	4	Nabuseri	4
Igikoshi	1	Gasahira	4	Imirundi	4
Nakarasi_2	1	Mbwayasaze	4	Imizariya	4
Solange	2	Kidihe_1	4	Maguruyinkware_3	4
Yongwe_1	2	Bunwa	4	Umutakabumba	4
Kibembe_1	2	Inarubono	4	Mugerera Yvonne_1	4
Criolina	2	Ntunduguru	4	Mugerera Yvonne_2	4
Matara	2	Kigoma	4	Kidihe_2	4
Sisiriya	2	Imijumbura	4	Nyawera	4
Ruvuna	2	Nyabisindu anastasie_1	4	Nyamugari sophie_1	4
Butoke	2	Kabumbe	4	Mukecuru	4
Kiganda	2	Gasasa	4	Fundiko	4
Ntabahungu	2	Yongwe_3	4	Umuhendangurube	4
Kibembe_2	2	Mutsindekwiburi	4	Sagarara	4
Munengera	3	Murozi	4	Imiduga_2	4
Mwotsi_2	3	Umusimbaruzi	4	Mwotsi_1	4
Berita	3	Bukarasi	4	Kavyiro	4
Ntegagakoko	3	-	-	-	-

1 = Imbo plain, 2 = Mumirwa slopes, 3 = East and north depressions, 4 = Central plateau

Table 3. 2. Introduced cassava germplasm in Burundi and their country of origin

Variety name	Country of origin
KBH2002/066	Tanzania
Pwani	Tanzania
Mkumba	Tanzania
KBH2006/026	Tanzania
Kizimbani	Tanzania
Kiroba	Tanzania
Albert	Tanzania
Okhumelela	Mozambique
Orera	Mozambique
Eyope	Mozambique
Tajirika	Kenya
F10-30-R2	Kenya
Kibandameno	Kenya
TZ 130	Uganda
Nase14	Uganda
Nase1	Uganda
Nase3	Uganda
MM96/5280	Burundi

3.3.2. Field layout and design

One hundred local landraces were collected from four major agro ecological zones in Burundi, namely Imbo plain, Mumirwa slopes, east and north depressions, and Central plateau, selected on the basis of their importance in growing cassava. Once collected, the landraces were planted in a randomized complete block design with two replications in field gene banks at two sites (Moso and Murongwe research stations). Eighteen elite cassava genotypes earlier introduced to Burundi were also planted at the same sites. A single row plot of five cassava cuttings was planted. Each cutting having between 4 and 5 nodes with viable buds from each of the local landraces was planted at a spacing of 1.0 m x 1.0 m between and within rows. No fertilizer or irrigation was provided, but was kept weed-free throughout the growing period. Landraces and introduced cassava genotypes were characterized using morphological and agronomic cassava descriptors developed by Fukuda et al. (2010).

3.3.2. Morphological Characterization

Seventeen qualitative traits were evaluated (Table 3.3) based on agro-morphological descriptors of cassava described by Fukuda et al. (2010). Data was collected at 3, 6, 9, 12 months after planting (MAP) from three central plants only to minimize border effects using 17 descriptors with score scales that varied between 0 and 10 (Table 3.3). Color and pubescence on apical leaves were recorded earlier at 3 MAP since the most frequent damage by cassava green mite might obscure the traits.

At 6 MAP, shape of central leaf lobe and color of petiole were recorded by taking the leaf from a mid-height position and color of leaf and petiole orientation were observed from the middle of the plant. At 9 MAP, prominence of foliar scars, color of stem cortex and color of stem exterior were recorded from the middle third of the plant. Color of stem cortex was visualized by shallow cut and peel back of the epidermis as described by Fukuda et al. (2010). The distance between leaf scars was measured from the middle of stem on the middle third of the plant, where scars are not flat. Measurement was made along the stem and distance was divided by number of nodes in the measured section to obtain the internode length. Stem's growth habit was recorded as either straight or zig-zag, and color of end branches of adult plant was observed on top 20 cm of plant.

At 12 MAP, observations on color of root cortex, color of root-pulp, external color of root and root taste were taken. Color of root cortex and color of root-pulp were visualized by removing the skin of the root and by transversal cutting of the root.

Table 3.3. Qualitative traits used to characterize 118 cassava genotypes

Trait observed	Trait acronym	Sore code	Data entry
Color of apical leaves	CAL	3 = light green; 5 = dark green; 7 = purplish green; 9 = purple	3 MAP
Pubescence on apical leaves	PAL	0 = absent, 1 = present	3 MAP
Shape of central leaflet	SCL	1 = ovoid; 2 = elliptical-lanceolate; 3 = obovate-lanceolate; 4 = oblong-lanceolate; 5 = lanceolate; 6 = linear; 7 = pandurate; 8 = linear-pyramidal; 9 = linear-pandurate; 10 = linear-hostatilobalate	6 MAP
Petiole color	PC	1 = yellowish-green, 2 = green, 3 = reddish-green, 5 = greenish-red, 7 = red, 9 = purple	6 MAP
Leaf color	LC	3 = light green; 5 = dark green; 7 = purple green; 9 = purple	6 MAP
Petiole orientation	PO	1 = inclined upwards, 3 = horizontal, 5 = inclined downwards, 7 =	6 MAP

		irregular	
Prominence of foliar scars	PFS	3 = semi-prominent, 5 = prominent	9 MAP
Color of stem cortex	CSC	1 = orange, 2 = light green, 3 = dark green	9 MAP
Color of stem epidermis	CSEp	1 = cream, 2 = light brown, 3 = dark brown, 4 = orange	9 MAP
Color of stem exterior	CSEx	3 = orange, 4 = green-yellowish, 5 = golden, 6 = light brown, 7 = silver, 8 = gray, 9 = dark brown	9 MAP
Distance between leaf cars	DBLS	3 = short (≤ 8 cm), 5 = medium (8–15 cm), 7 = long (≥15 cm)	9 MAP
Growth habit of stem	GHS	1 = Straight, 2 = Zig-zag	9 MAP
Color of end branches of adult plant	CEBAP	3 = Green, 5 = Green-purple, 7 = Purple	9 MAP
Color of root cortex	CRC	1 = White or cream, 2 = Yellow, 3 = Pink, 4 = Purple	12 MAP
Color of root-pulp	CRP	1 = white; 2 = cream; 3 = yellow; 4 = orange; 5 = pink	12 MAP
External color of storage root	ECSR	1 = white or cream; 2 = yellow; 3 = light brown; 4 = dark brown	12 MAP
Root taste	RT	1 = Sweet, 2 = Intermediate, 3 = Bitter	12 MAP

MAP = Months after planting

3.3.3. Molecular Characterization

To assess the genetic structure of cassava genotype, molecular marker techniques were applied. Microarray-based markers, DArT, were used to study the genetic diversity of 100 local and 18 introduced cassava germplasm by characterizing their polymorphisms.

3.3.3.1. DNA extraction

After two months of planting, 2 newly expanded apical leaf tissues approximately 6 cm from a single stem were collected and placed inside the tube containing silica gel. Genomic DNA of 118 cassava genotypes were extracted from leaf samples using the protocol described by Dellaporta et al. (1983). Fifty milligrams of each crushed leaf sample were placed in 400 µl of extraction buffer and placed at 65ºC water bath for 25 minutes with gentle shaking. To precipitate proteins and polysaccharides, 200 µl of ice-cold 5M potassium acetate was added to each sample and mixed by gentle inversions. A volume of 350 µl chloroform: isoamyl alcohol (24:1) was added, gently mixing with continuous rocking and centrifuged at 4000 g for 10 minutes. The upper layer was transferred to a new tube simply by pouring to the corresponding set of next tubes (tissue debris would have blocked the bottom chloroform: isoamyl alcohol layer). One volume (400 µl) of ice-cold isopropanol was added. After centrifugation at 4500 g for 20 minutes, the supernatant

was discarded carefully. A volume of 300 µl of 70% ethanol was added and the supernatant was decanted after centrifuging at 3500 g for 10 minutes. A volume of 100 µl of low salt TE (10mM Tris, 1 mM EDTA, pH 8.0) + 3 µl RNAse (10 mg/ml) was added. A gentle mixing was done and the DNA samples were incubated at 37 ºC for 30 minutes.

3.3.3.2. DNA quality check and preparation of DArT arrays for genotyping

DNA quality and quantity were checked on 0.8% agarose gel. Libraries were constructed according to DArTseq™ complexity reduction method through digestion of genomic DNA and ligation of barcoded adapters (Kilian et al., 2012). PCR reactions were performed according to the program described by Jaccoud et al. (2001) and Xia et al. (2005). Libraries were sequenced using Single Read sequencing runs for 46377 bases. Next generation sequencing was carried out using Hiseq2500. However, the technology used by Hiseq2500 machine is DArTSeq. DArT uses a genotyping by sequencing (GBS) DArTseq™ technology, providing rapid, high quality and affordable genome profiling, even from the most complex polyploid genomes (Kilian et al., 2012; Raman et al., 2014). DArTseq markers scoring was achieved using DArTsoft14 which is an in-house marker scoring pipeline based on algorithms (Kilian et al., 2012). Two types of DArTseq markers, SilicoDArT markers and SNP markers were both scored as binary fashion for presence or absence (1 and 0 respectively) of the restriction fragment with the marker sequence in genomic representation of the sample.

3.3.4. Data analysis

Morphological traits analysis

Botanical characterization was done to describe the cultivars using morphological and agronomic descriptors of cassava developed by Fukuda et al. (2010). Data was processed using IBM SPSS statistics software version 20. Dissimilarity matrix and principal component analysis (PCA) were used to determine the relationship among accessions and populations. Structure of morphological changeability was visualized using ascending hierarchical clustering (AHC) based on data and Ward's Method to plot a dendrogram (Nadjiam et al., 2016 and Karim et al., 2020). Distribution percentage of the morphological traits was determined using MS excel.

Molecular analysis

Generated SNP data from DArTseq were cleaned in MS Excel by removing all genotypes with >5% missing data and monomorphic SNPs. Genotypes Umutuburano and Gifunzo-caritas1 had 18% missing data hence removed. Hamming single distance (distance matrix) between genotypes was calculated using KDCompute, Version 1.5.2 beta and hierarchical clustering done using Ward's method to produce a dendrogram (https://www.rdocumentation.org/packages/dartR). Generated sequences were imported into DartR and then filtered for repeatability, monomorphic loci, call rate per locus, single locus per sequence tag and call rate per individual (Gruber et al., 2019). To better identify duplicated genotypes and determine cut-off, true duplicate cassava genotypes were added to the dataset as duplicate checks. Identity of genotypes was checked by adding true identities as controls. To assess the population statistics, the observed heterozygosity (Ho) was calculated using mean hobs function in the R package 'Adegenet' (Adamacket and Gruber, 2014). Expected heterozygosity (He) was calculated using Hs function in the R package "Adegenet". Hamming distance was calculated in DartR and exported as a comma separated values (csv) for use in Darwin. Pair wise fixation index (Fst) among populations was calculated using StAMPP package in R (https://www.rdocumentation.org/packages/dartR) and the output value indicated existence or not of differentiation between populations where <15% indicate low differentiation, 0.15<Fst<0.25 indicate moderate differentiation and >25% indicate high differentiation (Mousadik and Petit, 1996). Genetic relationships of landrace and introduced cassava genotypes were assessed by estimation of hamming distance between genotypes using dartR in KDcompute as described by Hoque and Rahman (2007). The single distance matrix was exported as a csv file and imported into DarWin to make dendrogram in order to estimate the genetic relationship (Perrier et al., 2006).

3.4. Results

3.4.1. Morphological characterization of local and elite germplasm

At six, nine and twelve MAP, some accessions or parts of the plants were completely destroyed by CMD and CBSD, making it difficult to characterize some traits. The number of destroyed accessions increased over time. Root cortex, root pulp color, external color of storage roots and root taste were scored after 12 months during harvesting. The traits used in the characterization of 118 accessions are shown in Table 3.3.

3.4.1.1. Leaf traits

Landraces

Apical leaves showed more than 76% of landraces with purplish green color, 12% shown purple color, 11% shown dark green color and 1% had light green color and less than 3% only had hair on apical leaves (Fig. 3.1). Leaf shape varied widely with more than 52% of landraces having elliptic-lanceolate leaf shape, 20% lanceolate leaf shape, 20% oblong-lanceolate leaf shape. The obovate lanceolate, pandurate, lanceolate-pandurate and linear-pyramidal leaf shapes were together observed in 8% of the local accessions (Fig. 3.1). Petiole color also varied between local accessions with 53% having purple color. The other colors observed included yellowish-green (4%), green (1%), red-green (28%) and red (10%) (Fig. 3.1). The color of leaves among the landraces was predominantly dark green (81%). However, landraces with light green (13 %) and purple green (6 %) leaves were observed. The orientation of the petioles was diverse, with 62% horizontally oriented, 14%, irregularly oriented, 15% inclined downwards, 7% inclined upwards petiole and 2% had a vertical petiole (Fig. 3.1). The color of the end of branches of adult plants was also very diverse the local landraces. Forty six percent of the landraces had greenish purple color at the end of the branches. The other colors observed were greenish (20%) and purplish (3%) (Fig. 3.1).

Elite germplasm

The apical leaves of 33.3% of 18 accessions were purple colored, 27.8% colored dark green, 22.2% shown purplish green color and 27.8% had light green color and the pubescence on apical leaves were found on only 27.7% of the accessions belonging to elite germplasm. The shape of the leaves was diverse among accessions where 55.6% of 18 accessions had lanceolate, 38.9% elliptic-lanceolate and 5.6% were lanceolate-pandurate (Fig. 3.2). The color of petioles varied where purple green color was found on 38.9% of the 18 genotypes, purplish color on 33.4% and greenish color on 11.1%. However, diverse colors were available, including red, greenish red and purple yellow on 17.8 % of the 18 genotypes (Fig. 3.2). Color of leaves diversely distributed among genotypes where 38.9% of 18 accessions were colored purplish green and 27.8% colored dark green. Light green and purple color of leaves were found each on 16.7% of 18 genotypes (Fig. 3.2).

Petiole orientation was diverse, where 22.2% were horizontally oriented, 50%, were irregularly oriented, 22.2% inclined downwards while only one clone namely TZ130 had a vertical petiole

(Fig. 3.2). Color of end of branches of adult plants was little diverse for the tolerant introduced clones. Thus, the end of branches of the adult plants for 55.6% and 44.4% of the 18 characterized accessions were colored greenish purple and green, respectively (Fig. 3.2).

Combined elite germplasm and local landraces

The accessions had purplish green color as the dominant color for the apical leaf with more than 67.8% of 118 accessions dominated by landrace (64.4%) while elite germplasm had 3.4% (Fig. 3.3). Accessions that shown purple color were 15%, 13% shown dark green color and 3% had light green color and less than 7% only had hair on apical leaves (Fig. 3.3). These results show a diversity of color of the apical leaves for all the cassava genotypes.

Leaf shape varied widely where 50% had elliptic-lanceolate as the dominant shape dominated by landraces accessions (44.1%), followed by lanceolate with 24.4% and then oblong-lanceolate with 15.2%. Elite germplasm accessions with elliptic-lanceolate shape had 5.9%. However, the obovate lanceolate, pandurate, lanceolate-pandurate and linear-pyramidal shapes were found and only occupied 4.2% (Fig. 3.3). Petiole color was also varied where purple color was dominant on 50% but mainly occupied by landraces with 44.9% while elite germplasm with 5.1%. Other diverse colors were available, including yellowish-green, green, green purple, purple yellow, red-green, and red (Fig. 3.3).

Most accessions had dark green color as the dominant color for their leaves (66.1%) mostly landraces accessions occupying 60.2%, but light green, purple and purple green were also noted. Orientation of petiole was diverse and dominant orientation were horizontally oriented on 56% of 118 accessions that were mostly landraces (52.5%). The irregularly oriented on 16.1%, 14.4% inclined downwards, 4.2% inclined upwards petiole and only one clone having vertical oriented petiole were also found (Fig. 3.3). The color of the end of branches of adult plants was diversely distributed and the greenish purple color was dominantly found on more than 45% of the 118 characterized accessions, mostly landraces. However, the green and purple colors of the end of branches were also available in the collection (Fig. 3.3).

3.4.1.2. Stem traits

Landraces

Diversity of the landraces in stem cortex color was dominated by light green color found on 42% of 100 landraces accessions, followed by dark green at 21% and orange stem cortex at 6% (Fig. 3.1). Epidermis color was also diverse, where 38% of the landraces accessions had light brown stem epidermis, 28% of accessions had stem epidermis colored dark brown and 3% of accessions had cream stem epidermis. Color of stem exterior was diversely grey for 24%, silver for 21% and dark brown for 15% but the golden, orange, light brown and green yellowish were also present (Fig. 3.1). Foliar scars of 47% of all landraces were prominent while 23% had semi prominent foliar scars. Distance between leaf scars varied within landraces where 64% had medium distance (8–15 cm), 2% had long distance (≥ 15 cm) and 3% had short (≤ 8 cm) while all 100% accessions had straight stems (Fig. 3.1).

Elite germplasm

The stem cortex of accessions belonging to elite germplasm was colored dark green and light green, respectively for 61.1% and 38.9% of the 18 genotypes (Fig. 3.2). Epidermis color was mostly light brown with 72.2% and the stem epidermis of 27.8% was colored dark brown while the color of stem exterior was diversely distributed with 44.4% having gray stem exterior color, 22.2% having silver color, 16.7% having green yellow color, 11.1% having dark brown color and 5.6% having green color (Fig. 3.2). Stem growth habit for all genotypes was straight except for Orera, which had a zigzag stem. (Fig. 3.2). Foliar scars of all accessions were prominent except Okhumelela and Eyope, which presented semi-prominent foliar scars. Medium distance (8–15 cm) between leaf scars was dominant occupying 77.8%, but short (≤ 8 cm) and long distance (≥ 15 cm) were also available on three genotypes (Nase1, Mkumba and Pwani) and one genotype (Tajirika), respectively (Fig. 3.2).

Combined elite germplasm and local landraces

The accessions had light green color as the dominant color for stem cortex with 41.5% mostly dominated by landrace (35.6%) while elite germplasm had 5.9%. Orange stem cortex was recorded on 5.1%, while dark green color was found on 27.1% (Fig. 3.3). Epidermis color was diverse, where more than 43% of the accessions had light brown color as the dominant color, mostly landraces (32.2%). Other colors such as dark brown and cream were also present (Fig. 3.3). The color of stem exterior was diversely gray as dominant color for 27.1% mostly landraces

(20.3%), silver for 21.2% mostly landraces (17.8%), dark brown for 14.4% mostly landraces (12.7%) and yellowish color for 3.4%. Nonetheless, other colors wer also present including golden, orange, light brown and green (Fig. 3.3). Foliar scars of 53.4% were prominent while 21.2% had semi prominent foliar scars. Accessions with prominent foliar scars were mainly landraces (39.8%) while elite germplasm with prominent foliar scars were 13.6%. However, within elite germplasm, all clones were prominent foliar scars except two accessions that had semi prominent foliar scars (Fig. 3.3). Distance between leaf scars varied within cassava accessions where 70% had medium distance (8–15 cm) mostly dominated by landraces, 2.5% had long distance (≥ 15 cm) and 4.2% had short (≤ 8 cm) while stem growth habit for all genotypes was straight except Orera (Fig. 3.3).

3.4.1.3. Root traits

Landraces

The accessions had cream color as the dominant color for root cortex with 37%. The dominant root pulp color was white for almost all accessions except for Solange accession. Accessions had dark brown color as the dominant color for external storage root with 37%. The accessions having external storage root colored light brown were 8%. Also, among characterized accessions for the trait, 81.8% tasted bitter while 18.2% had sweet taste (Fig. 3.1).

Elite germplasm

Cream color was the dominant color for root cortex with 94.4% of the 18 accessions. Pink root cortex was found only on Kiroba genotype while all 18 accessions had white root pulp (Fig. 3.2). External storage root color was diverse although dark brown and light brown were dominant at 50% and 44.4%, respectively. Cream color was found on only Tajirika genotype. Furthermore, two-thirds of accessions, or 66.7%, tasted bitter as dominant taste while 33.3% had sweet taste (Fig. 3.2).

Combined elite germplasm and local landraces

Cream color was the dominant color for root cortex and almost all accessions of elite germplasm fell to this group while the dominant color for the root pulp was white for all accessions except Solange (Fig. 3.3). Accessions having external of storage root colored dark brownish as dominant color, mostly landraces, were 39%. Of the all characterized accessions for the taste, 77.4% had

bitter as dominant taste and were predominantly landraces while other accessions, predominantly elite germplasm tasted sweet (Fig. 3.3).

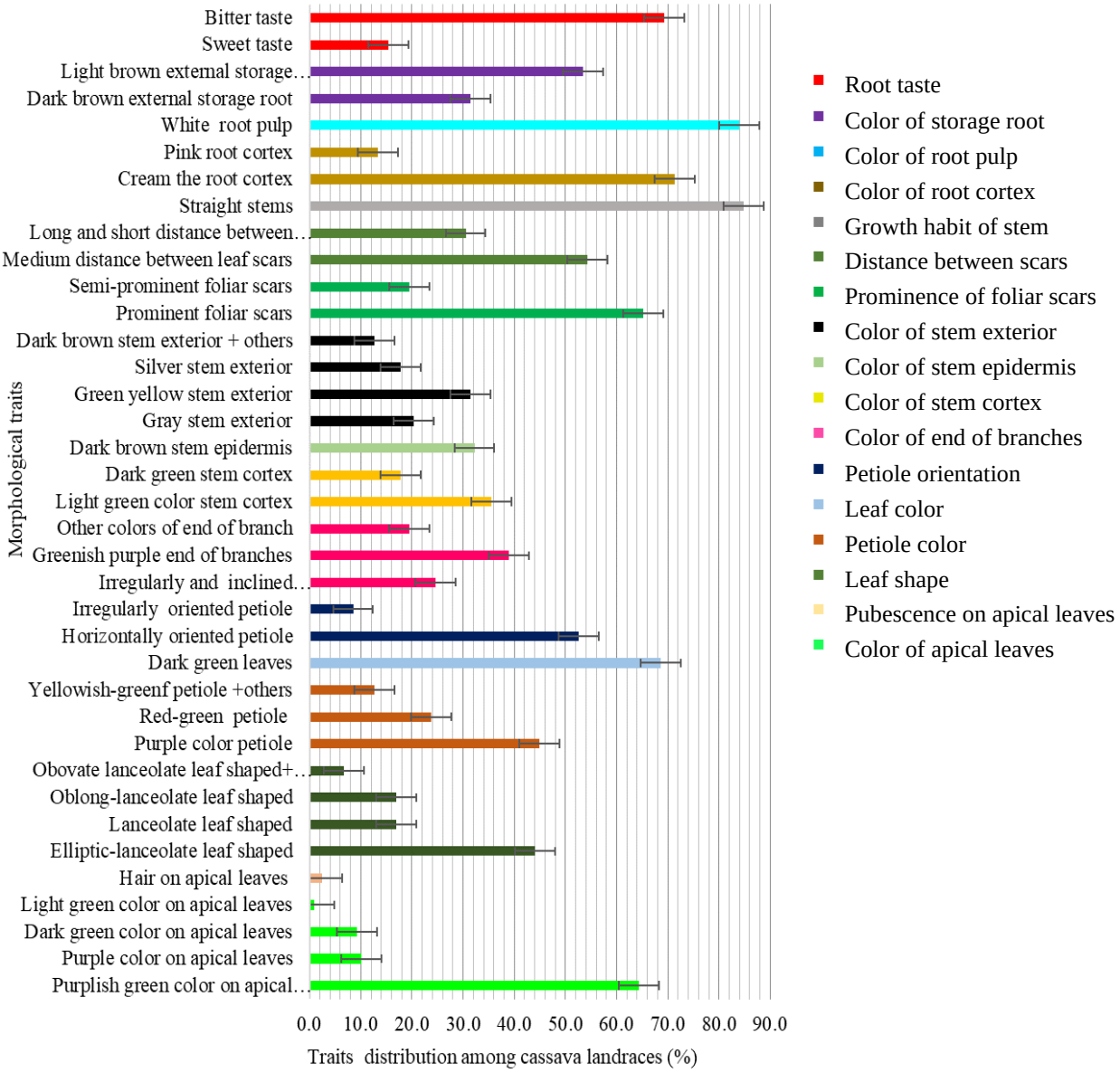

Figure 3.1. Morphological traits distribution among the cassava landraces with errors bars determining whether differences are statistically significant

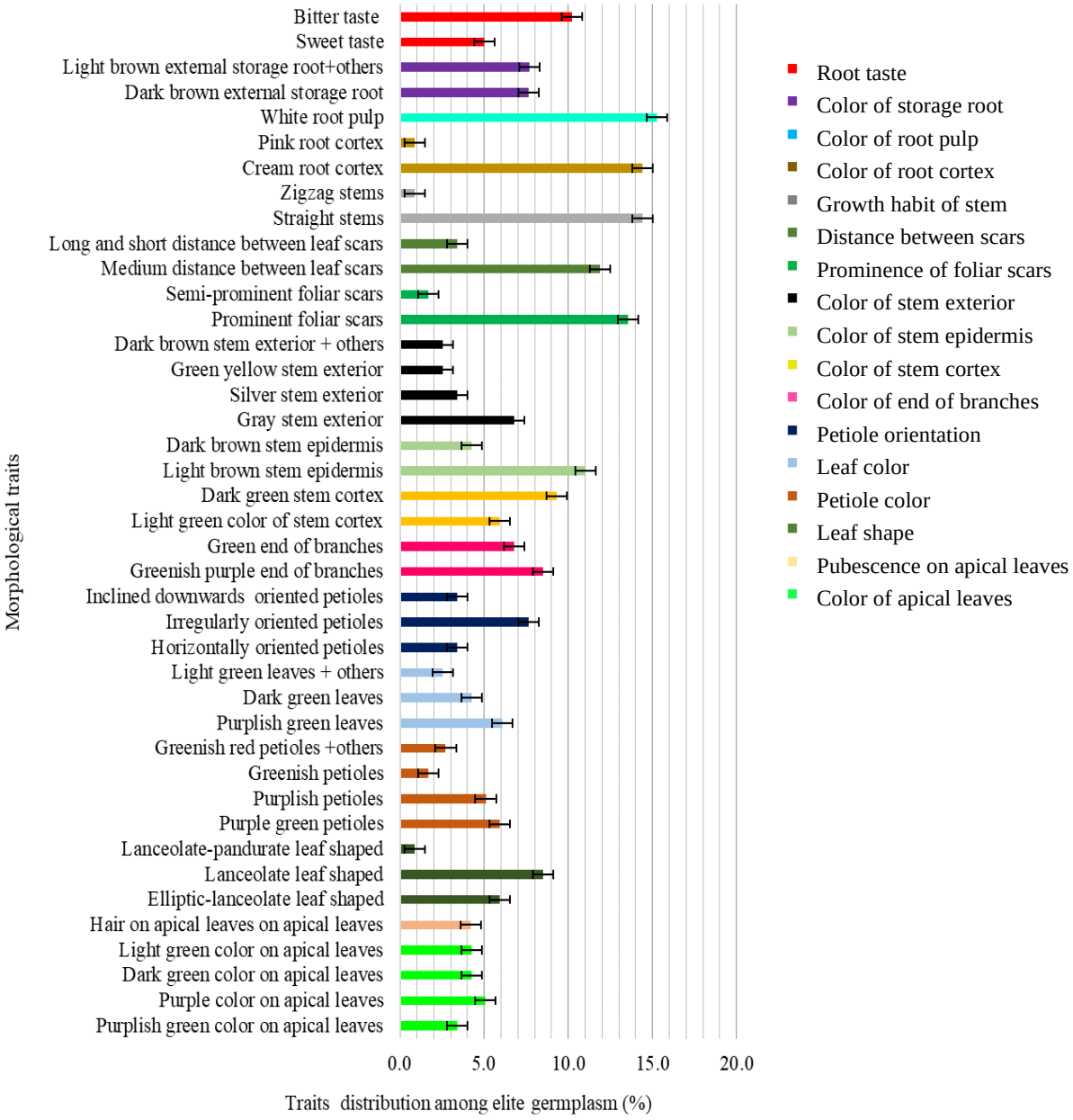

Figure 3.2. Morphological traits distribution among the elite germplasm with errors bars determining whether differences are statistically significant

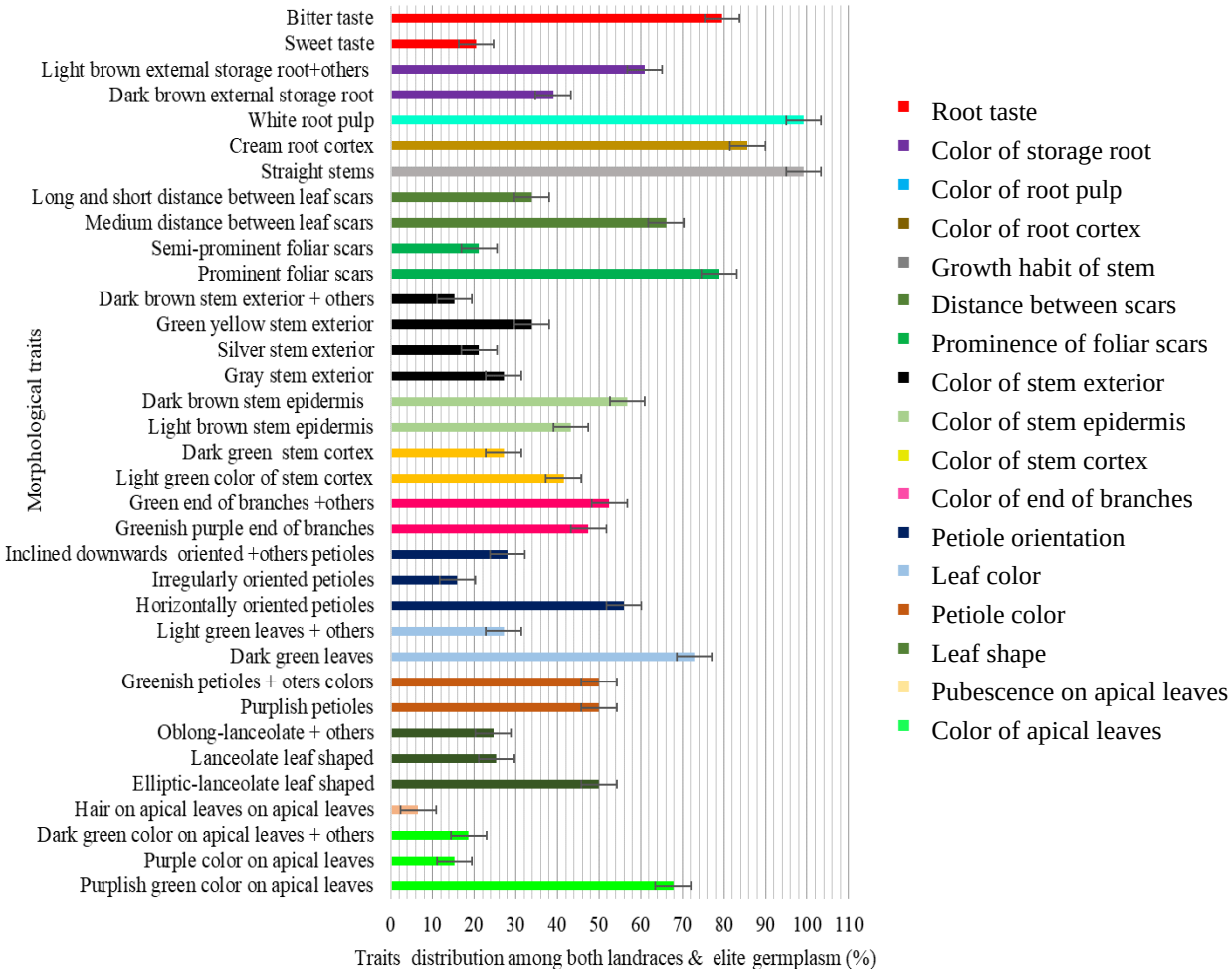

Figure 3.3. Morphological traits distribution among both landraces and elite germplasm with errors bars determining whether differences are statistically significant

3.4.2. Hierarchical clustering of 118 morphologically characterized cassava accessions

Ascending hierarchical clustering analysis based on morphological traits and ward's method showed three major clusters (Fig. 3.4) following the horizontal line at a dissimilarity level of 6. Cluster I containing 31 accessions, all local landraces, had two sub-clusters (A and D). Cluster II consisted of 26 accessions 3 sub-clusters and consistedof local landraces and elite germplasm. The first sub-cluster consisted 5 resistant genotypes, Tajirika, Nase 1, Nase 3, KBH2002/066 and Orera, 2 local landraces, Nakarasi and Igipila; the second sub-cluster was composed 3 resistant genotypes, Kizimbani, Kiroba and Eyope and the third sub-cluster consisted of 16 local landraces (Fig. 3.4)**.** Cluster III was the largest with 2 sub-clusters consisting of 51 local landraces and 10 resistant genotypes. Resistant genotypes under this category were KBH2006/026, Okhumelela, Mm96/5280, Nase 14, F10-30-R2, TZ130, Albet, Mkumba, Kibandameno and Pwani (Fig. 3.4). PCA on cassava qualitative morphological traits showed extensive variation among accessions at different levels, with no clear grouping of accessions (Fig. 3.5). Axes explained 62.2% of total variation having 50.1 and 12.1% for horizontal and vertical axes, respectively (Fig. 3.5). Among populations, 62% of the total variation was explained with 50% on horizontal axis while 12.1% on vertical axis (Fig. 3.6).

3.4.3. Genetic relationship among cassava genotypes using DArT analyses

Results from DArTR analysis showed 72 unique genotypes at dissimilarity level of 1.0 (red line), 43 genotypes presented similar SNP profile (Fig. 3.7) following the cut off (green line) calculated from the distance matrix based on an average value of known duplicates. Similar accessions were grouped in 16 classes, each of them with different clones (Fig. 3.7). Genotypic classification of accessions based on Ward's Method showed six major clusters (I, II, III, IV, V and VI) (Fig. 3.7). Cluster I contained two resistant genotypes, Pwani and Mkumba, 5 true identities and known duplicates checks, Pwani_2, Pwani_3_SB101, Mkumba_1, Pwani_1, Mkumba_2_SB102 (Fig. 3.7). Cluster II had 13 genotypes consisting of local landraces eand elite germplasm. Nine genotypes were resistant, including Eyope, Kiroba, KBH2006/026, Tajirika, KBH2002/066, Nase 3, Nase 1, Kizimbani and Okhumelela while 4 were landraces, Nakarasi ya congo, Rumonge, Munembwe and Gitamisi (Fig. 3.7). Cluster II also had 8 duplicates such as Eyope-1, Tajirika-2, KBH 2002/026/1, KBH 2002/026/2, Tajirika-5CP-Kephis, KBH 2002-066-SB103, Nase 3-1and Nase 1-1 (Fig. 3.7). Cluster III and V consisted of 8 and 7 accessions, respectively, all local landraces. Cluster IV was composed of 50 local landraces and 8 resistant genotypes including Orera, F10-30-R2, Kibandameno, Albert, Okhumelela, Mm96/5280, Nase 14 and TZ130 (Fig. 3.7). Cluster VI consisted of 33 local landrace accessions. Paired similar accessions that fell into

this category were igikoshi and Munengera, Sosomasi and Igipila, Mwotsi, Mwarabu and Mwzisita, Bunwa and Kigoma, Maguruyinkware-2 and Rumaramuntu, Ndoha and Imikabika, and Bugiga annociate 1 and gifunzo caritas 2 (Fig. 3.7).

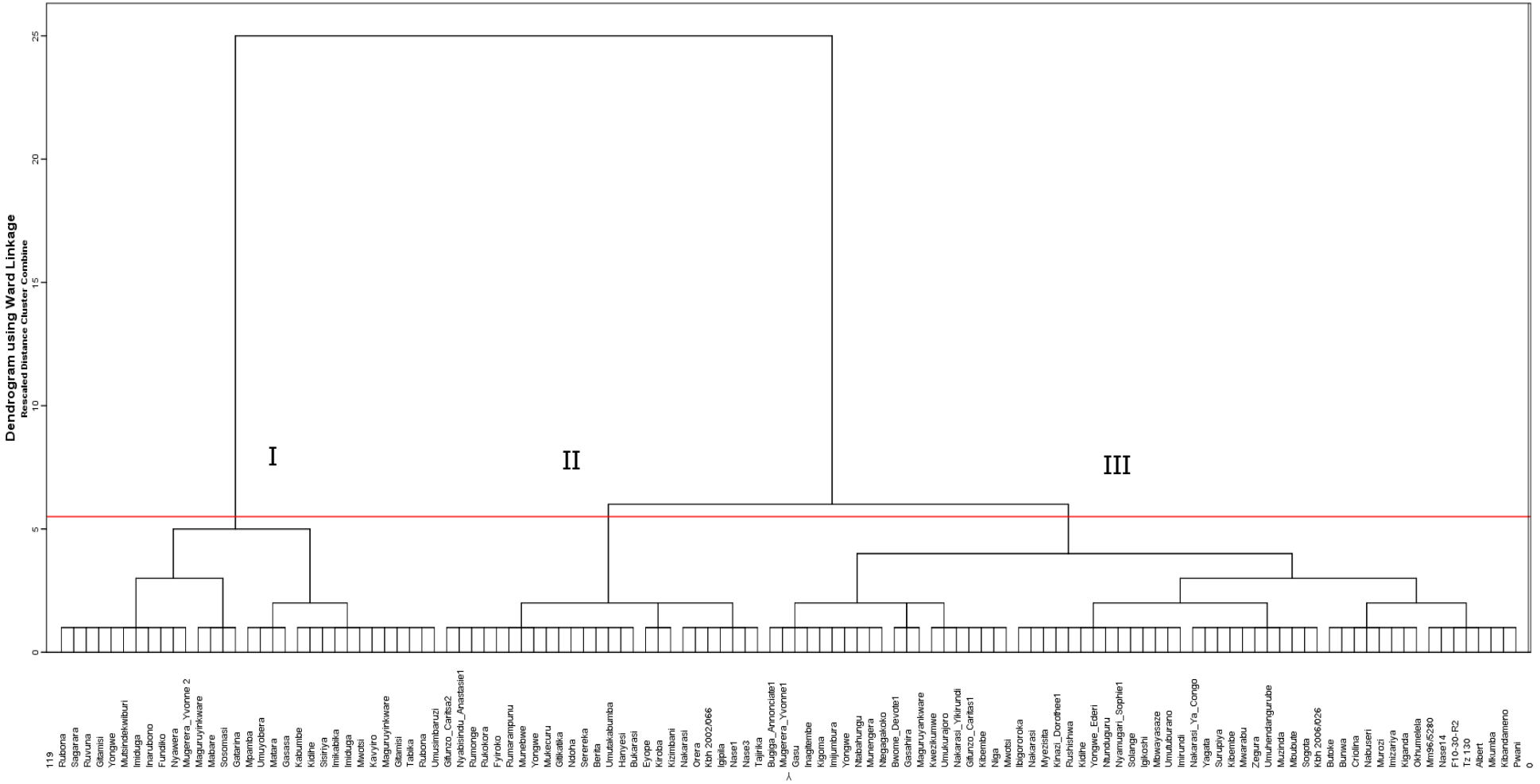

Figure 3. 4. Phenotypic classification of cassava accessions based on the Ward's method at a dissimilarity level of 6 (red line)

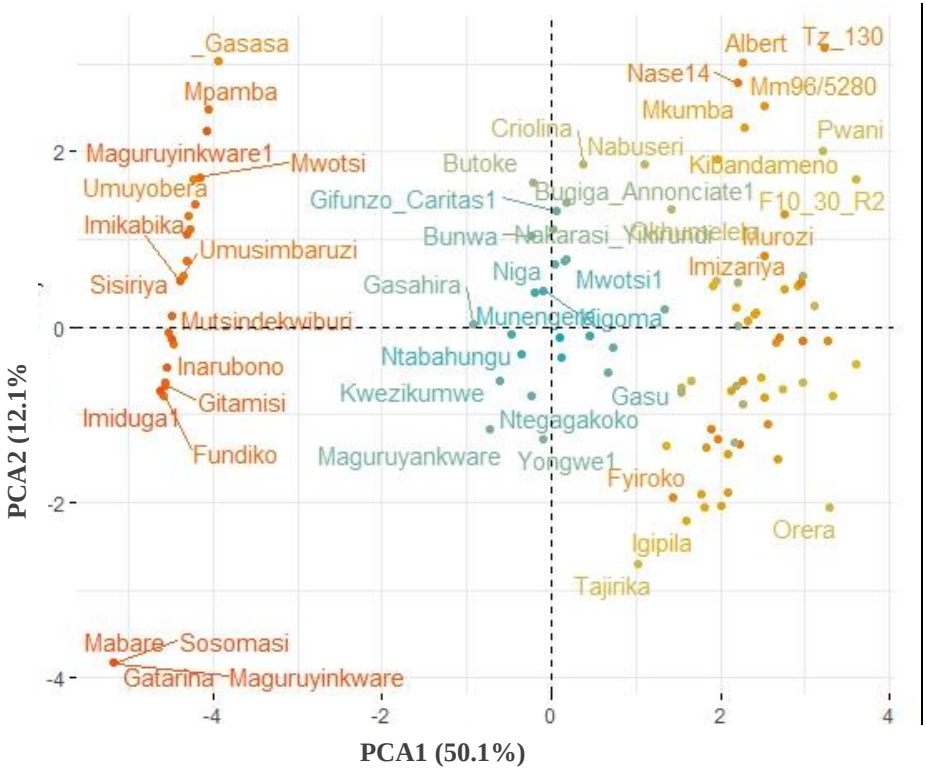

Figure 3.6. PCA showing phenotypic relationship between cassava accessions in Burundi

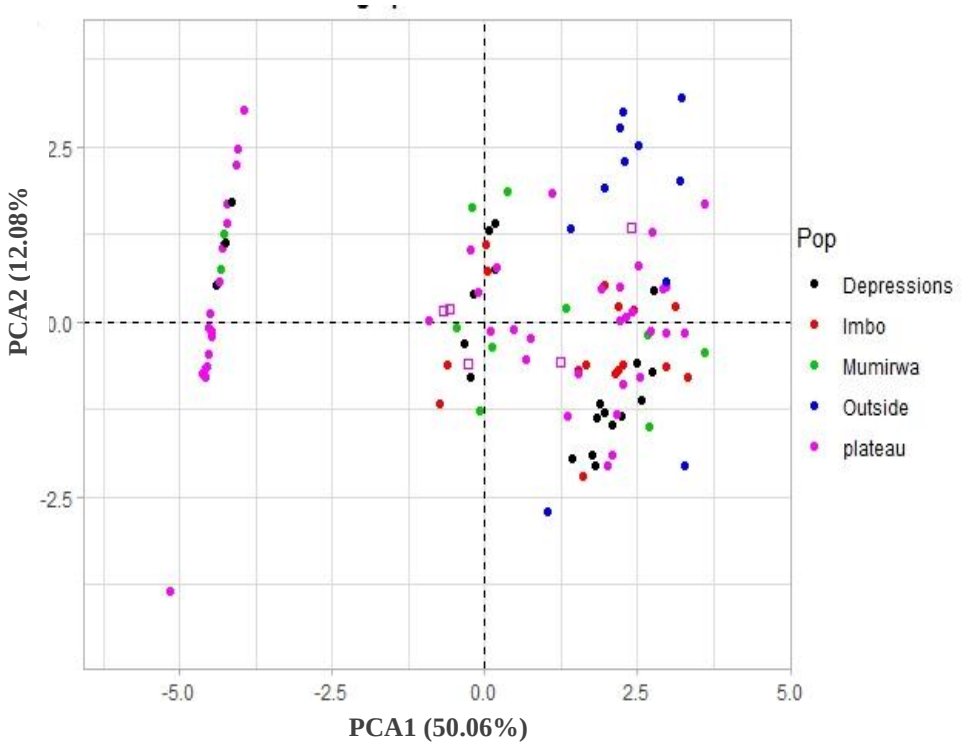

Figure 3.6. PCA showing phenotypic relationship between the five populations

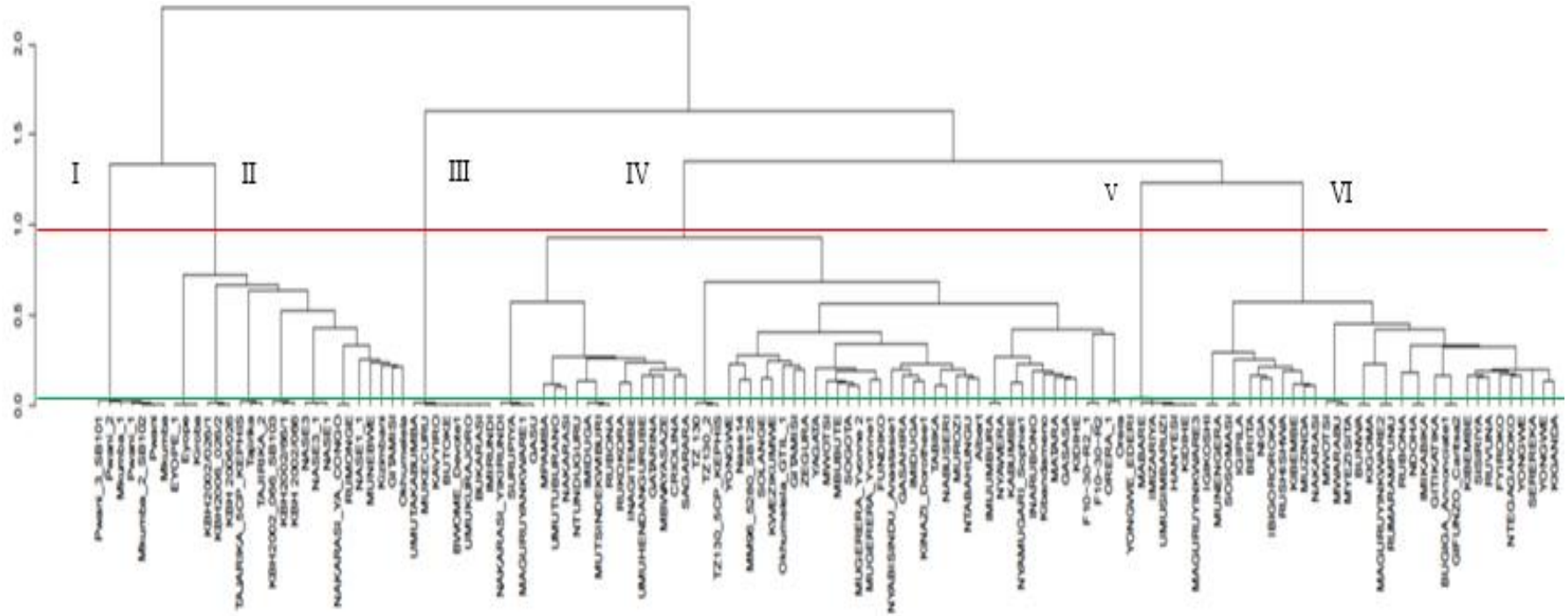

Figure 3.7. Genotypic classification of accessions based on Ward's method at dissimilarity level of 1.0 (red line), green line determining the threshold for putative and known duplicates

3.4.4. Assessment of the population statistics of the genotypes

Population statistics was assessed within and between populations to determine relationships present. The output values of calculated pair wise fixation index (Fst) among all populations were <15% indicating low differences between populations (Table 3.4). The results showed pair wise fixation index of 0.071, 0.095, 0.073, and 0.083 between resistant genotypes and local landraces of Imbo plain, landraces of Mumirwa slopes, landraces of North east depressions and landraces of Central plateau, respectively, that indicate little variation between them (Table 3.4). Also, between local landraces of Imbo plain and Mumirwa slopes, North East (NE) Depressions and Central plateau, the pair wise fixation index were 0.010, 0.023 and 0.020, respectively, indicating very low differentiation between the populations. Similarly, the pair wise fixation index between landraces of Mumirwa Slopes and NE Depressions, between landraces of Central plateau and NE Depressions were respectively 0.027 and 0.028 (Table 3.4).

Within population, the output values were greater than 25% for all populations indicating high differentiation between genotypes (Table 3.5). The results showed pair wise fixation index of 0.59, 0.60, 0.57, 0.59 and 0.56 within resistant genotypes, landraces of Imbo plain, landraces of Mumirwa slopes, landraces of NE depressions and landraces of Central plateau, respectively, indicating high variation between genotypes within population (Table 3.5). Heterozygosity was calculated per marker and population, where Ho was greater than He in all populations except resistant genotypes, indicating a suspected mixing of previously isolated populations (Table 3.5).

Table 3. 4. Pairwise fixation index between landraces from different locations

	Resistant genotypes	Landraces of Imbo Plain	Landraces of Mumirwa Slopes	Landraces of NE Depressions	Landraces of Central Plateau
Resistant genotypes	-				
Landraces of Imbo Plain	0.071	-			
Landraces of Mumirwa Slopes	0.095	0.010	-		
Landraces of NE_Depressions	0.073	0.023	0.027	-	
Landraces of Central_Plateau	0.083	0.020	0.001	0.028	-

Table 3. 5. Fixation index and heterozygosity within population

Population	Fixation Index F within population	Observed heterozygosity (Ho)	Expected heterozygosity (He)
Resistant genotypes	0.59	0.25	0.27
Landraces of Imbo Plain	0.60	0.27	0.25
Landraces of Mumirwa slopes	0.57	0.27	0.25
Landraces of NE Depressions	0.59	0.26	0.25
Landraces of Central Plateau	0.56	0.26	0.25

3.4.5. Comparison of the results from morphological and molecular dendograms

Morphological traits and classification based on Ward's method that grouped accessions into the clusters revealed existence of phenotypic variability among accessions. Morphological traits were diversely distributed, thus morphological classification clustered accessions into 3 main groups, indicating that the accessions were phenotypically diverse among themselves.

Molecular analysis clustered accessions into six groups indicating that they were genetically diverse. All genotypes in clusters I and II for morphological classification method and clusters III, V and VI for genetic classification methods were local landraces. Cluster I in the genetic classification method only consisted of resistant genotypes (Pwnani and Mkumba) while clusters II, IV and III for morphological clustering method contained local landraces and elite germplasm.

Morphological traits distribution analysis, morphological clustering and the molecular analysis facilitated assessment of germplasm diversity by establishing phenotypically and genetically similar accessions.

3.5. Discussion

Morphological traits

The analysis of morphological traits based on leaf traits, stem traits and root traits of the cassava landraces and the elite germplasm were diverse indicative of their use as criteria of selection for future production by farmers and by breeders for developing new and improved cultivars.

Leaf traits play an important role in cultivar identification and photosynthetic activity

According to Nkansah et al. (2013), leaf shape is considered an important trait since expresses the extent of leaf area for seasonal integral of light interception which can directly affect plant yield. In this study, examined cassava landraces and elite germplasm displayed phenotypic diversity in leaf shape and color, which are important variables to distinguish between accessions as reported by Asare et al. (2011). In addition, leaf traits play an important role in cultivar identification and are relevant for cassava selection in leafy vegetable markets where leaves are consumed. Color of cassava (storage root, root cortex, root pulp, apical leaves, whole leave, petiole, end of branches, stems) was the most representative and distinguishing trait in this study, which is known as an important trait for farmers to select cassava cultivars (Agre et al., 2016). Analysis revealed few accessions colored light green on apical leaves, having hairs and with central leaflet shaped linear- piramidal, which were comparable to those obtained by Nadjiam et al. (2016). However, plants with leaflets shaped linear- pyramidal decrease transpiration to limit water loss, hence the ability to survive during drought conditions while light green color is evident of photosynthetic activity for food production (Van der Vyver and Peters, 2017). According to Ehleringer and Mooney (1978), presence of hair reduces leaf absorptance, heat load, and consequently lower leaf temperatures and transpiration rates but it also lowers photosynthetic activity. At end of branches for many genotypes, they were colored greenish purple, suggesting presence of anthocyanin that absorbs green and yellow light. Similar results have been reported by Eze et al. (2016) in Nigeria. Anthocyanins are documented to prevent cardiovascular disease, obesity control and treatment of cancer (Lin et al., 2017).

Stem foliar scars are important as indicators of ease of propagation

The dominant color for stems cortex was light green color, mostly landraces, but others colors such as orange and dark green were present, suggesting that the color of stem cortex was diverse.

However, this dominant light green color is considered the least efficient wavelength in the visible spectrum for photosynthesis, but is still useful in photosynthetic activity and regulates plant development. Most landraces accessions had stem epidermis and stem exterior colored light brown and gray respectively as dominant colors in the collection suggesting that this study of diversity of cassava in Burundi highlighted groups of accessions characterized by color of stem stem epidermis and color of stem exterior. Similar results were found by Kosh-Komba et al. (2014) who studied the diversity of cassava in Central Africa Republic and underlined clusters of accessions characterized by the stems colored light brown and gray. Prominent foliar scars for the cassava were more than 50% while 20% had semi-prominent foliar scars indicative of ease of propagation at planting time. According to Adu et al. (2018) and Banoc et al. (1999), when scars planted they lead to development of roots and also lateral branches come from the foliar scars on lower stem parts. Furthermore, the distance between leaf scars determine the number of scars per unit stem length and indeed the number of lateral branches.

Root traits

Cream color on root cortex and root pulp on many accessions was indicative of the presence of a precursor of Vitamin A. Although this was the case, elite germplasm (with 94% cream color) seemed to have high numbers with the vitamin A precursor compared to the local landraces with 37%. Njenga et al. (2014) reported that cassava with yellow and cream roots have carotene content, a precursor of vitamin A. Yellow cassava roots are associated high value proteins levels in leaves, therefore, improving cassava for beta-carotene could also improve overall nutritional value of the crop (Njenga et al., 2014). Almost 50% of the accessions had bitter taste, suggesting that processing is required prior to consumption. Similar suggestions were made by Chiwona-Karltun et al. (2004) who studied the relationship between bitter taste in cassava roots and cyanogenic glucoside levels and recommended processing to reduce their levels.

Molecular characterization

Analysis based on molecular characterization clustered accessions into 6 main clusters indicative of their variation. Clusters I contained two resistant genotypes, Mkumba and Pwan,i that shared all genetic characteristics, suggesting that they were duplicate clones. Clusters III, V and VI contained 8, 7 and 33 local landraces, respectively, suggesting that the groups shared similar genetic characteristics. Cluster II, had 9 resistant genotypes (Okhumelela, Kizimbani, NASE1,

NASE3, KBH2002/066, Tajirika, KBH2006/026, Kiroba and Eyope) with 4 landraces (Gitamisi, Munembwe, Rumonge and Nakarasi) while cluster IV had 7 resistant genotypes (F-10-30-R2, Kibandameno, Orera, Albert, MM96/5280, Nase14 and TZ130) clustered together with 51 local landraces, suggesting that local landraces and elite germplasm in clustered together had similar genetic characteristics. Local landraces in clusters II and IV could therefore have possible partial resistance but this should be verified. Cluster II had 3 pairs of accessions (Eyope and Kiroba, Nase3 and Nase1, Nakarasi ya congo and Rumonge) that shared all genetic characteristics, indicating that they were duplicate clones.

Population genetic studies revealed a narrow genetic base

Population statistics analysis for the populations that determine existence of any relationships showed little variation between populations, introduced genotypes and local landraces of Imbo plain, Mumirwa slopes, North east depressions and Central plateau, suggestive of a narrow genetic base. This could have been due to a high previous breeding possible between isolated populatios leading to narrow genetic diversity (Neaves et al., 2015) or since cassava is mainly clonally propagated, variation limited (Pillay and Tenkouano, 2011). On the other hand within population, the analysis showed a high differences between genotypes indicating low previous breeding possible.

Variation between morphological and molecular analysis suggests environmental effect

The accessions that shared similar morphological characteristics were divergent at the molecular level indicating that clustering using morphological traits is less consistent. These results are in agreement with the findings of Sujii et al. (2013) and Feldberg et al. (2011) who reported that plants showing similar morphological characteristics could be very divergent at molecular level. Darkwa et al. (2020) and Sujii et al. (2013) reported that clustering using morphological traits is less reliable due to environmental influence and plant growth stage expression. According to Darkwa et al. (2020), this phenomenon could explain the changing and clustering observed in comparing membership of hierarchical cluster dendrograms originating from morphological and molecular characterization.

Accessions clustered in group II for morphological and molecular characterization including Eyope, Kizimbani, Kiroba, Rumonge, Munembwe, Tajirika, Nase3 and Nase1 suggested he possibility of morphological characterization of some accession without the need for molecular fingerprinting work (Benesi, 2005). Pwani and Mkumba, assumed to be duplicates clones were in cluster I in molecular characterization while sub-cluster of cluster III together with Kibandameno, Albert, TZ130, F10-30-R2, Nase14 and MM96/5280 in morphological characterization indicating the existence of divergence on clustering results using the two methods. The duplicates clones noted within clusters indicated that a single genotype could have multiple names such as Eyope and Kiroba, Pwani and Mkumba Nase1 and Nase3 from elite germplasm while local landraces like Imiduga, Mutsindekwiburi and Imiduga, Nakarasi ya congo and Rumonge. The difference of number of clusters between the 2 methods of characterization could have been due to number of specific traits used. This was due to the fact that phenotypic classification had 17 morphological traits while genotypic classification had more than 18 000 SNP's, hence genotypic classification showed more similarities between accessions. The presence of accessions with unique genotypes indicated that they were not duplicate clones and vice versa. Similar results were reported by Albuquerque et al. (2019) and Arnaud-Haond et al. (2007) while identifying duplicate accessions based on multilocus analysis and stated that accessions presenting similar SNP profile were assumed to be duplicates as each multi loci genotype corresponded to a single genotype.

3.6. Conclusion

The aim of this study was to characterize cassava genotypes and determine the relationship between the local landraces and the introduced resistant genotypes. Morphological and molecular characterization revealed low differences between introduced genotypes and local ladraces indicating little variation between them. Furthermore, the study showed classes of cultivars and within each class, sub classes with similar SNP's profile were identified. Accessions having very close similar characteristics namely Pwani and Mkumba, Eyope and Kiroba, and Imiduga, Mutsindekwiburi and Rubona were assumed to be duplicates, hence reason why they will be removed from the collection and the cassava breeding program. Despite the variabilities found within the collection, it was concluded that cassava landraces in Burundi and the introduced clones present a narrow genetic base.

CHAPTER FOUR

DETERMINATION OF DUAL RESISTANCE RESPONSE TO CASSAVA BROWN STREAK DISEASE AND CASSAVA MOSAIC DISEASE OF INTRODUCED CASSAVA GENOTYPES

4.1. Abstract

Cassava is the fourth most important staple food crop in the tropics after wheat, rice and maize. It is a cash crop in Eastern and Central Africa region, including Burundi. Cassava production in Burundi is constrained by biotic and abiotic stress leading to unavailability of resistant varieties. Cassava brown streak disease (CBSD) and cassava mosaic disease (CMD) are the most important biotic stresses to cassava production in Burundi. Countries like Uganda, Kenya and Tanzania have already identified CBSD/CMD dual resistant genotypes but Burundi. The objective of this study was to determine the effects of genotype by environment interaction on resistance to CBSD and CMD diseases in varied agroecological zones of Burundi. This study evaluated performance of 17 cassava elite genotypes introduced from neighboring countries and genotype MM96/5280 from Burundi with resistance to CMD disease. Field experiments were conducted at Moso, Mparambo and Murongwe research stations with known variation in climatic and environmental conditions between January and December 2019. The 18 treatments were arranged in alpha lattice design having 9 blocks per site and each block containing 6 plots. Plot data was recorded for number of sprouted cuttings, CBSD and CMD foliar severity, CBSD root necrosis, whiteflies population, growth parameters and root yield. Data was analysed using GenStat Discovery Software 14th edition. Results showed that 4 genotypes, F10-30-R2, KBH/2002/026, Nase-14 and Pwani had dual tolerance to CBSD and CMD on leaves while Mkumba had dual resistance. Secondly, 5 genotypes were tolerant to CMD while 5 genotypes were resistant. The dual resistant/tolerant genotypes found in this study could be used as genetic stocks to combine resistance to CBSD and CMD into a single genotype. The succeptible genotypes could be improved by incorporating resistant genes into their genome through breeding. Thirdly, 7 genotypes were tolerant to CBSD on leaves and stems while 2 were resistant. Eight cassava clones showed good performance in yield and 3 were tolerant to CMD and CBSD at all locations, thus can be considered as stable genotypes. Release of the three high yielding and tolerant genotypes to CMD and CBSD would most likely increase cassava production in Burundi. It is

therefore recommended that the high yielding and disease tolerrant genotypes be incorporated into the Burundi breeding program.

4.2. Introduction

The CBSD and CMD are the most damaging diseases of cassava in the tropical and subtropical lowlands countries (Houngue et al., 2019; Pariyo et al., 2015). Cassava is a high-energy root crop that tolerates drought and poor soil conditions where cereals and other crops do not grow (Nassar and Ortiz, 2007). Cassava is produced in several agro-ecological zones but production levels are dependent on genotype, environment and the interaction (Athanase et al., 2017). Production of cassava has declined significantly in recent years due to several diseases including CBSD and CMD (FAOSTAT, 2010-2019; IITA, 2014). The two diseases are transmitted by whiteflies (*Bemisia tabaci)* and use of infected planting materials resulting to production losses up to 100% (Ntawuruhunga et al., 2007; Busogoro, 2008; Abaca et al., 2012).

Reduction of production losses could be done using selection of tolerant/ resistant and high yielding genotypes with stable performance across environments using farmer-preferred traits for cassava farming systems (Benesi, 2005; Akinwale et al., 2011). Genotypes under investigation should be subjected to different environmental conditions to determine stable performers (İlker et al., 2009). The information generated is then applied to shape appropriate recommendations for specific genotypes and environments.

The objective of this study was to determine the effects of genotype x environment interaction on resistance to CBSD and CMD diseases in three agro ecological zones of Burundi. Deployment of dual resistant varieties will increase cassava productivity and householder's income that cultivate cassava in Burundi.

4.3. Materials and methods

4.3.1. Description of the experimental sites

The experiments were established at Moso, Murongwe and Mparambo research stations in Burundi for the 2018/2019 cropping season. These sites for the study varied in altitude, amounts of rainfall and pressure of CBSD and CMD (Table 4.1). Mparambo is located at -2°50′16″S and 29° 4' 16″E, 886 meters above sea level (m.a.s.l.) in Cibitoke province in north-western of Burundi, Moso is located at 03°59′ 54″ S and 30°4′49″ E, 1180.9 m.a.s.l. in Rutana province in

southeastern of Burundi while Murongwe located at 03°11′36″S and 29°53′47″E, 1523 m.a.s.l. in Gitega province in the central of Burundi.

Table 4. 1. Description of the three experimental sites

Parameter	Site		
	Mparambo	Murongwe	Moso
Soil parameters[c]			
pH	6.7	4.3	6.0
Available P (mg kg−1)	4.2	1.2	0.5
Exch K (meq/100g)	0.7	0.4	0.4
Total N (%)	0.1	0.1	0.13
Total Zn (mg kg−1)	33.4	30.8	50.0
Exch Zn (mg kg−1)	0.7	0.7	1.4
Organic C (%)	2.0	2.7	1.6
Exch Ca (meq/100g)	6.9	0.6	10.0
Exch Mg (meq/100g)	5.3	0.8	8.8
CEC (meq/100g)	19.6	18.6	20.6
Clay (%)	39.9	45.2	67.0
Sand (%)	27.9	37.4	17.6
Silt (%)	32.3	17.4	26.3
Climatic parameters			
Altitude (m.a.s.l.)[d]	886	1523	1180
Rainfall (mm)(Mean annual)[a]	688.2	1135,0	1108.6
Temperature (°C) (Mean annual)[a]	24.5	20.0	22.7
Disease pressure[b]			
CBSD pressure	HIGH	VERY LOW	HIGH
	Severity 2.9	Severity 1	Severity 2.8
	Incidence 54%	Incidence 0%	Incidence 37%
CMD pressure	HIGH	LOW	HIGH
	Severity 3	Severity 3.3	Severity 3
	Incidence 25%	Incidence 5.7%	Incidence 13%

[a] Source: ISTEEBU, 2017 and Eurostat, 2015, [b] Source: Bigirimana et al., 2004, [c]: analyses were done by soil and food products analysis laboratory at ISABU, [d]: Data taken by the author, Exch: Exchangeable, CEC: Cation exchange capacity, pH: Potential of hydrogen, meq: milliequivalents and m.a.s.l.: meters above sea level.

4.3.2. Plant materials

Eighteen cassava clones were used in the study where 15 were elite clones identified from "New Cassava Varieties and Clean Seed to Combat CBSD and CMD" project sourced from Kenya, Uganda, Mozambique and Tanzania with moderate to high tolerance levels to CBSD and CMD ((Brian et al., 2015; Table 4.2). Two clones, Kibandameno and Albert, were used as standard

susceptible checks for CMD and CBSD respectively while one local clone MM96/5280 was used as susceptible check for CBSD. To ensure uniformity of planting materials, 200 virus indexed plantlets per clone were introduced to the ISABU tissue culture laboratory at Regional Research Station of Gisozi in Burundi, acclimatized and multiplied in the field at a site with low disease pressure to obtain plantable cuttings which were used to establish the trials.

Table 4. 2. Characteristics of introduced elite germplasm for studies on response of resistance

Country of origin	Variety name	Fresh Root Yield (t/ha)	% DM content	Reaction to disease		Release status
				CMD resistance	CBSD resistance	
Tanzania	KBH2002/066	34.1	28.0	Moderate	Moderate	Released
Tanzania	Pwani	50.8	29.2	Moderate	Moderate	In pipeline
Tanzania	Mkumba	23.3	27	Weak	Moderate	In pipeline
Tanzania	KBH2006/026	30.0	29.0	Moderate	Moderate	Released
Tanzania	Kizimbani	28.6	28.0	Moderate	Moderate	Released
Tanzania	Kiroba	20.0	32.0	Weak	Moderate	Released
Tanzania	Albert	Fair	Good	Strong	Weak	Not released
Mozambique	Okhumelela	20.0	32.8	Moderate	Moderate	Released
Mozambique	Orera	23.0	32.0	Weak	Moderate	Released
Mozambique	Eyope	25.0	32.0	Moderate	Moderate	Released
Kenya	Tajirika	61	25.7	Moderate	Moderate	Released
Kenya	F10-30-R2	58	40	Moderate	Moderate	Adv. yield trial
Kenya	Kibandameno	26.1	40	Susceptible	Susceptible	Not released
Uganda	TZ 130	-	-	Strong	Moderate	Released
Uganda	Nase14	31.2	35.0	Strong	Moderate	Released
Uganda	Nase1	14.9	32.5	Strong	Moderate	Released
Uganda	Nase3	<10	30.0	Moderate	Moderate	Released
Burundi	MM96/5280	27.5	54.3	Moderate	unknown	Released

Source: Tumwegamire et al., 2018 and Brian et al., 2015

4.3.3. Field layout and design

The experiments were conducted at Moso, Murongwe and Mparambo research stations in Burundi between January and December 2019. Eighteen cassava clones comprising of 15 elite clones, two standard checks and one national check (MM96/5280) were used to estabilsh the experiment. The cassava clones were planted in a 42 m^2 plot arranged in alpha lattice design with nine blocks per site. Each treatment had 42 stem cuttings in a plot of 7 rows, each measuring 6 m long. There was 2 m spacing between plots and 1 m spacing within rows of the plot, while blocks were 2 m apart. Three plot replications per clone were used. The method of placing stem cutting into the ground was the same in all locations and consisted of inclined planting on mounds where two-thirds of the cutting were placed in the soil. The experiment was kept weed-free and neither inorganic fertilizers nor chemical pesticides were applied during the crop cycle. No plants were rogued out during the crop cycle; no supplementary irrigation was applied. To increase CBSD

and CMD inoculum pressure, two susceptible clones (Kibandameno and Albert) were included in the experiment as disease spread rows.

4.3.4. Data collection

The number of sprouted cuttings were recorded from 2 weeks to one month after planting (MAP). Severity of CBSD and CMD foliar and stem symptoms was recorded at 3, 6 and 9 MAP using 10 plants in two alternate rows of the net plot, for the first and third rows, where the first row was randomly selected. Scoring of CBSD and CMD diseases was done using previously described methods (Gondwe et al., 2003; Ntawuruhunga, 2009; Ntawuruhunga et al., 2009; Abaca et al., 2012). Symptoms of CBSD on the leaves and stems were recorded using the following scale 1 = absence of symptoms, 2 = light foliar mosaic, absence of lesions on the stem, 3 = presence of the mosaic on leaves, presence of slight lesions on the stem, without dieback, 4 = presence of the mosaic on leaves, presence of severe lesions on the stem, without dieback, 5 = presence of severe lesions on the stem, defoliation, dieback (Ntawuruhunga et al., 2009). Incidence of CBSD and CMD foliar and stem symptoms were recorded by counting plants with visible symptoms in the net plot.

Severity of root necrosis caused by CBSD was assessed using a score scale of 1 to 5 where 1 = absence of necrosis, 2 = presence of necrosis less than 5%, 3 = presence of necrosis of 5-10%, 4 = presence of necrosis 11-25%; mild root constriction, 5 = >25% root necrosis with severe root constriction (Gondwe et al., 2003, Ntawuruhunga, 2009; Abaca et al., 2012).

The severity of CMD was assessed using a score scale of: 1= No observed symptoms, 2 = Slight chlorotic appearance on all the young leaves or little deformation limited at the bases of most leaves while other leaves are green and healthy, 3 = Strong mosaic on most leaves and one-third of the lower leaves are deformed and narrow, 4 = Mosaic with two-thirds of the most leaves severely deformed; leaf size generally reduced and some stunting of shoots, 5 = all leaves very severely mosaic, twisting and reduction of most leaves (Gondwe et al., 2003; Ntawuruhunga, 2009). Adult whiteflies were counted at 3, 6 and 9 MAP on the five top most cassava leaves of the sampled plants (Ariyo et al., 2005). The counting was done during morning when conditions were relatively calm and whiteflies were immobile. At 12 MAP, the number of whiteflies was not

assessed since older cassava plants are not attractive to *Bemisia tabaci* (Kalyebi et al., 2018) and the numbers decrease during the growth period (Legg, 1994).

Data was also recorded for plant height and height to first branching by measuring vertical height from the ground to the top of canopy and measuring vertical height from ground to first primary branch respectively. Data on harvest index (HI) was recorded by uprooting 4 to 6 cassava plants per clone and roots and aboveground biomass (stems, branches, and leaves) weighed separately. Number of marketable root and fresh storage root was recorded from the net plot of 20 cassava plants with length greater than 20 cm for marketable root. The yield was calculated in tonnes per hectare (tons/ha). Soil analysis was done from composite samples taken during planting and analyzed to determine the nutrient status. The important parameters for analyses included soil pH, total nitrogen, available P, exchangeable bases, cation exchange capacity (CEC) and particle size. Plant establishment (PE) was calculated as a percentage of a ratio of the number of sprouted cuttings to the number of cuttings planted per plot (Ntawuruhunga, 2009).

$$\text{PE (\%)} = \frac{\text{Number of sprouted cuttings per plot}}{\text{Total number of planted cuttings per plot}} \text{ x } 100$$

Fresh storage root yield (FSRY) and biomass in tons/ha were calculated using the number of surviving plants in the net plot (Ntawuruhunga, 2009).

$$\text{FSRY (tons/ha)} = \frac{\text{Weight roots from harvested area (kg) x 10,000}}{\text{Harvested area (m}^2\text{) x 1,000}}$$

Harvest Index (HI) was calculated as a percentage of the fresh root yield to the total sum of root and aboveground biomass (Fukuda et al., 2010).

$$\text{HI} = \frac{\text{Weight of fresh roots}}{\text{Weight of fresh roots + weight of aboveground biomass}} \text{ x } 100$$

The root DM and starch content were estimated based on the principle of a linear relationship between specific gravity with DM and starch content (Fukuda et al., 2010). Percentage DM= 158.3x -142 while starch content = 112.1x -106.4 where “x” is a specific gravity.

Specific gravity was computed at Ww/ (Wa -Ww) where Wa and Ww are weight of sample in air and water, respectively.

The dry storage root yield was calculated as follow:

DSRY (tons/ha) = [DMC (%) x FSRY (tons/ha)] /100 (Nduwumuremyi et al., 2017)

Disease was measured in terms of intensity and was expressed either as disease incidence or severity. Disease incidence was recorded as percentage of diseased plants or parts in the sample or population of plants assessed and was calculated as follows:

$$\text{Disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plant assessed}} \text{ x } 100$$

The CBSD and CMD foliar severity during the cassava growth stage were utilized to compute the cumulative Area Under the Disease Progress Curve (AUDPC) using the method described by Shane and Finney (1977). This represented the magnitude of disease for the full growing period and was calculated according to the formula proposed by Shaner and Finney (1977):

$$\text{AUDPC} = \sum_{i=1}^{n} \left(\frac{x_i + x_{i+1}}{2}\right) t$$

Where, xi= is the score on date i of CBSD/CMD severity, n= is the number of assessments made and t= is the time (in months) between two assessments.

However, the AUDPC measure quantitatively the disease intensity in the timeline. It expressed the levels of resistance to CBSD and CMD diseases of the introduced cassava clones.

The additive main effect and multiplicative interaction (AMMI) stability value (ASV) was computed in Microsoft Excel using the formula developed by Purchase (1997).

$$\text{ASV} = \sqrt{\left[\frac{SS_{IPCA1}}{SS_{IPCA2}}(IPCA1_{SCORE})\right]^2 + (IPCA2_{SCORE})^2}$$

The ASV was used to rank the cassava clones in terms of stability in specific and across locations. Where ASV = the AMMI stability value, SS IPCA1 and SS IPCA2 = the sum of squares of the interaction principal component analysis one and two.

Genotype stability index (GSI) integrates both performance for a given trait and stability across environments into a single index, to select varieties. The GSI was calculated using the sum of the ranking based on performance for a given trait and ranking based on the AMMI stability value.

$$GSI = RY + RASV \text{ (Adjebeng-Danquah et al., 2017)}$$

where GSI = the genotype stability index, RASV = the rank of the genotypes based on the AMMI stability value and RY = the rank of the genotypes based on performance for a given trait across environments.

4.3.5. Statistical data analysis

Statistical GenStat Discovery 14th edition was used to perform ANOVA and the treatment means were separated using least significant difference (LSD) at 0.05 significant levels (VSN, 2010). Analyses of variance was done initially for each site and later for all sites combined.

Genotype by environment interaction was performed by using additive main effects and multiplicative interaction (AMMI) GenStat package. The AMMI stability value (ASV) was calculated to rank clones in each location and across locations. The AMMI model was important in this study, according to Falkenhagen (1996), because it can to compute the mean genotype by environment means and rank of genotype in specific and across environments. Lowest ASV score indicates a wide adaptability of specific genotypes for specific environments and vice-versa, consequently the most stable genotypes (Purchase, 1997). Nevertheless, for yield performance, according to Adjebeng-Danquah et al. (2017), stability alone cannot guarantee selection since a low yielding genotype can still be stable. In some cases, good stable genotypes do not always have good yield performance. Yield stability index (YSI) was calculated to rank the clones with good root yield and stability.

Pearson's phenotypic correlation coefficients were determined to estimate correlation between agronomic traits, diseases intensity and yield traits. In addition, paired test (Two-sample T-test) was computed to compare means parameters and differences were declared to be significant at 95% confidence level.

Where the coefficient of variation (CV) appeared high, the data were transformed using square root and logarithmic functions to normalize them and lower the CV.

4.4. Results

4.4.1. Sprouting, plant height and height to the first branching

The genotypes differed significantly (P<0.001) for plant height and height to the first branching (Table 4.3). There were high significant (p<0.001) differences in sprouting between cassava genotypes. At all locations, the genotypes showed high sprouting rate with average means of 84.3%, 82.1% and 82.3% at Moso, Mparambo and Murongwe, respectively. Genotypes were taller at Moso and Mparambo with mean height of 2 m compared to Murongwe with 1.3 m (Table 4.4). At Moso, the tallest genotype was Albert with height of 2.4 m while the shortest was MM96/5280 with 1.5 m. At Mparambo, the tallest genotype was KBH/2002/026 with height of 2.8 m while shortest genotype was Nase-3, 1.4 m. Orera was the tallest genotype at Mparambo with height of 1.8 m while shortest was MM96/5280. Kibandameno had the highest height to first branching at both Moso and Mparambo while Mkumba had the highest at Murongwe (Table 4.4).

Table 4.3. Mean squares for sprouting, plant height and height to first branching for the cassava genotypes on the three sites

Source of variation	Degree of freedom	Sprouting rate (%)	Plant height (m)	Height to 1st branching (m)
Block	2	91.7^{ns}	0.2^{ns}	0.01^{ns}
Site	2	76.1^{ns}	10.1^{***}	0.1^{**}
Genotype	17	930.3^{***}	0.5^{***}	0.2^{***}
Block x Site	3	162.9^{ns}	0.2^{ns}	0.1^{ns}
Site x Genotype	33	157.9^{ns}	0.2^{ns}	0.04^{*}
Block x Genotype	33	110.9^{ns}	0.1^{ns}	0.03^{ns}
Block x Site x Genotype	24	100.4^{ns}	0.1^{ns}	0.04^{*}
Residual	45	129.1	0.2	0.02

*** Very highly significant at p<0.001 probability level, *: Significant at p<0.05 probability level; ^{ns}: no significant.

Table 4.4. Performance of morphological traits of 18 cassava genotypes at Moso, Mparambo and Murongwe during 2018-2019 season

Genotype	Moso			Mparambo			Murongwe		
	Sprouting (%)	Plant height (m)	Height to first branch (m)	Sprouting (%)	Plant height (m)	Height to first branch (m)	Sprouting (%)	Plant height (m)	Height to first branch (m)
Albert	91.3	2.4	0.6	89.7	2.3	0.6	95.2	1.2	0.7
Eyope	78.6	2.0	1.0	91.3	1.5	0.4	91.3	1.1	0.7
F10-30-R2	89.7	2.3	0.9	80.2	2.7	0.7	87.3	1.4	0.7
KBH/2002/026	71.4	2.3	0.5	84.1	2.8	0.5	77.0	1.3	0.5
KBH/2002/066	96.0	1.9	0.6	63.5	2.0	0.7	73.0	0.9	0.4
Kibandameno	82.5	1.7	1.3	77.0	1.4	1.0	61.3	1.1	0.8
Kiroba	81.0	2.1	0.7	90.5	2.3	0.7	87.3	1.1	0.4
Kizimbani	76.2	2.1	0.4	89.7	2.1	0.4	92.1	1.3	0.4
Mkumba	100	2.2	0.7	97.6	2.4	0.8	99.2	1.5	0.8
MM96/5280	84.1	1.5	0.4	67.5	1.7	0.6	81.8	0.9	0.4
Nase-1	59.5	1.9	0.7	65.1	1.4	0.4	62.7	1.1	0.6
Nase-14	94.4	2.2	0.7	88.9	1.9	0.6	92.1	1.4	0.8
Nase-3	67.5	1.9	0.8	54.0	1.4	0.6	62.7	1.8	0.7
Okhumelela	81.8	1.9	0.5	77.8	2.0	0.5	84.9	1.2	0.5
Orera	91.3	1.7	0.4	90.5	1.8	0.4	87.3	1.8	0.5
Pwani	97.6	2.0	0.6	95.2	2.3	0.7	96.0	1.5	0.7
Tajirika	90.4	1.9	0.6	85.7	1.9	0.5	82.5	0.9	0.4
TZ-130	83.3	2.1	0.6	89.7	2.4	0.5	68.3	1.2	0.5
Mean	84.3	2.0	0.7	82.1	2.0	0.6	82.3	1.3	0.6
LSD (0.05)	13.9	ns	0.3	21.6	ns	0.3	22.2	ns	0.2
CV (%)	9.5	14.3	22.5	15.1	24.0	29.8	15.5	29.2	22.2
P-value	0.001	0.1	<.001	0.02	0.06	0.04	0.02	0.2	0.003

LSD: Least significant difference, CV: Coefficients of variation, ns: no significant, %: percentage and m: meter.

4.4.2. Performance of the 18 cassava genotypes against CBSD and CMD at 3 MAP

There were high significant differences ($p<0.001$) for whitefly populations between genotypes and sites. Interaction between sites and genotypes also showed significant ($P<0.05$) differences (Table 4.5). The reaction of genotypes to CBSD severity and incidence as well as CMD incidence was not significantly different. However, reaction of genotypes to CMD severity varied significantly ($P<0.01$) (Table 4.5). The CBSD on leaves was not observed at Murongwe while the incidence and severity were very high at Moso compared to Mparambo (Tables 4.6). The CBSD

severity scores ranged from 1.0-2.67, 1.0-1.67 and 1.0 for Moso, Mparambo and Murongwe, respectively, with an incidence ranging from 0-34% and 0-28% at Moso and Mparambo, respectively. Across sites, CBSD severity ranged from 1-1.6 while incidence from 0-11.5 % (Table 4.6). The CMD severity scores ranged from 1-4 at all sites and from 1-3 across sites with incidence ranging from 0-67% and 0-43 at all sites and across sites, respectively (Table 4.7).

Table 4.5. Mean squares for white flies' population, CBSD, and CMD across sites at 3 MAP

Source of variation	Degree of freedom	Whiteflies population	CBSD_ severity	CBSD_ incidence	CMD_ severity	CMD_ incidence
Block	2	12405^{ns}	0.5^{ns}	72.0^{ns}	2.7^{*}	4606.9^{**}
Site	2	3573800^{***}	3.4^{***}	1302.0^{**}	2.7^{*}	118.6^{ns}
Genotype	17	350668^{***}	0.3^{ns}	236.4^{ns}	2.3^{**}	727.9^{ns}
Block x Site	3	53589^{ns}	0.1^{ns}	80.4^{ns}	1.2^{ns}	385.0^{ns}
Site x Genotype	33	98297^{*}	0.3^{ns}	246.9^{ns}	1.1^{ns}	1174.3^{ns}
Block x Genotype	33	59292^{ns}	0.1^{ns}	215.4^{ns}	0.8^{ns}	1172.3^{ns}
Block x Site x Genotype	24	57156^{ns}	0.2^{ns}	277.0^{ns}	0.5^{ns}	629.1^{ns}
Residual	45	55611	0.3	226.4	0.8	805.4

*** Very highly significant at $p<0.001$ probability level, **: highly significant at $p<0.01$ probability level; *: Significant at $p<0.05$ probability level; ns: no significant difference, MAP: months after planting, CBSD: Cassava Brown Streak Disease and CMD: Cassava Mosaic Disease.

Table 4.6. Performance of 18 cassava genotypes at 3 sites against white flies and CBSD during 2018-2019, season, at 3 MAP

Genotype	Moso			Mparambo			Murongwe			Across sites		
	WFP	CBSD Sev	CBSD Inc (%)	WFP	CBSD Sev	CBSD Inc (%)	WFP	CBSD Sev	CBSD Inc (%)	WFP	CBSD Sev	CBSD Inc (%)
Albert	871.1	2.7	30.0	901.3	1.0	0.0	621.3	1.0	0.0	797.9	1.6	10.0
Eyope	606.4	1.7	2.9	850.7	1.3	28.6	378	1.0	0.0	611.7	1.3	10.5
F10-30-R2	678.7	2.0	34.2	1181.2	1.0	0.0	338.7	1.0	0.0	732.9	1.3	11.4
KBH/2002/026	483.5	1.7	23.1	1151.5	1.0	0.0	711.8	1.0	0.0	782.3	1.2	7.7
KBH/2002/066	633.5	1.0	0.0	1048.8	1.0	0.0	558.0	1.0	0.0	746.8	1.0	0.0
Kibandameno	427.9	2.0	6.1	833.8	1.0	0.0	145.3	1.0	0.0	469.0	1.3	2.0
Kiroba	435.5	1.0	0.0	705.7	1.0	0.0	426.0	1.0	0.0	522.4	1.0	0.0
Kizimbani	542.7	1.0	0.0	719.8	1.0	0.0	363.7	1.0	0.0	542.1	1.0	0.0
Mkumba	391.3	1.0	0.0	478.8	1.3	2.9	234.8	1.0	0.0	368.3	1.1	1.0
MM96/5280	916.2	1.0	0.0	1211.2	1.0	0.0	740.7	1.0	0.0	956.0	1.0	0.0
Nase1	412.7	1.0	0.0	755.7	1.3	0.9	563.5	1.0	0.0	577.3	1.1	0.3
Nase14	531.0	2.0	17.3	1026.0	1.0	0.0	252.2	1.0	0.0	603.1	1.3	5.8
Nase3	233.2	1.7	6.9	619.7	1.0	0.0	519.7	1.0	0.0	457.5	1.2	2.3
Okhumelela	468.8	1.3	33.3	1527.9	1.0	0.0	863.3	1.0	0.0	953.4	1.1	11.1
Orera	895.7	2.0	18.3	1059.2	1.3	24.4	465.7	1.0	0.0	806.8	1.4	14.2
Pwani	280.7	1.0	0.0	375.0	1.0	0.0	58.4	1.0	0.0	238.0	1.0	0.0
Tajirika	507.3	1.0	0.0	1333.2	1.3	7.0	635.3	1.0	0.0	825.3	1.1	2.3
TZ-130	410.7	1.7	3.8	1028.3	1.7	9.5	221.2	1.0	0.0	553.4	1.4	4.4
Mean	540.0	1.5	9.8	934.0	1.1	4.1	450.0	1.0	0.0	641.3	1.2	4.6
LSD (0.05)	313.8	ns	ns	434.2	ns	ns	406.0	-	-	223.9	ns	ns
CV (%)	35.0	38.1	42.7	26.7	35.0	37.0	29.1	0.0	0.00	36.8	33.0	37.0
P-value	0.002	0.4	0.5	0.002	0.7	0.5	0.02	-	-	<.001	0.3	0.4

LSD: Least significant difference, CV: Coefficients of variation, WFP: Whiteflies population, ns: no significant, Sev: severity, Inc: incidence and MAP: months after planting and CBSD: Cassava Brown Streak Disease.

Table 4.7. Performance of 18 cassava genotypes against CMD at Moso, Mparambo, Murongwe and across sites during 2018-2019, season, at 3 MAP

Genotype	Moso		Mparambo		Murongwe		Across sites	
	Sev	Inc (%)	Sev	Inc (%)	Sev	Inc (%)	Sev	Inc (%)
Albert	2.3	48.6	1.3	2.8	1.3	3.4	1.7	18.3
Eyope	1.0	0.0	1.0	0.0	1.7	22.8	1.2	7.6
F10-30-R2	1.7	19.5	1.7	33.3	2.3	11.0	1.9	21.3
KBH/2002/026	2.0	30.1	1.0	0.0	2.7	14.6	1.9	14.9
KBH/2002/066	2.7	30.2	2.3	34.9	4.0	64.2	3.0	43.1
Kibandameno	2.7	36.1	2.7	67.8	1.7	3.5	2.3	35.8
Kiroba	2.3	19.4	3.0	40.8	2.3	17.0	2.6	25.8
Kizimbani	3.0	22.2	2.3	33.0	2.0	12.4	2.4	22.5
Mkumba	1.0	0.0	1.0	0.0	1.0	0.0	1.0	0.0
MM96/5280	1.0	0.0	1.0	0.0	3.3	40.2	1.8	13.4
Nase-1	1.7	13.5	1.7	18.0	2.0	33.3	1.8	21.6
Nase-14	1.0	0.0	1.0	0.0	2.0	25.5	1.3	8.5
Nase-3	2.0	20.0	2.7	54.7	2.7	19.5	2.4	31.4
Okhumelela	1.7	8.0	2.3	39.5	2.3	21.5	2.1	23.0
Orera	2.0	31.2	1.7	32.2	1.0	0.0	1.6	21.1
Pwani	1.0	0.0	1.0	0.0	3.0	31.7	1.7	10.6
Tajirika	1.0	0.0	1.7	30.7	2.0	19.5	1.6	16.7
TZ-130	1.7	18.9	1.7	5.4	1.0	0.0	1.4	8.1
Mean	1.8	16.5	1.7	21.8	2.1	18.9	1.9	19.1
LSD (0.05)	ns	ns	1.2	ns	ns	ns	0.8	ns
CV (%)	27.5	38.1	38.7	39.4	31.1	33.3	28.3	32.8
P-value	0.08	0.6	0.02	0.2	0.2	0.6	0.002	0.4

LSD: Least significant difference, CV: Coefficients of variation, ns: no significant, Sev: Severity, Inc: Incidence and MAP: months after planting.

4.4.3. Performance of the 18 cassava genotypes against CBSD and CMD at 6 MAP

The CBSD severity and incidence on leaves, CMD severity and incidence and white flies' population differed significantly (P<.001) between genotypes (Table 4.8). Between sites and the interaction between site and genotype, there were high significant (P<.001) differences for both CBSD foliar severity and incidence, CMD severity and incidence and whiteflies' population (Table 4.8). The lowest and highest recorded score for CBSD severity at all sites and across sites were 1 and 3, respectively while incidence ranged from 0-100% (Table 4.9). Low mean severity and percentage of incidence for CBSD was recorded at Murongwe, followed by Mparambo and Moso (Table 4.9). The negative effect of the genotypes to CMD was found to be lower at

Murongwe compared to Mparambo and Moso (Table 4.10). Across sites, CMD severity and incidence ranged from 1-4.4 and from 0-99.4% with means score of 2.1 and 29.2%, respectively.

Table 4.8. Mean squares for white flies population, CBSD incidence and severity and CMD incidence and severity at 6 MAP

Source of variation	Df	White fly population	CBSD severity	CBSD incidence	CMD severity	CMD incidence
Block	2	62748^{ns}	0.7^{*}	344.8^{ns}	3.3^{***}	3628.6^{***}
Site	2	2040912^{***}	14.7^{***}	5090.5^{***}	3.5^{***}	12283.5^{***}
Genotype	17	116951^{***}	2.9^{***}	6162.8^{***}	8.2^{***}	8726.4^{***}
Block x Site	3	31051^{ns}	0.2^{ns}	285.4^{ns}	0.3^{ns}	154.1^{*}
Site x Genotype	33	65841^{***}	0.6^{***}	656.8^{**}	0.7^{***}	1160.8^{***}
Block x Genotype	33	20779^{ns}	0.3^{ns}	295.1^{ns}	0.4^{ns}	167.7^{***}
Block x Site x Genotype	24	19316^{ns}	0.2^{ns}	299.6^{ns}	0.4^{*}	132.4^{**}
Residual	45	20794	0.2	244.7	0.2	57.4

***:Very highly significant at $p<0.001$ probability level, **: highly significant at $p<0.01$ probability level; *: Significant at $p<0.05$ probability level; ^{ns}: no significant difference, CBSD: Cassava Brown Streak Disease, CMD: Cassava Mosaic Disease, df: degree of freedom and MAP: months after planting.

Table 4.9. Performance of the 18 cassava genotypes against whiteflies and CBSD at Moso, Mparambo, Murongwe and across sites during 2018-2019 season, at 6 MAP

Genotype	Moso			Mparambo			Murongwe			Across sites		
	WFP	CBSD Sev	CBSD Inc (%)	WFP	CBSD Sev	CBSD Inc (%)	WFP	CBSD Sev	CBSD Inc (%)	WFP	CBSD Sev	CBSD Inc (%)
Albert	588.0	3.0	84.0	72.5	3.0	95.1	407.5	1.7	20.0	356.0	2.6	66.4
Eyope	486.0	2.3	59.3	77.1	2.0	38.6	371.7	1.0	0.0	311.6	1.8	32.6
F10-30-R2	881.2	2.3	38.5	114.2	1.0	0.0	390.0	1.0	0.0	461.8	1.4	12.8
KBH/2002/026	267.8	2.3	8.6	201.7	1.0	0.0	293.5	1.0	0.0	254.3	1.4	2.9
KBH/2002/066	654.2	3.0	21.3	88.3	1.0	0.0	208.3	1.0	0.0	316.9	1.7	7.1
Kibandameno	907.5	2.0	6.8	142.5	1.7	18.2	213.3	1.0	0.0	421.1	1.6	8.3
Kiroba	488.2	1.7	6.3	84.2	1.0	0.0	345.8	1.0	0.0	306.1	1.2	2.1
Kizimbani	467.3	1.7	6.3	111.7	1.0	0.0	299.2	1.0	0.0	292.7	1.2	2.1
Mkumba	143.8	1.0	0.0	50.8	1.0	0.0	116.7	1.0	0.0	103.8	1.0	0.0
MM96/5280	759.5	3.0	95.0	217.3	3.0	100	735.8	3.0	100	570.9	3.0	98.3
Nase-1	346.3	2.0	21.2	175.0	1.0	0.0	415.8	1.0	0.0	312.4	1.3	7.1
Nase-14	568.6	2.3	6.2	167.5	1.3	4.6	365.8	1.0	0.0	367.3	1.6	3.6
Nase-3	482.8	1.0	0.0	79.2	1.0	0.0	313.0	1.0	0.0	291.7	1.0	0.0
Okhumelela	658.0	1.0	0.0	213.3	1.0	0.0	401.7	1.0	0.0	424.3	1.0	0.0
Orera	358.3	3.0	37.9	95.0	2.0	48.1	635.8	1.0	0.0	363.1	2.0	28.7
Pwani	105.7	1.7	5.8	43.3	1.0	0.0	135.0	1.0	0.0	94.7	1.2	1.9
Tajirika	350.5	3.0	20.8	135.8	2.7	42.3	690.0	1.0	0.0	392.1	2.2	21.0
TZ-130	515.3	3.0	22.2	90.0	2.7	55.0	413.4	1.0	0.0	339.6	2.2	25.7
Mean	502.0	2.2	24.4	120.0	1.6	22.3	375.0	1.1	6.7	332.2	1.6	17.8
LSD (0.05)	271.2	1.2	33.7	ns	0.7	34.3	309.2	0.0	0.0	136.9	0.4	14.9
CV (%)	30.9	32.4	21.2	27.9	25.9	25.3	21.6	0.0	0.0	21.1	28.1	27.1
P-value	<.001	0.02	<.001	0.2	<.001	<.001	0.02	<.001	<.001	<.001	<.001	<.001

LSD: Least significant difference, CV: Coefficients of variation, WFP: White flies' population, Sev: severity, Inc: incidence, ns: no significant, CBSD: Cassava Brown Streak Disease and MAP: months after planting.

Table 4.10. Performance of 18 cassava genotypes against CMD at Moso, Mparambo, Murongwe and across sites during 2018-2019, season, at 6 MAP

Genotype	Moso		Mparambo		Murongwe		Across sites	
	Sev	Inc (%)	Sev	Inc (%)	Sev	Inc (%)	Sev	Inc (%)
Albert	3.0	83.6	3.0	29.1	3.0	25.4	3.0	46.0
Eyope	1.0	0.0	1.0	0.0	1.0	0.0	1.0	0.0
F10-30-R2	1.7	2.9	1.7	2.1	2.3	5.4	1.9	3.5
KBH/2002/026	2.7	17.4	1.0	0.0	1.0	0.0	1.6	5.8
KBH/2002/066	3.0	56.6	2.7	50.6	2.3	14.0	2.7	40.4
Kibandameno	4.0	98.2	5.0	100	4.3	100	4.4	99.4
Kiroba	3.3	73.5	3.0	98.9	3.0	17.8	3.1	63.4
Kizimbani	3.0	76.7	3.0	76.8	1.7	5.1	2.6	52.9
Mkumba	1.0	0.0	1.0	0.0	1.0	0.0	1.0	0.0
MM96/5280	1.0	0.0	1.0	0.0	1.0	0.0	1.0	0.0
Nase-1	3.3	80.6	3.0	87.1	2.3	8.4	2.9	58.7
Nase-14	1.0	0.0	1.0	0.0	1.0	0.0	1.0	0.0
Nase-3	3.3	85.0	3.0	81.2	1.7	21.7	2.7	62.6
Okhumelela	3.0	89.4	3.0	92.1	1.7	15.2	2.6	65.6
Orera	3.0	22.3	1.7	4.4	1.7	2.0	2.1	9.6
Pwani	1.0	0.0	1.0	0.0	1.0	0.0	1.0	0.0
Tajirika	1.0	0.0	2.3	9.7	1.0	0.0	1.4	3.2
TZ-130	2.3	40.2	1.0	0.0	1.7	1.2	1.7	13.8
Mean	2.3	40.4	2.1	35.1	1.8	12.0	2.1	29.2
LSD (0.05)	0.7	19.3	0.5	10.5	1.2	8.0	0.5	7.2
CV (%)	18.3	27.3	13.1	17.2	36.9	38.4	23.5	26.0
P-value	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

LSD: Least significant difference, CV: Coefficients of variation, Sev: severity, Inc: incidence and MAP: months after planting.

4.4.4. Performance of cassava genotypes to CBSD and CMD based on foliar symptoms

The performance of genotypes to CBSD, CMD and whitefly number varied significantly ($P<0.001$) (Table 4.11). The lowest foliar symptoms of CBSD were recorded on genotypes F10-30-R2, KBH/2002/026, KBH/2002/066, Kiroba, Kizimbani, Mkumba, Nase-14, Nase-3, Okhumelela and Pwani with percentage of incidence ranging from 0-14% and severity ranging from 1-1.9 (Table 4.12). Two genotypes, Mkumba and Okhumelela, showed high performance to CBSD foliar symptoms at all sites with a score of 0% incidence and 1 severity (Table 4.12), while 3 genotypes Mkumba, Nase-14 and Pwani showed high performance to CMD symptoms at all sites with a score of 0 % incidence and severity of 1 (Table 4.13). Ten genotypes namely

Eyope, F10-30-R2, KBH/2002/026, Mkumba, MM96/5280, Nase-14, Orera, Pwani, Tajirika and TZ-130 showed good performance to CMD with severity ranging between 1-1.9 and incidence between 0 and 13% (Table 4.13). Genotypes F10-30-R2, KBH/2002/026, Mkumba, Nase-14, and Pwani showed good performance to both CBSD and CMD (Tables 4.12 and 4.13).

Table 4.11. Mean squares for whitefly number, CBSD severity and incidence; CMD incidence and severity and area under the disease progress curve of cassava genotypes at 9 MAP

Source of Variation	df	WFN	CBSD Sev	CBSD Inc	CMD sev	CMD Inc	AUDPC CBSD	AUDPC CMD
Block	2	312.3^{**}	0.7^{ns}	1067.6^{*}	4.2^{***}	1594.5^{**}	28.6^{ns}	254.8^{***}
Site	2	1762.4^{***}	19.4^{***}	9599.4^{***}	7.2^{***}	8276.4^{***}	462.4^{***}	23.4^{ns}
Genotype	17	487.8^{***}	2.9^{***}	7173.5^{***}	7.5^{***}	5053.6^{***}	83.3^{***}	330.5^{***}
Block x Site	3	72.7^{ns}	0.5^{ns}	153.4^{ns}	0.4ns	224.9^{ns}	6.9^{ns}	28.3^{*}
Site x Genotype	33	371.7^{***}	1.2^{**}	955.7^{***}	0.8^{***}	748.3^{**}	18.7^{*}	32.1^{***}
Block x Genotype	33	384.5^{***}	0.3^{ns}	487.7^{ns}	0.3^{ns}	177.5^{ns}	6.1^{ns}	14.9^{ns}
Block x Site x Genotype	24	176.3^{***}	0.3^{ns}	318.3^{ns}	0.3^{ns}	275.7^{ns}	7.1^{ns}	10.4^{ns}
Residual	45	40.0	0.4	311.1	0.2	295.5	10.6	10.2

*** Very highly significant at $p<0.001$ probability level, **: highly significant at $p<0.01$ probability level; *: Significant at $p<0.05$ probability level; ^{ns}: no significant difference, CBSD: Cassava Brown Streak Disease, CMD: Cassava Mosaic Disease, Sev: severity, Inc: Incidence, df: degree of freedom, WFN: whitefly number and MAP: months after planting.

Table 4.12. Whitefly number and CBSD mean performance reaction of 18 cassava genotypes at Moso, Mparambo and Murongwe and across sites during 2018-2019, season, at 9 MAP

Genotype	Moso				Mparambo				Murongwe				Across sites			
	WFN	CBSD Sev	CBSD Inc (%)	AUDPC	WFN	CBSD Sev	CBSD Inc (%)	AUDPC	WFN	CBSD Sev	CBSD Inc (%)	AUDPC	WFN	CBSD Sev	CBSD Inc (%)	AUDPC
Albert	0.5	3.0	98.3	23.5	2.5	3.0	100	18.5	3.2	2.3	37.5	15.5	2.0	2.8	78.6	19.2
Eyope	0.8	3.0	59.4	18.5	6.8	2.7	58.3	16.0	3.6	1.0	0.0	9.5	3.7	2.2	39.3	14.7
F10-30-R2	0.9	3.0	43.6	19.5	16.9	1.0	0.0	9.5	2.2	1.0	0.0	9.5	6.7	1.7	14.6	12.8
KBH/2002/026	1.6	2.3	15.2	17.5	8.8	1.7	1.8	10.5	6.8	1.7	2.7	10.5	5.7	1.9	6.6	12.8
KBH/2002/066	0.5	3.3	32.8	19.0	6.7	1.0	0.0	9.5	8.8	1.0	0.0	9.5	5.3	1.8	10.9	12.7
Kibandameno	24.9	3.0	100	18.5	2.4	3.0	74.2	14.5	1.3	1.0	0.0	9.5	9.6	2.3	58.1	14.2
Kiroba	1.6	1.7	10.5	12.5	5.0	1.7	14.0	10.5	5.5	1.0	0.0	9.5	4.0	1.4	8.2	10.8
Kizimbani	0.7	1.7	8.3	12.5	2.4	1.0	0.0	9.5	2.0	1.0	0.0	9.5	1.7	1.2	2.8	10.5
Mkumba	0.1	1.0	0.0	9.5	0.7	1.0	0.0	10.5	0.9	1.0	0.0	9.5	0.5	1.0	0.0	9.8
MM96/5280	2.1	3.0	100	18.5	64.7	3.0	100	20.5	16.5	3.0	100	20.5	27.8	3.0	100	19.8
Nase-1	0.2	3.0	30.7	15.5	12.0	2.3	7.0	12.5	4.1	1.0	0.0	9.5	5.4	2.1	12.6	12.5
Nase-14	0.5	3.0	7.2	20.2	8.0	1.7	3.0	11.5	3.9	1.0	0.0	11.5	4.1	1.9	3.4	14.4
Nase-3	0.5	1.0	0.0	11.5	3.7	3.0	44.4	12.5	7.2	1.0	0.0	9.5	3.8	1.7	14.8	11.2
Okhumelela	2.3	1.0	0.0	10.5	56.5	1.0	0.0	9.5	3.5	1.0	0.0	9.5	20.8	1.0	0.0	9.8
Orera	1.0	3.0	30.3	21.5	4.9	2.0	48.1	15.0	2.3	1.7	9.3	10.5	2.7	2.2	29.3	15.7
Pwani	0.2	2.0	6.6	13.0	0.8	1.0	0.0	9.5	1.8	1.0	0.0	9.5	0.9	1.3	2.2	10.7
Tajirika	0.8	3.0	22.1	18.5	31.9	3.0	57.3	18.5	11.4	1.0	0.0	9.5	14.7	2.3	26.5	15.5
TZ-130	0.6	3.0	25.1	20.5	2.6	3.0	26.4	19.5	3.7	1.0	0.0	13.5	2.3	2.3	17.2	17.8
Mean	2.2	2.4	32.8	16.7	13.2	2.0	29.7	13.2	4.9	1.3	8.3	10.9	6.8	1.9	23.6	13.6
LSD (0.05)	2.8	ns	36.0	ns	18.6	1.1	40.3	3.9	3.4	0.6	2.5	2.1	6.0	0.6	16.8	3.1
CV (%)	24.4	37.9	34.2	31.1	28.1	31.6	30.1	16.9	39.8	28.1	17.3	10.9	26.4	35.1	21.2	23.9
P-value	<.001	0.1	<.001	0.2	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

LSD: Least significant difference, CV: Coefficients of variation, %: Percentage, Sev: severity, Inc: incidence, AUDPC: Area under the disease progress curve, WFN: Whitefly number, MAP: months after planting and CBSD: Cassava brown streak disease.

Table 4.13. Performance of 18 cassava genotypes against CMD at Moso, Mparambo and Murongwe and across sites during 2018-2019, season, at 9 MAP

Genotype	Moso			Mparambo			Murongwe			Across sites		
	Sev	Inc (%)	AUDPC	Sev	Inc (%)	AUDPC	Sev	Inc (%)	AUDPC	Sev	Inc (%)	AUDPC
Albert	3.0	78.5	24.5	3.0	29.1	20.2	1.7	0.8	18.2	2.6	36.1	20.9
Eyope	1.0	0.0	9.5	1.0	0.0	9.5	1.0	0.0	11.5	1.0	0.0	10.2
F10-30-R2	1.7	3.9	14.5	1.7	1.5	14.5	1.0	0.0	18.5	1.7	1.8	15.8
KBH/2002/026	2.7	20.5	20.0	1.0	0.0	9.5	1.0	0.0	14.5	1.6	6.8	14.7
KBH/2002/066	3.0	44.6	24.2	2.7	40.8	21.0	1.0	0.0	23.8	2.2	28.5	23.0
Kibandameno	4.0	100	30.0	4.3	71.2	35.5	4.0	77.8	30.0	4.1	83.0	31.8
Kiroba	3.0	63.8	23.5	3.3	73.5	28.3	3.0	18.6	25.8	3.1	52.0	25.9
Kizimbani	3.0	49.0	25.2	3.0	15.7	22.5	2.3	4.7	16.5	2.8	23.1	21.4
Mkumba	1.0	0.0	9.5	1.0	0.0	9.5	1.0	0.0	9.5	1.0	0.0	9.5
MM96/5280	1.0	0.0	9.5	1.0	0.0	9.5	1.0	0.0	16.5	1.0	0.0	11.8
Nase-1	3.0	26.8	21.5	3.0	45.7	20.5	1.7	3.4	17.5	2.6	25.3	19.8
Nase-14	1.0	0.0	9.5	1.0	0.0	9.5	1.0	0.0	12.5	1.0	0.0	10.5
Nase-3	3.0	57.6	23.2	3.3	54.7	24.7	2.3	16.8	18.5	2.9	43.0	22.1
Okhumelela	3.0	72.4	23.2	4.0	70.5	25.3	2.0	3.6	18.3	3.0	48.8	22.3
Orera	3.0	7.8	22.2	1.7	2.4	14.5	1.0	0.0	11.5	1.9	3.4	16.1
Pwani	1.0	0.0	9.5	1.0	0.0	9.5	1.0	0.0	15.5	1.0	0.0	11.5
Tajirika	1.0	0.0	9.5	2.3	4.4	18.2	1.0	0.0	12.5	1.4	1.5	13.4
TZ-130	2.3	40.2	17.5	1.0	0.0	11.5	1.0	0.0	11.5	1.4	13.4	13.5
Mean	2.3	31.4	18.1	2.2	22.7	17.4	1.6	7.0	16.8	2.0	20.4	17.5
LSD (0.05)	0.7	25.3	6.9	0.9	40.5	4.5	0.8	20.6	5.2	0.4	16.3	3.0
CV (%)	16.7	26.4	21.7	22.6	27.0	14.7	30.1	33.1	17.9	22.6	22.1	18.3
P-value	<.001	<.001	<.001	<.001	0.003	<.001	<.001	<.001	<.001	<.001	<.001	<.001

LSD: Least significant difference, CV: Coefficients of variation, Sev: severity, Inc: incidence, AUDPC: Area under the disease progress curve and MAP: months after planting.

4.4.5. Reaction to Cassava brown streak disease based on symptoms on cassava roots

There were significant (P<0.001) differences in root necrosis between genotypes and sites. The genotypes varied significantly (P<0.01) for root necrosis severity while there were no significant differences in root necrosis incidence across sites (Table 4.14). The lowest symptoms of CBSD on cassava roots were recorded on genotypes KBH/2002/026, Kiroba, Mkumba, Okhumelela and Pwani with a severity mean score ranging from 1-1.8 and incidence ranging from 0-48.9 % (Table 4.15). Highest root necrosis mean severity and incidence was observed on two genotypes used as susceptible checks (Albert and Kibandameno) and genotype MM96/5280. Severity mean scores ranged from 3.3-4.2 with incidence ranging from 81.3-100 % across sites (Table 4.15).

Table 4.14. Mean squares for root necrosis severity and incidence

Source of variation	Degree of freedom	Root necrosis severity	Root necrosis incidence
Block	2	2.5^{*}	3965.0^{ns}
Site	2	17.7^{***}	13983.3^{***}
Genotype	17	5.2^{***}	6369.8^{***}
Block x Site	3	0.8^{ns}	1582.5^{ns}
Site x Genotype	33	1.2^{**}	1059.6^{ns}
Block x Genotype	33	0.7^{ns}	770.1^{ns}
Block x Site x Genotype	24	0.7^{ns}	224.1^{ns}
Residual	45	0.5	674.5

***: Very highly significant at P<0.001 probability level, **: highly significant at P<0.01 probability level, *: Significant at P<0.05 probability level, and ^{ns}: no significant difference.

4.4.5. Yield and yield components

Yield and yield components for the genotypes on the 3 sites were highly significantly (P<0.001) different for root number per plant, marketable root percentage, fresh storage root yield, dry storage root yield, dry matter, starch content and harvest index. Interaction between genotypes and site was significantly (P<0.001) different for root number per plant, fresh storage root yield, dry storage root yield, dry matter and starch content. There were also high significant (P<0.001) differences between sites in root number per plant, marketable roots percentage, fresh storage root yield, dry storage root yield, dry matter content, starch content and harvest index (Table

4.16). Interaction between site, block and genotypes was highly significant (P<0.001) for fresh storage root yield and dry storage root yield (Table 4.16).

Table 4.15. Performance of 18 cassava genotypes on root necrosis at Moso, Mparambo and Murongwe and across sites during 2018-2019 season

Genotype	Moso		Mparambo		Murongwe		Across sites	
	Sev	Inc (%)	Sev	Inc (%)	Sev	Inc (%)	Sev	Inc (%)
Albert	5.0	100	4.0	100	3.7	50.0	4.2	83.3
Eyope	4.0	73.3	2.3	100	1.7	13.3	2.7	62.2
F10-30-R2	3.0	73.3	2.7	58.3	1.3	16.7	2.3	49.4
KBH/2002/026	1.3	6.7	1.7	20.0	1.0	0.0	1.3	8.9
KBH/2002/066	3.0	70.0	2.3	40.0	1.7	20.0	2.3	43.3
Kibandameno	3.7	100	4.3	100	2.0	100	3.3	100
Kiroba	1.0	0.0	1.0	0.0	1.0	0.0	1.0	0.0
Kizimbani	3.7	53.3	1.3	8.3	1.3	6.7	2.1	22.8
Mkumba	2.0	66.7	2.3	80.0	1.0	0.0	1.8	48.9
MM96/5280	3.3	86.7	3.3	63.9	3.3	93.3	3.3	81.3
Nase-1	1.7	6.7	3.3	47.2	1.0	0.0	2.0	18.0
Nase-14	2.7	45.0	2.7	63.3	2.0	46.7	2.4	51.7
Nase-3	3.0	33.3	2.7	75.0	2.0	46.7	2.6	51.7
Okhumelela	2.0	13.3	1.7	35.0	1.7	6.7	1.8	18.3
Orera	3.7	86.7	1.7	55.6	2.3	50.0	2.6	64.1
Pwani	2.0	46.7	1.0	0.0	1.3	13.3	1.4	20.0
Tajirika	3.7	73.3	3.0	68.3	1.0	0.0	2.6	47.2
TZ-130	2.3	46.7	2.3	73.3	1.3	20.0	2.0	46.7
Mean	2.8	54.5	2.4	54.9	1.7	26.9	2.3	45.4
LSD (0.05)	1.8	48.0	0.9	57.8	0.9	24.7	0.7	24.7
CV (%)	36.1	37.5	20.8	30.2	29.4	33.1	30.5	28.6
P-value	0.02	0.005	<.001	0.04	<.001	<.001	<.001	<.001

LSD: Least significant difference, CV: Coefficients of variation, Sev: severity, Inc: incidence.

Table 4.16. Mean squares for storage root number, marketable roots percentage, fresh storage root yield, dry matter content, starch content and harvest index of evaluated genotypes

Source of Variation	df	Mean Squares for						
		No. of roots plant^{-1}	Marketable roots (%)	Fresh storage root yield (t/ha)	Dry storage root yield (t/ha)	Dry matter content (%)	Starch content (%)	Harvest index
Block (B)	2	7.7**	1078.4*	80.3**	10.6**	6.4***	3.2***	376.7*
Site (S)	2	16.2***	1647.6***	142.8***	208.9***	546.1***	273.8***	795.1***
Genotype (G)	17	210.0***	2921.8***	2162.6***	17.2***	1.0*	0.5*	937.3***
Bx S	3	6.8***	429.1*	96.4***	3.3^{ns}	0.7^{ns}	0.3^{ns}	193.8**
S x G	33	2.3*	176.7^{ns}	28.0**	7.7***	1.4***	0.7***	82.7^{ns}
B x G	33	0.7^{ns}	162.9^{ns}	36.8*	4.1*	0.5^{ns}	0.3^{ns}	105.7^{ns}
B x S x G	24	1.7^{ns}	219.8^{ns}	50.2***	5.1***	0.3^{ns}	0.1^{ns}	112.8^{ns}
Residual	45	1.2	248	11.4	1.6	0.4	0.2	75.7

*** Very highly significant difference at P<0.001 probability level, ** highly significant at P<0.01 probability level; * Significant at P<0.05 probability level, df: degree of freedom, ns: no significant difference, t/ha: Tonnes per hectare,.

Higher fresh storage root yield was found at Moso with mean of 17.2 tons/ha than at Mparambo and Murongwe with 10.6 and 4.5 tons/ha, respectively (Table 4.17). On the 3 sites, fresh storage root yield ranged from 0.4-16.6 tons/ha with mean of 10.8 tons/ha; the highest fresh storage root yield was recorded on genotypes Nase-1 and Okhumelela, with 16.6 tons/ha; while Kibandameno showed the lowest yield of 0.4 tons/ha (Table 4.17). Genotypes KBH/2002/026, Kiroba, Nase-1, Nase-14, Nase-3, Okhumelela and TZ-130 had the highest fresh storage root yield at Moso with a range of 18.2 to 40.0 tons/ha (Table 4.17). Genotype, Okhumelela, had highest fresh storage root yield at Mparambo while Kibandameno had lowest with 0.3 tons/ha. In Murongwe, Nase-14 had the highest fresh storage root yield while Kiroba the lowest yield, 0.3 tons/ha (Table 4.17).

Higher dry storage root yield was reported at Moso with 5.4 tons/ha while Mparambo and Murongwe had 3.3 and 1.4 tons/ha, respectively (Table 4.17). On the 3 sites, dry storage root yield ranged from 0.2-5.4 tons/ha with 3.4 tons/ha where highest dry storage root yield of 5.2, 5.3 and 5.4 was reported for Nase-1, Nase-14 and Okhumelela, respectively. Kibandameno showed the lowest yield for dry and fresh storage root (Table 4.17). Genotypes TZ-130, Okhumelera, KBH/2002/026, Nase-1, the highest dry storage root yield at Moso ranging from 7.1-12 tons/ha. Genotypes, Nase-14 and Okhumelera had highest dry storage root yield at Murongwe Mparambo respectively (Table 4.17). The dry matter content recorded at Moso ranged from 15.0-54.3%

compared to Mparambo and Murongwe from 15.0-54.0% and 15.0-54.1%, respectively. Dry matter content ranged from 15.0-54.1% with an average of 31.7 % across sites (Table 4.17). Starch content was 16.7, 16.6 and 16.5% for Mparambo, Murongwe and Moso, respectively. Genotype MM96/5280 recorded the highest mean starch content in all sites and across sites while genotype Albert had the lowest mean starch content of 6.2 % (Table 4.17).

Among sites, highest mean number of roots per plant was recorded at Moso, followed by Mparambo and Murongwe with site mean score of 6.7, 4.1 and 2.8, respectively (Table 4.18). Across sites, the number of roots per plant ranged from 0.6-6.5 for genotypes Kibandameno and Eyope, respectively (Table 4.18). Highest number of roots per plant was recorded on genotypes Eyope, F10-30-R2, KBH/2002/026, Nase-1, Nase-3, Okhumelela, and TZ-130 at Moso and on genotypes Eyope, F10-30-R2, KBH/2002/026, KBH/2002/066, Kizimbani, Mkumba, Nase-14, Okhumelela and TZ-130 at Mparambo (Table 4.18). At Murongwe, the highest number of roots per plant was observed on genotypes Eyope, F10-30-R2, KBH/2002/066, Nase-1, Nase-14, Nase-3, Orera, Pwani, Tajirika and TZ-130 (Table 4.18).

Higher marketable roots were observed at Moso than at Mparambo and Murongwe with 46.1, 40.7 and 31.6%, respectively (Table 4.18). Genotype Nase-1 had higher percentage of marketable roots than the others on all sites while the lowest were genotypes KBH/2002/026, Kibandameno and Kiroba having no marketable roots (Table 4.18). At Moso, F10-30-R2, KBH/2002/026, Kiroba, Mkumba, Nase-1, Nase-14, Nase-3, Okhumelela, Pwani, Tajirika and TZ-130 showed high percentage of marketable roots (Table 4.18). Genotypes F10-30-R2, KBH/2002/026, KBH/2002/066, Kiroba, Kizimbani, Mkumba, MM96/5280, Nase-3, Okhumelela, Pwani and TZ-130 had higher percentage of marketable root at Mparambo and Murongwe with the top 5 being F10-30-R2, Kizimbani, MM96/5280, Nase-1 and Nase-14 (Table 4.18). Across sites, Nase-3 and Kibandameno had high and low number of marketable roots, respectively (Table 4.18). Harvest index for the 3 sites ranged between 6.3 and 43.3 with the highest for genotype Eyope while the lowest on Kibandameno (Table 4.18).

Table 4.17. Fresh storage roots yield, dry storage root yield, percentage of dry matter and starch content in root mean performance of evaluated cassava genotypes at Moso, Mparambo and Murongwe and across sites during 2018-2019

Genotype	Moso				Mparambo				Murongwe				Across sites			
	FSRY (tons/ha)	DMC (%)	SC (%)	DSRY (tons/ha)	FSRY (tons/ha)	DMC (%)	SC (%)	DSRY (tons/ha)	FSRY (tons/ha)	DMC (%)	SC (%)	DSRY (tons/ha)	FSRY (tons/ha)	DM (%)	SC (%)	DSRY (tons/ha)
Albert	8.9	15.0	4.8	1.3	7.8	15.0	4.8	1.2	1.8	15.0	4.8	0.3	6.2	15.0	4.8	0.9
Eyope	15.6	31.6	16.5	4.9	4.7	31.0	16.1	1.5	5.0	32.1	16.9	1.6	8.4	31.5	16.5	2.6
F10-30-R2	17.1	39.9	22.4	6.8	10.9	40.6	22.9	4.4	6.7	40.2	22.7	2.7	11.5	40.2	22.7	4.6
KBH/2002/026	26.0	29.2	14.8	7.6	13.2	29.1	14.8	3.8	1.0	27.8	13.8	0.3	13.4	28.7	14.5	3.8
KBH/2002/066	10.0	28.2	14.1	2.8	19.1	27.7	13.8	5.3	6.3	28.0	14.0	1.8	11.8	28.0	14.0	3.3
Kibandameno	0.3	38.9	21.7	0.1	0.3	39.7	22.3	0.1	0.4	39.9	22.4	0.2	0.4	39.5	22.1	0.2
Kiroba	18.2	31.3	16.3	5.7	8.3	32.0	16.8	2.7	0.3	31.4	16.4	0.1	8.9	31.6	16.5	2.8
Kizimbani	14.6	28.0	14.0	4.1	15.5	28.5	14.3	4.4	1.6	28.0	14.0	0.4	10.5	28.2	14.1	3.0
Mkumba	13.4	26.5	13.0	3.6	12.1	27.0	13.3	3.3	1.5	27.0	13.3	0.4	9.0	26.9	13.2	2.4
MM96/5280	12.4	54.3	32.6	6.7	9.0	54.1	32.5	4.9	2.0	54.0	32.4	1.1	7.8	54.1	32.5	4.2
Nase-1	40.0	29.9	15.3	12	5.4	31.4	16.4	1.7	4.3	32.5	17.1	1.4	16.6	31.2	16.3	5.2
Nase-14	20.3	34.2	18.4	6.9	12.9	35.4	19.2	4.6	12.6	34.9	18.9	4.4	15.3	34.8	18.8	5.3
Nase-3	22.8	29.8	15.2	6.8	10.0	30.0	15.4	3.0	8.5	30.0	15.4	2.6	13.8	29.9	15.3	4.1
Okhumelela	22.9	32.7	17.3	7.5	24.5	32.8	17.4	8.0	2.3	32.7	17.3	0.8	16.6	32.8	17.4	5.4
Orera	16.6	32.1	16.9	5.3	4.6	32.0	16.8	1.5	7.0	31.8	16.7	2.2	9.4	31.9	16.8	3.0
Pwani	13.9	29.2	14.8	4.1	13.3	28.8	14.6	3.8	4.4	29.1	14.8	1.3	10.5	29.0	14.7	3.0
Tajirika	13.5	25.6	12.3	3.5	5.5	26.0	12.6	1.4	7.2	25.2	12.0	1.8	8.7	25.6	12.3	2.2
TZ-130	22.5	31.6	16.5	7.1	14.0	31.6	16.6	4.4	8.2	31.8	16.7	2.6	14.9	31.7	16.6	4.7
Mean	17.2	31.6	16.5	5.4	10.6	31.8	16.7	3.3	4.5	31.7	16.6	1.4	10.8	31.7	16.6	3.4
LSD (0.05)	7.9	1.1	0.8	2.7	6.5	1.5	1.1	2.7	1.4	0.5	0.4	0.5	3.2	0.6	0.4	2.1
CV (%)	26.5	2.0	2.7	29.5	35.4	2.7	3.6	37.9	17.9	1.0	1.3	18.2	31.3	2.0	2.7	27.8
P-value	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.003	<.001	<.001	<.001	<.001	<.001	<.05	<.05	<.001

FSRY: Fresh storage root yield, DMC: Dry matter content, SC: Starch content, DSRY: Dry storage root yield, LSD: Least significant difference, CV: Coefficients of variation, tons/ha : Tonnes per hectare, %: Percentage.

Table 4.18. Number of roots per plant, percentage of marketable roots percentage and harvest index mean performance of evaluated cassava genotypes at Moso, Mparambo and Murongwe sites during 2018-2019 season

Genotype	Moso			Mparambo			Murongwe			Across sites		
	NRP	PMR (%)	HI	NRP	PMR (%)	HI	NRP	PMR (%)	HI	NRP	PMR (%)	HI
Albert	5.5	30.7	24.6	2.6	37.3	31.6	1.7	8.1	32.6	3.3	25.4	29.6
Eyope	9.7	38.5	36.7	5.1	20.5	33.0	4.8	30.2	60.1	6.5	29.7	43.3
F10-30-R2	7.4	48.3	34.4	5.1	49.2	26.7	3.0	53.5	43.5	5.1	50.3	34.9
KBH/2002/026	10.1	52.9	32.0	5.6	41.6	22.5	0.9	0.0	20.4	5.5	31.5	25.0
KBH/2002/066	5.3	36.2	38.1	6.8	46.0	42.8	5.7	19.6	36.5	5.9	33.9	39.1
Kibandameno	0.5	0.0	7.2	0.6	0.0	5.9	0.7	0.0	5.9	0.6	0.0	6.3
Kiroba	6.2	58.8	34.0	3.0	46.2	25.7	0.5	0.0	12.2	3.2	35.0	24.0
Kizimbani	8.9	43.5	33.2	5.3	45.0	33.1	1.8	43.9	33.9	5.3	44.2	33.4
Mkumba	6.5	52.4	40.3	4.7	45.5	30.2	1.4	28.3	36.7	4.2	42.1	35.7
MM96/5280	6.4	34.9	38.9	1.5	53.2	29.8	1.6	51.0	20.5	3.2	46.4	29.8
Nase-1	8.2	69.6	57.4	2.5	38.4	26.3	2.9	46.3	50.8	4.5	51.4	44.8
Nase-14	5.5	53.9	35.2	6.2	29.1	36.8	4.9	48.5	55.7	5.5	43.8	42.6
Nase-3	7.4	60.1	49.0	3.4	66.1	40.6	3.9	64.5	42.2	4.9	63.6	44.0
Okhumelela	7.4	62.6	33.6	5.7	60.5	38.6	1.9	35.0	49.5	5.0	52.7	40.6
Orera	5.7	33.8	31.1	2.3	28.2	16.7	4.4	32.8	38.0	4.1	31.6	28.6
Pwani	6.5	49.1	36.3	3.9	51.0	32.9	3.4	33.7	48.6	4.6	44.6	39.3
Tajirika	4.6	51.0	36.5	4.1	27.9	30.1	3.4	26.1	53.1	4.0	35.0	39.9
TZ-130	8.3	54.3	45.8	5.9	46.9	34.6	3.5	46.9	42.3	5.9	49.4	40.9
Mean	6.7	46.1	35.8	4.1	40.7	29.9	2.8	31.6	37.9	4.5	39.5	34.5
LSD (0.05)	2.7	23.5	10.0	1.6	23.2	16.0	1.2	27.2	17.8	1.0	15.0	8.3
CV (%)	23.3	29.1	15.9	22.2	32.8	30.8	24.2	52.0	27.1	24.1	39.9	25.2
P-value	<.001	0.007	<.001	<.001	0.005	0.03	<.001	0.02	<.001	<.001	<.001	<.001

NRP: Number of root per plant, PMR: Percentage of marketable roots, HI: Harvest index, LSD: Least significant difference, CV: Coefficients of variation, %: Percentage.

4.4.6. Correlation among agronomic traits, disease expression and yield

Correlations coefficients from the 3 sites were determined and reported on Table 4.19. Positive significant correlation ($r>0$, $P<0.001$) was observed between marketable root percentage and number of roots per plant ($r = 0.4$) and between harvest index and fresh storage root yield ($r = 0.4$). Marketable roots were negatively influenced by root necrosis ($r = -0.2$) and progression of CMD ($r = -0.2$) (Table 4.19). Similarly, root number per plant and fresh storage root yield were highly positively correlated ($r = 0.8$, $P<0.001$). Harvest index was negatively correlated by CMD and CBSD while root necrosis incidence and severity were significantly influenced by CBSD on

leaves ($r = 0.6$, $p<0.001$). The results showed that harvest index was negatively associated by progression of CBSD ($r = -0.2$) and CMD ($r = -0.3$) (Table 4.19).

Equally, dry matter content and incidence of CBSD on leaves were positively influenced by number of whitefly ($r = 0.03$, $r = 0.2$), while number of root per plant and the harvest index were negatively influenced the number of whitefly ($r = -0.2$) (Table 4.19).

Two-sample T-test (paired test) computed for incidence and severity on shoots for periods between 3 and 6 months and then 6 to 9 months showed significant ($P<0.001$) differences between genotypes on CBSD incidence. Similarly, genotypes differed highly for CMD incidence between 3 and 6 months as well as 6 to 9 months but not for CMD severity between 6 to 9 months ($P>0.05$). Similarly, for periods of 3 and 6 months, and periods of 6 and 9 months, genotypes differed ($P<0.001$) significantly for severity of CBSD on shoots.

Table 4.19. Pearson's correlation coefficients among agronomic, disease intensity and yield traits evaluated on 18 cassava genotypes at Moso, Mparambo and Murongwe

	Fresh storage root yield	Harvest index	Root number per plant	Marketable root %	Dry matter content%	Root necrosis sev	Root necrosis inc	CMD sev	CMD inc	CBSD sev	CBSD inc	AUD PC /CBSD	AUD PC/ CMD	WFP
Fresh storage root yield	-													
Harvest index	0.4***	-												
Root number per plant	0.8***	0.4***	-											
Marketable root %	0.6***	0.5***	0.4***	-										
Dry matter content%	-0.04	-0.1	-0.1	0.1	-									
Root necrosis sev	0.01	-0.2**	0.1	-0.2*	0.04	-								
Root necrosis inc	-0.1	-0.3***	0.02	-0.6*	0.2*	0.7***	-							
CMD sev	0.1	-0.3***	-0.1	-0.04	-0.1	0.1	0.1	-						
CMD inc	0.1	-0.3***	0.01	-0.04	-0.04	0.2*	0.2*	0.8***	-					
CBSD sev	0.1	-0.2**	0.1	-0.03	0.1	0.6***	0.5***	0.1	0.1	-				
CBSD inc	-0.2	-0.3***	-0.1	-0.2*	0.2**	0.6***	0.6***	0.1	0.2*	0.7***	-			
AUDPC/CBSD	0.1	-0.2*	0.1	-0.01	0.1	0.6***	0.5***	-0.02	0.04	0.9***	0.7***	-		
AUDPC/CMC	-0.04	-0.3***	-0.2	-0.2*	-0.1	0.1	0.1	0.8***	0.7***	0.04	0.1	-0.02	-	
WFP	-0.1	-0.2*	-0.2*	0.03	0.03***	0.0	-0.01	0.1	0.1	0.1	0.2*	0.1	0.1	-

*, **, ***= Significant difference at P<0.05, P<0.01 and P<0.001; inc: incidence, sev: severity, AUDPC/CBSD: Area under the disease progress curve for cassava brown streak disease, AUDPC/CMD: Area under the disease progress curve for cassava mosaic disease and WFP: Whiteflies' population.

4.4.7. Genotype rank and stability based on cassava mosaic disease and root necrosis

Analysis of the main effects and multiplicative interaction (AMMI) for the cassava genotypes showed that the effect on both fresh storage root yield, root necrosis and CMD due to genotypes, environments and interactions between genotypes and environments (G × E) were significant (P<0.001) (Table 4.20).

Table 4.20. ANOVA for AMMI for fresh storage root yield, root necrosis and CMD of 18 evaluated cassava genotypes

Source of variation	df	Fresh storage root yield	CBSD roots necrosis	Cassava mosaic disease
Treatments (G, E, G x E)	53	191.4***	3.6***	3.3****
Genotypes (G)	17	147.4***	5.5***	7.8***
Environments (E)	2	2162.6***	17.7***	7.2***
Block	6	35.9ns	1.2ns	0.4ns
Interactions (G x E)	33	97.5***	1.2**	0.8***
IPCA1	18	126.3***	1.4**	0.9***
IPCA2	16	65.1**	0.9ns	0.7**
Error	102	25.6	0.6	0.3

IPCA1 and IPCA2 = interaction principal component axis 1 and 2, ns = not significant, *= significant, **= highly significant, ***= very highly significant at P>0.05, <0.01, <0.001, respectively, G: Genotype, E: Environment and GxE: Genotype by environment.

Interaction PCA for environment (IPCAe) of 4.2, 2.9 and 1.3 and environmental mean for fresh storage root yield of 17.2, 10.6 and 4.5 tons/ha were recorded for Moso, Mparambo and Murongwe, respectively (Table 4.21). Genotypes Nase-1, KBH/2002/066, Kibandameno and KBH/2002/026 had higher IPCA score values of 4.0, 2.1, 1.3 and 1.2 and higher AMMI stability values (ASV) of 8.8, 4.5, 3.1 and 3.0 repectively. Genotypes Nase-1 and KBH/2002/026 had IPCA scores of 4.0 and 1.2 while ASV score of 8.8 and 3.0, respectively and they also recorded high storage fresh root yield of 40 and 26 tons/ha, respectively) in Moso. KBH/2002/066 having IPCA score and ASV of 2.1 and 4.5, respectively, recorded higher storage fresh root yield (19.1 tons/ha) in Mparambo. Genotype Kibandameno recorded high higher storage fresh root yield in Murongwe (Table 4.21). Across sites, regarding genotype stability index (GSI) for the yield that integrates yield and stability, the three top AMMI selection genotypes were TZ-130, Nase-14 and F10-30-R2 (Table 4.21).

Root necrosis caused by CBSD for genotypes, Nase-1, Kizimbani, Orera, Eyope, KBH/2002/026, Mkumba and Pwani recorded high IPCA score ranging between 0.9 and 0.3 and high ASV score between 1.5 and 0.5 (Table 4.22). Genotype Nase-1 had IPCA score of 0.9 and ASV score of 1.5 recorded low root necrosis score of 1.0 and 1.7, respectively, in Murongwe and Moso. Genotype Kizimbani having IPCA score of 0.8 and ASV score of 1.3 had low root necrosis score of 1.3 in Mparambo and Murongwe. Genotypes, Orera, Eyope and Mkumba had IPCA score of 0.7, 0.4 and 0.3, respectively, recorded low root necrosis score in Mparambo and Murongwe respectively. Genotype, KBH/2002/026 having IPCA score of 0.3 and ASV of 0.5 recorded low root necrosis in all the sites. Genotype, Pwani, had IPCA and ASV scores of 0.3 and 0.6 respectively, recorded low root necrosis of 1.0 and 1.3 respectively in Mparambo and Murongwe (Table 4.22). Across sites, the top six AMMI selections for root necrosis having low GSI score that integrates root necrosis severity score and stability into a single index were KBH/2002/026, Kiroba, Mkumba, Okhumelela and Pwani (Table 4.22).

Regarding the CMD disease, genotypes, KBH/2002/026, KBH/2002/066, Orera, Tajirika and TZ-130 recorded high IPCA score of 0.7, 0.5, 0.7, 0.5 and 0.5 and high ASV of 0.9, 0.7, 0.8, 0.7 and 0.7 respectively. The top AMMI selection genotypes for CMD with low genotype mean scores were KBH/2002/026, Orera and TZ-130 in Murongwe and Mparambo, Tajirika in Moso and Murongwe and KBH/2002/066 in Murongwe (Table 4.23). Similarly, the top 6 AMMI selections genotypes across sites for CMD having low GSI score were Eyope, F10-30-R2, Mkumba, MM96/5280, Nase-14 and Pwani (Table 4.23).

Table 4.21. Ranking, IPCA scores, AMMI stability values and GSI for fresh storage root yield (in tons/hectare) of 18 evaluated cassava genotypes

Genotypes	Environment			Genotype mean (Gm)	Gm rank (A)	IPCAg[1] score	IPCAg[2] score	ASV	ASV rank (B)	GSI (A+B)	GSI rank
	Moso	Mparambo	Murongwe								
Albert	8.9	7.8	1.8	6.2	17	0.8	-0.2	1.8	9	26	10
Eyope	15.6	4.7	5.0	8.4	15	-0.4	-1.0	1.2	5	20	8
F10-30-R2	17.1	10.9	6.7	11.5	8	0.1	-0.4	0.5	1	9	3
KBH/2002/026	26.0	13.2	1.0	13.4	6	-1.2	1.3	3.0	12	18	6
KBH/2002/066	10.0	19.1	6.3	11.8	7	2.1	0.6	4.5	14	21	9
Kibandameno	0.3	0.3	0.4	0.4	18	1.3	-1.4	3.1	13	31	11
Kiroba	18.2	8.3	0.3	8.9	13	-0.6	0.5	1.4	6	19	7
Kizimbani	14.6	15.5	1.6	10.5	9	0.7	1.1	2.0	10	19	7
Mkumba	13.4	12.1	1.5	9.0	12	0.6	0.6	1.4	6	18	6
MM96/5280	12.4	9.0	2.0	7.8	16	0.4	0.0	1.0	3	19	7
Nase-1	40.0	5.4	4.3	16.6	1	-4.0	0.2	8.8	15	16	5
Nase-14	20.3	12.9	12.6	15.3	3	0.2	-1.0	1.1	4	7	2
Nase-3	22.8	10.0	8.5	13.8	5	-0.7	-0.6	1.7	8	13	4
Okhumelela	22.9	24.5	2.3	16.6	2	0.5	2.6	2.8	11	13	4
Orera	16.6	4.6	7.0	9.4	11	-0.4	-1.3	1.6	7	18	6
Pwani	13.9	13.3	4.3	10.5	10	0.8	0.3	1.7	8	18	6
Tajirika	13.5	5.5	7.2	8.7	14	0.2	-1.3	1.4	6	20	8
TZ-130	22.5	14.0	8.2	14.9	4	-0.3	0.0	0.6	2	6	1
E Mean	17.2	10.6	4.5	10.8	-	-	-	-	-	-	-
IPCAe[1]	-4.2	2.9	1.3	-	-	-	-	-	-	-	-
IPCAe[2]	0.7	2.6	-3.4	-	-	-	-	-	-	-	-

Gm: Genotype mean, ASV: AMMI stability values, IPCAg: Interaction principal component analysis for the genotype, E mean: environment mean, IPCAe[1], IPCAe[2]: Interaction principal component analysis for the environment axis one and two and GSI: Genotype stability index.

Table 4.22. Ranking, IPCA scores and AMMI stability values and GSI index for root necrosis severity of 18 evaluated cassava genotypes

Genotypes	Environment			Genotype mean (Gm)	Gm rank (A)	IPCAg[1] score	IPCAg[2] score	ASV	ASV rank (B)	GSI (A+B)	GSI rank
	Moso	Mparambo	Murongwe								
Albert	5.0	4.0	3.7	4.2	13	-0.3	0.0	0.4	4	17	8
Eyope	4.0	2.3	1.7	2.7	10	-0.4	0.4	0.9	8	18	9
F10-30-R2	3.0	2.7	1.3	2.3	7	0.1	0.3	0.3	3	10	5
KBH/2002/026	1.3	1.7	1.0	1.3	2	0.3	-0.3	0.5	5	7	2
KBH/2002/066	3.0	2.3	1.7	2.3	7	-0.1	0.1	0.2	2	9	4
Kibandameno	3.7	4.3	2.0	3.3	11	0.6	0.5	1.0	9	20	11
Kiroba	1.0	1.0	1.0	1.0	1	0.1	-0.5	0.5	5	6	1
Kizimbani	3.7	1.3	1.3	2.1	6	-0.8	0.3	1.3	11	17	8
Mkumba	2.0	2.3	1.0	1.8	4	0.3	0.0	0.5	5	9	4
MM96/5280	3.3	3.3	3.3	3.3	12	0.1	-0.5	0.5	5	17	8
Nase-1	1.7	3.3	1.0	2.0	5	0.9	0.1	1.5	12	17	8
Nase-14	2.7	2.7	2.0	2.4	8	0.1	-0.2	0.3	3	11	6
Nase-3	3.0	2.7	2.0	2.6	9	0.0	-0.1	0.0	1	10	5
Okhumelela	2.0	1.7	1.7	1.8	4	-0.1	-0.4	0.4	4	8	3
Orera	3.7	1.7	2.3	2.6	9	-0.7	-0.1	1.2	10	19	10
Pwani	2.0	1.0	1.3	1.4	3	-0.3	-0.3	0.6	6	9	4
Tajirika	3.7	3.0	1.0	2.6	9	0.1	0.8	0.8	7	16	7
TZ-130	2.3	2.3	1.3	2.0	5	0.2	0.0	0.3	3	8	3
E Mean	2.8	2.4	1.7	2.3	-	-	-	-	-	-	-
IPCAe[1]	-1.0	1.3	0.3	-	-	-	-	-	-	-	-
IPCAe[2]	-0.8	0.3	-1.2	-	-	-	-	-	-	-	-

Gm: Genotype mean, ASV: Analysis of the main effects and multiplicative interaction stability values, IPCAg: Interaction principal component analysis for the genotype, E mean: Environment mean, IPCAe[1] and IPCAe[2]: Interaction principal component analysis for the environment axis one and two, GSI: Genotype stability index.

Table 4.23. Ranking, IPCA analysis scores and AMMI stability values and GSI for CMD of 18 evaluated cassava genotypes

Genotypes	Environment			Genotype mean (Gm)	Gm rank (A)	IPCAg[1] score	IPCAg[2] score	ASV	ASV rank (B)	GSI (A+B)	GSI rank
	Moso	Mparambo	Murongwe								
Albert	3.0	3.0	1.7	2.6	6	-0.2	-0.3	0.4	3	9	4
Eyope	1.0	1.0	1.0	1.0	1	0.2	0.3	0.4	3	4	1
F10-30-R2	1.7	1.7	1.0	1.7	3	0.2	0.3	0.4	3	6	2
KBH/2002/026	2.7	1.0	1.0	1.6	2	-0.7	0.4	0.9	6	8	3
KBH/2002/066	3.0	2.7	1.0	2.2	5	-0.5	-0.5	0.7	4	9	4
Kibandameno	4.0	4.3	4.0	4.1	11	0.3	0.1	0.4	3	14	8
Kiroba	3.0	3.3	3.0	3.1	10	0.3	0.1	0.4	3	13	6
Kizimbani	3.0	3.0	2.3	2.8	7	0.0	0.0	0.0	1	8	3
Mkumba	1.0	1.0	1.0	1.0	1	0.2	0.3	0.4	3	4	1
MM96/5280	1.0	1.0	1.0	1.0	1	0.2	0.3	0.4	3	4	1
Nase-1	3.0	3.0	1.7	2.6	6	-0.2	-0.3	0.4	3	9	4
Nase-14	1.0	1.0	1.0	1.0	1	0.2	0.3	0.4	3	4	1
Nase-3	3.0	3.3	2.3	2.9	8	0.1	-0.2	0.2	2	10	5
Okhumelela	3.0	4.0	2.0	3.0	9	0.1	-0.7	0.7	4	13	7
Orera	3.0	1.7	1.0	1.9	4	-0.7	0.1	0.8	5	9	4
Pwani	1.0	1.0	1.0	1.0	1	0.2	0.3	0.4	3	4	1
Tajirika	1.0	2.3	1.0	1.4	2	0.5	-0.4	0.7	4	6	2
TZ-130	2.3	1.0	1.0	1.4	2	-0.5	0.4	0.7	4	6	2
E Mean	2.3	2.2	1.6	2.0	-	-	-	-	-	-	-
IPCAe[1]	-1.2	0.5	0.7	-	-	-	-	-	-	-	-
IPCAe[2]	0.1	-1.1	0.9	-	-	-	-	-	-	-	-

Gm: Genotype mean, ASV: Analysis of the main effects and multiplicative interaction stability values, IPCAg: Interaction principal component analysis for the genotype, E mean: Environment mean, IPCAe[1] and IPCAe[2]: Interaction principal component analysis for the environment one and two, GSI: Genotype stability index.

4.5. Discussion

Genotypic and Environment effects on sprouting, plant height and height to first branching

Variability was observed among genotypes for sprouting rate, plant height and height to the first branching indicative of their influence by genotype and environment. Sprouting seemed to be genetically controlled due to high differences among genotypes but no significant difference for different sites. Mdenye (2016) reported similar results by relating sprouting rate to physiological differences among stem structure from different genotypes. For plant height and height to the first branching, the environment varied significantly indicative of its influence to performance of the cassava genotypes. Tallest genotypes were found in Mparambo at 2.0 m while shortest genotypes in Murongwe at 1.3 m could be attributed to differences in soil fertility, altitude, temperature and rainfall among locations. Similar results were reported that poor soil fertility, water stress and lower temperatures led to lower branching height (Irikura et al., 1979; IITA, 1990).

Genotypic and environmental effects on CBSD and CMD's severity and incidence

Performance of cassava genotypes against CMD and CBSD was dependent on genotype and environmental effects. Variation in disease status determined by various parameters among the cassava genotypes demonstrated differences in susceptibility and tolerance to CBSD and CMD infections. The AUDPC variability observed among genotypes for CMD and CBSD incidence and severity on leaves indicated genetic dissimilarities between genotypes.

Highest severity for CBSD and CMD observed at 9 MAP with maximum incidence of 100% at Mparambo and Moso could be attributed to high temperature, low altitude and rainfall obseved in Moso and Mparambo compared to Murongwe. According to Bigirimana et al.(2004), Moso and Mparambo record high CBSD and CMD disease pressure while Murongwe has low pressure. In Murongwe, 77.8% of genotypes did not show infection for CBSD. High differences ($P<0.001$) observed between genotypes on CBSD incidence between 3 and 6 months, and between 6 and 9 months was due the fact that at3 months, the number of whitefly was low but it increased at 6 months and decreased by 9 months. Results indicate that genotypes F10-30-R2, KBH/2002/026, KBH/2002/066, Kiroba, Kizimbani, Mkumba, Nase-14, Nase-3, Okhumelela and Pwani had low symptoms on leaves for CBSD with incidence ranging from 0-14 % and severity from 1-1.9, indicating their tolerance against CBSD on shoots. Genotypes Mkumba and Okhumelela had CBSD severity of 1.0 and incidence of zero throughout the evaluation period.

The response of cassava genotypes to CMD incidence differed for 3-6 months, and then at 6-9 months with higher values at 6 to 9 months, suggesting that progression of CMD and CBSD depends on age of cassava crops. Masinde et al. (2018) in Tanzania found similar results and reported that about 50% of cassava genotypes that had symptoms on leaves at 3 months, also had symptoms at 6 and 9 months for CMD and CBSD. Similarly, genotypes Eyope, F10-30-R2, KBH/2002/026, Mkumba, MM96/5280, Nase-14, Orera, Pwani, Tajirika and TZ-130 had low CMD severity between 1-1.9 while incidence between 0-13 % suggesting their good performance against CMD. Genotypes Mkumba, Nase-14 and Pwani had CMD severity of 1.00 and incidence of zero throughout evaluation period. Results showed that genotypes F10-30-R2, KBH/2002/026, Mkumba, Nase-14, and Pwani showed dual performance for CBSD on leaves and CMD.

The differences observed among sites in high disease incidence and severity for susceptible and moderately tolerant genotypes was due to differences in whitefly population among sites. The low number of whiteflies in Murongwe was accompanied with low disease incidence and severity. These results are in agreement with findings of Katono et al. (2015) who reported that disease spread depends on the number of whiteflies. However, the number of whiteflies was low at early stage, then increased at 6 months but then decreased by 9 months, which could be due to the fact that, whiteflies prefer the succulent period of plant growth observed around 6 months but at 9 months, the crop had started senescing hence reduced whitefly population. According to Katono et al. (2015), occurrence of adult whitefly on cassava is closely related to the crop's age. Fishpool et al. (1987) reported that young established crops do not attract whiteflies while rapid vegetative growth produces large succulent leaves are preferred by whiteflies. At the advanced growth stage, leaves dry-out, prompting whiteflies to seek new growth for feeding and oviposition.

The differences observed on incidence and severity of the roots necrosis were due to genetics of the plant and disease pressure of a given site. Kibandameno and Albert had high root incidence coupled with high severity scores for CBSD, thus are not suitable for use in high disease pressure environment. In contrast, genotype Kiroba had zero incidence and severity on root with CBSD symptoms on leaves suggsting that there is no direct relationship between symptoms on leaves and roots. Similar results were noted by Mohammed et al. (2012) who stated that some genotypes like Kiroba show foliar symptoms but without or delayed root symptoms. However, Kaweesi et al. (2014) indicated that shoot symptoms could cause higher yield reduction than losses caused

by root necrosis. Hence, cassava breeding program should focus on foliar symptoms and root necrosis symptoms (Kaweesi et al., 2014). The genotypes KBH/2002/026, Mkumba, Okhumelela and Pwani had low incidence and low severity on roots.

Genotypic and Environment effects on yield and yield components

The differences reported among genotypes for number of root per plant, percentage of marketable roots, fresh storage root yield, dry matter content, dry storage root yield, harvest index and starch content highlighted broad genetic differences among genotypes and environment for the 3 sites. Harvest index varied significantly having values ranging between 6.3 and 43.3 %.

Low harvest index recorded was due to the low number of roots produced and vice versa. The differences observed on number of roots per plant and number of marketable roots were influenced by differences in soil fertility between the 3 environments and their interaction with the genotypes. The lowest yields were recorded in Murongwe compared to Moso and Mparambo suggesting that Murongwe is less suitable to cassava than the two others sites. It was observed that genotypes that yielded above 10 tons/ha were 83.3%, 44.4% and 5.5% in Moso, Mparambo and Murongwe, respectively. This may have been due to differences in soil fertility, temperature and rainfall between the 3 environments. According to Howeler (2012), low soil fertility, temperatures and rainfall significantly reduce cassava root yields.

Differences observed for the interaction between sites and genotypes on dry matter and starch content indicated that combined effects of environment and genotype affected expression of the traits. Similar results were reported on the environmental effect on dry matter and starch content (Ssemakula and Dixon, 2007; Tan and Mak, 1995). Combined environment and genotype effects were different for root number per plant and fresh storage root yield, indicating the variation in performance of the genotypes. In contrast, number of marketable root and harvest index were not affected by the combined effects of environment and genotypes.

Effect of CBSD and CMD on yield and yield components

High significant positive correlation between fresh storage root yield and number of roots per plant, marketable roots and harvest index indicates that each trait could be used to predict performance of the other. Athanase et al. (2017) reported similar results where harvest index, number of root per plant and number of marketable roots were used to explain and predict fresh

storage root yield. For breeders, a strong and positive correlation means that the traits could be selected simultaneously.

There were negative relationships between traits where root necrosis incidence and CBSD severity affected yield. Similar findings were reported by Okechukwu and Dixon (2009) who found a negative correlation between CMD and yield. The significant and positive relationship between CBSD incidence on shoots and CBSD root necrosis severity and incidence indicates that the incidence on leaves could be used to identify susceptible or tolerant genotype against root necrosis. Similar results were reported by Abaca et al. (2012) on susceptible and tolerant genotypes in Uganda, suggesting that a simultaneous increase of leaf severity could be used to determine extent of root severity and therefore avoid uprooting plants for assessment. Contrary reports by Kaweesi et al. (2014) and Valentor et al. (2018) showed that genotypes without CBSD foliar symptoms might or not show high levels of root necrosis incidence and severity. This disagreement between results could be attributed to genetic differences between genotypes.

Effect of genotypes and environment on adaptability and stability of cassava

Suitability and stability of genotypes were based on fresh storage root yield, root necrosis and CMD. AMMI analysis are used to determine suitability and stability of clones to specific and all environments using PCA scores and AMMI stability values (Hagos and Abay, 2013). Genotypes with higher IPCA score are more adapted to specific locations while those with smaller IPCA scores are more adapted genotypes across locations (Purchase, 1997). Genotypes with smaller GSI scores are more stable across sites for yield while genotypes with smaller ASV scores are more stable genotype across sites for other traits (Adjebeng-Danquah et al., 2017).

Accordingly, the sum of the yield and stability rankings (GSI) ranked TZ-130, Nase-14 and F10-30-R2 as the genotype that combined high yield with stability across sites. High IPCA and ASV scores observed on genotypes Nase-1, KBH/2002/026, KBH/2002/066 and Kibandameno for fresh storage root yield sugests that these genotypes are adapted to specific sites. Therefore, Nase-1, and KBH/2002/026 suited in Moso, KBH/2002/066 in Mparambo and in Kibandameno in Murongwe.

With regards to root necrosis, GSI score, IPCA score and Gm ranked genotypes KBH/2002/026, Kiroba, Mkumba, Okhumelela and Pwani as the most stable genotypes across sites. The top 7

genotypes with high IPCA score and high ASV were Nase-1, Kizimbani, Orera, Eyope, KBH/2002/026, Mkumba and Pwani, suggesting that they are more adapted to specific sites for the trait. Genotype Pwani is considered suitable for root necrosis in Mparambo and Murongwe; Nase-1 in Murongwe and Moso; Kizimbani in Mparambo and Murongwe; Orera in Mparambo, Eyope and Mkumba in Murongwe while KBH/2002/026 at all sites.

Genotypes Eyope, Mkumba, F10-30-R2, MM96/5280, Nase-14 and Pwani had low GSI score and low IPCA score for CMD suggesting their suitability in all the sites. The higher IPCA score and the higher ASV observed for CMD on genotypes, KBH/2002/026, KBH/2002/066, Orera, Tajirika and TZ-130 indicated that these genotypes are adapted for specific sites for the trait. Therefore, KBH/2002/026, Orera and TZ-130 were suitable in Murongwe and Mparambo, Tajirika in Moso and Murongwe and KBH/2002/066 in only Murongwe.

4.6. Conclusion

The aim of this study was to identify among introduced cassava genotypes, high yielding genotypes having also resistance/tolerance to CBSD and CMD. Considering the virus diseases and yield, evaluated cassava genotypes had different responses to CBSD, CMD and to cassava root yield within and across sites. They were either resistant, tolerant or susceptibles.

Resistant/tolerant genotypes identified in this study can be incorporated in the core collection and used in cassava breeding programs for transferring resistance/tolerance to farmer-preferred varieties. Genotype Mkumba showed dual totally resistance and therefore, might be used as genetic stock that could combine resistance to CBSD and CMD into one genotype. Kiroba had CBSD symptoms on leaves but totally resistant to root necrosis. Therefore, this genotype could be used to improve susceptible genotypes to root necrosis. Similarly, Eyope, MM96/5280, Nase-14 and Pwani were also totally resistant to CMD, thus, they could be used to improve susceptible genotypes to CMD. Furthermore, high yielding genotypes were identified within the sites and across sites indicating that their release would likely increase the productivity of cassava. Pearson correlation analysis revealed that the yield can be explained and predicted by the harvest index, the number of root per plant and number of marketable roots. All these traits were strongly and positively correlated, and thus could be selected simultaneously.

CHAPTER FIVE

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1. General discussion

According to morphological traits, the fact that leaf traits distribution differed among accessions as well as between landraces and elite germplasm suggests that there was variability. Similarly, stems traits including were diversely distributed among accessions. Furthermore, root traits that include the color of the root cortex, the color of the root pulp, the color of external storage root and the taste were also diversely distributed. Equally, the hierarchical clustering based on morphological traits and ward's method grouped accessions into three clusters indicating the existence of phenotypic variability among themselves that could be utilized for breeding and varietal improvement. Furthermore, the molecular analysis clustered accessions into six groups indicating presence of the genetic diversity among themselves. Some accessions shared all genetic characteristics indicating that they are duplicates clones that will certainly contribute to decreasing the cost of conserving cassava collection. Similarly, cluster analysis showed landraces accessions grouped together with the elite germplasm indicating that they could have possible partial resistance. According to Lohani et al. (2012), grouping of accessions in clusters suggests the relative diversity of accessions that allows selection of the materials contained by each group as core collection. However, morphological clustering helped to identify accessions that shared the same characteristics and closely related. Similar findings have been found by Ghebreslassie (2017) who characterized the potato cultivars in Eritrea. This study revealed that the landraces and the introduced cassava genotypes presented differences but with narrow genetic variability, suggesting a previous breeding possible between genotypes. Bhandari et al. (2017) reported that narrow genetic base in many crops was found in the released varieties. The duplicates detected by both morphological and molecular analysis let assume that some accessions present multiple names in the collection that confirms variety nomenclature of the farmer. According to Elias et al. (2001), on farm, a single genotype of cassava could have multiple names or two cultivars of cassava or more could present same name. This could lead to an overvaluation or underestimation of the varietal cassava diversity in the collection or on farm. In addition, the observed difference in cluster number between the two clustering methods confirms the power of molecular analysis compared to clustering using morphological characters, generally less

consistent. Similar findings were found by Feldberg et al. (2011) who stated that *Syzygiella concreta* and *S. perfoliata* were morphologically similar but genetically distinct.

Considering the GxE results, sprouting, plant height and height to first branching were influenced by the genotype and environmental effects as reported by Mdenye (2016), Irikura et al. (1979) and IITA (1990). The shorter genotypes in Murongwe are due to poor soil fertility, low temperature and high altitude from that site compared to those of Moso and Mparambo where the genotypes were taller, indicating that the differeces in plant height among locations could be attributed to differences in soil fertility, temperature and altitude among themselves. The performance of the cassava genotypes against CMD and CBSD was dependent on both genotype and environmental effects. The variation observed among genotypes for CMD and CBSD incidence and severity on leaves indicated genetic dissimilarities between genotypes. The genotypes F10-30-R2, KBH/2002/026, KBH/2002/066, Kiroba, Kizimbani, Mkumba, Nase-14, Nase-3, Okhumelela and Pwani showed low symptoms on leaves for CBSD indicating their tolerance against CBSD on shoots. Genotypes Mkumba and Okhumelela had CBSD severity of 1.0 and incidence of zero throughout the evaluation period on shoots indicating their resistance against CBSD on shoots. Similarly, the genotypes Eyope, F10-30-R2, KBH/2002/026, Mkumba, MM96/5280, Nase-14, Orera, Pwani, Tajirika and TZ-130 had low CMD severity and incidence suggesting their tolerance against CMD. The genotypes Mkumba, Nase-14 and Pwani had CMD severity of one and incidence of zero throughout the evaluation period indicating their resistance againstet CMD. Dual performance for both CBSD on shoots and CMD was found on genotypes F10-30-R2, KBH/2002/026, Mkumba, Nase-14 and Pwani. This result suggests that the disease incidence and severity was due to the genetic of the plant, the pressure of the disease caused by the number of whitefly and is closely related to cassava crop age. Katono et al. (2015) reported similar results. The high root incidence coupled with high severity scores for CBSD found in some genotype, while others genotypes like Kiroba exhibited CBSD symptoms on leaves without symptoms on the roots suggests that there is no direct relationship between symptoms on the leaves and the symptoms on the roots. Similar results were reported by Mohammed et al. (2012) who stated that some plants exhibite symptoms on the leaves but do not exhibit symptoms on the roots. Accordingly, the genotypes KBH/2002/026, Mkumba, Okhumelela and Pwani had low incidence and low severity on roots. The genotypes TZ-130, Nase-14 and F10-30-R2 combined high yield with stability across sites while genotypes Nase-1 and KBH/2002/026 suited in Moso,

KBH/2002/066 in Mparambo and Kibandameno in Murongwe. However, theier IPCA and ASV score was high. Purchase (1997) reported that the higher IPCA score in absolute value, more the genotype is adapted to specific environment, and smaller IPCA scores suggest that the genotype is more adapted across environments. Moreover, some genotypes were considered suitable for a given trait in specific site and across sites. Adjebeng-Danquah et al. (2017) reported that genotypes with smaller GSI scores are more stable genotype across sites for the yield and the genotypes with smaller ASV scores are more stable genotype across sites for other traits.

5.2. Conclusions

The first objecive of this study was to assess the genetic diversity among cassava landraces and introduced genotypes using morphological and molecular markers. However, the assessment of genetic diversity of cassava germplasm is the first study of its kind carried out in Burundi. Morphological and molecular characterization showed distinct classes of cultivars and within each class, sub classes with similar SNP profiles were identified. Accessions having very close similar characteristics namely Pwani and Mkumba, Kiroba and Eyope, Nase1 and Nase3 and Imiduga, Mutsindekwiburi and Rubona should be considered as putative duplicates, hence, need to be pooled together as one cultivar. Despite the fact that the grouping of accessions highlighted the genetic variability among cassava accessions that can be exploited by breeding programs, it was concluded that cassava landraces in Burundi as well as the introduced clones present a narrow genetic base.

The second objective was to determine the effects of genotype x environment interaction on resistance to CBSD and CMD diseases in varied agroecological zones of Burundi. The resistant/tolerant genotypes identified in this study will be incorporated in cassava breeding program for transferring the genes for resistance/tolerance to farmer-preferred varieties. Identified dually genotypes like Mkumba and Pwani, also identified as duplicates clones, might be used as genetic stock that could combine resistance to CBSD and CMD into one genotype. The identified resistant genotypes to root necrosis like Kiroba could be used to improve susceptible genotypes to root necrosis. Identified resistant genotypes, MM96/5280 and Nase-14 to CMD could be used to improve susceptible genotypes to CMD. The high yielding and disease tolerrant genotypes identified indicated that their release would likely increase the productivity of cassava.

5.3. Recommendations

(1) The genotypic classification showed a lot of differences between accessions compared to morphological classification. It is therefore recommended to use of molecular analysis method to assess the diversity in cassava.

(2) The narrow genetic basis of cassava germplasm in Burundi suggests the need to enrich the germplasm by imported others germplasm.

(3) High yielding and disease tolerant genotypes have been identified. It is therefore recommended that these genotypes be released and incorporated into the Burundi breeding program.

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APPENDICE

Appendix 1. Morphological traits of 118 characterized cassava clones

Clone	CAL	PAL	SCL	PC	LC	PO	PFS	CSC	CSEp	CSEx	DBLS	GHS	CEBAP	CRC	CRP	ECSR	RT
Nakarasi_1	Purplish green	Absent	Ellip-lanc	Purple	Dark green	Horizontal	Prominent	Light green	D br	Silver	Medium	Straight	G-p	White	White	Li- br	Bitter
Nakarasi_2	Purplish green	Absent	Lanceolate	Purple	Dark green	Horizontal	Semi-prom	Light green	Li-br	Silver	Medium	Straight	G-p	-	-	-	-
Gitamisi	Purplish green	Absent	Lanceolate	Purple	Dark green	Horizontal	-	-	-	-	-	-	-	-	-	-	-
Solange	Purplish green	Absent	Obl-lanc	Purple	Dark green	Horizontal	Prominent	Light green	D br	Gray	Medium	Straight	G-p	Cream	Yellow	D br	Bitter
Yongwe	Dark green	Absent	Ellip-lanc	Green- red	Dark green	Inc up	Prominent	Light green	D br	Silver	Medium	Straight	Green	-	-	-	-
Kibembe	Purplish green	Absent	Ellip-lanc	Purple	Dark green	Inc up	Semi-prom	Light green	D br	Gray	Medium	Straight	G-p	-	-	-	-
Criolina	Purplish green	Absent	Obl-lanc	Purple	Dark green	Irregular	Semi-prom	Light green	D br	Silver	Medium	Straight	G-p	-	-	-	-
Matara	Purplish green	Absent	Ellip-lanc	Red	Dark green	Irregular	-	-	-	-	-	-	-	-	-	-	-
Sisiriya	Purplish green	Absent	Ellip-lanc	Purple	Dark green	Horizontal	-	-	-	-	-	-	-	-	-	-	-
Ruvuna	Purplish green	Absent	Ellip-lanc	Green- red	Dark green	Horizontal	-	-	-	-	-	-	-	-	-	-	-
Butoke	Dark green	Absent	Obl-lanc	Purple	Dark green	Irregular	Semi-prom	Light green	Li-br	Silver	Medium	Straight	Green				
Kiganda	Light green	Absent	Ellip-lanc	Purple	Dark green	Irregular	Semi-prom	Light green	Li-br	Gray	Medium	Straight	Green	White	White	D br	Sweet
Ntabahungu	Dark green	Absent	Obl-lanc	Green- red	Dark green	Horizontal	Semi-prom	Light green	Li-br	Silver	Medium	Straight	Green	-	-	-	-
Kibembe	Purplish green	Absent	Ellip-lanc	Purple	Dark green	Inc dow	Prominent	Light green	D br	D br	Medium	Straight	G-p	Cream	White	D br	Bitter
Muzinda	Dark green	Absent	Ellip-lanc	Purple	Dark green	Horizontal	Prominent	Light green	Li-br	Gray	Medium	Straight	Green	Cream	White	D br	Sweet
Kwezikumwe	Dark green	Absent	Ellip-lanc	Purple	Light green	Horizontal	Semi-prom	Light green	Li-br	Silver	Medium	Straight	Green	-	-	-	-
Rumonge	Purple	Absent	Obl-lanc	Purple	Dark green	Irregular	Prominent	Orange	Cream	Golden	Medium	Straight	G-p	Pink	White	D br	Bitter
Mbubute	Dark green	Absent	Ellip-lanc	Purple	Dark green	Horizontal	Prominent	Light green	D br	Gray	Medium	Straight	Green	Pink	White	D br	Bitter
Yagata	Purplish green	Absent	Obl-lanc	Green- red	Dark green	Horizontal	Prominent	Light green	D br	Silver	Medium	Straight	Green	Cream	White	D br	Bitter
Niga	Purplish green	Absent	Ellip-lanc	Purple	Dark green	Horizontal	Semi-prom	Light green	D br	Gray	Medium	Straight	Green	-	-	-	-
Ibigororoka	Purple	Absent	Obl-lanc	Purple	Dark green	Horizontal	Prominent	Light green	D br	Silver	Medium	Straight	G-p	Cream	White	Li-br	Bitter
Maguruyankware	Purplish green	Absent	Ellip-lanc	Purple	Dark green	Horizontal	Prominent	Orange	D br	Orange	Medium	Straight	Green	-	-	-	-
Mwarabu	Purplish green	Absent	Ellip-lanc	Purple	Dark green	Horizontal	Semi-prom	Orange	Li-br	Gray	Medium	Straight	G-p	Cream	White	D br	Bitter
Rushishwa	Purple	Absent	Lanceolate	Purple	Dark green	Horizontal	Semi-prom	Light green	Li-br	Gray	Medium	Straight	Purple	Pink	White	D br	Bitter
Sosomasi	Purplish green	Absent	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Myezisita	Purple	Absent	Lanceolate	Purple	Dark green	Horizontal	Prominent	Light green	Cream	Silver	Medium	Straight	G-p	Cream	White	Li-br	Bitter

Appendix 1. Morphological traits of 118 characterized clones (continued)

Clone	CAL	PAL	SCL	PC	LC	PO	PFS	CSC	CSEp	CSEx	DBLS	GHS	CEBAP	CRC	CRP	ECSR	RT
Zegura	Purple	Absent	Ellip-lanc	Purple	Dark green	Horizontal	Prominent	Light green	D br	D br	Medium	Straight	G-p	Pink	White	D br	Bitter
Igipila	Dark green	Absent	Ellip-lanc	Yelow green	Dark green	Horizontal	Semi-prom	Dark green	Li-br	Silver	Medium	Straight	Green	Cream	White	D br	Bitter
Igikoshi	Purplish green	Absent	Lanceolate	Purple	Dark green	Horizontal	Semi-prom	Light green	Li-br	D br	Medium	Straight	G-p	Cream	White	D br	Bitter
Nakarasi	Dark green	Absent	Ellip-lanc	Red-green	Dark green	Horizontal	Prominent	Light green	Li-br	Gray	Medium	Straight	Green	White	White	D br	Bitter
Munengera	Purplish green	Absent	Obl-lanc	Red-green	Dark green	Inc dow	Semi-prom	Light green	Li-br	Li-br	Medium	Straight	G-p	-	-	-	-
Mwotsi	Purple	Absent	Lanceolate	Purple	Dark green	Inc up	Semi-prom	Light green	Li-br	D br	Medium	Straight	G-p	-	-	-	-
Berita	Purplish green	Absent	Obl-lanc	Red-green	Dark green	Horizontal	Prominent	Light green	D br	D br	Medium	Straight	G-p	Cream	White	D br	Bitter
Ntegagakoko	Purplish green	Absent	Ellip-lanc	Red-green	Dark green	Horizontal	Semi-prom	Light green	D br	Gray	Short	Straight	G-p	-	-	-	-
Gatarina	Purplish green	Absent	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Serereka	Purple	Absent	Ellip-lanc	Green- red	Dark green	Inc dow	Prominent	Dark green	Li-br	D br	Medium	Straight	G-p	Cream	White	Li-br	Bitter
Bugiga_annonciate1	Purplish green	Absent	Lanceolate	Red	Dark green	Inc dow	Prominent	Orange	Li-br	Golden	Medium	Straight	Purple	-	-	-	-
Yongwe	Purple	Absent	Ellip-lanc	Red-green	Dark green	Horizontal	Prominent	Dark green	D br	Gray	Medium	Straight	G-p	Cream	White	D br	Bitter
Gitikatika	Purplish green	Absent	Ellip-lanc	Green- red	Dark green	Horizontal	Prominent	Light green	Li-br	D br	Medium	Straight	G-p	Cream	White	Li-br	Intermediate
Gifunzo_caritas1	Purplish green	Present	Lanceolate	Purple	Dark green	Horizontal	Semi-prom	Light green	Cream	Silver	Medium	Straight	G-p	-	-	-	-
Gifunzo_caritsa2	Purplish green	Absent	Ellip-lanc	Green- red	Dark green	Horizontal	Prominent	Light green	Li-br	Li-br	Medium	Straight	G-p	Cream	White	D br	Bitter
Fyiroko	Purplish green	Absent	Ellip-lanc	Green- red	Light green	Horizontal	Semi-prom	Light green	Li-br	Gray	Medium	Straight	Green	Cream	White	D br	Bitter
Munebwe	Purplish green	Absent	Ellip-lanc	Green- red	Light green	Inc dow	Prominent	Dark green	Li-br	Gray	Medium	Straight	Green	Cream	White	D br	Bitter
Ndoha	Purplish green	Absent	Ellip-lanc	Green- red	Dark green	Horizontal	Prominent	Light green	Li-br	D br	Medium	Straight	G-p	Cream	White	D br	Bitter
Maguruyinkware	Purplish green	Absent	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rumarampunu	Purplish green	Absent	Ellip-lanc	Green- red	Dark green	Horizontal	Semi-prom	Dark green	Li-br	Gray	Medium	Straight	Green	Cream	White	D br	Bitter
Imikabika	Purplish green	Absent	Ellip-lanc	Purple	Dark green	Horizontal	-	-	-	-	-	-	-	-	-	-	-
Hanyesi	Purplish green	Absent	Obl-lanc	Yelow green	Light green	Horizontal	Semi-prom	Dark green	Li-br	D br	Medium	Straight	Green	Cream	White	D br	Bitter
Rubona	Purplish green	Absent	Obl-lanc	Purple	Dark green	Horizontal	-	-	-	-	-	-	-	-	-	-	-
Bwome_devote1	Purplish green	Absent	Ellip-lanc	Purple	Light green	Horizontal	Prominent	Orange	D br	Orange	Medium	Straight	G-p				
Gasahira	Purplish green	Absent	Ellip-lanc	Red	Dark green	Horizontal	Semi-prom	Orange	Li-br	Orange	Medium	Straight	G-p	-	-	-	-
Mbwayasaze	Purplish green	Absent	Lanceolate	Purple	Dark green	Horizontal	Semi-prom	Dark green	Li-br	Silver	Medium	Straight	G-p	Cream	White	D br	Sweet

Appendix 1. Morphological taits of 118 characterized clones (continued)

Clone	CAL	PAL	SCL	PC	LC	PO	PFS	CSC	CSEp	CSEx	DBLS	GHS	CEBAP	CRC	CRP	ECSR	RT
Kidihe	Purplish green	Absent	Obl-lanc	Purple	Dark green	Horizontal	Prominent	Dark green	D br	D br	Medium	Straight	G-p	Cream	White	D br	Bitter
Bunwa	Dark green	Absent	Ellip-lanc	Purple	Dark green	Irregular	Semi-prom	Light green	Li-br	Gray	Medium	Straight	Green	-	-	-	-
Inarubono	Purplish green	Absent	Ellip-lanc	Green	Dark green	Horizontal	-	-	-	-	-	-	-	-	-	-	-
Ntunduguru	Purplish green	Absent	Obl-lanc	Purple	Dark green	Horizontal	Prominent	Dark green	D br	D br	Short	Straight	G-p	Cream	White	D br	Sweet
Kigoma	Purplish green	Absent	Ellip-lanc	Red	Dark green	Inc dow	Prominent	Light green	Li-br	Silver	Short	Straight	G-p	-	-	-	-
Imijumbura	Purplish green	Absent	Ellip-lanc	Red	Dark green	Horizontal	Prominent	Light green	Li-br	Silver	Medium	Straight	G-p	-	-	-	-
Nyabisindu_anastasie1	Purplish green	Absent	Ellip-lanc	Red-green	Dark green	Horizontal	Prominent	Light green	Li-br	Gree-ye	Medium	Straight	G-p	Cream	White	D br	Sweet
Kabumbe	Purplish green	Absent	Ellip-lanc	Purple	Dark green	Inc dow	-	-	-	-	-	-	-	-	-	-	-
Gasasa	Purplish green	Absent	Lin-pira	Red	Dark green	Irregular	-	-	-	-	-	-	-	-	-	-	-
Yongwe	Purplish green	Absent	Lanceolate	Green- red	Dark green	Horizontal	-	-	-	-	-	-	-	-	-	-	-
Mutsindekwiburi	Purplish green	Absent	Ellip-lanc	Red	Light green	Inc dow	-	-	-	-	-	-	-	-	-	-	-
Murozi	Purplish green	Absent	Lanceolate	Purple	Dark green	Irregular	Prominent	Dark green	Li-br	Silver	Medium	Straight	G-p	Cream	White	D br	Bitter
Umusimbaruzi	Purplish green	Absent	Obl-lanc	Purple	Dark green	Inc up	-	-	-	-	-	-	-	-	-	-	-
Bukarasi	Purplish green	Absent	Pandurate	Red-green	Light green	Irregular	Prominent	Light green	D br	Silver	Medium	Straight	G-p	Cream	White	D br	Sweet
Mpamba	Purplish green	Absent	Lanceolate	Purple	Dark green	Irregular	-	-	-	-	-	-	-	-	-	-	-
Mabare	Purplish green	Absent	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Imiduga	Purplish green	Absent	Ellip-lanc	Purple	Light green	Horizontal	-	-	-	-	-	-	-	-	-	-	-
Tabika	Purplish green	Absent	Lanceolate	Purple	Dark green	Horizontal	-	-	-	-	-	-	-	-	-	-	-
Yongwe_ederi	Purplish green	Absent	Obl-lanc	Purple	Dark green	Horizontal	Prominent	Light green	D br	D br	Medium	Straight	G-p	Cream	White	D br	Bitter
Umuyobera	Purplish green	Absent	Obov-lanc	Purple	Dark green	Irregular	-	-	-	-	-	-	-	-	-	-	-
Umukurajoro	Dark green	Absent	Ellip-lanc	Purple	Dark green	Horizontal	Prominent	Dark green	Li-br	Gray	Long	Straight	Green	-	-	-	-
Rukokora	Purple	Absent	Ellip-lanc	Red	Light green	Inc dow	Semi-prom	Light green	Li-br	Gree-ye	Medium	Straight	G-p	Cream	White	D br	Bitter
Kinazi_dorothee1	Purple	Absent	Lanceolate	Purple	Dark green	Horizontal	Prominent	Dark green	Li-br	Silver	Medium	Straight	Purple	Cream	White	D br	Bitter
Gasu	Purplish green	Absent	Ellip-lanc	Green- red	Dark green	Horizontal	Prominent	Dark green	D br	Gray	Medium	Straight	G-p	-	-	-	-
Inagitembe	Purplish green	Absent	Obov-lanc	Green- red	Dark green	Horizontal	Prominent	Dark green	D br	Gray	Medium	Straight	G-p	-	-	-	-
Umutuburano	Purplish green	Present	Lanceolate	Purple	Light green	Horizontal	Prominent	Light green	Li-br	Gray	Medium	Straight	G-p	Cream	White	D br	Sweet

Appendix 1. Morphological traits of 118 characterized clones (continued)

Clone	CAL	PAL	SCL	PC	LC	PO	PFS	CSC	CSEp	CSEx	DBLS	GHS	CEBAP	CRC	CRP	ECSR	RT
Gitamisi	Purplish green	Absent	Ellip-lanc	Green- red	Dark green	Inc up	-	-	-	-	-	-	-	-	-	-	-
Rubona	Purplish green	Absent	Ellip-lanc	Green- red	Dark green	Horizontal	-	-	-	-	-	-	-	-	-	-	-
Nakarasi	Purple	Absent	Lanceolate	Purple	Dark green	Horizontal	Prominent	Dark green	D br	Silver	Medium	Straight	G-p	Cream	White	Li-br	Bitter
Surupiya	Purplish green	Absent	Ellip-lanc	Purple	Dark green	Horizontal	Prominent	Dark green	Li-br	Silver	Medium	Straight	G-p	Pink	White	D br	Sweet
Sogota	Dark green	Absent	Ellip-lanc	Red	Light green	Horizontal	Prominent	Light green	D br	D br	Medium	Straight	Green	Cream	White	D br	Bitter
Nabuseri	Purplish green	Absent	Obl-lanc	Purple	Purple green	Inc dow	Prominent	Dark green	D br	Gray	Medium	Straight	G-p	-	-	-	-
Imirundi	Purplish green	Absent	Lanceolate	Purple	Dark green	Horizontal	Prominent	Dark green	Li-br	Gray	Medium	Straight	Green	Cream	White	D br	Bitter
Imizariya	Purplish green	Present	Lanceolate	Purple	Dark green	Inc dow	Prominent	Dark green	Li-br	Silver	Medium	Straight	G-p	Cream	White	Li-br	Bitter
Maguruyinkware	Purplish green	Absent	Obl-lanc	Purple	Purple green	Inc dow	-	-	-	-	-	-	-	-	-	-	-
Umutakabumba	Purplish green	Absent	Lanceolate	Red-green	Dark green	Horizontal	Prominent	Light green	D br	Gray	Medium	Straight	G-p	Cream	White	D br	Bitter
Mugerera_yvonne1	Purplish green	Absent	Lanceolate	Red	Dark green	Horizontal	Prominent	Light green	Li-br	Li-br	Medium	Straight	G-p	-	-	-	-
Mugerera_yvonne2	Purplish green	Absent	Obl-lanc	Yelow green	Dark green	Horizontal	-	-	-	-	-	-	-	-	-	-	-
Kidihe	Purplish green	Absent	Ellip-lanc	Purple	Dark green	Inc dow	-	-	-	-	-	-	-	-	-	-	-
Nyawera	Purplish green	Absent	Ellip-lanc	Red-green	Dark green	Horizontal	-	-	-	-	-	-	-	-	-	-	-
Nyamugari_sophie1	Purplish green	Absent	Obl-lanc	Purple	Purple green	Horizontal	Prominent	Dark green	D br	D br	Medium	Straight	G-p	Cream	White	Li-br	Bitter
Mukecuru	Purple	Absent	Ellip-lanc	Red-green	Light green	Horizontal	Prominent	Light green	Li-br	Gray	Medium	Straight	G-p	Cream	White	D br	Bitter
Fundiko	Purplish green	Absent	Ellip-lanc	Yelow green	Dark green	Horizontal	-	-	-	-	-	-	-	-	-	-	-
Umuhendangurube	Purplish green	Absent	Ellip-lanc	Purple	Purple green	Horizontal	Prominent	Dark green	D br	D br	Long	Straight	G-p	Cream	White	D br	Bitter
Sagarara	Purplish green	Absent	Ellip-lanc	Green- red	Dark green	Horizontal	-	-	-	-	-	-	-	-	-	-	-
Imiduga	Purplish green	Absent	Ellip-lanc	Green- red	Light green	Horizontal	-	-	-	-	-	-	-	-	-	-	-
Mwotsi	Purplish green	Absent	Obl-lanc	Purple	Purple green	Horizontal	-	-	-	-	-	-	-	-	-	-	-
Kavyiro	Purplish green	Absent	Ellip-lanc	Purple	Purple green	Horizontal	-	-	-	-	-	-	-	-	-	-	-
Albert	Purple	Absent	Lanceolate	Purple	Purple green	Irregular	Prominent	Dark green	Li-br	Green	Medium	Straight	G-p	Cream	White	Li-br	Sweet
Eyope	Purplish green	Absent	Lanceolate	Green purple	Purple geen	Irregular	Semi-prom	Dark green	Li-br	Silver	Medium	Straight	Green	Cream	White	Li-br	Bitter
F10-30-R2	Purplish green	Present	Ellip-lanc	Purple green	Purple green	Irregular	Prominent	Dark green	D br	D br	Medium	Straight	G-p	Cream	White	D br	Bitter
KBH2002/066	Dark green	Present	Ellip-lanc	Green purple	Dark green	Inc dow	Prominent	Dark green	Li-br	Silver	Medium	Straight	Green	Cream	White	Li-br	Bitter

Appendix 1. Morphological traits of 118 characterized clones (continued)

Name	CAL	PAL	SCL	PC	LC	PO	PFS	CSC	CSEp	CSEx	DBLS	GHS	CEBAP	CRC	CRP	ECSR	RT
KBH2006/026	Light green	Absent	Lanceolate	Purple	Light green	Horizontal	Prominent	Dark green	Li-br	D br	Medium	Straight	Green	Cream	White	D br	Bitter
Kibandameno	Purplish green	Absent	Lanceolate	Red	Purple green	Inc dow	Prominent	Light green	Li-br	Gree-ye	Medium	Straight	G-p	Cream	White	D br	Sweet
Kiroba	Purple	Absent	Ellip-lanc	Greenish	Purple	Irregular	Prominent	Dark green	Li-br	Gray	Medium	Straight	Green	Pink	White	D br	Bitter
Kizimbani	Purple	Present	Lanceolate	Greenish	Purple	Horizontal	Prominent	Dark green	D br	Gray	Medium	Straight	Green	Cream	White	Li-br	Bitter
Mkumba	Purple	Absent	Lanceolate	Purple	Purple	Inc dow	Prominent	Light green	Li-br	Gree-ye	Medium	Straight	G-p	Cream	White	Li-br	Bitter
Mm96/5280	Dark green	Absent	Lanceolate	Purple	Purple green	Irregular	Prominent	Light green	D br	Gray	Medium	Straight	G-p	Cream	White	Li-br	Sweet
NASE1	Dark green	Absent	Lanceolate	Green purple	Dark green	Inc dow	Prominent	Dark green	Li-br	Gray	Short	Straight	Green	Cream	White	D br	Bitter
NASE14	Purplish green	Absent	Lanceolate	Purplish	Purple green	Irregular	Prominent	Light green	Li-br	Silver	Medium	Straight	G-p	Cream	White	Li-br	Sweet
NASE3	Light green	Absent	Ellip-lanc	Greenish red	Dark green	Irregular	Prominent	Light green	Li-br	Gray	Medium	Straight	Green	Cream	White	D br	Sweet
Okhumelela	Dark green	Absent	Lanceolate	Purple green	Dark geen	Irregular	Semi-prom	Light green	Li-br	Gray	Medium	Straight	Green	Cream	White	Li-br	Sweet
Orera	Dark green	Present	Ellip-lanc	Green purple	Dark green	Horizontal	Prominent	Dark green	D br	Gray	Medium	Zig zag	Green	Cream	White	D br	Bitter
Pwani	Purple	Present	Ellip-lanc	Purple	Purple	Irregular	Prominent	Light green	D br	Gree-ye	Long	Straight	G-p	Cream	White	D br	Bitter
Tajirika	Light green	Absent	Ellip-lanc	Green purple	Light green	Horizontal	Prominent	Dark green	Li-br	Silver	Short	Straight	Green	Cream	White	Cream	Bitter
TZ 130	Purple	Absent	Lanc-pand	Purple yellow	Purple green	Vertical	Prominent	Dark green	Li-br	Gray	Medium	Straight	G-p	Cream	White	D br	Bitter

CAL: Color of apical leaves, PAL: Pubescence on apical leaves, SCL: Shape of central leaflet, PC: Petiole color, LC: Leaf color, NLL: Number of leaf lobes, CLVL: Color of leaf vein in the lobe, PO: Petiole Orientation, PFS: Prominence of foliar scars, CSC: Color of stem cortex, CSEp: Color of stem epidermis, CSEx: Color of stem exterior, DBLS: Distance between leaf scars, GHS: Growth habit of stem, CEBAP: Color of end branches of adult plant, CRC: Color of root cortex, CRP: Color of root-pulp, ECSR: External color of storage root, RT: Root taste, Inc up: Inclined upwards, Inc dow: Inclined down, Obl-lanc: Oblong-lanceolate, Ellip-lanc: Elliptic-lanceolate, Lin-pira: Linear-pyramidal, Lanc-pand: Lanceolate-pandurate, Semi-prom: Semi-prominent, G-p: Green-purple, D br: Dark brown, Li-br: Light brown, Gree-ye: Green yellow. The blank areas indicate that the plants or parts of plant to be assessed were completely destroyed by the diseases, hence there is missing data.