ASSOCIATION MAPPING AND STABILITY ANALYSIS OF RESISTANCE TO CASSAVA GREEN MITE IN CASSAVA (*Manihot esculenta* Crantz)

By

EZENWAKA, LYDIA CHIDIMMA
(10496576)

THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY DEGREE IN PLANT BREEDING.

WEST AFRICA CENTRE FOR CROP IMPROVEMENT
COLLEGE OF BASIC AND APPLIED SCIENCES
UNIVERSITY OF GHANA
LEGON

DECEMBER 2017

DECLARATION

I hereby declare that except for references to work of other researchers, which have been duly cited, this work is my original research and that neither part nor whole has been presented elsewhere for the award of a degree.

…………………………………………….

Ezenwaka, Lydia Chidimma

(Student)

……………………………………………..

Prof. Eric Y. Danquah

(Supervisor)

……………………………………………

Prof. Essie T. Blay

(Supervisor)

……………………………………………

Prof. Isaac K. Asante

(Supervisor)

……………………………………………

Dr Agyemang Danquah

(Supervisor)

…………………………………………

Prof. Chiedozie N. Egesi

(In-country supervisor)

ABSTRACT

Cassava is a unique crop, because it is the primary source of carbohydrate for more than 1 billion people. In Nigeria, cassava has become a major sustenance crop and currently and is the world's largest producer of cassava. However, the yields on smallholder farms are relatively low largely due to pests' infections and other various cassava production constraints. A participatory rural appraisal study was therefore conducted to gather information on farmers' preferences, perception and knowledge of cassava green mite (CGM) and other production constraints and to lay the foundation for the development of CGM resistant cultivars in Nigeria. Individual interviews and focus group discussions involved 360 farmers in Abia, Anambra and Benue states. Termites, CGM and whitefly were recognized as the major pests that contribute to low yields and abandonment of some cassava cultivars by farmers. Majority of the farmers in the surveyed areas had little or no knowledge of CGM. However, farmers depend on traditional cultural practices such as weeding, selective pruning, use of barriers and setting traps as effective measures to reduce pest populations in their fields. These methods interfere with the survival of natural enemies of CGM. There is need to educate the farmers about the importance of CGM and the benefit of natural enemies. Resistance to pests and diseases, high yield and early bulking were highly ranked by farmers as their preferred traits. Cultivars which lack in most of these traits have been abandoned by farmers. Therefore, there is need to look for genetic resources for these farmers desired traits and incorporate them into new cultivars through plant breeding methods. To evaluate the presence of resistance genes in the available germplasm, a diverse panel of 845 advanced breeding lines obtained from the International Institute of Tropical Agriculture (IITA), the International Center for Tropical Agriculture (CIAT) and National Root Crops Research Institute (NRCRI) were evaluated for cassava green mite severity (CGMS), leaf pubescence (LP), leaf retention (LR), stay green (SG), shoot tip compactness (STC) and shoot tip size (STS). A genome-wide association mapping using mixed linear models detected 35 single nucleotide polymorphisms (SNPs) markers significantly associated with CGMS, LP and LR on chromosome 8. Co-localization of the most significant SNP associated with CGMS, LP and LR at chromosome 8 is possibly an indication of the presence of pleiotropic effects or closely linked genes that regulate these traits. Seventeen candidate genes were found to be directly linked to cassava green mite resistance. These candidate genes were subdivided into seven categories according to their protein structure: zinc finger, pentatricopeptide, MYB, MADS, homeodomain, trichome birefringence related protein and ethylene-responsive transcription factor genes. Genome wide association study revealed the presence of CGM resistant genes, which might represent new sources of resistance for the on-going effort to develop improved cassava cultivars.

A combined additive main effect and multiplicative interaction (AMMI) analysis of variance revealed that there were significant genotypic variations for all the traits indicating opportunity for selection and prospects for the improvement of cassava for the traits. The impact of environment was highly significant for cassava green mite severity at 6 months after planting (MAP) (CGMS6), leaf pubescence at 9 MAP (LP9), leaf retention at 9 MAP (LR9), stay green at 9 MAP (SG9), shoot tip compactness at 9 MAP (STC9), shoot tip size at 9 MAP (STS9), fresh root yield (FRY), root dry matter content (RDMC) and biomass justifying the need for multilocational testing to identify good performers for specific locations. There were significant responses of genotype by environment interaction for CGMS6, LP6, LP9, LR6, LR9, SG6, STC6, STC9, STS6, FRY, RDMC and biomass. This implies different adaptation by the different genotypes suggesting the need to identify and select location specific genotypes for different environments. Genotypes with wide or specific adaptability for these traits have been identified, and should be recommended for general or localized production and for use as sources of desired genes in crop improvement. Stable high yielding genotypes such as IBA131866, IBA131746, IBA131872, IBA131767 and IBA131770 which combine high FRY and resistance to CGM were identified, suggesting it is possible to combine these traits in cassava as desired by farmers.

DEDICATION

To God almighty and the loving memory of my late father, Chief Lawrence Chikwado Ezenwaka.

ACKNOWLEDGEMENTS

My deepest appreciation to the Almighty God for giving me such a wonderful supervisors, Prof. Eric Danquah, Prof. Chiedozie Egesi, Prof. Isaac Asante, Prof. Essie Blay and Dr Ayemang Danquah, whose encouragement and support fostered the completion of my PhD. Research.

I am grateful to Prof. Jean-Luc Jannink, Dr Dunia Pino Del Carpio, Dr Marnin Wolfe, Dr Joseph Onyeka and Roberto Lozano for their input, which improved the quality of this work. Special thanks go to:

- The entire staff of WACCI for their encouragement and moral support that kept me going during the tough moments of my study.
- NEXTGEN cassava breeding project through the funds from Bill and Melinda Gates Foundation and UKAid granted me full sponsorship of my studies.
- National Root Crops Research Institute (NRCRI), Umudike for providing a suitable research environments and planting materials for my work.
- International Institute of Tropical Agriculture (IITA), Ibadan for providing plant materials for my work.

My appreciation is extended to the entire staff of biotechnology programme of NRCRI, Umudike especially Mr Ahamefula Nwaogu, Mr Simon-Peter Abah, Mrs Favour Okeakpu, Mr Ugochukwu Ikeogu, Mr Amarachi Uzoechi, Mrs Onyinye Onyebule, Mr Tega Ogbe, Mr Kelechi Njoku and Mr Emma Okah for dedicating their time and strength to ensure a remarkable field research.

I count myself lucky to have been in a class of cheerful and motivating friends such as 7th WACCI cohort students.

I want to appreciate my mother, Mrs Comfort Ezenwaka, siblings and in-laws for their supports.

My greatest thanks goes to my precious husband, Dr Jiwuba, Peter-Damian Chukwunomso who has given me his whole hearted support and to our children, Rita Chidiebube and Laura Ifechukwu. I thank them for their patience and understanding.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AMMI	Additive Main Effects and Multiplicative Interactions
ASV	AMMI Stability Value
CGMS	Cassava Green Mite Severity
CIAT	Centro Internacional de Agricultura Tropical
CMD	Cassava Mosaic Disease
FAO	Food and Agriculture Organisation
FGD	Focus Group Discussion
FRY	Fresh Root Yield
GWAS	Genome-wide Association Studies
IITA	International Institute for Tropical Agriculture
LGA	Local Government Area
LP	Leaf Pubescence
LR	Leaf Retention
NGN	Nigerian naira
NRCRI	National Root Crops Research Institute
PCA	Principal Component Analysis
QTL	Quantitative Trait Loci
RDMC	Root Dry Matter Content
SG	Stay Green
SNP	Single Nucleotide Polymorphism
STC	Shoot Tip Compactness
STS	Shoot Tip Size
YSI	Yield Stability Index

CHAPTER ONE

1.0 GENERAL INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a perennial shrub that belongs to the family Euphorbiaceae. It is an outbreeding species possessing 2n=36 chromosomes and it is grown throughout the tropical regions of the world (El-Sharkawy, 2003). Cassava originated in South America, but has spread throughout tropical areas of Africa during the period of slave trade by the Portuguese explorers in the sixteenth century (Adeniji *et al.*, 2007). However, cassava did not become important until in the late nineteenth century when processing techniques were introduced by more slaves who returned to their homeland (Ekanem *et al.*, 2010). The crop is an important staple food and animal feed in tropical and sub-tropical Africa, Asia and Latin America countries. The ever increasing human population quickly adopted cassava because it was easy to grow under a wide range of agronomic conditions (Yaninek and Hanna, 2003). Today, cassava is the primary source of carbohydrates for more than 1 billion people (Lebot, 2009; Ospina and Ceballos, 2002) including the poorest on the continent. It provides food security and economic means to a majority of subsistence farmers and an increasing number of entrepreneurs interested in commercializing new cassava products.

In Nigeria, the world's largest producer, cassava has become a major sustenance crop. Cassava is produced in 24 out of the 36 states with an annual production of over 54.8 million tonnes of tuberous roots (FAO, 2014). Cassava production dominates the southern part of the country, both in terms of area covered and number of farmers growing the crop. The major states of Nigeria which produce cassava are Anambra, Delta, Edo, Benue, Cross river, Imo, Oyo and Rivers, and to a lesser extent Kwara, Kogi, Plateau, Taraba, Nasarawa, Niger and Ondo (Fig. 1.1) (Muojiama, 2017). Cassava is consumed in many processed forms in Nigeria. Most of the cassava produced in Nigeria is mainly used for human consumption and livestock feed (Fig. 1.2), although, its use in the industry is gradually increasing, especially as import substitution becomes prominent in the industrial sector of the economy. As a cash crop, cassava generates cash income for the largest number of households in Nigeria. As food crop, cassava has some inherent characteristics, which makes it very attractive to farmers. Firstly, cassava is the chief source of carbohydrates (Echebiri and Edaba, 2008) and the young leaves are consumed as vegetables which have protein, vitamins and mineral supplements (Ravindran, 1993). Secondly, it has a flexible harvesting date making it available all year round allowing farmers to keep the storage roots in the ground until needed. Thirdly, cassava has the ability to grow and give reasonable yields in low soil fertility, adapt to climate change, resistant to drought, pests and diseases.

Cassava is an important crop in Nigeria, therefore, it is necessary to ensure sustainability and increase in cassava production throughout the regions of Nigeria. In this sense, plant breeding has one of the highest rates of return among the investments in agricultural research (Ceballos *et al.*, 2004). Cassava genetic improvement has had an impact on the production system, but still faces challenges such as long breeding cycle, cross-pollinated species, low multiplication rate, poor flowering, limited genetic diversity and production of botanical seeds from crosses is expensive.

Since cassava has a long growth cycle of 8 – 12 months, its production is hampered with abiotic and biotic stresses, an important constraint to production is the combined effect of pests, diseases and weeds that together can substantially reduce cassava yields; the effect of cassava green mite (CGM) alone can be as large as 80% yield losses (Janssen and Yaninek, 1993). Early reports indicate that CGM was accidentally introduced in Africa when cassava was imported from South America. It was first reported in Uganda during the 1970s, from where it spread everywhere in Africa (Megevand *et al*., 1987; Yaninek and Herren, 1988). The introduction of this pest into Africa prompted IITA and national breeding programmes to initate a campaign in the 1980s to control CGM by using biological control (Yaninek and Hanna, 2003). Several predatory mites of the family Phytoseiidae, *Neoseiulus idaeus, Typhlodromalus manihoti* and *Typhlodromalus aripo* were introduced into Africa from Brazil. Of these three predators, *T. aripo* is the most widely used (Yaninek and Hanna, 2003). Unfortunately, the post-release investigations showed that the predatory mites failed to establish, particularly in north-western Zambia (Chalwe *et al*., 2015). No matter which predator species is used, *T. aripo* tends to disappear from cassava apices during the dry season (Mebelo *et al*., 2002). Some workers have reported that in some parts of Uganda and Cameroon, *T. aripo* seems to establish well only during the rainy season and tends to disappear from cassava shoot apices during the dry and cold seasons (Hanna *et al*., 2005), resulting in increased populations and attack of cassava green mite in cassava fields during the dry seasons (Onzo *et al*., 2003; Yaninek *et al*., 1989). Although several chemicals like Dimethoate and Dicofol can control CGM they are dangerous to the agricultural land and environment, human health, negatively impacting on agricultural production and reducing agricultural sustainability (Wilson and Tisdell, 2001). These chemicals are often too expensive for farmers and may be dangerous if not well applied particularly in countries where leaves are consumed as vegetables (Chalwe *et al*., 2015). Cultural methods for control of CGM such as adjusting planting time for the crop to escape severe damage at young age, mixing varieties to avoid genetic uniformity and removing infested tips have been tried but without much success. There have been limited work on the genetic control of CGM to determine the gene(s) conferring resistance. Most studies on CGM have focused on conventional breeding with limited work on molecular breeding (Nzuki *et al.*, 2017). Two SSR markers, NS 1099 and NS 346 showed high association with CGM resistance (Ceballos *et al.*, 2010; Choperena *et al.*, 2012). Recently, two quantitative trait loci (QTL) qCGMc5Ar and qCGMc10Ar were reported to be linked to CGM resistance (Nzuki *et al.*, 2017). The genetic mechanisms underlying the resistance to CGM have not been studied using the modern genome-wide association mapping. Identifying the genetic basis of CGM resistance will provide important insights for breeding resistant varieties for sustainable agriculture. Genome wide association studies (GWAS) makes it possible to simultaneously screen a vast number of accessions for genetic variation underlying complex traits at an unprecedented rapid rate (Consortium *et al.*, 2007; Consortium *et al.*, 2007; Altshuler *et al.*, 2008). However, GWAS have not been largely applied to the dissection of complex traits in crop plants (Nordborg and Weigel, 2008; Atwell *et al.*, 2010; Gore *et al.*, 2009) because of the lack effective genotyping techniques and limited resources for developing high-density haplotype maps like those seen in well-developed systems such as the human genome HapMap project (Consortium *et al.*, 2007). Recently, the potential of GWAS has been used in cassava to unravel the genetic architecture of resistance to cassava mosaic disease (Wolfe *et al.*, 2016), beta carotene content (Esuma *et al.*, 2016a), shoot weight, fresh root yield, starch fraction amylose content, dry matter content and starch yield (de Oliveira *et al.*, 2012). This approach has been particularly productive for rice, where major associations have been detected for important traits such as flowering time, disease resistance, grain size, panicle size, grain weight, abiotic stress tolerance (Famoso *et al.*, 2011; Huang *et al.*, 2010; Zhao *et al.*, 2011; Huang *et al.*, 2012). Major- effect loci discovered via QTL mapping and GWAS have been successfully introgressed from wild and exotic genetic resources into elite, high-yielding varieties via marker-assisted selection (Collard and Mackill, 2008; Septiningsih *et al.*, 2012; Jiang *et al.*, 2013). GWAS is used to identify significant associations that uncover useful QTL and candidate genes.

Clones with pubescent leaves, large compact shoot apices, enhanced leaf retention and stay green in cassava offer excellent levels of resistance to cassava green mite (Bellotti *et al.*, 2002). These plant morphological traits are associated with CGM resistance. They provide shelter for the predatory mite and enhance the ability of the predator to find the prey (Bellotti *et al.*, 2002). Plant breeders need to improve these traits for CGM resistance. However, there is very little information on the stability of cassava genotypes for such morphological traits. There is a need to know how such important traits are influenced by environment so that stable genotypes can be identified which could be used as sources of stable genes for CGM resistance breeding. Moreover, information pertaining to farmers' perception and knowledge of CGM needs to be captured and considered in breeding in order to accelerate adoption of new cultivars by farmers. This can be achieved through participatory breeding or participatory rural appraisal which involves interviews and visits to farms.

The main objective of the study was to use the genome-wide association mapping to identify genes conferring resistance to CGM and plant morphological traits that offer excellent levels of resistance to CGM.

The specific objectives were to:

1. assess farmers' perception of major cassava pests and traditional coping strategies as well as the plant morphological traits that are associated with reduced pest population and damage in cassava fields
2. estimate heritability of CGM and associated traits
3. identify significant SNP markers associated with CGM and other associated traits
4. identify candidate genes associated with CGM resistance
5. evaluate the adaptability and stability of cassava genotypes.

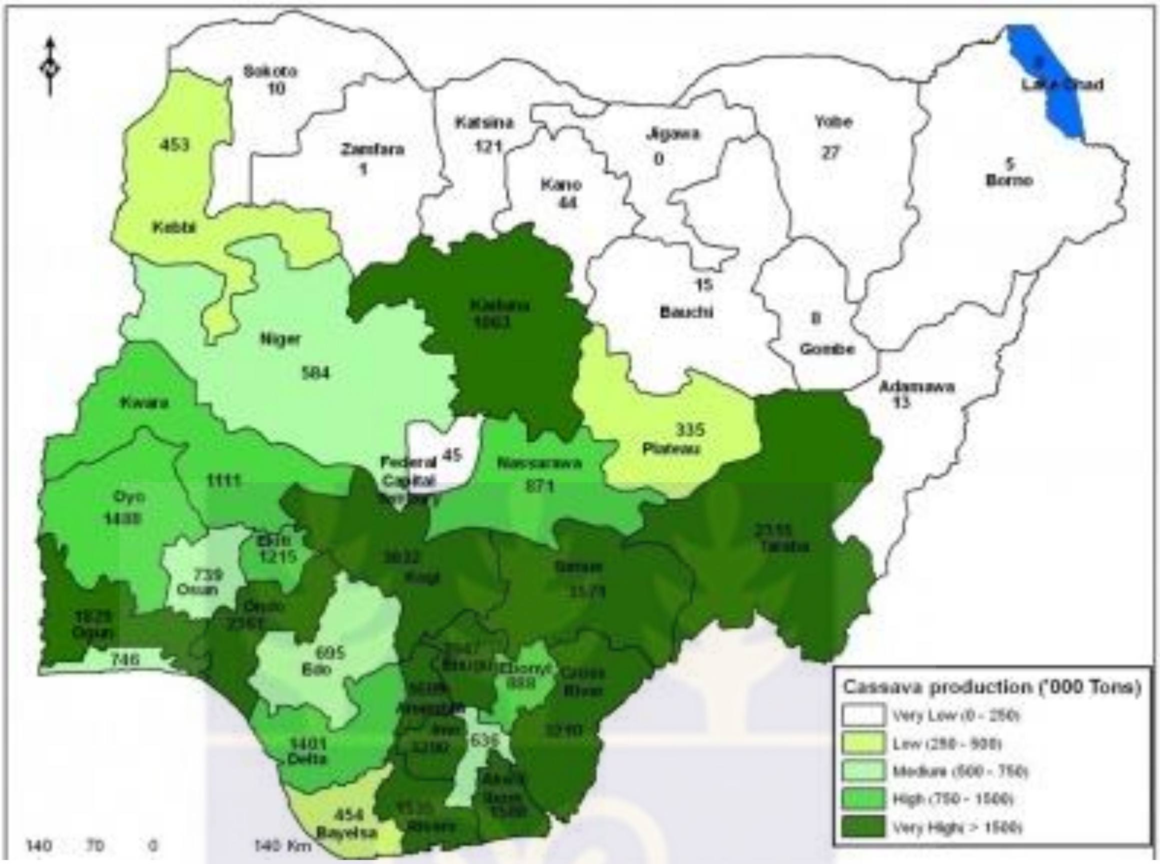


Fig 1. 1 Map of cassava growing areas in Nigeria

(FAO, 2014)

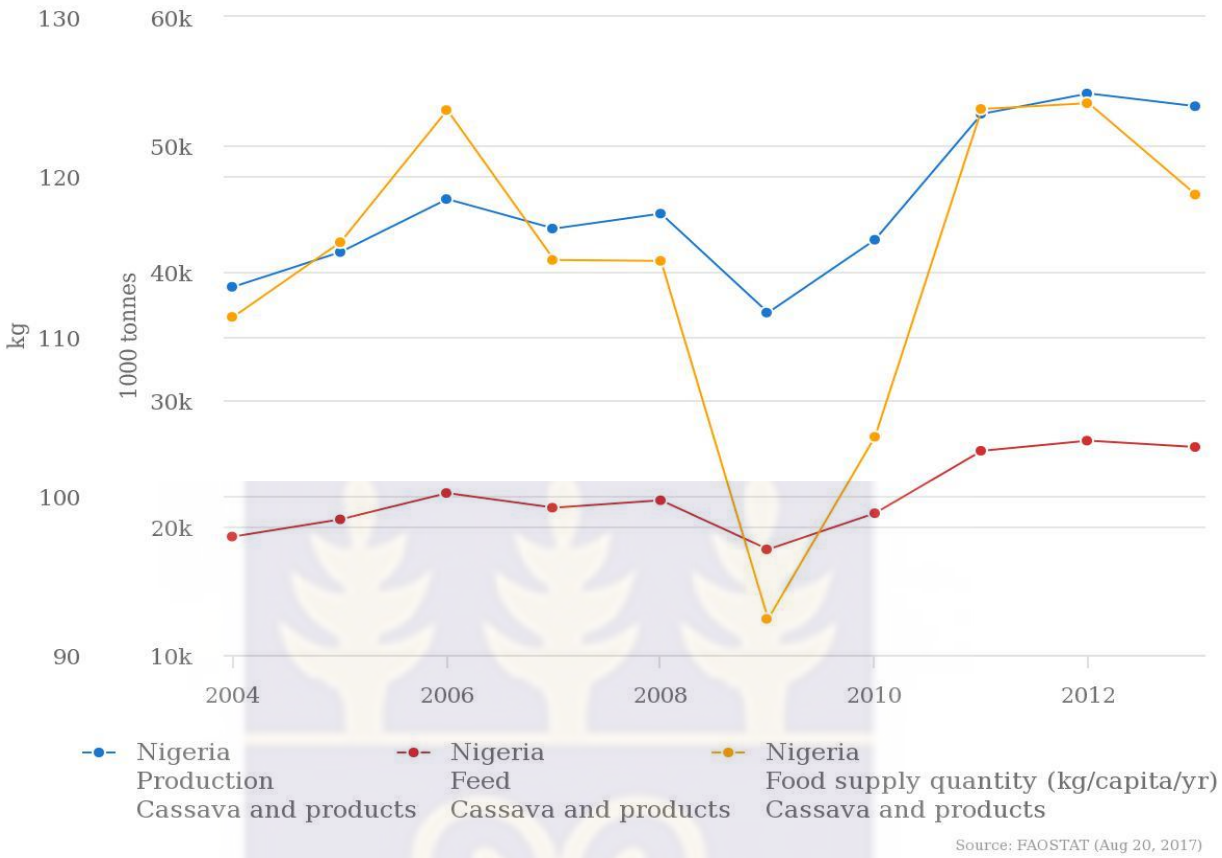


Fig 1. 2 Production and consumption levels of cassava in Nigeria, 2004 – 2012 (FAOSTAT, 2017)

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and adaptation of cassava

The center of origin of the 98 species of the genus Manihot are found in the neotropics (South America) with a main center of diversity in Brazil, and a secondary center in Meso-America (Central America) (Allem, 2002).

Although cassava is considered a polyploidy species, analyses carried out during diakinesis and metaphase I showed the presence of eighteen small and similar bivalents in cassava (Hahn *et al.*, 1990). In some cases occurrence of univalents/trivalents and late bivalent chromosome pairing has been observed. Cassava is, therefore, a functional diploid with chromosome number of $2n = 36$ (Jennings, 1963; Nassar and Ortiz, 2009). Magoon *et al.*, (1970), suggested that certain portions of the genome may be duplicated and cassava may be a segmental allopolyploid origin.

Cassava crop is grown mainly between 30^0 S and 30^0 N latitude with altitudes up to 2000 m near the equator. When the crop moves to the north or south of the equator, the maximum altitude at which it grows and produces will decrease. Most cassava are not found in areas where the average temperature is less than about 20^oC, although in areas near the equator where seasonal temperature variations are small it can be found growing in areas with low temperatures up to 17^oC (Cock, 1982, 1985).

Cassava is widely grown in areas with rainfall average of over 1000 mm per year, although it is has been found in areas where rainfall average is below 750 mm a year and with some years with as little as 600 mm/year distributed over as little as five months. The crop can be found in areas with up to 3000 mm of rainfall per year, but it does not stand poor drainage and flooding.

Cassava is well adapted to low fertility soils and very tolerant to low soil pH that predominate in large areas of the tropics. It is often cultivated on the highly weathered and leached Oxisols, Ultisols and Alfisols, with smaller areas found on Inceptisols (especially in India) and Entisols (Cock, 1982, 1985; Howeler, 2002).

2.2 Genetic resources of *Manihot* species

Genetic resources of cassava comprise local or introduced landraces, improved cultivars, genetic stocks and related wild species (Nassar *et al.*, 2009). All known cultivars of cassava belong to the species *M. esculenta*.

In 2002, Allem, suggested that there are three *M. esculenta* subspecies: *escultenta* (cultivated cassava) *flabellifolia*, and *peruviana*. These three subspecies along with the closest wild relative (*M. pruinosa*) constitute the primary gene pool. The secondary gene pool are *M. triphylla, M. pilosa, M. brachyloba, M. anomala, M. epruinosa, M. gracilis, M. tripartita, M. leptophylla, M. pohlii, M. glaziovii, M. dichotoma, M. aesculifolia and M. chlorosticta* (Allem, 2002).

The largest *ex situ* cassava collections are held *in vitro* by CIAT with about 6,500 accessions, Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) - Brazil holds about 4,000 accessions and IITA with about 3,700 accessions. Other cassava genebanks are those in Central Tuber Crops Research Institute (CTCRI) - India, National Agricultural Research Institute (INIA) - Peru, National Root Crops Research Institute (NRCRI) - Nigeria, Instituto Agronomico Nacional (IAN)

- Paraguay, and Plant Genetic Resources Centre/ Crop Research Institute (PGRC/CRI) – Ghana (Nassar and Ortiz, 2009).

2.3 Recent trends in production and utilization of cassava

Cassava is an exceptional crop because all parts of the cassava plant are used. Cassava leaves can be used to make soup or for livestock feed; the stems can be used for planting, for mushroom production or as firewood. The root can be cooked or eaten fresh which are processed for starch, flour, gari and bio-ethanol production.

Cassava originated in South America and was domesticated less than 10,000 years ago, with evidence of early cultivation in Brazil, Peru, Colombia and Venezuela (Allem, 2002; Elias *et al.*, 2001; Nassar and Ortiz, 2009). Early European merchants recognized the value of the crop and carried it from Brazil to West Africa. By the end of the 16th Century, cassava began to spread in Eastern Africa and a century later had reached the islands of Réunion, Madagascar and Zanzibar (Janssens, 2001). From there, the merchants later introduced it to Asia to be grown as a food security crop and for extraction of starch. Cassava does not grow in temperate regions and so far its products are not well known outside the tropical and subtropical regions where it is grown and consumed.

Out of the 105 cassava producing countries, those in Africa produce the most (50%) cassava worldwide while 30% and 20% come from Asia and Latin America respectively (FAOSTAT, 2017). The top ten cassava producing countries in the world today are Nigeria (54.8 million tonnes), Thailand (30.0 million tonnes), Indonesia (23.4 million tonnes), Brazil (23.2 million tonnes), Ghana (16.5 million tonnes), Democratic Republic Congo (14.7 million tonnes), Viet Nam (10.2 million tonnes), Cambodia (8.3 million tonnes), India (8.1 million tonnes) and Angola (7.6

million tonnes) (FAOSTAT, 2017) (Fig 2.1). In the early 1990s, Nigeria replaced Brazil as the leading producing country globally (FAOSTAT, 2017) (Fig 2.2) and currently accounts for a large proportion of the cassava area (Fig. 2.3). There are forces that explain this dramatic growth. First, demand for cassava has expanded because of rapid population growth and increased poverty therefore encouraging consumers to search for cheaper sources of calories. Second, the supply of cassava has expanded because genetic research and better agronomic practices have boosted cassava yields. Collaborative studies of cassava in Africa found that in Nigeria availability of improved processing equipment like mechanized cassava graters was related to an increase in the area planted to cassava. Although, Nigeria is the largest producer of cassava, it is not the highest yielding cassava country. The highest yields (more than 20t/ha) are obtained in Asia with India ranking first (35.6 t/ha) (FAO, 2014) (Fig 2.4). In Africa, average yields are low (less than 12 t/ha), mainly because the crop is almost exclusively grown by smallholders in a slash and burn system without agricultural mechanization and chemical fertilizers or organic manure application.

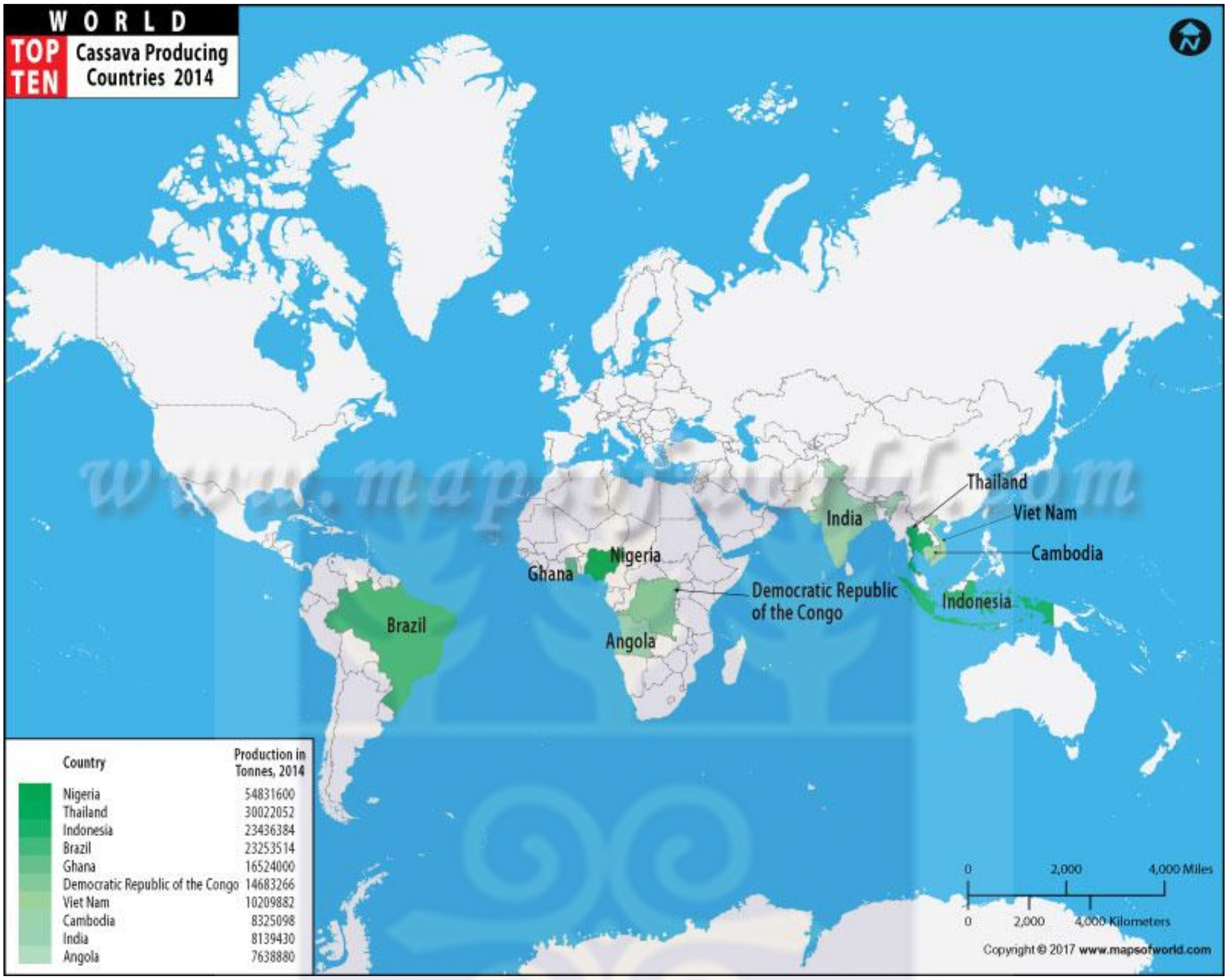


Fig 2. 1 Top ten cassava producing countries in 2014

(FAOSTAT, 2017).

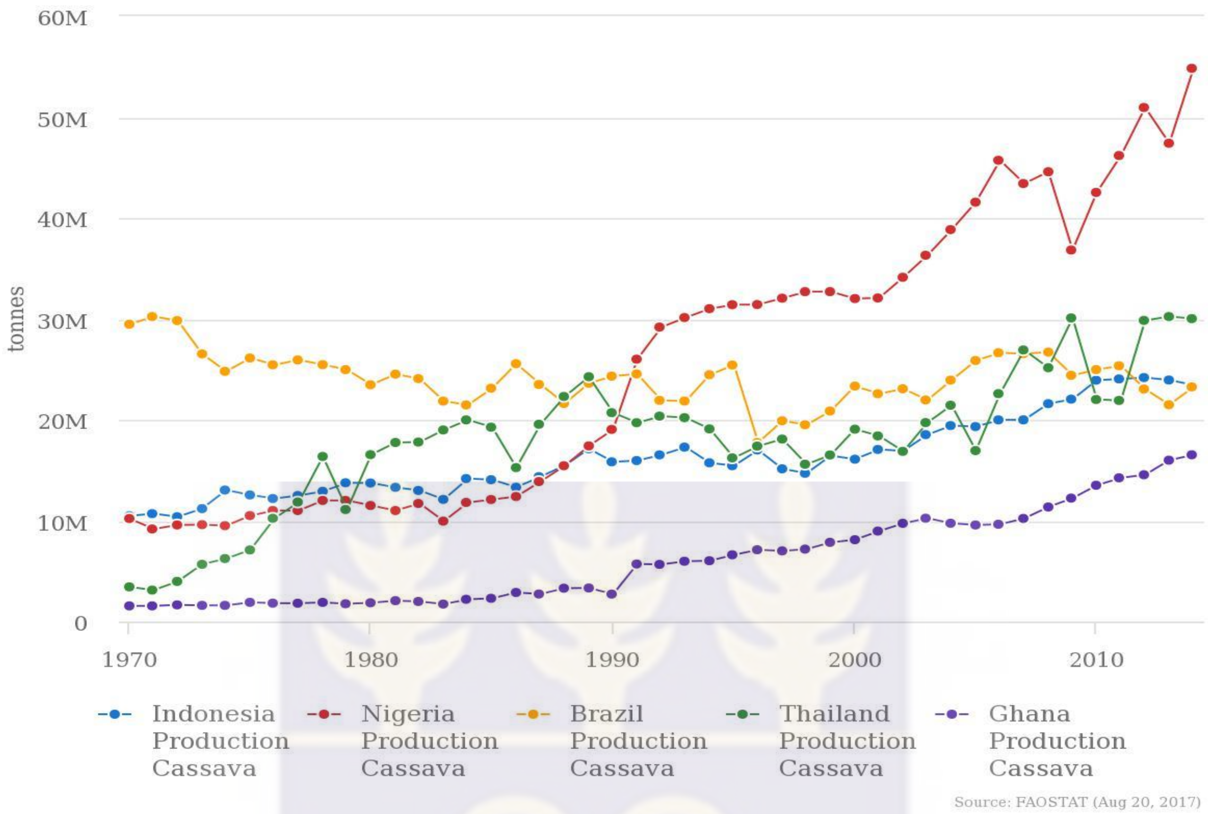
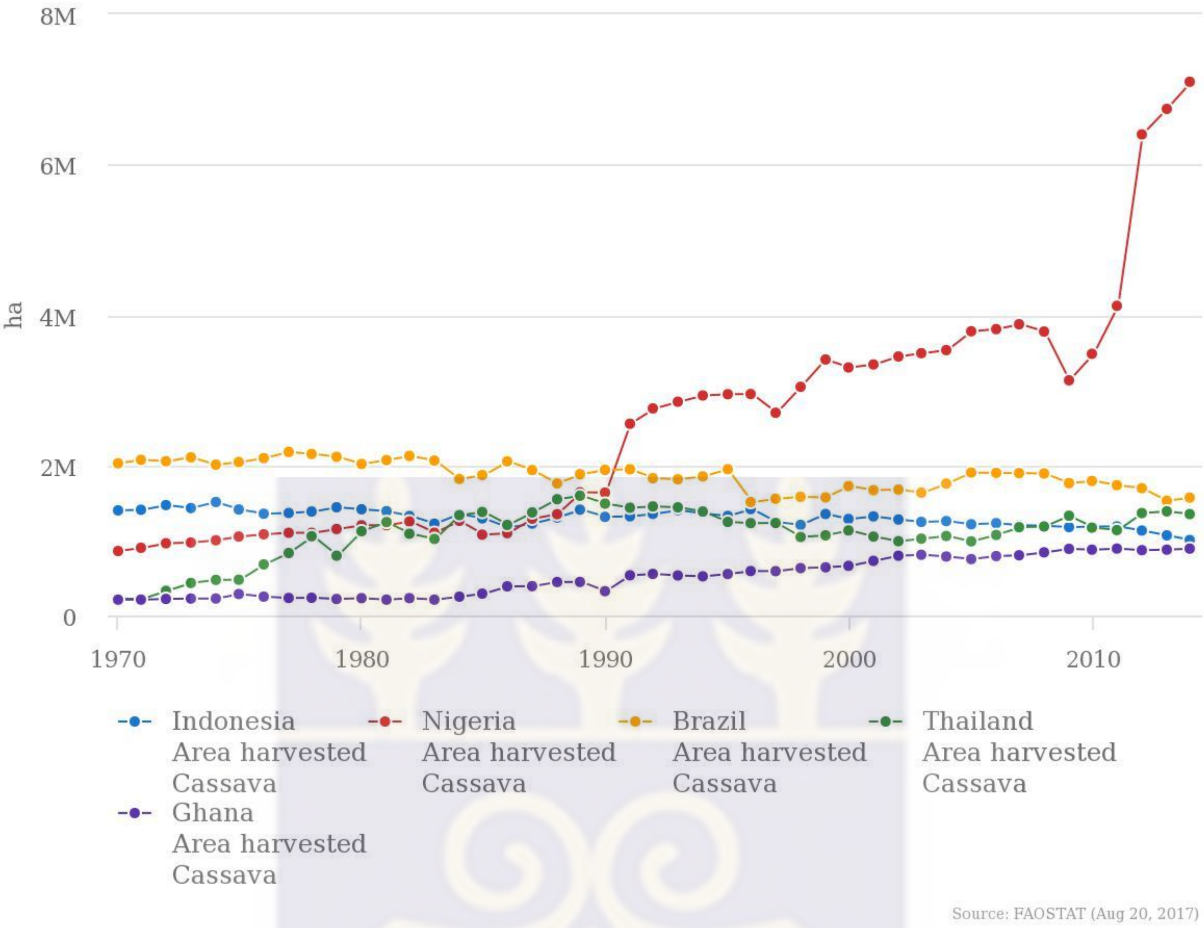


Fig 2. 2 Annual Cassava production (tons) for the top five producing countries over the period 1970 – 2014.

(FAOSTAT, 2017).



Source: FAOSTAT (Aug 20, 2017)

Fig 2. 3 Area cultivated with cassava (ha) for the top five producing countries over the period 1970 – 2014.

(FAOSTAT, 2017).

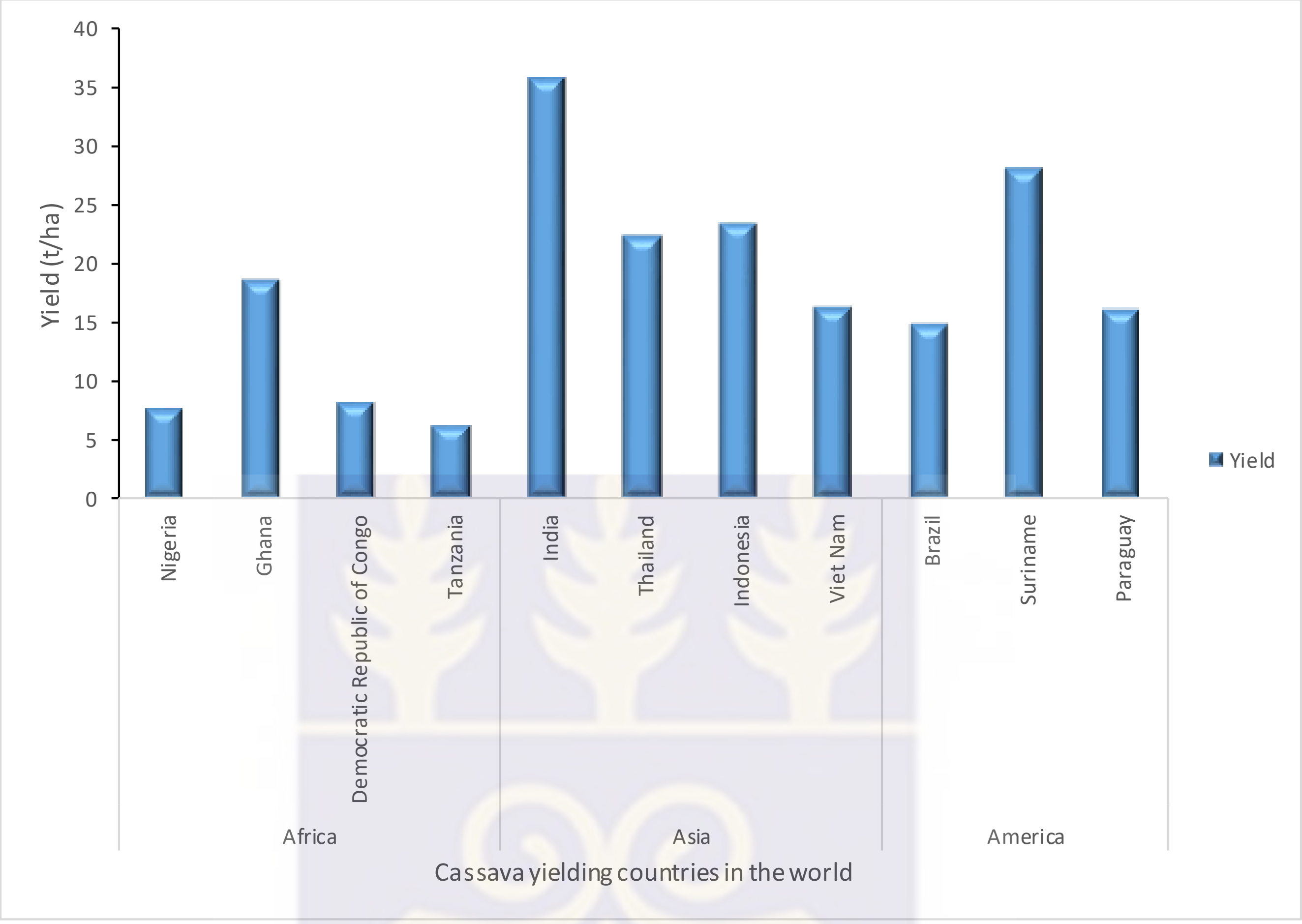


Fig 2. 4 Average cassava yielding (t/ha) in selected countries in the world (FAO, 2014).

2.4 Production constraints in Nigeria

High and stable productivity of cassava depends on adaptation to biotic and abiotic stresses specific to the cassava-growing environment (Ceballos *et al.*, 2012). In Nigeria, cassava mosaic disease caused by geminiviruses and transmitted from one cassava plant to another by the whitefly, *Bemisia tabaci*, bacterial blight, caused by *Xanthomonas axonopodis* pv. Manihotis, anthracnose disease caused by *Colletotrichum gloesporioides* f.sp Manihotis (William, 2012), root rot disease caused by *Botryodiplodia theobromae* (Onyeka *et al.*, 2005) and brown leaf spot (*Cercosporidium henningsii* Allesch (Unartngam, 2012) are having devastating effects on yield and the availability of planting material (Hillocks and Wydra, 2002). Pests that particularly decrease cassava productivity in Nigeria include cassava green mite (CGM) (*Mononychellus tanajoa*), mealybugs (*Phenacoccus manihotis*), termites (*Microtermus sp*.) and rodents. In addition to the biotic and abiotic stresses, cassava production is also influenced by the socio-economic factors such as size of farmland, labour, land revenue system, farm mechanization and equipment, transportation facilities and marketing facilities (Fischer *et al.*, 2005).

2.5 Biology, origin and ecology of cassava green mite

2.5.1 Cassava mites

Approximately 45 species of phytophagous mites have been reported to feed on cassava in the Americas, Africa and Asia (Bellotti *et al.*, 2012; Bellotti, 2008). The most important are *Mononychellus tanajoa*, (a synonym of *M. progresivus*), *M. caribbeanae, M. mcgregori, Tetranychus cinnabarinus, T. urticae* (also reported as *T. bimaculatus* and *T. telarius*), *T. truncates, T. kanzawai, T. neocalidonicus, Oligonychus biharensis* and *O. peruvianus*. Cassava is the major host for the *Mononychellus* species, while the *Tetranychus* species tends to have a wide host range. *M. tanajoa*, the Cassava Green Mite (CGM), is native to the Neotropics and is the most important mite species, causing crop losses in both the Americas and Africa. *M. tanajoa* has not been reported in any of the Asian cassava-producing countries, but the closely related species have been reported.

2.5.2 Bioecology of cassava green mite

The life cycle of CGM follows a pattern typical of many *Tetranychinae*. Reproduction is arrhenotokous (Roy *et al*., 2003); it is a form of parthogenesis in which unfertilized eggs develop into males. There are four active stages: a six-legged larva, two nymphal stages (proto- and deutonymph) and the adult stage (Fig. 2.5). The growth rate and development of *M. tanajoa*, depends on temperature, rainfall, humidity, host plant and sex (Yaninek and Hanna, 2003). In a study with the CGM in Nigeria, temperature of 27°C, with relative humidity of 70% and a photoperiod of 12 hours light and 12 hours darkness, the developmental times of egg, larva, protonymph and deutonymph on leaves of cassava (variety TMS 30572) were 5.4, 3.0, 1.1 and 2.8 days, respectively (Yaninek *et al*., 1989). At this temperature, the adult female mite lives for 11.6 days including a day for preoviposition and 9.8 days of oviposition and lays an average of 62.8 eggs over a period of 9.8 days with a maximum reproduction rate of 43.2 progeny. Egg to adult developmental periods were estimated to be 21.3, 15.5, 12.3, 7.7 and 6.9 days at 20, 24, 27, 31 and 34°C, respectively. The average life span of these females is 24.4 days. Studies in Nigeria also show that CGM populations have two peaks in a year: there is one peak at the end of the wet season (November- December) and another at the start of the rainy season (March-April). CGM is a dry-season pest and thrive in the lowland where high temperatures prevail. Low temperatures and constant rainfall increase the mortality rate of the mites and population density.

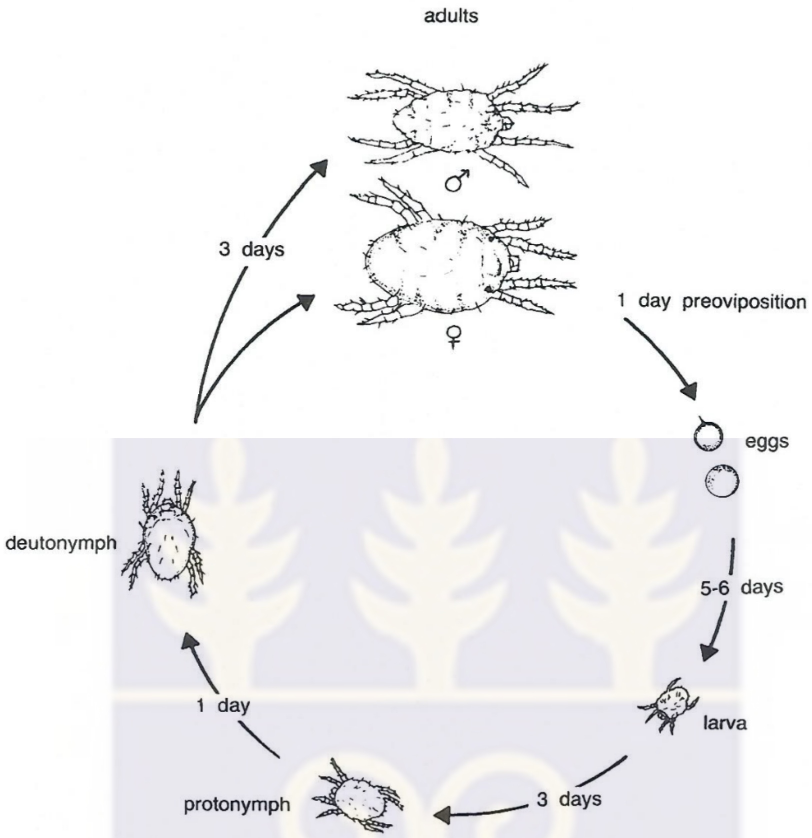


Fig 2. 5 Life cycle of CGM at temperature of 27^{0}C with relative humidity of 70%. Egg to adult – 12.5 days, adult life span – 24 days, fecundity – 60 days.

(Yaninek *et al.*, 1989)

2.5.3 First report and spread of CGM in Africa

In 1971, a species of mite was seen attacking cassava in Uganda, East Africa. The mite was identified as *Mononychellus tanajoa* (Bondar), an exotic species of Neotropical origin (Nyiira, 1972; Lyon, 2009). This mite was introduced into Uganda on cassava cuttings imported from Colombia, South America (Nyiira, 1972). By 1974, the mite had spread to all countries bordering

Uganda (Ndayiragije, 1984; Shukla, 1976). It continued to spread from Tanzania into Zambia, Malawi and Mozambique (Bellotti *et al*., 1987; INGRAM, 1982; Nyiira, 1972). By 1977, it had infested most of the cassava in East Africa (Yaninek *et al*., 1993). Spreading west from Uganda, *M.tanajoa* made a sudden leap across much of central Africa to Congo (Nyiira, 1982). *M.tanajoa* was first found in West Africa in Nigeria in 1979 (Yaninek *et al*., 1989). In West Africa, *M. tanajoa* moved rapidly across the broadly similar vegetation from Nigeria to Benin, Togo, Ghana, Ivory coast at an average rate of 600km/year (Yaninek *et al*., 1989). The speed of spread decreased to 250km/year as the mite moved through the rain forest in Ivory coast, Liberia, Sierra Leone and Guinea Conakry by 1985 (Yaninek *et al*., 1989). The mite has spread throughout the African cassava belt and threatening a crop that is often the last major food source available for harvest during drought condition (Herren, 1989). CGM is spread by infested planting material and by wind, but it is not clear how the mites are carried on the planting material. However, the most important method by which CGM is dispersed is by wind. In the morning, adult mites lower themselves from the leaves on silken threads so that even low wind currents can carry them over long distances. This may account for the rapid spread of the mite (300 km/ year) (Mcgregor, 2016).

2.5.4 Damage and yield losses caused by CGM

Cassava green mites are green to yellowish in colour and can be barely seen with naked eyes (Yaninek and Hanna, 2003). They have piercing and sucking feeding habits. They insert their chelicerae (stylets) into the abaxial surface of cassava leaves and suck out the fluid content of palisade and spony mesophyll cells (Yaninek *et al*., 1989). This causes chlorosis which increase from a few whitish to yellowish appearance to complete loss of green pigment (Bellotti *et al*., 2012).

Heavy infestations of CGM can cause defoliation starting from the apical tip of plant and lateral buds down to the shoots, resulting in severe candlesticks and often times die back might occur. CGM diminishes the plant's photosynthetic capacity and growth rate, by reducing the leaf area of the plant (Tomkiewicz *et al.*, 1993). Damage by the mite affects the quantity and quality of planting material, increases weed infestation and root rot disease in cassava (Yaninek *et al.*, 1989).

Yield losses caused by CGM have been estimated at 10 to 80% in the Neotropics and Africa (Yaninek and Hanna, 2003). CGM- induced losses are more severe when dry season is longer (3 to 6 months) and less severe when the dry season is shorter (Byrne *et al.*, 1983b). Yaninek, (1994) found out that increasing populations of CGM during the dry season significantly reduced the dry matter content found in the leaves, stems and roots. From that study, CGM had reduced the dry matter content in the roots by 10 to 30% during the dry season and this increased to 25 to 45% gains during the wet season. Environmental stress such as drought was an additional reason for reduction in the dry matter content (Byrne *et al.*, 1983a). Byrne *et al.* (1983a), found an average 73% root yield loss and 67% reduction in stem yield in susceptible cultivars. However, Cock, (1982) reported that a 10% drop in the photosynthetic rate of cassava caused a 20% decline in dry matter production. Schulthess *et al.* (1987) found that the dry matter lost during the dry season depended on the amount of stress caused by drought, other pests or pathogens. From that study, the presence of CGM on drought stressed plants significantly increased dry matter losses in cassava. A strong negative correlation was observed between plant height and CGM severity (Egesi *et al.*, 2007). Cassava plants recover from drought stress during the subsequent rainy season, but not from mite damage (Yaninek and Hanna, 2003). New plant growth is triggered by rainfall and mites are washed off the leaves during rainy seasons. Mites can survive on leaves, stems and cuttings removed from the field for a period of up to 60 days (Yaninek and Hanna, 2003) .

2.5.5 Alternative plant hosts of cassava green mite

Cassava green mites do not only affect cassava (*Manihot esculenta*), it also affects other *Manihot* species such as *M. glaziovii* and *M. dichotoma* (Euphorbiaceace) (Bastos *et al*., 1985; Ezulike *et al*., 1993). De Moraes *et al*. (1995) reported the occurrence of CGM on summer squash *Cucurbita pepo* L. (Cucurbitaceae), Tomato *Lycopersicum esculentum* Mill (Solanaceae) and fig leaf squash *Sechium edule* (Jacq.) (Cucurbitaceae) in the northeastern Brazil. However, under field observations in northeastern Brazil the same authors also reported the presence of CGM on wild passion fruit *Passiflora cincinnata* Mart (Passifloraceae), wild cassava *M. pseudoglaziovii* Pax. et K. Hoffm. (Euphorbiaceae), swampmallows *Pavonia cancellata* Cav. (Malvaceae), potato tree *Solanum erianthum* D. Don. (Solanaceae), Brazilian orchid tree *Bauhinia forficata* Link (Caesalpiniaceae), shrubby false button weed *Borreria verticillata* G.F.W. Meyer (Rubiaceae), wild-bush bean *Macroptilium martii* Benth. (Fabaceae), Juruebba *Solanum paniculatum* L. (Solanaceae), Red tasselflower *Emilia sonchifolia* DC. (Asteraceae) and Jack beans *Canavalia brasiliensis* Mart. ex Benth. (Fabaceae).

CGM was only temporarily found on most of those plants after reaching very high numbers on cassava and dispersing by the wind to nearby vegetation. However, CGM developed to the adult stages and lay eggs on *P. cincinnata* and *M. pseudoglaziovii*, indicating that they are the major alternative host plants (De Moraes *et al*., 1995).

2.5.6 Measures of controlling CGM

2.5.6.1 Chemical control

Cassava is a long-cycle crop and mostly grown by small-scale farmers with few resources. Chemical control, although technically possible, is not economically feasible for low-income farmers (Bellotti *et al.*, 2012). Even low doses of pesticides have adverse effects on natural enemies and reduced the yield by 33%. Chemical treatments usually cause secondary pest outbreaks and posed a threat of pest resurgence due to rapidly induced pesticide resistance in the long term (Nyiira, 1982).

2.5.6.2 Cultural/agronomic practices

Early research on CGM concentrated on modifying cultural / agronomic practices to reduce mite losses. Most of the recommendations are still useful, but their impact is limited owing to technical, social and economic factors (Bellotti *et al.*, 1999; Byrne *et al.*, 1983b; Nyiira, 1982). Cassava plants aged 2-9 months are the most vulnerable to infestation (Bellotti *et al.*, 2012). Adjusting the planting times, the way the cuttings were planted, intercropping cassava, detopping and removal of infested leaves have been the principal forms of protecting cassava plant from CGM attack. Ezulike *et al.* (1993) reported that the cuttings planted in a slanting position had mites on the leaves soon after sprouting, but those planted horizontally did not. Cassava intercropped with pigeon pea suffered less damage from CGM and gave higher yield than those grown on a pure stand (Ezulike and Egwuatu, 1990). Detopping of the infested shoot tip has been recommended (Lyon, 2009), but that aggravates the problem since the resulting lateral shoot growth produces even more new leaves.

2.5.6.3 Host-plant resistance

Host plant resistance is defined as those plant attributes that enables a plant to avoid, tolerate or recover from injury by insect populations that would cause greater damage to other plants of the same species under similar environmental conditions (Chalwe, 2012). The aim of host plant strategy is to develop varieties that have low to moderate levels of resistance to the CGM (Bellotti *et al*., 2012). Certain plant attributes like stay green ability, leaf pubescence and retention have been reported to suppress the initial buildup of CGM population. The importance of leaf pubescence as a possible mechanism of resistance is disputed (Ayanru and Sharma, 1983; Hahn *et al.*, 1989). Immunity to CGM is not yet known (Bellotti *et al*., 1999). More recently, studies on leaf pubescence have been shown to favour the colonization of biological control agents such as *T. aripo*.

2.5.6.4 Biological control

Biological control method involves the use of CGM natural enemies to control the population of CGM. A complex of indigenous natural enemies were found associated with CGM in Africa, but it was not considered sufficiently effective to control the pest (Nyiira and Mutinga, 1977). Natural enemies of CGM are found in the families of Chrysopidae, Cecidomyiidae, Syrphidae, Anthocoridae, Lygaeidae, Staphylinidae, Coccinellidae and Phytoseiidae (Byrne *et al*., 1983a; Murphy, 1984; Yaseen and Bennett, 1977). However, Phytoseiidae are the most common predators of mite in the Neotropical region (Bellotti *et al*., 2012; Byrne *et al*., 1983b; Yaseen and Bennett, 1977). The first introduction of a natural enemy of CGM into Africa was by scientists from the commonwealth Institute of Biological Control. Later in 1980, IITA began the Africa-wide Biological Control Project (ABCP) to control exotic cassava pests using enemies introduced from the Neotropical Region. More than ten species of phytoseiids were shipped from Colombia and Brazil to Africa. The Colombian species were *Galendromus annectens* (De Leon), *Euseius concordis* (Chant) and *Amblyseius limonicus* Garman and McGregor, *Euseius ho*, *Typhlodromalus tenuiscutus*, *Neoseiulus californicus*, and *Galendromus annectens* while those from Brazilian were *Neoseiulus idaeus*, *Typhlodromalus aripo* and *Typhlodromalus manihoti*. None of the Colombian species survived in Africa but the three Brazilian species did. In 1993, *T. aripo* was reported to be the most successful species released in Africa. Post-release survey reported by Yaninek *et al.* (1993), that the natural enemies associated temporally and spatially with CGM. The disappearance of *T. aripo* increased the severity of CGM on cassava plant. Cassava green mite is still a serious arthropod pest causing considerable damage to cassava in Nigeria, so there is need to look for genetic source of resistance of CGM.

2.6 Integration of molecular marker into cassava breeding

Molecular breeding involves the use molecular marker technology as the novel genetic tool for developing high yielding, pest and disease resistant cultivars (Landjeva *et al.*, 2007; Varshney *et al.*, 2007). Molecular markers offer a spectacular improvement in the efficiency and sophistication of plant breeding:

i. They allow plant breeders /geneticists to identify the resistance genes rapidly and accurately by tagging the presence of important resistance genes.
ii. They also provide significant assistance for increasing selection efficiency through indirect selection for valuable traits via marker-assisted selection (MAS).

MAS offers a potential tool for assisting conventional plant breeding approaches to select phenotypic traits for screening disease resistant crop plants (Todorovska *et al.*, 2009). Therefore, existing plant breeding techniques along with available molecular markers (Gupta *et al.*, 2010) and functional genomic tools (Gupta *et al.*, 2008) can help a breeder for developing superior cassava cultivars resistant against cassava green mite in order to minimize yield losses (Goyal and Prasad, 2010). DNA-based molecular markers like Restriction Fragment Length Polymorphism (RFLP), Random Amplification of Polymorphic DNA (RAPD), sequence-tagged site (STS), Single nucleotide polymorphisms (SNP), Simple Sequence Repeat (SSR), Cleaved Amplified Polymorphic Sequences (CAPS), Amplified fragment length polymorphism (AFLP) and Sequence Characterized Amplified Region (SCAR) have made an immense contribution to cassava breeding and genetics. Interestingly, two SSR markers (NS1009 and NS346) (Ceballos *et al.*, 2010; Choperena, 2007) and two SNPs markers (qCGMc5Ar and qCGMc10Ar10) (Nzuki *et al.*, 2017) were found to be associated with the resistance to CGM. According to Ceballos *et al.* (2015), another important application of molecular biology for cassava is in diversity studies (Asante and Offei, 2003; Kawuki *et al.*, 2009), ethnobotany, evolutionary and hybridization studies (Duputié *et al.*, 2007; Pujol *et al.*, 2005) and basic research on the origin of cultivated cassava and its taxonomy (Duputié *et al.*, 2011).

According to Ceballos *et al.* (2015), molecular markers research has also explored areas related to nutrition such as cyanogenic glucosides (Balyejusa *et al.*, 2007), carotenoids content in the roots (Esuma *et al.*, 2016b; Welsch *et al.*, 2010) and post-harvest physiological deterioration (PPD) in cassava roots (Reilly *et al.*, 2007). Plant architecture, early bulking and root yields have also been linked to different types of markers (Boonchanawiwat *et al.*, 2011; Okogbenin and Fregene, 2003; Okogbenin *et al.*, 2008).

Cassava has witnessed the same evolution of molecular markers in relation to genetic improvement observed for other crops, although lagging well behind crops with a much higher commercial breeding investment, such as rice or maize. Earlier markers such as random amplified polymorphisms, restriction length polymorphisms, and amplified fragment length polymorphism have been gradually replaced by simple sequence repeat (SSR) markers and single nucleotide polymorphisms (SNPs). The cassava genome has been recently sequenced (Prochnik *et al.*, 2012). Consolidated information can be found at http://cassavabase. org. Sequencing the cassava genome is expected to increase knowledge on the molecular interactions mediating growth and development processes in cassava.

2.6.1 Quantitative trait locus (QTL) mapping

Quantitative trait locus (QTL) mapping is a powerful and well-established tool for studying the genetic basis of complex quantitative traits in plants and animals (Yan *et al.*, 2011) .The association of phenotypic trait values with segregating alleles of molecular markers in a mapping population is referred to as QTL mapping. QTL mapping detects genomic regions that explain phenotypic variation in a trait of interest and the subsequent identification of potential causal genes in that region (Collard *et al.*, 2005).

QTL are regions on the chromosomes, which are physically linked to a molecular marker allele. With the recent advances in the development and success in the applications of association mapping in dissecting a number of simple to complex traits in many crop species demonstrate powerful gene tagging tool for crops in the plant genomics era of 21st century.

2.6.2 Association Mapping

Association mapping is a high-resolution method for mapping QTL based on principle of linkage disequilibrium that holds a great promise for the dissection of complex genetic traits (Buckler and

Thornsberry, 2002). It is a powerful tool for the dissection of complex agronomic traits and for the identification of alleles that can contribute to the enhancement of a target trait. The power of association studies is determined by the size of the experimental population, the magnitude of the target allele effect, the density of markers used, and the rate of linkage disequilubrum decay between marker and target allele as well as errors in phenotyping and genotyping data and the desired resultant statistical significance level (Gordon and Finch, 2005). Association mapping is a very efficient and effective method for confirming candidate genes or for identifying new genes (Altshuler *et al.*, 2008). It is now being increasingly used in a wide range of plants (Rafalski, 2010), where it appears to be more powerful than in humans or animals (Zhu *et al.*, 2008). Though association mapping is widely used, it has a lower power to detect rare alleles in a population, even those with large effects, than linkage mapping (Visscher, 2008). Association mapping is a useful alternative to standard QTL mapping approaches which involves the correlation of molecular polymorphisms with phenotypic variation in a diverse assemblage of individuals. The comparatively high-resolution provided by association mapping is dependent upon the structure of linkage disequilibrium (LD) across the genome. Association studies can be divided into two broad categories:

(i) Candidate gene association mapping

(ii) Genome Wide Association mapping

2.6.2.1 Candidate gene mapping

The candidate gene mapping is aimed at identifying the most important alleles/genes. It involves genotyping or resequencing the genes considered to have a high probability of association with the phenotype(s) of interest within the germplasm being tested. This method of association analysis is used to identify the candidate gene and level of confidence, the researcher has the belief that a given gene is important for the target trait (Kushwaha *et al.*, 2017)

2.6.2.2 Genome Wide Association Studies

A Genome Wide Association Studies (GWAS) also known as whole genome association study (WGAS) is an approach that involves rapidly scanning molecular markers across the complete sets of DNA or genomes of different individuals to find genetic variations associated with a trait (Altshuler *et al.*, 2008). With the recent development of highthroughput genotyping technologies, genetic variation in many model organisms such as mice, arabidopsis, and maize is being discovered on a genome wide scale (Flint-Garcia *et al.*, 2005). Genome wide association mapping in model organisms has great potential to identify risk factors for complex traits related to human diseases.

QTL mapping and association mapping are the most commonly used tools for dissecting the genetic basis of phenotypic trait variation (Balasubramanian *et al.*, 2009; Brotman *et al.*, 2011; Dobón *et al.*, 2011). In QTL mapping only a limited number of recombination events that have occurred within families and pedigrees can be studied, whereas with association mapping the recombination events that have accumulated over thousands of generations can be exploited (Zhu *et al.*, 2008). QTL mapping has been used most frequently since the 1980s, but association mapping is a promising alternative method for dissecting complex traits and it is currently replacing QTL mapping (Chan *et al.*, 2011, 2010). Increased mapping resolution, reduced research time and larger allele numbers have been put forward as main advantages over traditional QTL mapping (Chan *et al.*, 2011, 2010).

2.6.3 Factors affecting association mapping

Factors that adversely affect marker-trait associations include complex population structure, relationship among parents (kinship), small sample size, quality of phenotypic data, genotype by environment interaction, epistasis effect among genes and low frequency of specific alleles that may increase the detection of false positive associations.

Population structure can cause some allele frequencies to differ significantly between subpopulations, which can create unexpected LD between unlinked loci across the genome (Myles *et al.*, 2009).

Several statistical approaches have been used to control the effect of population structure and genetic relatedness in association analyses. This include structured association (Pritchard *et al.*, 2000), principal components analysis (PCA) (Patterson *et al.*, 2006; Price *et al.*, 2006), non-metric multi-dimensional scaling (nMDS) (Zhu and Yu, 2009), and the unified mixed-model approach that combines both population structure information (Q-matrix) and level of pairwise relatedness coefficients—"kinship" (K-matrix) in the analysis (Flint-Garcia *et al.*, 2005). One most powerful strategy is the unified mixed model approach (mixed linear model, MLM), which captures the multiple levels of relatedness to maximize the rejection of false positives while maximizing the statistical power to identify real associations (Yang *et al.*, 2010; Zhu and Yu, 2009).

2.6.4 General Procedure of Association mapping

- **Association mapping population**

The association mapping population consist of a diverse germplasm including cultivars, breeding lines and landraces. The populations should include a diverse genetic diversity present in the population collection. This population constitutes the association mapping population, association mapping panel, or association panel (Barabaschi *et al.*, 2016).

- **Phenotyping**

The association panel are grown in the field and various traits of interest are evaluated. Phenotyping should be done on replicated trials conducted over locations and years to minimize environmental effects. The trials should be conducted using a suitable experimental design like randomized block design, augmented design, nested design, etc. A precise and reliable phenotyping is critical to any mapping effort (Barabaschi *et al.*, 2016). Unfortunately, this is often ignored and the quality of data is below the standards required.

- **Genotyping**

Genotyping of the populations can be done with SNP markers using the genotyping by sequencing (GBS) procedure (Elshire *et al.*, 2011) using the ApeKI restriction enzyme recommended by (Hamblin and Rabbi, 2014) and read lengths of 100 bp. Marker genotypes are called with the TASSEL GBS pipeline (Glaubitz *et al.*, 2014) and aligned to the reference genome available on Phytozome (http://phytozome.jgi. doe.gov) and described by the International Cassava Genetic Map Consortium (2014).

- **Population structure and kinship analysis**

The marker data is then analyzed to detect and estimate the population structure of the sample using the STRUCTURE program and the extent of kinship among the individuals of the sample using the TASSEL program (Barabaschi *et al.*, 2016)

- **Association mapping and linkage disequilibrium analyses**

A model-based analysis of relatedness of marker-trait associations are done to detect and quantify LD between the markers and the genes/QTL governing the traits of interest.

Linkage disequilibrium (LD) refers to the non-random association of alleles between genetic loci (Barabaschi *et al.*, 2016). LD is a population-based phenomenon, it is generally observed that there tends to be a higher LD between alleles that are located more closely together (Abdurakhmonov and Abdukarimov, 2008). Thus, linkage disequilibrium is an important factor in association mapping. Several statistical parameters can be used to estimate the extent of LD (Hedrick, 1987), most commonly $r^{2,}$ which estimates the correlation between allelic states of two given polymorphic loci. Linkage disequilibrium can be greatly over estimated when sample sizes smaller than 50 individuals are used (Yan *et al.*, 2009).

The estimates of population structure and kinship are used as covariates in the model to minimize false associations between the markers and the genes/QTL of interest. It is necessary to note that these analyses are computationally intensive and therefore require suitable computer programs for their implementation. The most common software used for marker-trait association is the “Trait Analysis by aSSociation, Evolution and Linkage” (TASSEL) Mixed linear model (Yan *et al.*, 2009).

2.6.5 Advantages and applications of association mapping

GWAS is a valuable tool for the detection of novel genes or QTL of important agronomic characteristics in crop plants. This approach as resulted in the establishment of the novel high-throughput genotyping and sequencing technologies (Mackay and Powell, 2007; Oraguzie *et al.*, 2007). In crop plants, major marker-trait association have been detected for important traits in rice (Huang *et al*., 2010; McCouch *et al*., 2016), maize (Yu and Buckler, 2006), wheat (Ravel *et al*., 2006; Tommasini *et al*., 2007; Barabaschi *et al*., 2016), barley (Cockram *et al*., 2010; Kraakman *et al*., 2006; Kraakman *et al*., 2004), sorghum (Hamblin *et al*., 2004), soybean (Hyten *et al*., 2007), arabidopsis (Aranzana *et al*., 2005), potato (Gebhardt *et al*., 2004; Urbany *et al*., 2011), rapeseed and sugar beet (Stich and Melchinger, 2009). GWAS have been particularly productive for cassava, where major associations have been detected for important traits and numerous associated candidate genes were recognized. The traits include including the genetic architecture of cassava mosaic disease (Wolfe *et al*., 2016), beta carotene content (Esuma *et al*., 2016a), shoot weight, fresh root yield, dry matter content and starch yield (de Oliveira *et al*., 2012).

GWAS provide a bridge between genomics and applied plant breeding (McCouch *et al*., 2016). GWAS results can be incorporated as fixed variables into genomic selection models to improve the accuracy of genomic estimated breeding values (Zhang *et al*., 2010; Zhou and Stephens, 2012)

2.6.6 Genotype by environment interaction, performance and stability analysis

Genotype by environment interaction (GEI) refers to the differential response of genotypes, when evaluated in different environments (Fox *et al*., 1997). Multi-location evaluation trials help to reveal GEI, which enables plant breeders to identify superior genotypes and locations that best represent production environments.

Breeders are primarily concerned with high yielding and stable cultivars as much possible as since variety development is time consuming. Developed new variety should have a stable performance broad adaptation over a wide range of environments in addition to high yielding potential.

Evaluating stability of performance and range of adaptation has become increasingly important for breeding programmes. Successful varieties must have good yield and other essential agronomic characters. Besides, their performance should be reliable over a wide range of environmental conditions. The basic cause of differences in stability between genotypes is a wide occurrence of GEI.

In analysing the stability of performance, it is important to describe the basic components of phenotypic variability, the genotype, the environment, and the interaction of the genotype and environment. Genotype refers to any of pure-line variety, clone, inbred-line, hybrid variety, open-pollinated variety, composite variety, synthetic variety, elite breeding lines and others on which the breeder collects performance and trait information (da Silveira *et al*., 2013). Environment refers to the combination of physical attributes of a location and the climatic and other attributes of a specific season (i.e. soil type, fertility, topography, relative humidity, temperature, rainfall, pest/disease challenge) that affect the plant growth in the growing season and a specific location. The genotype by environment interaction refers to the deviation in performance of any attributes of genotypes within the growing environments (Van *et al*., 2016). The most important consequence of GEI is that the different traits under consideration show a change in rank in different environments. Such changes of rank in the genotypes which is called crossover GEI (Kang, 2002) creates inconvenience in plant breeding. Analysis of GEI was used to estimate how much adaptability and stability of a variety if planted in the different environment (Masinde *et al*., 2018).

Adaptability is the ability of plants to adapt to the environmental conditions of growth. Varieties are able to adapt to the broad environment, means that the GEI are small. While the narrow adaptation the variety performance is good in such environment but not in other environments. This means that there is GEI is large (Abate *et al.*, 2015).

Phenotype stability is dependent upon plant's ability to determine its response in different environments. A number of statistical methods are used for estimation of phenotypic stability. These includes; simple linear regression (regression coefficient b-value) across environments (Finlay and Wilkinson, 1963), regression coefficient in conjunction with deviations from regression (Sd^2) models (Eberhart and Russell, 1966) , univariate parametric stability statistics (Francis and Kannenberg, 1978; Lin and Binns, 1988; Shukla, 1972) and nonparametric stability statistics (Akcura and Kaya, 2008).

Other methods includes; multivariate statistical methods; multivariate ANOVA, multiple regression, principal component analysis (PCA), factor analysis, clustering and ordination and Additive Main effect and Multiplicative Interaction (AMMI) model. Multivariate approaches extract more information from GEI components by exploring the multi-directional aspects (Miranda *et al.*, 2009). AMMI analysis is one of the most effective multivariate techniques. AMMI model has widely been used for analyzing GEI in cassava (Adjebeng-Danquah *et al.*, 2017; Esuma *et al.*, 2016), rice (Islam *et al.*, 2014), wheat (Castillo *et al.*, 2012), and cotton (Campbell and Jones, 2005; Naveed *et al.*, 2007). With the AMMI model, additive main effects (genotypes and environments) are first accounted for a regular analysis of variance and the multiplicative effect of GEI is analyzed by PCA (Gauch Jr, 1992) leading to a more exhaustive data analysis, accurate yield estimates and reliable selections.

There are three main benefits of the use of AMMI analysis. First, it can be used as a preliminary analysis to find a more appropriate model (Gauch Jr, 1988). Second, it clarifies the GEI and summarizes patterns and relationships of genotypes and environments (Crossa *et al.*, 1990; Zobel *et al.*, 1988). Third, it improves the accuracy of yield estimates. Gains have been obtained in the accuracy of yield estimates that are equivalent to increasing the number of replicates by a factor of two to five (Crossa, 1990; Zobel *et al.*, 1988).

AMMI Stability Value (ASV)

The ASV is the distance from the coordinate point to the origin in a two-dimensional scatter gram of interaction principal component axis one (IPCA1) scores against interaction principal component axis two (IPCA2) scores in the AMMI model (Purchase, 1997). Because the IPCA1 score contributes more to the GEI sum of squares, a weighted value is needed. This weight is calculated for each genotype and each environment according to the relative contribution of IPCA1 to IPCA2 to the interaction sum of squares (SS). The genotypes with larger IPCA score, either negative or positive, are the more specifically adapted to certain environments and those with smaller IPCA scores indicate a more stable genotype across environments.

Yield Stability Index (YSI)

Farshadfar *et al.* (2011) developed YSI which is similar to genotype selection index developed by (Farshadfar, 2008). It is recommended as a measure of stability. YSI is calculated by summing the rank of mean yield across environments and rank of AMMI stability value of genotypes. The lowest ASV takes the rank one, while the highest yield mean takes the rank one and then the ranks are summed in a single simultaneous selection index of yield and yield stability. The genotypes with lowest value of this parameter are desirable genotypes with high mean yield and stability.

CHAPTER THREE

3.0 FARMERS’ PERCEPTION OF CASSAVA GREEN MITE PEST IMPACT AND PRODUCTION CONSTRAINTS IN NIGERIA

3.1 INTRODUCTION

Cassava is a hardy and reliable crop, which tolerates a wide range of climatic conditions and is able to grow under marginal soil fertility. In Nigeria, cassava is mostly grown by small – scale farmers in traditional farming systems mainly on low fertile soils under low or unpredictable rainfall, using few purchased inputs such as fertilizers and pesticides. Because of the low yields in these systems, pest control has a low priority due to the high costs and long growth cycle that may require use of pesticides.

The dynamics of cassava production are changing as the production is moving towards larger – scale production. In this production system, the cassava crop is found at different growth stages in the same or surrounding fields. Current evidence indicates that pest problems will be increase in these overlapping production systems. Populations of certain pests such as mites, hornworms, whiteflies and mealybugs tend to increase when there is a steady food supply (e.g., young cassava foliage). Under such population conditions cassava green mite (CGM) (*Mononychellus tanajoa*) becomes the key herbivorous arthropod pest, causing economic damage or yield losses. The pest in Nigeria is prevalent in most of the farmers’ field, affecting the local and improved cultivars. National programmes aimed at controlling CGM through resistance breeding and biological control have been carried out as independent units and without active participation of farmers. According to den Berg and Jiggins, (2007) participation of farmers in research is thought to empower local farmers by enhancing local management capacity, increasing confidence in their own abilities. This kind of empowerment increases the adaptation and adoption of a newly developed technology. Knowledge and perception of farmers is necessary for the development of appropriate pest control management strategies in line with farmers' needs (Ojwang *et al*., 2009).

Farmers are continuously innovating in order to cope with the ever-changing environmental, ecological, policy, and market situations, and over the years they have become the custodians of traditional knowledge on many aspects of crop production including pests and coping strategies (Sleper *et al.*, 2006). In the longer term this will be translated into increased rates of adoption and retention of new technologies, and ultimately into a greater and more accelerated reduction in food insecurity and poverty (Vom *et al*., 2010) .

Participatory rural appraisal (PRA) enables close farmer – researcher collaborations to bring about cassava genetic improvement. The participatory appraisal improves the rate of adoption of new technologies and release of new cultivars. PRA was used to identify farmers' preferences in Nigeria, enhanced shelf life, high storage root yield, low level of hydrogen cyanide in cassava processed products, pests and disease resistance, and early maturity (Agwu and Anyaeche, 2007). In Ghana, Manu-Aduening *et al.* (2007) used PRA to describe the characteristics needed for cassava varieties and reported that farmers preferred those cassava varieties that had early growth and vigour to suppress weeds, early maturity, high yield, good cooking quality and suitability for intercropping. Mutisya *et al.* (2015) used PRA to investigate farmers' perceptions on CGM presence on cassava in Kenya. Therefore, a PRA was conducted to assess farmers' preferences, perception and knowledge of CGM and other production constraints and to lay the foundation for the development of CGM resistant cultivars in Nigeria.

The objectives of this study were to:

i. assess farmers’ knowledge and perceptions of CGM
ii. evaluate farmers’ knowledge on the management of CGM
iii. assess traditional knowledge on plant attributes that are associated with reduced pest population
iv. identify farmers’ preferred traits and various constraints to cassava production

3.2 MATERIALS AND METHODS

3.2.1 Study sites

This study was conducted in three major cassava growing states in Nigeria; Anambra, Benue and Abia. Anambra state lies at 6^{0}20’N 7^{0}00’E located in south-eastern part of Nigeria, Benue, 7^{0}47’N 10^{0} 0’E located in north central and Abia, 5^{0}28’33”N 32’ 56”E located in south-eastern Nigeria. In Anambra, farmers were interviewed in Anambra East, Awka South, and Aguata Local Government Areas (LGAs) as shown in fig 3.1. In Abia, the areas included Ohafia, Ikwuano and Osisioma-ngwa (fig. 3.2). In Benue, farmers were interviewed in Ogbadibo, Otukpo and Gwer LGAs (fig. 3.3).

The farmers in these states have a long history of growing cassava as a staple crop. The states have a long history of on-farm research activities, which have over the years afforded a good number of local farmers exposure to improved technologies and cultural practices. Cassava in these states is consumed in different forms. In Anambra, it is consumed mainly in the form of fufu; in Abia, it is consumed mainly in the form of gari, and in Benue, the leaves are used as vegetable. In addition, the production of cassava in these areas is largely concentrated among smallholder farmers whose farming conditions are diverse. The farmers are resource-poor and encounter several production constraints.

Fig 3. 1 Map of Anambra state showing the three study areas (www.anambrastate.gov.ng)

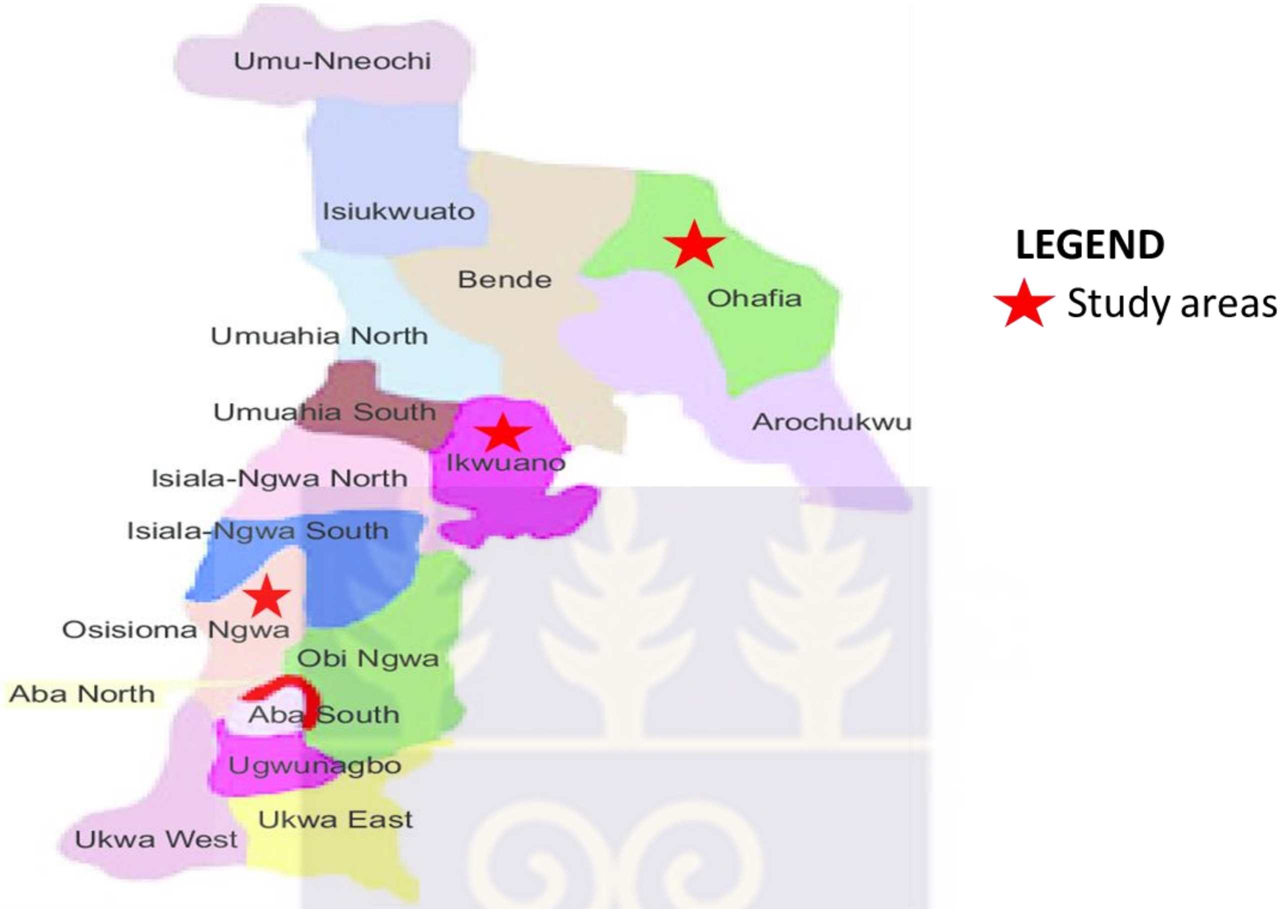


Fig 3. 2 Map of Abia state showing the three study areas (www.abiastate.gov.ng)

Fig 3. 3 Map of Benue state showing the three study areas (www.benuestate.gov.ng)

3.2.2 Data collection and sampling method

A state was used as the basis for sampling. From each state, 120 cassava farmers were sampled. A multistage sampling method was used in selection of farmers to participate in the interviews. The first stage involved purposive selection of states to participate in interviews, which was based on relative importance of cassava production in those states. The second stage involved random sampling of three LGAs, giving a total of 9 LGAs. The last stage involved random sampling of 40 cassava growing households per LGA, making a total of 360 household respondents. The identification of farmer – households was facilitated by the government agricultural extension officers at the LGA headquarters and also by the local council officials. Subsequently, the survey team comprises four agricultural extension officers and a breeder administered questionnaire – led interviews to the farmers. Each of the survey team members interviewed one individual farmer at a time and also made field visits to assess pest distribution and scoring of CGM severity. Cassava Green Mite Severity (CGMS) was evaluated at the visual rating of the damage caused by cassava green mite. A 1 to 5 scoring scale was used to rate symptoms: 1= highly resistant; no symptoms observed, 2= resistant; moderate damage, no reduction in leaf size, scattered chlorotic spots on young leaves, 3= moderately resistant; severe chlorotic symptoms, slight reduction in leaf size, 4 = susceptible; severe chlorotic symptoms and severe reduction in leaf size of young shoot, 5 = highly susceptible; very severe chlorosis, extensive defoliation, candlestick appearance of young shoots.

A focus group discussion (FGD) was also conducted in each of the selected areas. FGD consisted of three groups of 8 members each; Adult men (>40years), Adult women (>40years) and youth (<40years). Each group was assigned a trained extension officer who served as a guide. Farmers were asked to describe symptoms of damage caused by pests affecting cassava, and to provide a list of plant attributes that were considered to confer some level of plant resistance and also the cultural practices that are used to manage pests in their areas (fig 3.4). Pictures of the major pests and their damage symptoms as well as live infested plants were provided to serve as a guideline for the farmers in matching their description with the names of pests.

Fig 3. 4 Farmers conducting preference scoring and ranking of desirable plant attributes

3.2.3 Data analysis

Data collected were subjected to descriptive statistics analysis using a Statistical Package for Social Scientists (SPSS), 16th version (Carver and Nash, 2009).

3.3 RESULTS

3.3.1 Socio – economic characteristics of cassava farmers

3.3.1.1 Gender

Women in Nigeria dominate cassava production and processing. Results of this study showed that 70% of the farmers were women while only 30% were men (fig. 3.5). Benue state has the highest percentage of women cassava farmers (88%); closely followed by Anambra state (86%) and then Abia state (66%). Men see cassava as a women's crop in these surveyed areas.

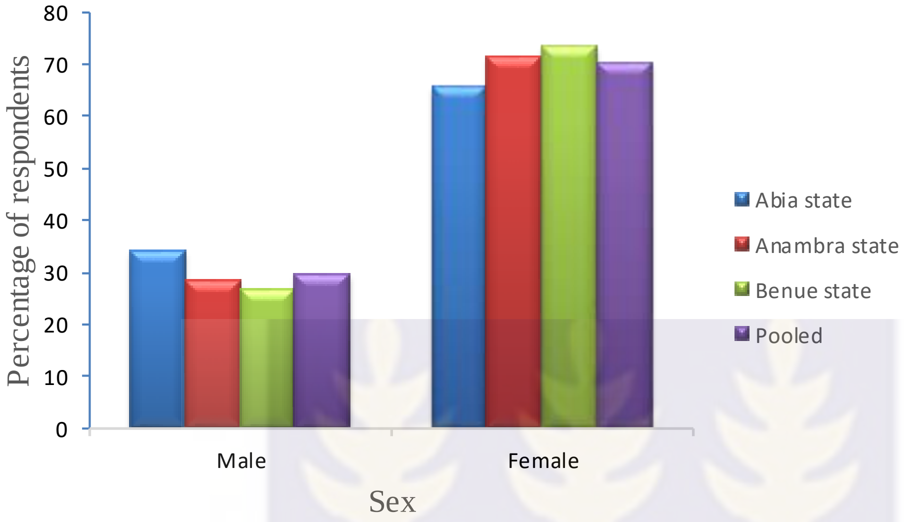


Fig 3. 5 Percent distribution of the respondents by sex

3.3.1.2 Age and marital status

Survey result presented in table 3.1 showed that the modal class of the cassava farmers in all surveyed states was between 25 and 45 years. This represents about 94% of the cassava farmers and is an indication that majority of the cassava farmers were in their middle and active age of life. In Abia and Anambra states respectively, 2% and 4% were young (< 25 years). The majority (98%) of the cassava farmers are aged between 25 and 45 years in Abia and Benue states while 86% was found in Anambra state. In Anambra and Benue states respectively, 10% and 2% could be regarded as fairly old i.e. > 45 years. During the FGD in Anambra state, farmers reported that the youths prefer trading to farming because the state has one of the largest markets in West Africa.

The table also showed that about 77% of the cassava farmers are in marriage, 16% are single while 1% and 6% of them were divorced and widowed respectively. This implies that majority of the farmers have additional responsibilities to their spouses and children.

Table 3. 1 Percentage respondents of Cassava farmers with respect to their age and marital status

	Age			Marital status			
State	< 25yrs	25 - 45 yrs	> 45 yrs	Single	Married	Divorced	Widowed
Abia	2	98	0	7	80	2	12
Anambra	4	86	10	15	78	0	7
Benue	0	98	2	27	73	0	0
Total	2	94	4	16	77	1	6

3.3.1.3 Educational level

Most (89.4%) of the respondents had one form of education or the other. Only 10.6% of the respondents did not have any form of education. In Abia state, 32% of the farmers had primary education, 45% had secondary education, 15% had tertiary education while 8% had no formal education. In Anambra state, 43% of the farmers interviewed had primary education, 33% had secondary education, 20% had tertiary education while 3% had no formal education. In Benue state, 30% of the farmers had primary education and secondary education, 20% had tertiary education while 20% had no formal education (fig. 3.6). This shows that majority of the farmers were literate which might enhance adoption of improved farm practices and production.

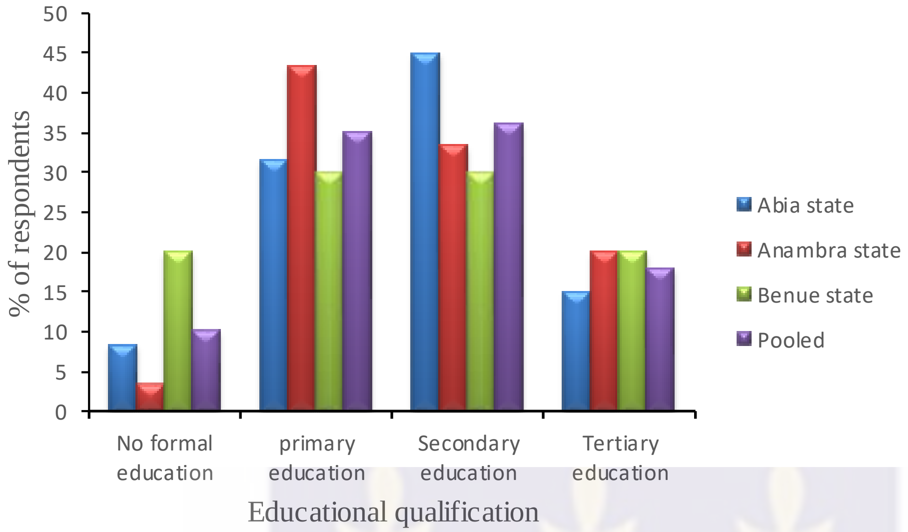


Fig 3. 6 Distribution of the respondents by level of education

3.3.1.4 Household size and monthly income

The majority (59.4%) of the cassava farmers in the surveyed areas had between 6 and 10 members with mean size of 7.23 (Table 3.2). In Abia, Anambra and Benue states respectively 20%, 42% and 17% of the cassava farmers were below 5 members, 63%, 42% and 73% had between 6 and 10 members, 7%, 17% and 13% had between 11 and 15 members. Only Benue state (7%) had above 16 members in a family. During the individual interview in Benue state, a respondent reported that the larger the family size, the increase in labour productivity.

The income level was also investigated and the result shows that most (48.6%) of the cassava farmers in the three states earn within the range of NGN101,000 - NGN500,000 with mean value of NGN437,395.6 (Table 3.2). In Abia, Anambra and Benue state respectively 48%, 18% and 20% earn below NGN50,000 from farm produce. In Anambra and Benue state, 23% of cassava farmers earn below NGN100,000 while only 8% in Abia state. Anambra state had the most (59%) cassava farmers that earn below NGN500,000 followed by (53%) then Abia state (33%). In Abia and Benue states respectively 12% and 18.6% earn between NGN501,000 and NGN1,000,000 while none in Anambra state. This implies that farmers get sufficient income from their farm produce.

Table 3. 2 Percentage distribution of household size and monthly income

	Household size				Monthly income			
State	1-5	6-10	11-15	16-20	NGN5,000 - NGN 50,000	NGN 51,000 - NGN 100,000	NGN 101,000 - NGN 500,000	NGN 501,000 - NGN 1,000,000
Abia	20	63	7	0	48	8	33	10
Anambra	42	42	17	0	18	23	59	0
Benue	17	73	13	7	20	23	53	3
Total	26	59	12	2	29	18	49	4

3.3.1.5 Years of farming experience

None of the respondents had below 10 years of farming experience in the three surveyed states. Only Abia state had respondents (17%) had over 40 years of farming experience. Majority (51.7%) of the farmers had cultivated cassava between 11 years and 20 years. Benue state had the highest (57%) percentage of respondents that had between 11 years and 20 years farming experience, closely followed by Anambra state (55%) then Abia state (43%).

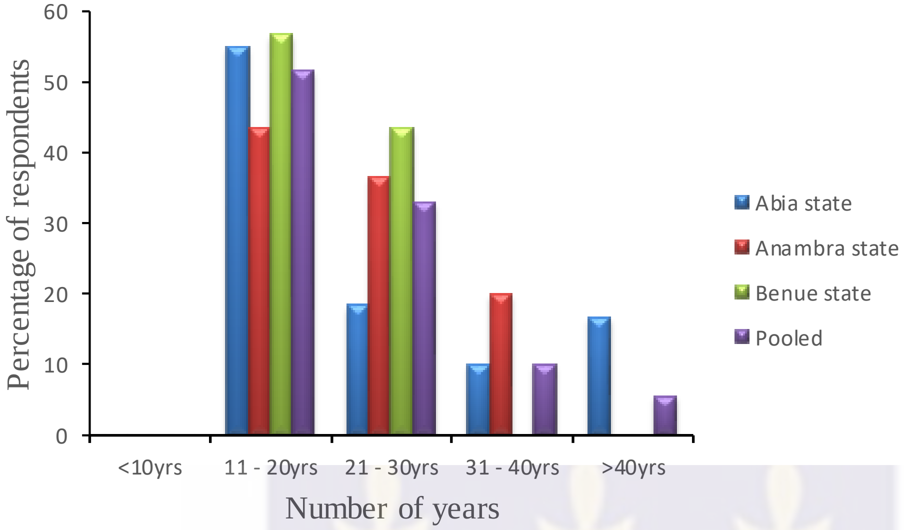


Fig 3. 7 Years of cassava farming experience

3.3.1.6 Farm size

This result in fig. 3.8 showed that most (58.3%) of the respondents cultivated between 1.1 and 2.9 ha with the mean value of 2.5 ha. Also the result showed that quite a sizeable proportion of the respondents (34.4%) cultivated farm land between 3.0 and 4.9 hectares. This implies that of the respondents are small scale farmers. The implication of this to food security is that food production will remain at a subsistence level and this can lead the respondents to diversity into nonfarm activities in other to be food secure.

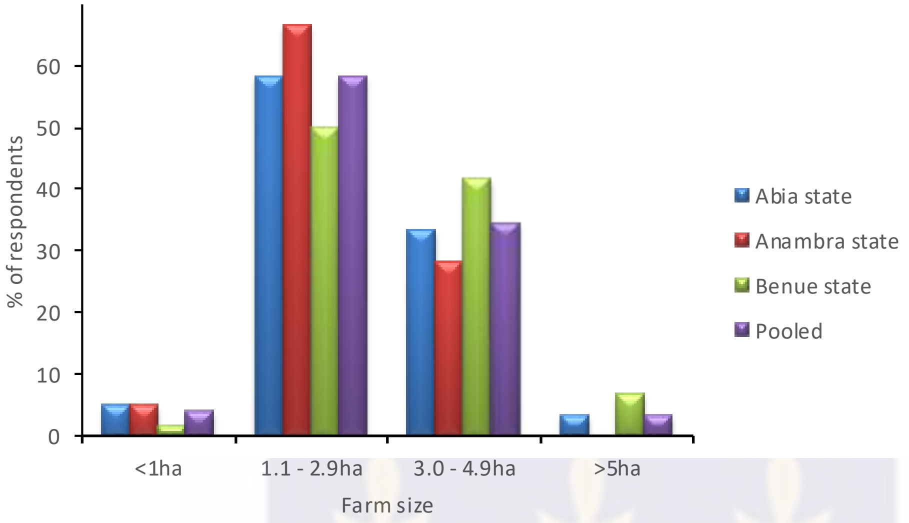


Fig 3. 8 Percentage distribution of the respondents with respect to their farm size

3.3.1.7 Percentage of the farm land grown cassava

Figure 3.9 show that 88.6% of the respondents grow cassava between 51- 100% of their farmland in the three surveyed states. In the essence, 66% and 61% of the respondents in Abia and Anambra state respectively grow cassava between 91 - 100% while only 28% of the respondents in Benue state grow cassava between 91 – 100%. In other words, the percentage of farm land cultivated cassava in south- eastern states (Abia and Anambra states) is more than the percentage of farm land grown cassava in the north- central state (Benue state). This result implies that cassava is important, not just as a food crop but also a cash crop

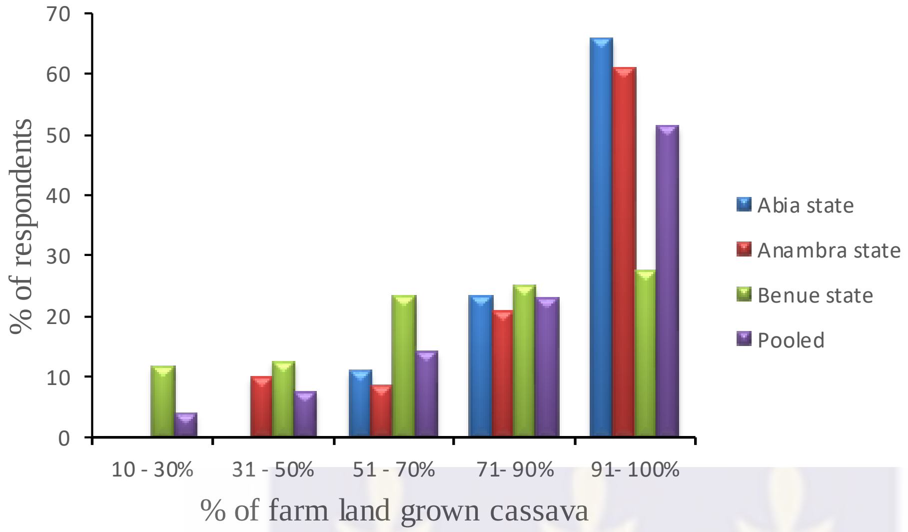


Fig 3. 9 Percentage of farm land grown cassava

3.3.1.8 Sources of cassava stem cuttings for planting

Most of the farmers in the three surveyed states get their planting materials from their own farms, fellow farms and research institute (Fig 3.10). In Abia state, 33% of the farmers obtained stem, while 30% of the farmers from the same state accessed cuttings for planting from their own fields. Only 17% of farmers in Abia state seem to receive planting materials from the Agricultural Development Programmes (ADPs). In Anambra state, 36% of the farmers accessed cuttings for planting from fellow farmers and 23% of the farmers obtained cuttings for planting from a research institute. In Benue state, 39.0% of the farmers obtained cuttings for planting from their colleagues, while the rest obtained cuttings from a research institute (28%) and own fields (33%)

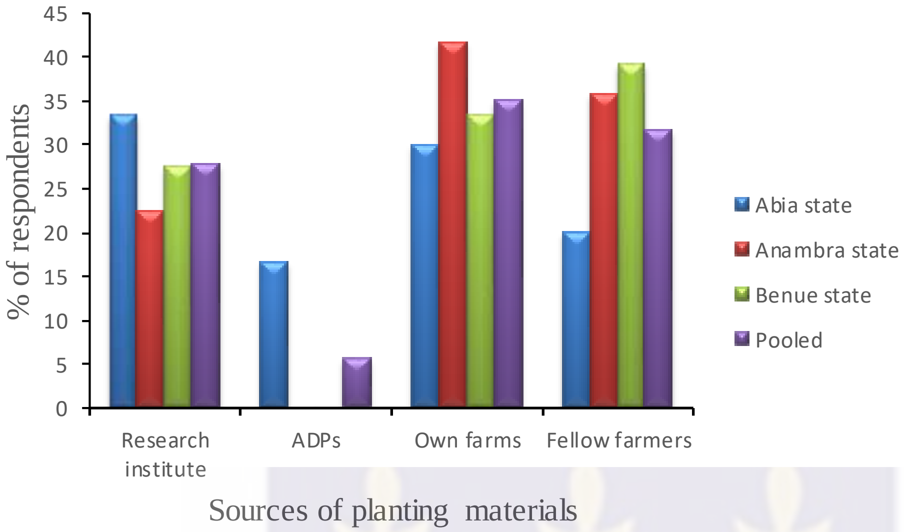


Fig 3. 10 Percentage distribution of the respondents with respect to sources of planting materials

3.3.1.9 Cassava cultivars grown

In each of the surveyed states more than 10 cultivars were identified (Table 3.3). In Abia state, Nwabibi was the most common cultivar grown by 83% of the farmers and the least common was Agric grown by 4% of the farmers. In Anambra state, Akpuocha was the most common cultivar, grown by 100% of the farmers and the least was Government grown by 8% of the farmers. The farmers in Anambra state said that they prefer Akpuocha to any other cultivar because of its whitish colour, which is very good for fufu. In Benue state, Owonono was the most commonly (92%) grown cultivar while Agric was the least (33%) grown cultivar. Onyewarri, Akpuocha and Panya were the most common local cultivars across the three states while yellow root, TMS1368 and Government were the popular improved cultivars across the three states. Farmers grew these cultivars because they are high yielding, have prolonged underground storability and resistance to diseases and pests. Farmers indicated that the improved cultivars were not readily available in their areas.

Table 3. 3 Common cassava cultivars grown in Abia, Anambra and Benue states in Nigeria

	Surveyed states				
Cassava cultivars	Abia (%)	Anambra (%)	Benue (%)	Remarks	Type of Cultivar
Nwabibi	83	67	0	High yielding, prolonged underground storability, high dry matter content	Landrace
Onyewarri	75	75	79	High yielding, disease and pest resistance, more planting materials	Landrace
Nkwo-igwe	71	71	0	High yielding, disease and pest resistance	Landrace
Nwaopoto	63	0	0	High yielding	Landrace
Akpuocha	58	100	83	Whitish colour, early maturity, high yielding	Landrace
Lagos	42	0	0	High yielding	Landrace
Yellow root	29	17	33	Source of vitamin A, prolonged underground storability	Landrace
TME419	25	0	58	High yielding	Improved
TMS1368	13	25	33	High yielding	Improved
Government	8	8	47	High yielding	Landrace
Panya	8	17	42	Boil and eat cassava	Landrace
Otorokwem	8	0	0	High yielding, disease and pest resistance	Landrace
Agric	4	0	33	High yielding	Improved
Owonono	0	0	92	High yielding	Landrace
TMS980505	0	42	83	High yielding	Improved
Dango	0	0	83	High yielding	Landrace
Anurika	0	67	0	High yielding	Landrace
Okoiyawo	0	58	0	High yielding	Landrace

3.3.1.10 Farmers' preferred traits

Farmers suggested a number of traits they would like to see incorporated into CGM resistance cassava cultivars (Table 3.4). Their suggestions did not differ much across the three study areas. The majority of the farmers (86%) preferred high yielding followed by early maturity (81%) and white fufu (76%). High yielding was ranked first by 83%, 83% and 92%, early maturity by 83%, 75% and 83%, white fufu cassava by 71%, 83% and 75% of farmers in Abia, Anambra and Benue states, respectively.

Table 3. 4 Traits preferred by farmers

	Surveyed states				
Farmers' preferred traits	Abia (%)	Anambra (%)	Benue (%)	Mean	Rank
High yielding	83	83	92	86	1
Early maturity	83	75	83	81	2
White fufu	71	83	75	76	3
High dry matter content	75	68	71	71	4
Resistance pests and diseases	68	71	67	69	5
More planting materials	46	38	21	35	6
White flour	25	38	40	34	7
Prolonged underground storability	21	25	32	26	8
Low cyanide	23	21	28	24	9

3.3.1.11 Uses of cassava

All the farmers across the three states indicated that food and income were the major benefits derived from cassava products (Fig 3.11). The result also revealed that 100% of the respondents in the three states ate cassava in the *gari* form. One hundred percent of respondents in Anambra and Benue states ate cassava in the form of *fufu* while 80% was in Abia state. In Abia state, 88% of the respondents consumed cassava as snacks whereas only 3% in Anambra and Benue states. Majority (72%) of the respondents in Anambra state liked cassava products in form of Abacha. Only 3% of the respondent in Abia state ate cassava product in bread form. In Benue and Anambra states, 30% and 3% of the respondents respectively used cassava leaves as vegetables. Only 7% of the respondents in Abia state consumed cassava product in Tapioca form. Only respondents in Benue state (40%) ate cassava in *elubo* (cassava flour) form.

A majority of the farmers (52%) ate cassava in these forms twice daily, 29% of the farmers ate cassava thrice daily whereas 27% ate it once a day. This indicates the importance of these cassava products among Nigerians (fig 3.12).

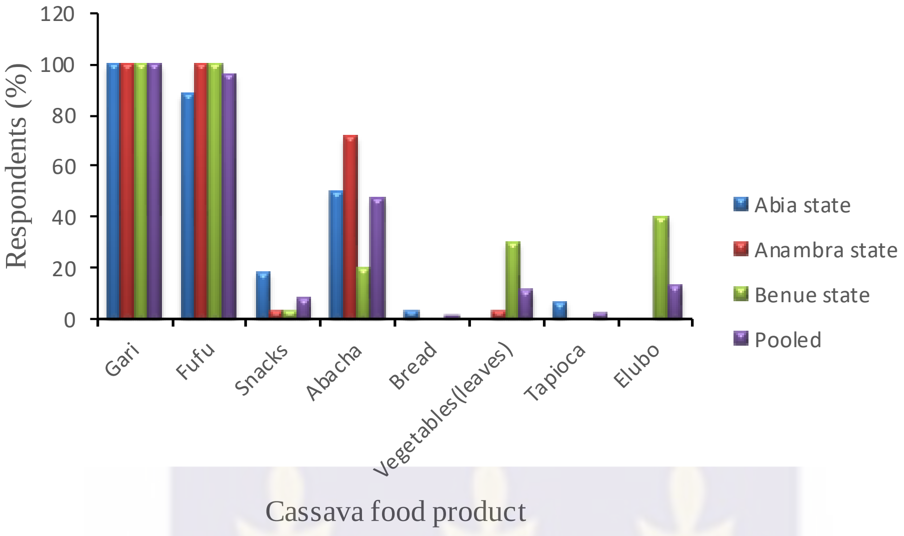


Fig 3. 11 Distribution of cassava products eaten

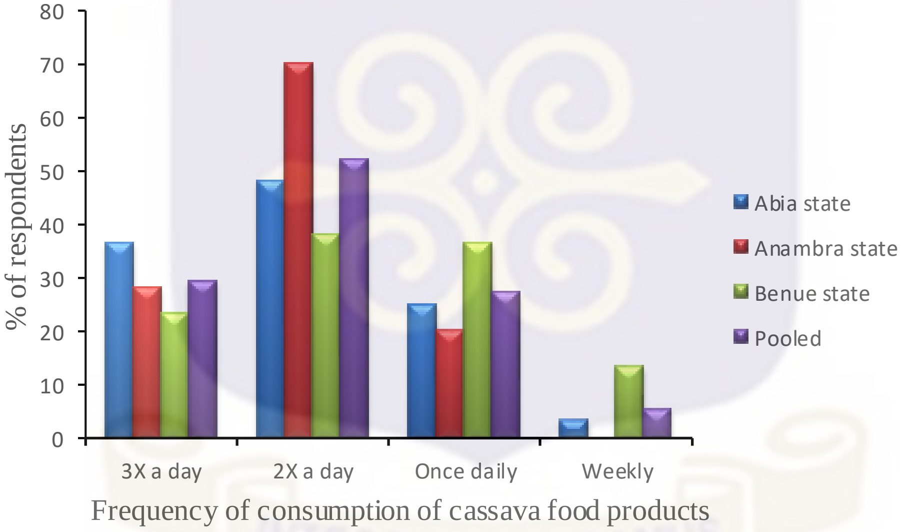


Fig 3. 12 Percentage distribution of cassava farmers with respect to their eating habit

3.3.1.12 Cassava production constraints

A number of cassava production constraints were identified in the states (Table 3.5). No access to farm inputs like fertilizers, agricultural credit facilities and farm implements were the most common problems in the surveyed states, indicated by 100% of the farmers in Benue, 100% in Anambra and 80% in Abia. Insect and disease damage were the second most important problem reported by 78% of farmers in Abia, 83.33% in Anambra and 86.67% in Benue. The key insect pests identified were termites, cassava green mite, cassava mealybug and whiteflies. Cassava mosaic disease and cassava bacterial blight were the major diseases identified by the farmers. Also, rodents, especially mole rats (*Cryptomys hottentotus*) and squirrels (*Sciurus carolinensis*) were identified as an important problem. High cost of labour was the third most important problem. Poor underground storability, high storage root fibre, weeds and flooding were identified as minor constraints to cassava production.

Table 3. 5 Cassava production constraints as identified by farmers in Abia, Anambra and Benue states in Nigeria

Production constraints	**Abia (%)**	**Anambra (%)**	**Benue (%)**	**Mean (%)**	**Ranking**
No access to farm inputs	80	100	100	93.33	1
Prone to disease and pest damage	78	83.33	86.67	82.67	2
High cost of labour	55	96.67	76.67	76.11	3
Low fresh root yield	55	86.67	73.33	71.67	4
Lack of extension services	75	62.5	72.5	70	5
High cost of transportation of farm produce	60	70	53.33	61.11	6
Cattle attack	50	45	66.67	53.89	7
Labour unavailability	8.33	13.33	3.33	8.33	8
Poor underground storability	11.33	5.5	3.33	6.72	9
High storage root fibre	11.67	0	3.33	5	10
Weeds (Climbers)	0	3.33	0	1.11	11
Flooding	0	3.33	0	1.11	12

3.3.2 Farmers' perception of cassava green mite

3.3.2.1 Farmers' awareness about cassava green mite

Majority (66.4%) of the farmers in the surveyed areas had little or no knowledge of cassava green mite. However, Amawom and Gwer East recorded the highest number (47.5%) of farmers who had knowledge about CGM, followed by Okpokwu (45%), Otukpo (42.5%) and Osisioma (40%). However, 35%, 25% and 20% of the farmers in Anambra East, Ohafia and Orumba North respectively had knowledge about CGM. None of the farmers in Awka North had knowledge about CGM. The results also showed that Benue state has the highest (45%) number of farmers who had some knowledge about CGM, followed by Abia state (37.5%) then 18.3% in Anambra state. Those farmers who had some knowledge about CGM make use of the cassava leaves as vegetables and had interacted with researchers either during on farm research or farmer training. These farmers were able to recognize and describe symptoms of the pest and were even able to distinguish CGM from cassava mealybug and cassava mosaic disease symptoms.

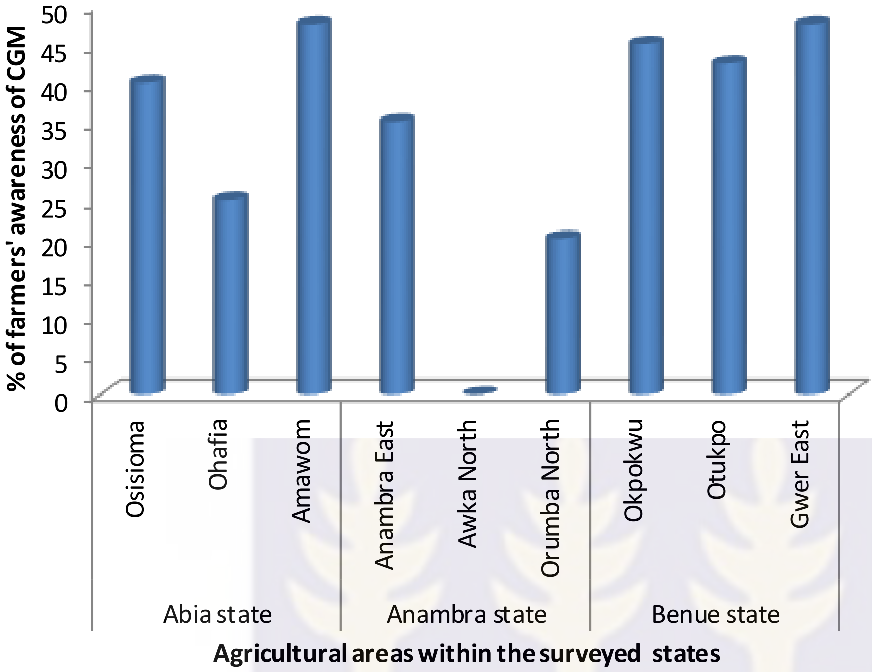


Fig 3. 13 Farmers awareness about cassava green mite

3.3.2.2 Incidence and severity of CGM in farmers' fields

Although most of the farmers were not aware of CGM, symptoms of the pest attack were present in most of the fields. The average incidence of CGM across the three districts was 73.9%. Benue state (77.8%) had the highest CGM incidence followed by Abia (72.6%) and Anambra (71.2%). The CGM severity was moderately resistance with severe chlorosis and slight reduction in leaf size with an overall mean of 3.0. Benue state scored 3.5 of CGM severity, followed by Abia state (3.0) and then Anambra state scored (2.5).

Table 3. 6 Cassava Green Mite (CGM) incidence and severity in farmers' field in Abia, Benue and Anambra states

States	**CGM incidence (%)**	**CGM severity**
Abia	72.6	2.5
Anambra	71.2	3.0
Benue	77.8	3.5
Mean	**73.9**	**3.0**

3.3.2.3 Distribution of insect pests of cassava in farmers' field

All the fields visited had cassava green mite, cassava mealybug (CM), termites and whitefly infestations. Farmers in the three surveyed states were able to estimate the importance of pests experienced in their own fields. Major pests of cassava included termites, CGM, whitefly, and CM. Data obtained from FGD indicated that CGM and termites (*Microtermes* sp) are the most widely distributed pests. Farmers also mentioned that most losses in planting materials and leaves were caused by termites and CGM respectively. These pests were said to be most serious in the dry season (November-March), while whitefly was reported to be found in all cassava fields mostly in the rainy season. Across the three states, termites (39%) were regarded as the most important pest, followed by CGM (23%), then whitefly and CM (19%) in the farmers fields. (Figure 3.14).

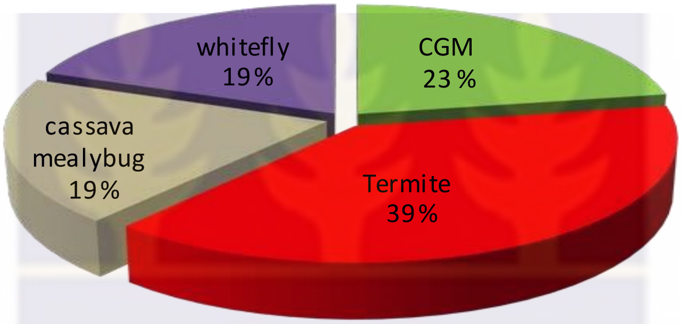


Fig 3. 14 Distribution of major cassava pests in farmers' field as estimated by the farmers

3.3.2.4 Farmers associated plant attributes with reduced damage of cassava green mite

Among the plant attributes that were mentioned by farmers as being associated with reduced pest damage, large and compacted shoot apices, leaf pubescence (hairiness), and extended leaf retention and stay green were highly associated with reduced damage caused by foliar pests such as CGM (Table 3.7). Broad hairy leaves were the most common plant attribute mentioned by 88.33% of the farmers in Abia state, 50.00% in Anambra state and 70.00% in Benue state. Large and hairy shoot tips were the second most plant attribute reported by 81.67% of the farmers in Abia state, 36.67% in Anambra state and 60.00% in Benue state. High retention of the green leaves was third most important plant attribute mentioned by 81.67% of the farmers in Abia state, 40.00% in Anambra state and 54.65% in Benue state. The farmers reported that leaf hairiness limits the movement of the CGM thereby reducing its severity on the cassava leaves. Canopy size was also said to be highly associated with reduced damage due to termites in cassava fields. Farmers were aware of differences in response to pest damage among cultivars. Cassava cultivars that had red or purple leaves, petiole and stems which farmers called "purple or red cassava" was said to be not attacked by CGM, as such leaf type was considered as a good vegetable.

Table 3. 7 Plant attributes associated with reduced damage of cassava green mite

Plant attributes	Abia (%)	Anambra (%)	Benue (%)	Mean (%)
Broad hairy leaves	88.33	50.00	70.00	69.44
Big and hairy shoot tips	81.67	36.67	60.00	59.44
High retention of green leaves	81.67	40.00	54.65	58.77
Highly dense canopy	78.33	18.33	50.00	51.11
Highly branching	10.00	5.00	6.67	7.22

3.3.2.5 Traditional cultural practices associated with reduced pest population/ damage in cassava

During FGD, farmers listed cultural practices that are associated with reduced pest population and damage in cassava fields (Figure 3.15). Majority (40.6%) of the farmers weed frequently to reduce pest infestation and damage in their cassava farms. A total of 31.7% of the farmers interviewed said they practice selective pruning of infested plant shoots, 24.4% of the farmers also mentioned the use of barriers such as planting border rows around the cassava field, 21.1% of the farmers reported that they reduce the population of pest by setting traps. Detopping (14.4%), crop rotation (11.7%), intercropping (12.5%) and early planting (10.2%) are among the strategies that reduce pest infestation and damage in cassava. Bush burning before planting is a practice carried out by 4.4% of the farmers to erase build-up of pests in the field. More than 10% of the farmers said they do not practice any control measures against any pest in cassava fields.

Fig 3. 15 Traditional cultural practices to reduce pest damage in cassava fields as identified by farmers in Abia, Anambra and Benue states in Nigeria

3.3.2.6 Group ranking of the effectiveness of cultural pest management practices

From the information gathered from the FGD, it was learnt that the effectiveness of the above-mentioned cultural practices varies with the kind of pest. Irrigation was considered as the most effective measures against CGM, CM and termite attack. Selective pruning was also cited as an effective measure in reducing the population of whitefly, CGM and CM. Farmers detop the infested plants to ameliorate CGM and CM problem. Early planting on the onset of the rains was reported by the farmers to encourage vigourous growth and thereby increase tolerance to CGM, CM and termite attack. The farmers also said that cassava planted aged between 2 and 9 months are the most vulnerable to infection. Crop rotation and use of healthy planting materials were said to play an important role in reducing termite attack. The farmers affirmed that planting the same crop on the same land year after year reduces soil fertility and structure, therefore crops grown on such conditions will be weaker and susceptible to termites. Barriers and traps prevent rodents from reaching the cassava plants in the field.

Table 3. 8 Ranking of traditional cultural practices associated with reduced damage of cassava by CGM, CM, whitefly, termites and rodents by farmers.

	Pests				
Cultural practices	CGM	CM	Whitefly	Termite	Rodent
Irrigation	1	1	9	1	5
Selective prunning	2	2	3	5	9
De-topping	2	2	5	9	9
Intercropping	2	2	2	1	5
Crop rotation	2	2	3	4	5
Early planting	2	2	7	2	7
Weeding	5	5	8	8	1
Bush burning	6	6	6	3	3
Use of barriers	9	9	9	9	2
Setting traps	9	9	9	9	1

3.4 DISCUSSION

The findings of this study revealed that no access to farm inputs like fertilizers, agricultural credit facilities and farm implements were the most common problems in the Abia, Anambra and Benue states. High fresh root yield, resistance to pests and diseases and early bulking cassava cultivars are the important attributes that determine adoption and retention of new cassava cultivars by farmers in the three surveyed states.

Farmers in Benue state put more emphasis on factors affecting the quality of leaves and roots while farmers in Abia and Anambra states are more concerned with factors affecting the root yield and healthy planting materials. The farmers in Benue state are interested in healthy cassava leaves which are preferred for eating as vegetables. Farmers in Abia and Anambra states are interested in high root yield which they normally process into *gari*, abacha and fufu and there is readily available market for the cassava products.

This study also showed that the key insect pests identified were termites, CGM, CM and whiteflies. CMD and CBB were the major diseases identified by farmers. Farmers are aware that pests and diseases are the major contributing factors to yield loss and abandonment of cassava cultivars in Nigeria. Generally, farmers pay more attention to the conspicuous than non-conspicuous pests. The non-conspicuous nature of CGM had made it difficult for the farmers to clearly identify it; therefore, farmers underrate its effect. Farmers are very observant of the impact of various traditional cultural practices on pests. During the field tours conducted with individual farmers revealed that the cassava is mainly grown as intercrop, often with maize, cowpea or pigeon pea. The popularity of intercropping cassava is widely used by farmers to alleviate the problems of pests and diseases and increase soil fertility. This agrees with Ezulike *et al.* (1993), which reported that cassava intercropped with pigeon pea suffered less damage from CGM and has higher yield those grown on a pure stand. Ezulike and Egwuatu, (1990) found that the way a mite-infested cutting is planted influences presence of the mites on the leaves soon after sprouting. Cuttings planted in a slanting position had mites on the leaves soon after sprouting, but those planted horizontally did not. Moreover, growing of more than one crop in a field assured the farmers of food security. This is one of the reasons why intercropping is practiced by the farmers in the three states.

Agreeing with the observation, farmers complained that short and early branching cassava varieties suffer more damage from CGM attack as compared to tall and late- or non- branching cassava varieties. This agrees with the report by Egesi *et al*. (2007) that early branching cultivars suffer more damage of CGM and also makes weeding difficult than the tall cultivars. The short distances between branching levels in short cassava varieties aid CGM movement between branches and leaves within the cassava plants.

To protect cassava against CGM, farmers carried out cultural practices such as detopping, selective pruning, harvesting of tender leaves and bush burning. However, there are adverse effects of such practices as they interfere with the survival of predatory mites *Typhlodromalus manihoti* Moraes, *Typhlodromalus aripo* DeLeon, *Sthethorus* and *Holobus* (= Oligota) genera. Nweke *et al.* (2002) reported that frequent harvesting of cassava leaves and de-topping of cassava plants may lead to loss to shelter for the natural enemies and decrease in photosynthesis which may have retarding effect on the plant growth leading to yield loss of root, stem and leaves and this may not provide complete control of CGM.

Farmers selected preferred cassava cultivars based on the plant attributes that are linked to resistance to pests and diseases and high yielding. Agreeing with the observation, farmers reported that CGM tend to feed on younger leaves than the older leaves. This reason could be that the older leaves have thicker cell walls, which makes it difficult for the mite to penetrate its stylet. Some cassava cultivars defend themselves through morphological mechanism to deter feeding and damage by insect pests. Plant breeders are using this information to develop pest-resistant cassava cultivars. Farmers in Benue state are aware that the pubescent cultivars reduce the attack and damage of CGM. Leaf pubescence have been claimed to confer tolerance to *M. tanajoa* (Leuschner, 1982). Further studies of Markham *et al.* (1987) revealed that leaf pubescent cultivars have lesser damage symptoms and higher yields than glabrous cultivars. High pubescent cassava cultivars tend to have more and tender leaves, which is preferred for vegetables. Women normally prefer young and fresh cassava leaves for vegetables. Enhancement of leaf pubescence in cassava cultivars will not only reduce CGM attack but it will improve the quality and quantity of cassava leaves as vegetables for consumers.

The majority of the farmers get their planting materials from either their own fields or from fellow farmers. The exchange of cassava stem cuttings is not restricted to within farming communities but also occurs across states. These planting materials are often susceptible to pests and diseases. This practice of exchanging cassava stem cutting for planting is a way of spreading diseases and pests.

None of the farmers applied fertilizers to their cassava. Some of the farmers complained that fertilizers decrease the dry matter content in the root. This is similar to the practice growing of cassava without fertilizer application among small-scale farmers in Democratic Republic of Congo (Muengula-Manyi *et al.*, 2012).

3.5 CONCLUSION

The study established that the farmers had little knowledge of CGM. Farmers associated CGM with cassava mosaic disease and mealybug infestation. The inadequate knowledge of CGM by the farmers could be attributed to perceived damage to cassava plants. There is need to educate farmers about CGM and its damage, the importance of which has been underestimated, due to its non-conspicuous nature.

Resistance to pests and diseases, high yield and early bulking were widely mentioned by farmers as their preferred traits, so these traits have to be given attention in the breeding programmes.

Most of the farmers prefer to grow landraces because they have the desired attributes and are adapted to low-input conditions.

Knowledge and perception of farmers is necessary for the development of appropriate pest control management strategies in line with farmers' needs. Finally, participation of farmers in breeding programmes, from the early to advanced stages, will facilitate the adoption of new improved cultivars.

CHAPTER FOUR

4.0 GENOME-WIDE ASSOCIATION STUDY OF CASSAVA GREEN MITE RESISTANCE AND OTHER ASSOCIATED TRAITS IN *MANIHOT ESCULENTA*

4.1 INTRODUCTION

Cassava Green Mite (CGM) (*Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae) is a dry season pest which usually feeds by inserting their piercing and sucking mouthparts (chelicerae) into the abaxial surface of cassava leaves and extracting the cell contents (Yaninek and Hanna, 2003). As a result, the pest poses a major threat to cassava production and present serious economic and social impacts on the agricultural community. The feeding habit of the pest results in chlorosis, total defoliation, stunted growth, root yield reduction up to 80%, which can lead to candle stick appearance and low accumulation of starch in the storage roots. Nigeria is the world's largest producer of cassava and thus its production is vital to the economy.

There have been limited work on the genetic control of CGM to determine the gene(s) conferring resistance. Most studies on CGM have focused on conventional breeding with limited work on molecular breeding (Nzuki *et al.*, 2017). Two SSR markers, NS 1099 and NS 346 showed high association with CGM resistance (Ceballos *et al.*, 2010; Choperena *et al.*, 2012). Recently, two quantitative trait loci (QTL) qCGMc5Ar and qCGMc10Ar were reported to be linked to CGM resistance (Nzuki *et al.*, 2017). The genetic mechanisms underlying the resistance to CGM have not been studied using the modern genome-wide association mapping. QTL has not previously been identified for the associated traits (leaf pubescence, leaf retention, stay green, shoot tip size and compactness). Identifying the genetic basis of CGM resistance will provide important insights for breeding resistant varieties for sustainable agriculture.

Conventional phenotype-based recurrent selection to breed CGM resistant varieties is lengthy and resource intensive due several biological aspects associated with cassava including low seed set, slow multiplication rate of planting materials and 12 month growing cycle (Ceballos *et al.*, 2012). Moreover, phenotypic selection for CGM resistance requires dry environmental conditions that favour *M. tanajoa* infestation. When and where such conditions occur irregularly or non-uniformly, screening for the trait can be difficult. These challenges can be overcome though use of genomic-assisted and marker-assisted breeding tools that can facilitate indirect selection (Wolfe *et al.*, 2016). This study therefore carried out a Genome-Wide Association Study (GWAS) to uncover genomic regions associated with natural resistance to *M. tanajoa*.

GWAS is an efficient and effective method for confirming candidate genes and identifying new genes in complex phenotypic traits (Altshuler *et al.*, 2008). This approach has been particularly useful for mapping major loci for various other traits in cassava. Some of the studied traits include: genetic architecture of cassava mosaic disease (Wolfe *et al.*, 2016), beta-carotene and dry matter content (Esuma *et al.*, 2016; Rabbi *et al.*, 2017), shoot weight, fresh root yield, starch fraction amylose content, dry matter content and starch yield (de Oliveira *et al.*, 2012). Against this background, the current study was conducted to:

i. identify genomic regions and candidate genes linked to CGM resistance and other useful traits
ii. assess the genetic diversity in cassava
iii. identify stable traits across environments
iv. estimate the heritability of the traits

4.2 MATERIALS AND METHODS

4.2.1 Germplasm collection

A diverse panel also referred to as training population was used for this study. The panel consists of eight hundred and forty-five advanced breeding lines that have been selected and maintained clonally. Three hundred and seventy-two were obtained from IITA cassava gene pool, eighty-two were clones from the Centro Internacional de Agricultura Tropical (CIAT) in Cali, Columbia and three hundred and ninety-three were gotten from the germplasm of National Root Crops Research Institute (NRCRI) Umudike, Nigeria.

4.2.2 Experimental sites

The trial was evaluated in three locations (Umudike, Otobi and Kano) in Nigeria. Umudike (annual rainfall of 2200 mm; altitude 120 m; mean annual temperature of 22 to 31^{o}C; coordinates 7^{o}24'E, 5^{o}29' N; Dystric Luvisol soils; humid forest); Kano (annual rainfall of 830 mm; altitude 470 m; mean annual temperature of 29 to 38^{o}C; coordinates 12° 0′ 0″ N, 8° 31′ 0″ E; Calcic Luvisol soils; sudan–savanna); and Otobi (annual rainfall of 1500 mm; altitude 319 m; mean annual temperature of 24 to 35^{o}C; coordinates 7^{o}20' E, 8^{o}41' N; Ferric Luvisol soils; southern Guinea savanna). These sites represent the hot spots of CGM in the country.

4.2.3 Field layout and experimental design

The trial was laid out in a randomized incomplete block design with three replications per location per year. Each plot consisted of 5 plants per row on ridges. Spacing between ridges was 1m and also between plants within the row giving a total population of 10 000 plants ha^{-1}. The trial was evaluated in 2013/2014, 2014/2015 and 2015/2016 cropping seasons. The populations were systematically phenotyped for Cassava Green Mite Severity (CGMS), Leaf Pubescence (LP), Leaf Retention (LR), Stay Green (SG), Shoot Tip Compactness (STC) and Shoot Tip Size (STS).

4.2.4 Agronomic data

The traits were evaluated as follows:

(A) Cassava Green Mite Severity (CGMS) was evaluated at the visual rating of the damage caused by cassava green mite, *Mononychellus tanajoa* at six months after planting in January (peak of dry season). A 1 to 5 scoring scale was used to rate symptoms: 1= highly resistant; no symptoms observed, 2= resistant; moderate damage, no reduction in leaf size, scattered chlorotic spots on young leaves, 3= moderately resistant; severe chlorotic symptoms, slight reduction in leaf size, 4 = susceptible; severe chlorotic symptoms and severe reduction in leaf size of young shoot, 5 = highly susceptible; very severe chlorosis, extensive defoliation, candlestick appearance of young shoots.

(B) Leaf Pubescence (LP) was characterized visually for the degree of hairiness on the young leaf with 0 = glabrous; 3 = little pubescence; 5 = moderate pubescence and 7 = high pubescence.

(C) Leaf Retention (LR) was evaluated at the visual rating of leaf longevity using a scale of 1= very poor retention; 2 = poor retention; 3 = fair retention; 4 = good retention; 5 = outstanding retention.

(D) Stay Green (SG) was scored visually based on a 1-3 scoring scale where: 1 = poor (<50% of the leaves are live and green); 2 = moderately good (50-74% of the leaves are live and green); 3 = very good (≥75% of the leaves are live and green). Leaf longevity was assessed by scoring for LR and SG.

(E) Shoot Tip Size (STS) was a visual assessment of shoot apices based on how large or small the shoot apices are and categorized on a scale of 1-3 where 1 = small; 2= medium and 3 = large.

(F) Shoot Tip Compactness (STC) was also based on visual assessment of the compactness of shoot apices based on how closely the shoot apices are; with 1 = loose; 2 = moderately compact; 3 = compact.

All the traits were evaluated during the peak of dry season (January) at six months after planting.

4.2.5 Phenotypic data analyses

Phenotypic data obtained from three experimental years were initially analysed separately and subsequently pooled across years, using lme4 (Linear Mixed-Effects Models using 'Eigen' and S4) package implemented in R software (R Development Core Team, 2016).

Clones (c) were evaluated in replicated (r) trials in different environments (e). Heritability in the broad sense based on clone means was estimated as $\boldsymbol{H} = \frac{\sigma_c^2}{\frac{\sigma_e^2}{re}+\frac{\sigma_{ce}^2}{e}+\sigma_c^2}$ according to Hallauer *et al.* (2010). Variance components were estimated in R v. 3.0.1 (R Development Core Team, 2010) using the lmer package in lme4 (Bates and Maechler, 2010). Phenotypic mean best linear unbiased prediction (BLUP) was estimated taking into account of the random effects, which represents an estimate of the total genetic value (EGV) for each individual. To calculate the predictor error variance (PEV), the BLUPs were de-regressed using the equation:

$$\mathbf{de-regressed\ BLUP} = \frac{\mathrm{BLUP}}{1-\frac{\mathrm{PEV}}{\sigma_i^2}}$$

Where PEV is the prediction error variance for each clone and $\boldsymbol{\sigma}_{\mathbf{i}}^{\mathbf{2}}$ is the clonal variance component (Wolfe *et al*; 2016, 2017).

The mixed models were fitted considering the location and year effect using the lmer function of the lme4 r package (Bates et al., 2014).

$$\boldsymbol{Y_{ijk}} = \mu + c_i + \beta_j + r_{k(i)} + \varepsilon_{ijk}$$

Where, $\boldsymbol{Y_{ijk}}$ represents the raw phenotypic observations, μ is the grand mean, c_i is a random effects term for clone with c_i ~ N (0, σ_{i}^2), β_j is a fixed effect for the combination of location and year harvested, $r_{k(i)}$ is a random effect for replication nested within location–year combination assumed to be distributed N (0, σ_{r}^2), and finally, ε_{ijk} is the residual variance, assumed to be random and distributed N (0, σ_{e}^2). The de-regressed BLUP were used for the association analysis, this is to help reduce noise variation in GWAS.

Pearson correlation was calculated between traits using trait means of the accessions was also performed with R software

4.2.6 DNA extraction and SNP genotyping

DNA extraction was performed using DNeasy Plant Mini Kit (Qiagen) with a slight modification. The young fresh leaf samples were harvested from the apical part of cassava plant in the field. About 3-5 tender leaves, weighing about 100 mg – 900 mg, were put in well labelled extraction tubes arranged in a labelled 96-well box and placed on ice to maintain DNA integrity. From the field the leaf samples were transferred to the NRCRI molecular laboratory and stored in a -80ºC freezer. Before the commencement of the extraction process, the stored samples were lyophilized for 48 hours. With the use of a tissuelyser running with a 1X speed at 1500 strokes/min rate, the samples were ground to a fine powder. Genomic DNA was extracted and quantified using a NanoDrop 1000 (Thermo Scientific) while the molecular weight was assessed with agarose gel electrophoresis.

After successful DNA extraction, the samples were sent to the Institute of Genomic Diversity (IGD), Cornell University, Ithaca, New York, United States of America for SNP genotyping. At IGD, the SNP genotyping was performed following the genotyping-by-sequencing protocol of Elshire *et al*. (2011) using the ApeK1 restriction enzyme recommended by Hamblin and Rabbi (2014). Genotypes were called using the TASSEL 5.0 GBS pipeline Version 2 (Glaubitz *et al*., 2014) and aligned to the cassava reference genome, version 6 (http://phytozome.jgi. doe.gov). SNPs with low quality (that is markers with extreme deviation from Hardy-Weinberg equilibrium (chi-square > 20) across all samples) were removed from the dataset. Only biallelic SNP markers with call rate > 70% and minor allele frequency (MAF) of 0.05 were used for the analyses. Finally, imputation was done with Beagle version 4.0 (Browning and Browning, 2009)

4.2.7 Population structure and Genome – Wide Association Analysis

A total of 61,307 SNP markers with MAF $\geq$ 0.05 was used for the assessment of population structure and GWAS. Principal Component Analysis (PCA) on SNP markers was used to identify major patterns of relatedness within and among the populations (NRCRI, IITA and CIAT) using the prcomp function in R.

In the association analysis, three different statistical models comprising both general linear models (GLM) and mixed linear models (MLM) were used to calculate *P*-values for associating each marker with the traits evaluated using TASSEL 5.0 (Bradbury *et al*., 2007). These following models were tested (i) Naïve model: GLM without any correction for population structure, (ii) PK-model: MLM with PCs from PCA and K-matrix as correction for population structure and (iii) K-model: MLM with K-matrix as correction for population structure. Results were compared to determine the best model for the analysis.

The statistical formula for the GLM is $y = Xb + e$, and for the MLM is $y = Xb + Zu + e$ (Yu *et al*., 2006)

Where y is the vector of the phenotypic observations, *X* and *Z* are the known design matrices, *b* is a vector containing the fixed effects [genetic marker information and the population structure (the Q matrix)], *u* is a vector containing random additive genetic effects and *e* is the vector of random residues. In MLM, the variance structure of random vectors *u* and *e* is:

$$\mathrm{Var}\begin{bmatrix}u\\e\end{bmatrix} = \begin{bmatrix}G & 0\\0 & R\end{bmatrix}$$

Where $G = o'^{2}_{a}K$ with *K* as the kinship matrix and o'^{2}_{a} as the additive genetic variance, $R = Io'^{2}_{e}$ with *I* as an identity matrix and o'^{2}_{e} as the residual variance. In MLM, PCs from principal component analysis and kinship (*K*) matrix analyses were used as covariate (Bradbury *et al*., 2007; Hao *et al*., 2012). For the GLM analyses, a permutation test was run and the number of permutations was 1000. The correcting ability of these models was tested through the evaluation of the quantile- quantile (Q-Q) plots of the observed – $\log_{10}$ (*p*-values) versus expected $-\log_{10}$ (*p*-values) (Wang *et al*., 2012b). Manhattan and quantile-quantile plots (qqplots) were generated using the R package qqman (Turner, 2014). In order to avoid false positives Bonferroni correction was used to set the significance cut-off at $-\log_{10}(\alpha/n)$. where α is 0.05 which is the standard significance threshold and n is the number of SNPs. In this study, the Bonferroni correction significance cut-off was $-\log_{10}(0.05/61{,}307) = 6.23$.

4.2.8 Candidate Genes Identification

Significant SNPs from the GWAS results were selected for the analysis. SNP markers that were above Bonferroni threshold (6.23) were mapped on genes with the gene interval position using the annotation list from Phytozome 11. Gene ontology annotation for each time point and combining all the datasets was done using Panther (http://go. Pantherdb.org/). These sequences were aligned against the cassava V6 reference genome assembly using the intersect function from bedtools (Quinlan and Hall, 2010).

4.3 RESULTS

4.3.1 Phenotypic evaluations

4.3.1.1 Descriptive statistics and broad-sense heritability across three locations for three years

Summary statistics of phenotypic data obtained for three growing seasons in the three locations Umudike, Otobi and Kano are presented in Table 4.1. The highest mean (3.22 of a maximum of 5) was obtained for CGM at Kano and the lowest (2.51 of a maximum 5) was obtained for CGM at Umudike. The highest mean for leaf pubescence and retention was obtained in Umudike while the lowest was found in Kano. This implies that the more the severity of CGM the lesser the leaf pubescent and retention. For heritability estimates, broad sense heritability estimates h^2_B was generally low to moderate, with highest h^2_B for CGM (0.30) and lowest h^2_B for LR (0.15). Variabilities for the traits estimated by coefficient of variation ranged from 30.60% for STC to 82.63% for LP.

Table 4. 1 Descriptive statistics (mean ± standard deviation (SD), coefficient of variation (CV) and broad-sense heritability (h^2_B ± approximate standard error mean (s.e.m) for all the traits.

Traits	Umudike			Otobi			Kano			Pooled		
	Mean ± s.d	CV (%)	Heritability (h^2_B±s.e.m)	Mean ± s.d	CV (%)	Heritability (h^2_B±s.e.m)	Mean ± s.d	CV (%)	Heritability (h^2_B±s.e.m)	Mean ± s.d	CV (%)	Heritability (h^2_B±s.e.m)
CGMS	2.81±0.93	33.10	0.30±0.01	2.81±0.89	31.53	0.16±0.01	3.22±1.15	35.66	0.08±0.01	2.91±1.00	40.00	0.30±0.01
LP	2.58±2.61	101.00	0.29±0.03	2.39±2.50	104.5	0.15±0.03	2.62±2.56	97.44	0.12±0.04	2.53±2.60	101.30	0.29±0.01
LR	3.02±0.86	28.50	0.13±0.01	3.05±0.86	28.26	0.05±0.01	2.93±0.80	27.31	0.05±0.01	3.01±0.80	28.20	0.15±0.01
SG	2.24±0.61	27.08	0.15±0.01	2.23±0.63	28.37	0.07±0.01	2.22±0.57	25.68	0.06±0.01	2.23±0.60	27.20	0.17±0.01
STC	2.05±0.63	30.69	0.22±0.01	2.03±0.62	30.69	0.11±0.01	2.00±0.60	30.20	0.12±0.01	2.03±0.60	30.60	0.17±0.01
STS	2.22±0.71	31.86	0.16±0.01	2.18±0.72	33.19	0.08±0.01	2.19±0.68	31.00	0.06±0.01	2.20±0.70	32.10	0.23±0.01

CGMS= Cassava Green Mite; LP = Leaf Pubescence; LR = Leaf retention; SG= Stay green; STC = Shoot tip compactness; STS = Shoot tip size.

4.3.1.2 Analysis of variance for all the traits

The analysis of variance (ANOVA) revealed highly significant differences among the genotypes for all the traits (P<0.001), thereby indicating the presence of substantial phenotypic variance (Table 4.2). The results also indicated that the effects of location and year were significant (P<0.001) and (P<0.0.5) for all the traits. Genotype by location interactions were not significantly different for all the traits. Apart from CGMS, all other traits were significantly different for genotype by year interactions. Genotype by location by year interactions were significantly different for CGMS, LP, LR and STC. SG and STS showed no significant different for genotype by location by year interactions.

Table 4. 2 Analysis of variance (ANOVA) for all traits evaluated in the three different locations across three years.

Traits	Source of variations	Df	Sum Sq	Mean Sq	F value	Pr(>F)
CGMS	Genotype	955	4892.01	5.12	6.78	***
	Location	2	492.92	246.46	326.28	***
	Year	2	56.50	28.25	31.52	***
	Genotype: Location	1830	867.70	0.47	0.70	ns
	Genotype: Year	874	418.70	0.48	0.71	ns
	Genotype: Location: Year	1321	1611.10	1.22	1.81	***
LP	Genotype	955	38793.00	40.62	8.25	***
	Location	2	197.00	98.41	19.99	***
	Year	2	3399.18	1699.58	457.03	***
	Genotype: Location	1830	3193.00	1.75	0.35	ns
	Genotype: Year	1743	20419.91	11.71	3.15	***
	Genotype: Location: Year	1321	9056.00	6.86	1.39	***
LR	Genotype	955	4197.73	4.40	7.90	***
	Location	2	9.15	4.58	8.23	***
	Year	2	20.93	10.46	22.60	***
	Genotype: Location	1830	366.10	0.20	0.35	ns
	Genotype: Year	1743	1776.44	1.02	2.20	***
	Genotype: Location: Year	1321	715.60	0.54	0.94	***
SG	Genotype	955	1953.57	2.04	6.93	***
	Location	2	2.00	0.98	3.28	*
	Year	2	5.08	2.54	10.46	***
	Genotype: Location	1830	210.70	0.12	0.38	ns
	Genotype: Year	1743	1006.25	0.57	2.38	***
	Genotype: Location: Year	1321	413.40	0.31	1.04	ns
STC	Genotype	955	2020.16	2.11	6.84	***
	Location	2	1.80	0.89	2.81	*
	Year	2	63.17	31.59	123.21	***
	Genotype: Location	1830	215.50	0.12	0.37	ns
	Genotype: Year	1743	961.48	0.55	2.15	***
	Genotype: Location: Year	1321	440.70	0.33	1.06	*
STS	Genotype	955	2633.36	2.75	6.87	***
	Location	2	7.72	3.90	9.61	***
	Year	2	206.15	103.07	313.99	***
	Genotype: Location	1830	265.20	0.14	0.35	ns
	Genotype: Year	1743	1182.43	0.68	2.07	***
	Genotype: Location: Year	1321	529.30	0.40	0.98	ns

DF = degrees of freedom, SS = sum of squares. MS = Mean square, F-probabilities are indicated by symbols: *P<0.05, **P<0.01, ***P<0.001, ns (non-significant). CGMS= Cassava Green Mite Severity, LP = Leaf Pubescence, SG = Stay Green, STC = Shoot Tip Compactness, STS = Shoot Tip Size

4.3.1.3 Distribution and correlation of the traits

The correlation among the different traits evaluated in three environments are presented in fig. 4.1. Result showed that LP (r = -0.94), LR (r = -0.63), SG (r = -0.61), STS (r = -0.52), STC (r = -0.65) significantly and negatively correlated with CGMS at the 0.01 and 0.001 levels of probability while LR (r = 0.65), SG (r = 0.55), STS (r = 0.58) and STC (r = 0.68) significantly and positively correlated with LP. A wide range of variation was observed among the genotypes for all traits measured. Most traits evaluated exhibited a near normal distribution (fig. 4.1). The results also showed that the performance of plants that were moderately resistance to CGMS were more in number than the resistant and susceptible plants. Moderately pubescent plants tend to show good leaf retention and stay green.

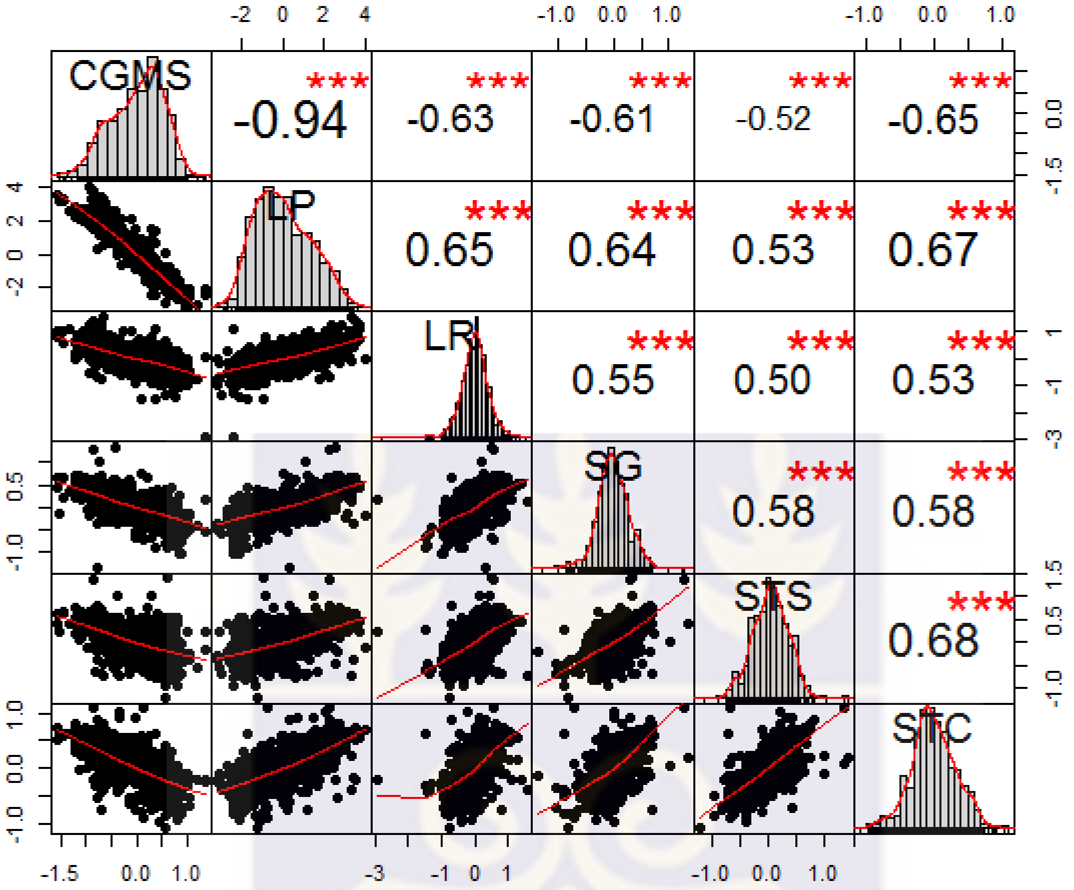


Fig 4. 1 Phenotypic correlation coefficient analysis and distribution of all traits evaluated across the three locations for three years

4.3.2 Population structure and genetic diversity

A total of 84,585 SNP markers were examined genome-wide for the selection of polymorphisms. Of these 23,278 SNPs (27.5%) were excluded because of a minor allele frequency (MAF) below 0.05. The remaining 61,307 polymorphic SNPs with MAF greater than 5% were used for this study. Using principal component analysis (PCA) to summarize genetic variation in the accessions, a subtle differentiation was observed in the collection of germplasm (fig 4.2). PCA of the accessions revealed the first, second, third and fourth principle components (PCs) accounted for 0.31, 0.17, 0.06 and 0.05 of the genotypic variability in the accession respectively (table 4.3). The two top axes of PCA accounted for only 48% of the variation (fig. 4.2). This indicates a low level of genetic diversity in the *M. esculenta* germplasm used in the study.

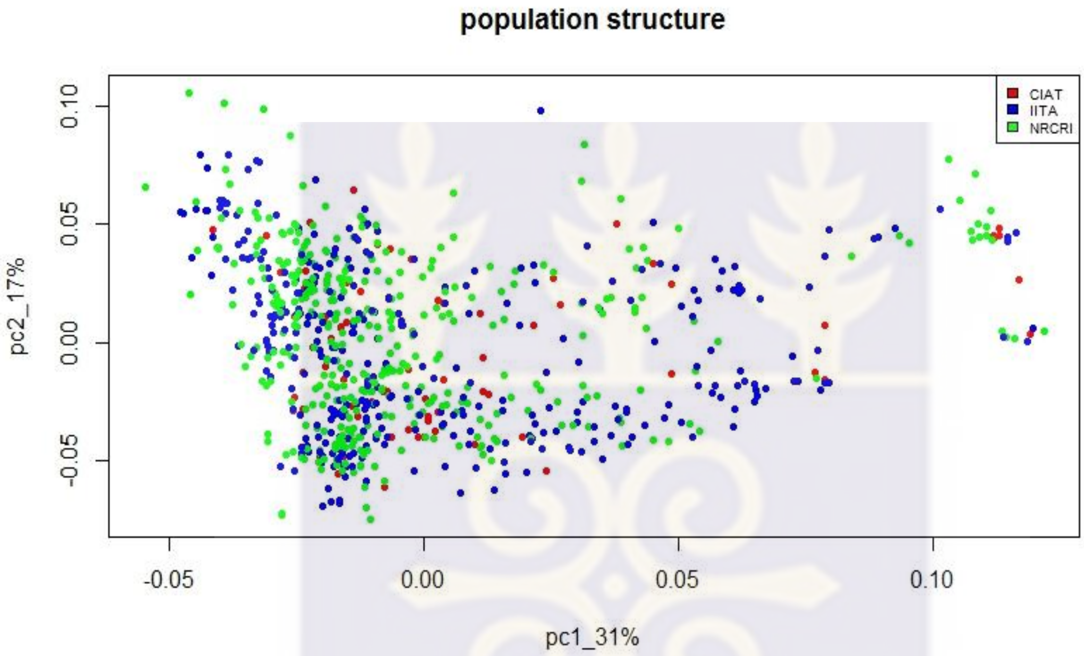


Fig 4. 2 Principal component analysis (PCA) pot of PC1 against PC2 illustrating population structure in the *M. esculenta* diversity panel genotyped with the SNP markers. The red, blue and green rectangles represent *M. esculenta* with CIAT, IITA and NRCRI origins

Table 4. 3 PCA of training populations genotyped with SNPs marker

PCs	Standard Deviation	Variance components	Cumulative proportion
1	1.63	0.31	0.31
2	1.22	0.17	0.48
3	0.73	0.06	0.54
4	0.68	0.05	0.60
5	0.63	0.05	0.65

4.3.3. Association analysis

4.3.3.1 Comparison of models

To identify the most suitable model to conduct GWAS for the dataset, three different standard GWAS models were evaluated as shown in the quantile-quantile plots (Fig. 4.3). As depicted in the quantile-quantile plots, numerous false-positive associations were observed for all traits using the naïve model for association analysis. When the kinship (K-model) and principal component plus kinship (PK-model) effects were incorporated, potential spurious associations were filtered out and the *p*-values were closer to the expected distribution for all the traits analysed. The K and PK showed a good fit for *p*-values, while the naïve model was characterized by the excess of small *p*-values which is corresponding to the abundance of spurious associations (fig.4.3). This indicated that the K and PK were consistent for reducing $-\log_{10}$ (*p*-values) toward the expected level, thereby controlling for false-positives. On the other hand, the K-model performed similar to the PK model in displaying a highly uniform distribution of *P*-values. All subsequent results are, therefore, based on K- model.

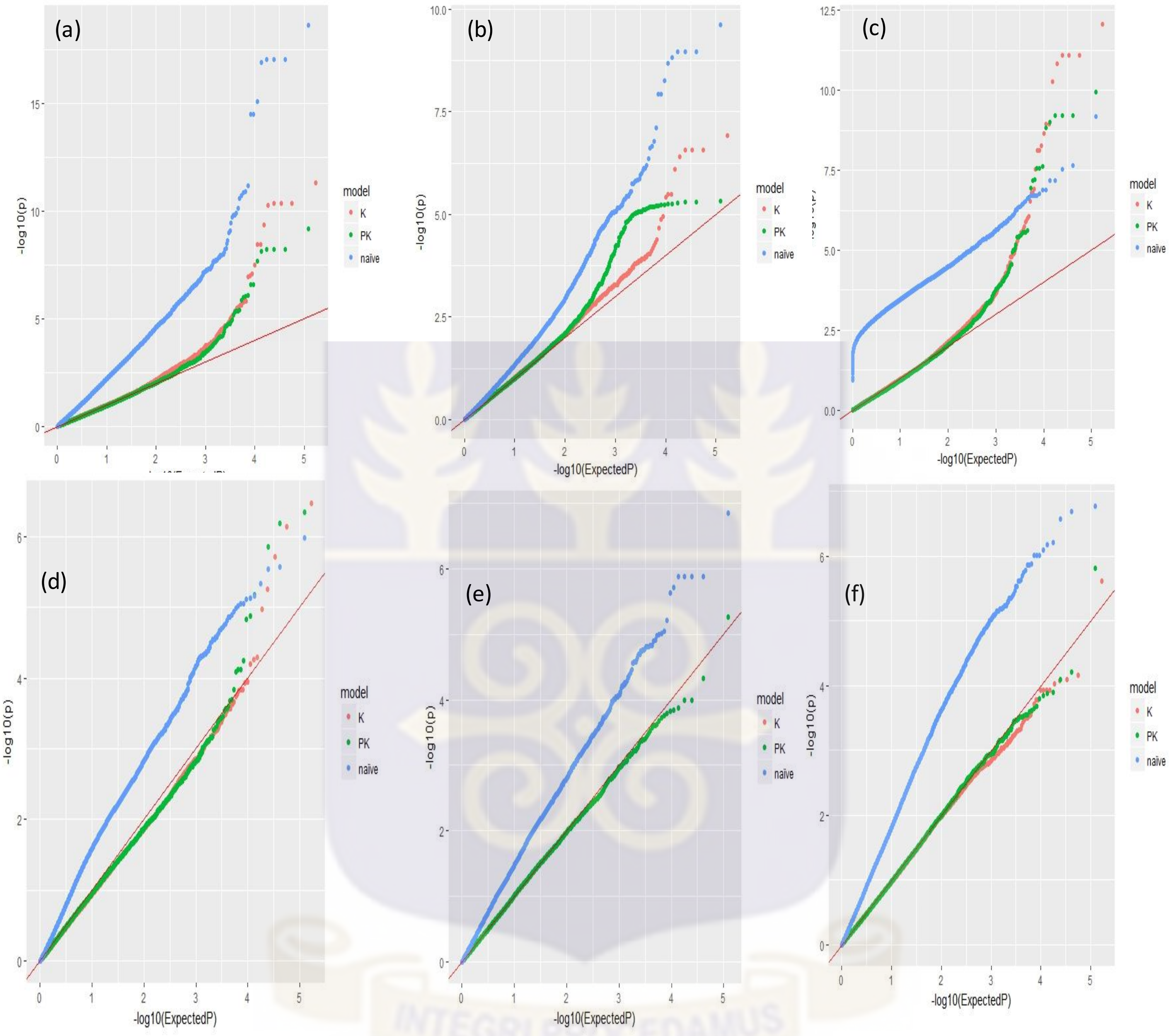


Fig 4. 3 Comparison of different GWA models for traits (a) cassava green mite severity (b) leaf pubescence (c) leaf retention (d) stay green (e) shoot tip compactness (f) shoot tip size. The more uniform the distribution of p-values, the better is the model

4.3.3.2 Marker-trait association

Association tests were performed for all traits in an analysis that combined all years and locations. Thirty-five SNP markers in total passed the Bonferroni significance threshold (Table 4.4). GWAS identified 12, 17, 5 and 1 significant SNP markers for CGMS, LP, LR and SG respectively. The most significant SNP marker (S8_5962253) has a $-\log_{10}$ (p-value) of 13% and explained 7% of the observed phenotypic variation. No significant SNP markers was observed for STS and STC in the combined dataset. The contribution of any single SNP to the phenotypic variation ranged from 4 to 7% across the traits (Table 4.4).

Cassava green mite

Twelve markers were significantly associated with CGM resistance. The significant markers associated with the trait mostly concentrated at a single region on chromosome 8. The top significant SNP marker (S8_5962253) explained 7% of the phenotypic variance (Table 4.4).

Leaf pubescence

Seventeen markers were found to be significantly associated with leaf pubescence. The significant markers associated with this trait also lies on chromosome 8. Variance explained by the significant markers ranged from 4 to 7% (Table 4.4).

Leaf retention

Five markers displayed significant associations with leaf retention. These markers (S8_5962253, S8_6439483, S8_6439519, S8_6439891 and S8_6439935) were found on chromosome 8. Each of these markers explained 4% of phenotypic variation of this trait (Table 4.4).

Stay green

A marker was found to be significantly associated with stay green. This marker (S13_692620) was found on Chromosome 13. The marker explains 4% of the phenotypic variation (Table 4.4).

Table 4. 4 SNP marker loci significantly associated with the traits and their explained proportion of phenotypic variation by marker (R^2)

Trait	SNP Marker	Chromosome	SNP position	p-value	R^2
LP	S8_5962253	8	5962253	8.69E-13	0.06965
CGMS	S8_5962253	8	5962253	5.08E-12	0.06508
LP	S8_6439483	8	6439483	8.25E-12	0.06383
LP	S8_6439519	8	6439519	8.25E-12	0.06383
LP	S8_6439891	8	6439891	8.25E-12	0.06383
LP	S8_6439935	8	6439935	1.50E-11	0.06228
CGMS	S8_6439483	8	6439483	4.61E-11	0.0594
CGMS	S8_6439519	8	6439519	4.61E-11	0.0594
CGMS	S8_6439891	8	6439891	4.61E-11	0.0594
LP	S8_6125583	8	6125583	5.44E-11	0.05897
CGMS	S8_6439935	8	6439935	5.79E-11	0.05881
CGMS	S8_6125583	8	6125583	4.52E-10	0.05355
LP	S8_6061415	8	6061415	1.12E-09	0.05123
LP	S8_6061421	8	6061421	1.12E-09	0.05123
LP	S8_5962010	8	5962010	2.25E-09	0.04945
CGMS	S8_6061415	8	6061415	3.54E-09	0.04831
CGMS	S8_6061421	8	6061421	3.54E-09	0.04831
LP	S8_5962464	8	5962464	5.23E-09	0.04732
LP	S8_6494548	8	6494548	7.37E-09	0.04644
LP	S8_5389240	8	5389240	7.70E-09	0.04633
LP	S8_5191862	8	5191862	2.88E-08	0.043
CGMS	S8_6494548	8	6494548	3.18E-08	0.04274
CGMS	S8_5962464	8	5962464	8.59E-08	0.04023
CGMS	S8_5962010	8	5962010	1.02E-07	0.0398
CGMS	S8_5389240	8	5389240	1.12E-07	0.03957
LR	S8_5962253	8	5962253	1.19E-07	0.03941
LP	S8_5389232	8	5389232	1.24E-07	0.03931
LP	S8_5388872	8	5388872	1.56E-07	0.03873
LP	S8_5389151	8	5389151	1.56E-07	0.03873
LR	S8_6439483	8	6439483	2.64E-07	0.0374
LR	S8_6439519	8	6439519	2.64E-07	0.0374
LR	S8_6439891	8	6439891	2.64E-07	0.0374
LP	S8_5745904	8	5745904	3.00E-07	0.03709
SG	S13_692620	13	692620	3.37E-07	0.03679
LR	S8_6439935	8	6439935	3.83E-07	0.03647

4.3.3.3 Genotype by environment effects

To investigate how environmental variation affected the performance of GWAS, these traits (CGMS, LP, LR, SG, STS and STC) were evaluated in three locations (Umudike, Otobi and Kano) across the three years and results were compared. The GWAS peaks explained between 4% to 8% of the observed phenotypic variation for all traits in Kano, Otobi and Umudike respectively (Appendix 4.1 a, b, c). As seen in figure 4.4, 4.5 and 4.6, a well-defined peak on chromosome 8 was observed for CGMS, LP and LR in the different locations and most significant signal marker (S8_5962253) was observed for the traits across the locations (Appendix 4.3). This indicates that the gene was stable across the locations.

Also, the effect of genotype by environment (G x E) interaction for all the traits was demonstrated by comparing GWAS results in three years (2013, 2014 and 2015) across the three locations (Table 4.5, 4.6 and 4.7). In this case, different number of significant markers were observed for all traits. In 2013, 34 significant SNP markers were observed (11, 13, 8, 1 and 1 for CGMS, LP, LR, SG and STC respectively). In 2014, 11 SNPs were found to be associated the two traits (eight for LP and three for STS) on chromosome 8 and 9 respectively. In 2015, GWAS identified 60 SNPs on chromosome 8 for five traits (CGMS (15), LP (19), LR (14), SG (8) and STC (3)). Here, a year to year variation was observed among the three years evaluated, mostly due to the different weather patterns experienced during the three growing seasons. The most signal SNP marker (S8_5962253) was the top hit reference significant SNPs for years 2013 and 2015, but not in 2014, though it was significant using the average from the three years.

However, the same SNPs on chromosome 8 was significantly associated with more than one trait when mapping across all accessions in the panel. This could be as a result of pleiotropy or closely linked genes, Here, in this study, chromosome 8 had significant associations for CGMS, LP and LR.

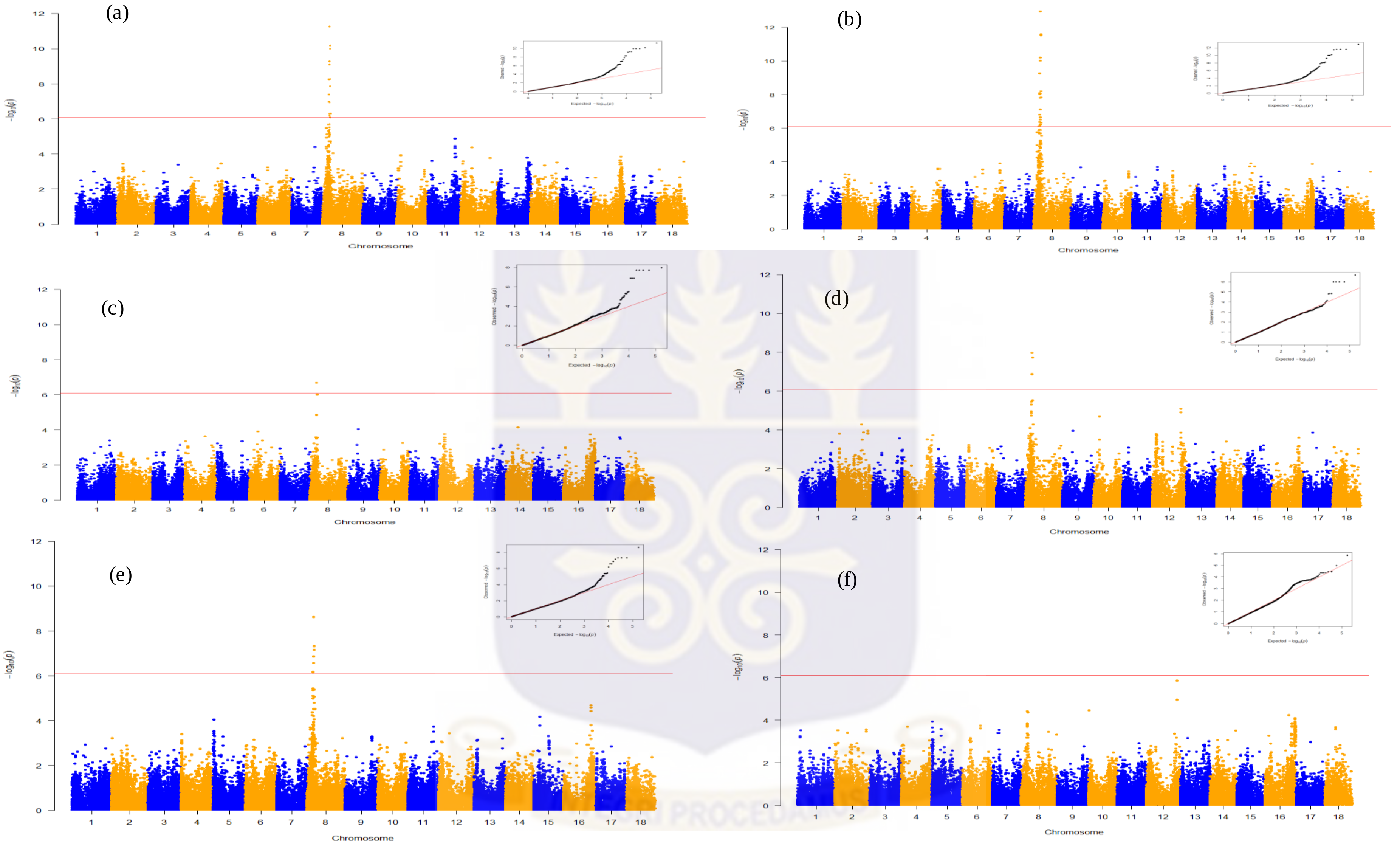


Fig 4. 4 Manhattan plots summarizing genome-wide association results for (a) CGMS (b) LP (c) LR (d) SG (e) STC (f) STS in Kano across the three years. Bonferroni significance threshold is shown in red. Q-Q plot is shown inset to access the number and magnitude of observed associations between genotyped SNPs and the traits.

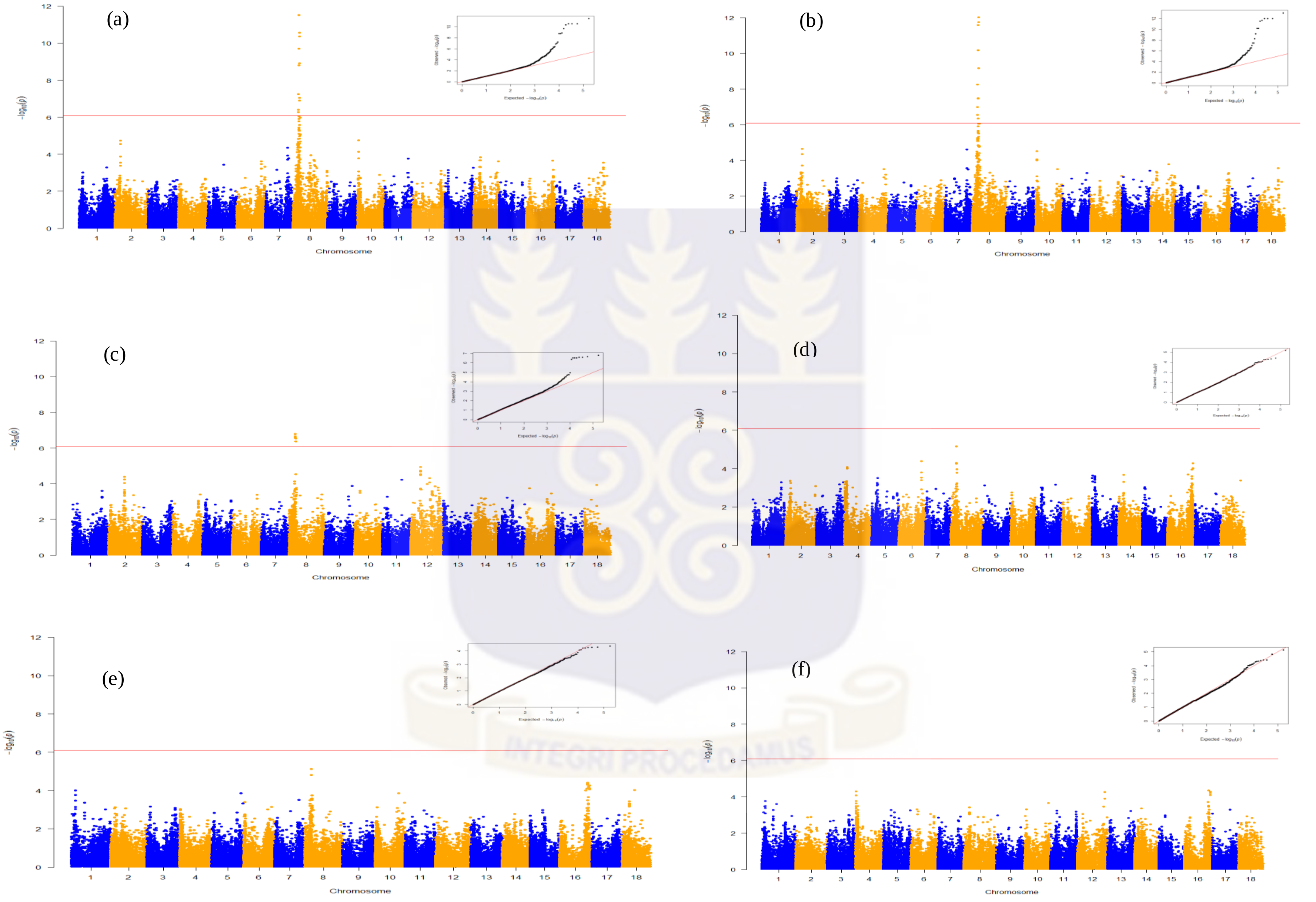


Fig 4. 5 Manhattan plots summarizing genome-wide association results for (a) CGMS (b) LP (c) LR (d) SG (e) STC (f) STS in Otobi across the three years. Bonferroni significance threshold is shown in red. Q-Q plot is shown inset to access the number and magnitude of observed associations between genotyped SNPs and the traits.

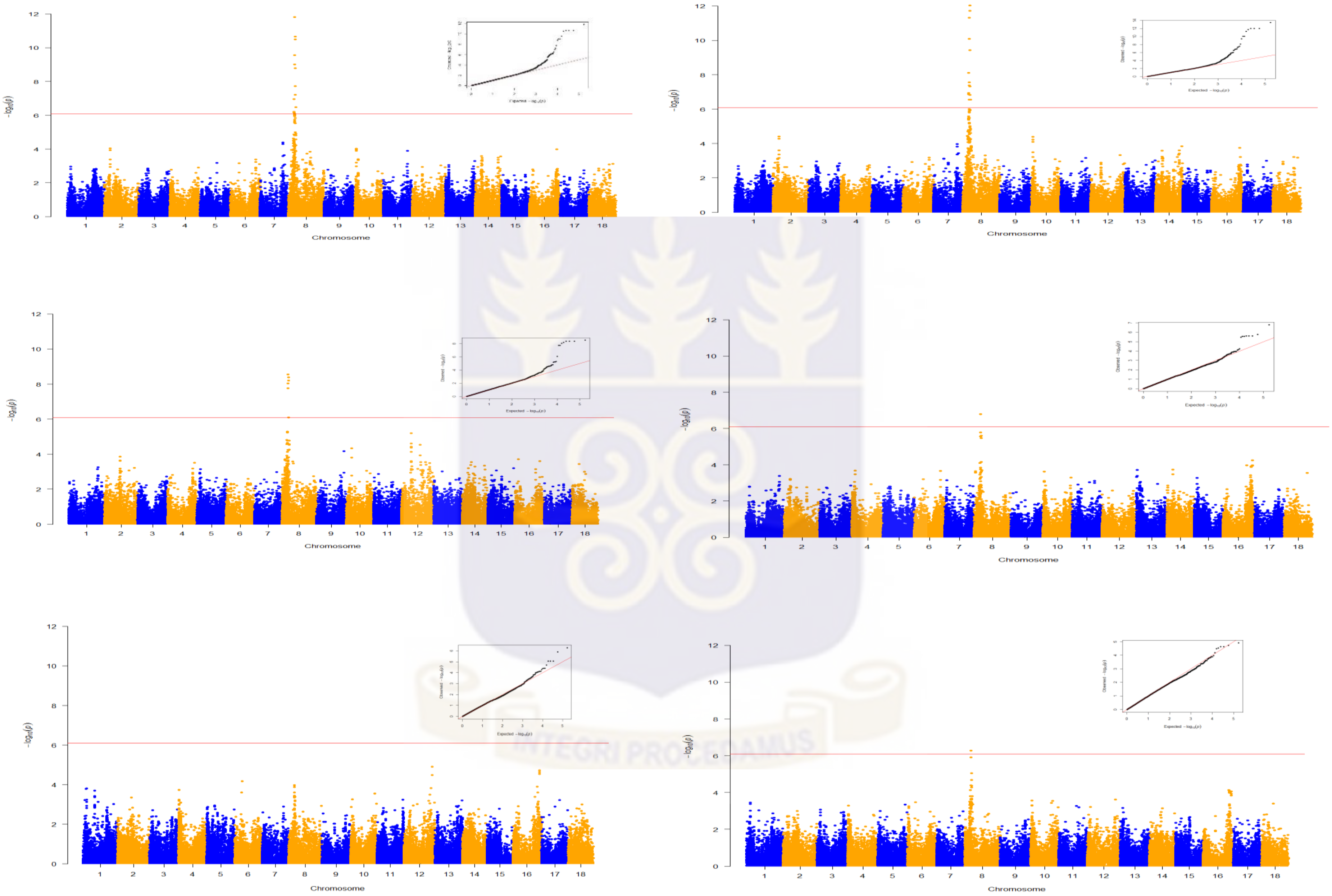


Fig 4. 6 Manhattan plots summarizing genome-wide association results for (a) CGMS (b) LP (c) LR (d) SG (e) STC (f) STS in Umudike across the three years. Bonferroni significance threshold is shown in red. Q-Q plot is shown inset to access the number and magnitude of observed associations between genotyped SNPs and the traits.

Table 4. 5 GWAS results for year 2013

Trait	SNP Marker	Chromosome	SNP Position	p-value	R^2
LP	S8_5962253	8	5962253	3.87E-12	0.0657
CGMS	S8_5962253	8	5962253	9.92E-12	0.06327
LP	S8_6439483	8	6439483	5.97E-11	0.05866
LP	S8_6439519	8	6439519	5.97E-11	0.05866
LP	S8_6439891	8	6439891	5.97E-11	0.05866
CGMS	S8_6439483	8	6439483	8.50E-11	0.05776
CGMS	S8_6439519	8	6439519	8.50E-11	0.05776
CGMS	S8_6439891	8	6439891	8.50E-11	0.05776
LP	S8_6439935	8	6439935	1.09E-10	0.05713
CGMS	S8_6439935	8	6439935	1.16E-10	0.05697
LP	S8_6125583	8	6125583	6.46E-10	0.05257
CGMS	S8_6125583	8	6125583	1.05E-09	0.05134
LR	S8_5962253	8	5962253	1.35E-09	0.0507
LR	S8_6439483	8	6439483	1.61E-09	0.05024
LR	S8_6439519	8	6439519	1.61E-09	0.05024
LR	S8_6439891	8	6439891	1.61E-09	0.05024
CGMS	S8_6061415	8	6061415	1.87E-09	0.04987
CGMS	S8_6061421	8	6061421	1.87E-09	0.04987
LR	S8_6439935	8	6439935	2.43E-09	0.0492
LP	S8_6494548	8	6494548	3.08E-09	0.0486
LP	S8_6061415	8	6061415	3.48E-09	0.04829
LP	S8_6061421	8	6061421	3.48E-09	0.04829
CGMS	S8_6494548	8	6494548	4.25E-09	0.04778
LR	S8_6125583	8	6125583	1.38E-08	0.0448
LR	S8_6061415	8	6061415	2.56E-08	0.04323
LR	S8_6061421	8	6061421	2.56E-08	0.04323
SG	S8_5962253	8	5962253	1.01E-07	0.03977
LP	S8_5389240	8	5389240	1.21E-07	0.03932
CGMS	S8_6576192	8	6576192	1.37E-07	0.039
LP	S8_5962464	8	5962464	1.45E-07	0.03886
LP	S8_5962010	8	5962010	1.47E-07	0.03882
STC	S8_5962253	8	5962253	3.71E-07	0.0365
LP	S8_6576192	8	6576192	5.39E-07	0.03557
CGMS	S8_6508308	8	6508308	5.43E-07	0.03555

Significant markers associated for the traits in year 2013, *P*-value of association and variance explained by marker (R^2)

Table 4.6 GWAS results for year 2014

Trait	SNP Marker	Chromosome	SNP position	p-value	R^2
LP	S8_6125583	8	6125583	3.57E-08	0.03875
STS	S9_28106721	9	28106721	4.13E-08	0.03842
STS	S9_28106726	9	28106726	4.13E-08	0.03842
STS	S9_28106732	9	28106732	4.13E-08	0.03842
LP	S8_6439483	8	6439483	6.79E-08	0.03728
LP	S8_6439519	8	6439519	6.79E-08	0.03728
LP	S8_6439891	8	6439891	6.79E-08	0.03728
LP	S8_6439935	8	6439935	8.53E-08	0.03675
LP	S8_6061415	8	6061415	1.95E-07	0.03485
LP	S8_6061421	8	6061421	1.95E-07	0.03485
LP	S8_5962253	8	5962253	3.87E-07	0.03328

Significant markers associated for the traits in year 2014, *P*-value of association and variance explained by marker (R^2)

Table 4. 7 GWAS results for year 2015

Trait	SNP Marker	Chromosome	SNP Position	p-value	R^2
LP	S8_5962253	8	5962253	7.89E-14	0.07579
CGMS	S8_5962253	8	5962253	1.13E-12	0.06887
LP	S8_6439483	8	6439483	6.19E-12	0.06449
LP	S8_6439519	8	6439519	6.19E-12	0.06449
LR	S8_5962253	8	5962253	2.58E-11	0.06082
CGMS	S8_6439483	8	6439483	2.95E-11	0.06047
CGMS	S8_6439519	8	6439519	2.95E-11	0.06047
LP	S8_6439891	8	6439891	3.56E-11	0.05999
LP	S8_6439935	8	6439935	3.56E-11	0.05999
CGMS	S8_6439891	8	6439891	1.31E-10	0.05666
CGMS	S8_6439935	8	6439935	1.31E-10	0.05666
LP	S8_6061415	8	6061415	1.47E-10	0.05635
LP	S8_6061421	8	6061421	1.47E-10	0.05635
CGMS	S8_6061415	8	6061415	1.93E-10	0.05566
CGMS	S8_6061421	8	6061421	1.93E-10	0.05566
LP	S8_6125583	8	6125583	2.41E-10	0.0551
LR	S8_6439483	8	6439483	2.53E-10	0.05497
LR	S8_6439519	8	6439519	2.53E-10	0.05497
CGMS	S8_6125583	8	6125583	4.65E-10	0.05341
LR	S8_6439891	8	6439891	6.36E-10	0.05261
LR	S8_6439935	8	6439935	6.36E-10	0.05261
LP	S8_6494548	8	6494548	1.45E-09	0.05052
LR	S8_6125583	8	6125583	1.65E-09	0.05018
LR	S8_6061415	8	6061415	2.00E-09	0.0497
LR	S8_6061421	8	6061421	2.00E-09	0.0497
CGMS	S8_6494548	8	6494548	2.78E-09	0.04886
SG	S8_5962253	8	5962253	5.61E-09	0.04708
LP	S8_6576192	8	6576192	9.90E-09	0.04564
LP	S8_6508308	8	6508308	1.79E-08	0.04414
CGMS	S8_6576192	8	6576192	2.06E-08	0.04379
STC	S8_5962253	8	5962253	2.67E-08	0.04313
CGMS	S8_6508308	8	6508308	3.46E-08	0.04248
LP	S8_5389240	8	5389240	3.76E-08	0.04227
SG	S8_6061415	8	6061415	5.58E-08	0.04127
SG	S8_6061421	8	6061421	5.58E-08	0.04127
LP	S8_5962010	8	5962010	9.99E-08	0.0398
SG	S8_6439483	8	6439483	1.06E-07	0.03966
SG	S8_6439519	8	6439519	1.06E-07	0.03966
CGMS	S8_5389240	8	5389240	1.20E-07	0.03933

Table 4.7 continued

Trait	SNP Marker	Chromosome	SNP Position	p-value	R^2
SG	S8_6125583	8	6125583	1.24E-07	0.03926
LR	S8_4525999	8	4525999	1.24E-07	0.03925
SG	S8_6439891	8	6439891	1.25E-07	0.03924
SG	S8_6439935	8	6439935	1.25E-07	0.03924
LR	S8_5389240	8	5389240	1.33E-07	0.03908
LP	S8_5962464	8	5962464	1.36E-07	0.03903
LP	S8_5389232	8	5389232	1.37E-07	0.039
STC	S8_6439483	8	6439483	1.95E-07	0.03812
STC	S8_6439519	8	6439519	1.95E-07	0.03812
LR	S8_4525986	8	4525986	2.00E-07	0.03805
LP	S8_5388872	8	5388872	2.15E-07	0.03788
CGMS	S8_5389232	8	5389232	2.56E-07	0.03744
LP	S8_6939658	8	6939658	3.37E-07	0.03675
LR	S8_5388872	8	5388872	3.51E-07	0.03664
CGMS	S8_5388872	8	5388872	4.20E-07	0.0362
LR	S8_6939658	8	6939658	4.26E-07	0.03616
CGMS	S8_6939658	8	6939658	4.56E-07	0.03598
LP	S8_5389151	8	5389151	5.23E-07	0.03564
LP	S8_5745904	8	5745904	5.29E-07	0.03561
LR	S8_6494548	8	6494548	5.33E-07	0.0356

Significant markers associated for the traits in year 2015, *P*-value of association and variance explained by marker (R^2)

4.3.3.4 Candidate Genes

The association results were intersected with the gene annotations and 35 unique genes were identified within SNPs associated with CGMS, LP and LR at 4 – 8MB on chromosome 8 (Fig 4.7, Appendix 4.2). Most of the annotated genes are classes of membrane proteins that are involved in diverse functions ranging from plant growth and development to stress tolerance.

Among these genes, 17 candidate genes were found to be directly linked to cassava green mite resistance. In table 4.8, the 17 candidate genes are subdivided into seven categories according to the protein structure: zinc finger, pentatricopeptide, MYB, MADS, homeodomain, trichome birefringence related protein and ethylene-responsive transcription factor genes.

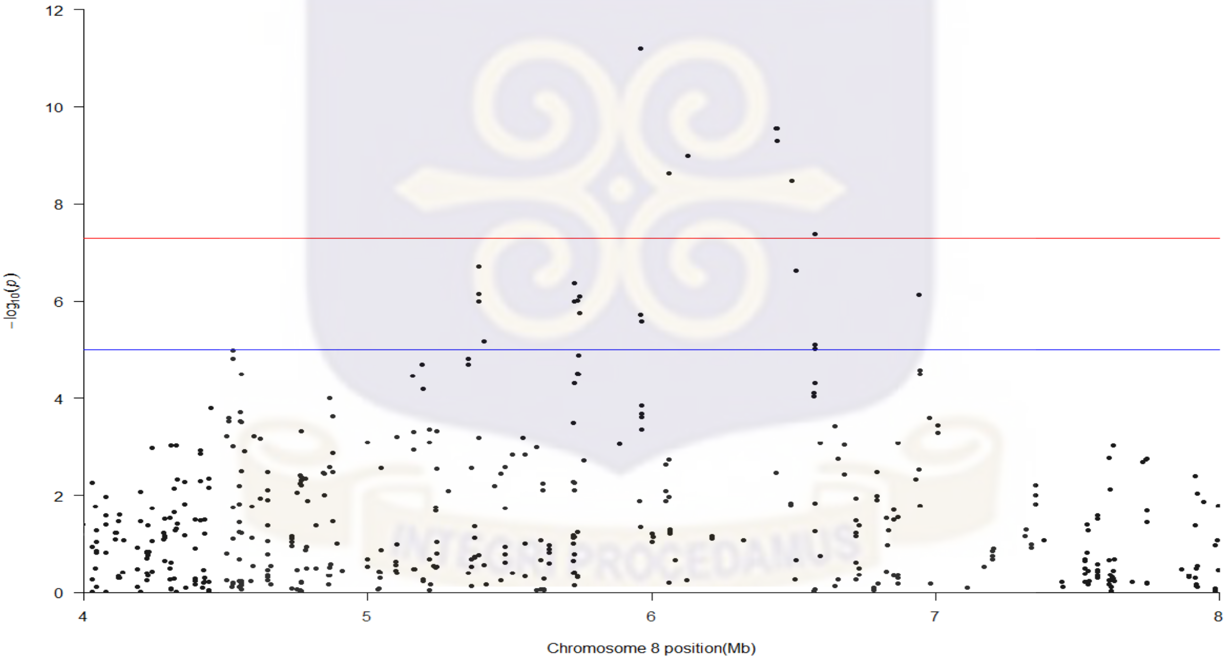


Fig 4. 7 Manhattan plot for genome-wide diagnosis of association for chromosome 8, zooming out the region with association signal for cassava green mite. Horizontal blue and red lines are the suggestive and Bonferroni threshold lines, respectively

Table 4. 8 Genes annotated as candidates for association with CGM resistance.

Gene	Chromosome	Gene description
Zinc finger transcription factor genes		
Manes.08G058500	8	C2H2-like-zinc-finger-protein
Manes.08G048200	8	C2H2-type-zinc-finger-family-protein
Manes.08G048800	8	CCCH-type-zinc-finger-protein-with-ARM-repeat-domain
Manes.08G034200	8	Dof-type-zinc-finger-DNA-binding-family-protein
Manes.08G046400	8	KH-domain-containing-protein-/-zinc-finger-(CCCH-type)-family-protein
Manes.08G041900	8	zinc-finger-protein-8
Pentatricopeptide repeat family		
Manes.08G026500	8	Pentatricopeptide-repeat-(PPR)-superfamily-protein
Manes.08G053900	8	Pentatricopeptide-repeat-(PPR-like)-superfamily-protein
Manes.08G060500	8	PENTATRICOPEPTIDE-REPEAT-596
MYB-domain transcription factor genes		
Manes.08G058000	8	myb-domain-protein-106
Manes.08G045400	8	myb-like-HTH-transcriptional-regulator-family-protein
MADS-domain transcription factor genes		
Manes.08G035100	8	AGAMOUS-like-80
Homeodomain transcription factor genes		
Manes.08G043900	8	homeobox-from-Arabidopsis-thaliana
Manes.08G024700	8	Basic-leucine-zipper-(bZIP)-transcription-factor-family-protein
Manes.08G046700	8	bZIP-transcription-factor-family-protein
Trichome Birefringence related protein		
Manes.08G044000	8	PROTEIN TRICHOME BIREFRINGENCE-RELATED
Ethylene-responsive transcription factors		
Manes.08G026900	8	SAUR-like-auxin-responsive-protein-family-

4.4 DISCUSSION

This study described the application of genome-wide association on improving CGM resistance on *M. esculenta* accessions. The analysis of variance clearly indicated effects of genotypes, locations and years variations. The average monthly temperature of 27 – 29^{o}C and relative humidity of 52-72% experienced during the seasons seem to coincide with the optimum temperature of 27^{o}C and relative humidity of 50-70% (Hahn *et al.*, 1989; Yaninek *et al.*, 1989) and average daily temperatures of 28^{o}C and relative humidity of 72 – 75% (Chalwe, 2012) reported for maximum oviposition of CGM. More rains are normally experienced in Umudike and Otobi than Kano. This is a reason for the highest CGMS mean found in Kano. Consistent with the observation of Yaninek *et al.* (1989), new plant growth are triggered by rainfall and mites are washed off the leaves during the rainy seasons.

The estimates of the broad-sense heritability for the traits evaluated ranged from 0.15 - 0.30, heritability was generally low to moderate. As might be expected, h^2_B estimates in this study is lower than the narrow sense heritability estimates reported by (Chalwe *et al.*, 2015) for cassava green mite population density and leaf damage, tip size, tip compactness and leaf retention. The heritability estimates indicate that selection of these traits should result in significant gains in cassava germplasm improvement (Clark and Watkins, 2012).

Correlation estimates in crop breeding helps to determine the success of indirectly selecting one trait for another (Falconer *et al.*, 1996). The result of this study showed that LP, LR, SG, STS and STC significantly and negatively correlated with CGMS. This means that genotypes with high pubescent leaves, outstanding leaf retention, stay green ability, compacted shoot tip and large shoot tip size tend to be resistant/tolerant to cassava green mite attack. The association of leaf and shoot pubescence and cassava green mite has been reported by several workers (Byrne *et al.*, 1983a; Yaninek and Hanna, 2003, Chalwe *et al.*, 2015; Molo *et al.*, 2016). Pubescence most likely works as protective barriers for the young shoot and expanding leaves where the mite prefer to feed. A distance between the trichomes of 0.3 mm or less makes it difficult for the mites to feed and rest (Yaninek and Hanna, 2003). Pubescence, therefore, acts to protect the most susceptible part of the plant from the *Mononychellus* mites to genetically overcome this barrier (Hershey, 1987). It has also been suggested that pubescence helps the biological control agents (*T. aripo*) to colonize the plant.

Many crops have long, complex histories of domestication and breeding, including cassava. Relatedness amongst cultivars can lead to population structure which can confound the results of GWAS (Yu *et al.*, 2006; Price *et al.*, 2010). Population structure analysis allowed the assignment of accessions to discrete sub-populations based on their genetic similarities from a subset of the SNPs assayed, thereby providing information about the number of sub-populations underlying the observed genetic diversity (Gapare *et al.*, 2017a). The population structure analysis in this study showed a subtle differentiation and occupies similar genetic space which supports (Wolfe *et al.*, 2016) findings. This may be consistent with a history of germplasm sharing and recurrent use of elite parents among the breeding institutes (Wolfe *et al.*, 2016).

In spite of the advantages of GWAS for revealing genetic polymorphisms underlying agronomic traits, this approach is prone to introduction of false positives due to population structure (Lander *et al.*, 1994; Zhang *et al.*, 2010; Kang *et al.*, 2008). False-positive associations were therefore examined using three different standard GWAS models. These models showed significant corrections when both K and PK models were incorporated in GWAS while the naïve model has high false positive rates. This confirms the findings by Gapare *et al.* (2017b); Pasam *et al.* (2012); Crowell *et al.* (2016). The incorporation of both K and PK matrices into a mixed linear model (MLM) has been successfully used previously in other species that exhibited significant population structure and relatedness (Gajardo *et al.*, 2015; Zhou *et al.*, 2016). Furthermore, the K-model computational time is faster and no additional steps like identifying population structure and principal components in the germplasm are required (Pasam *et al.*, 2012). In an exploratory analysis carried out by Pasam *et al.* (2012), mostly consistent results were obtained for all three approaches, the K-model was used in the complete data analysis of all the traits to avoid redundancy of data.

Most reports have aimed at studying the cultural control, host-plant resistance, biological and chemical control (Chalwe *et al.*, 2015; Chipeta *et al.*, 2013, Mutisya *et al.*, 2015; Mcgregor, 2016, Yaninek *et al.*, 1993) but the possibility of using genetic resistance to *M. tanajoa* on cassava has not been considered, particularly using the modern genome-wide association approach. Successfully, 35 unique genes were identified within SNPs associated with cassava green mite resistance, leaf pubescence and leaf retention at 4-8 MB on chromosome 8. Previous QTL mapping studies have reported a few number of QTL for cassava green mite. Nzuki *et al.* (2017), identified two QTL linked to CGM resistance (qCGMc5Ar and qCGMc10Ar) on chromosomes V and X respectively, with the maximum LOD of 20.19 at C1 (with PVE 6.48) and 24.03 at C2 (with PVE of 4.11). Ceballos *et al.* (2010) and Choperena, (2007) detected two SSR markers (NS1009 and NS346) that showed high association with CGM resistance. These results if validated and translated into marker-assisted breeding strategies will complement conventional breeding approaches to improve cassava varieties for resistance to cassava green mite.

The effect of genotype by environment (G x E) interaction was demonstrated for all the traits over three years across the locations by comparing GWAS results. Here, the GWAS peak associated with the candidate genes are significant in years 2013 and 2015, but not in year 2014. This could largely be due to different weather patterns experienced during the growing seasons (Zhao *et al.*, 2011). The effect of G x E interactions was also demonstrated for all the traits in three different locations (Umudike, Otobi and Kano states) across the years. In this case, there was no effect of G x E interactions for all traits. The same significant SNP marker was detected (CGMS, LP and LR) in all locations. The traits were insensitive to the location and, therefore, could be amenable to selection. G x E effects improve the ability to detect moderate strength and rare alleles as well as provide better resolution for the hit found in this study.

Pleiotropic effects of a target gene can be either beneficial or harmful in the context of plant breeding (Boerma and Walker, 2005; Brown, 2002) and it helps to understand the underlying genetic cause of multiple trait associations. In this study, pleiotropic effects may be beneficial as genes conferring resistance to CGM is linked to leaf pubescence and leaf retention. However, these desirable traits (leaf pubescence and leaf retention) may be co-introduced along with the pest resistance into susceptible, glabrous cassava varieties. There are cases where pleiotropic effects may be harmful for example in the case of blast resistance in rice, many late maturity varieties that are resistant to blast disease are used as donors to introduce disease resistance into susceptible, early maturity *temperate japonica* varieties (Boerma and Walker, 2005; Brown, 2002; Zhao *et al.*, 2010). Such undesirable traits may be co-introduced along with the disease resistance.

The use of many accessions in GWAS not only serves as mapping population between traits and DNA polymorphisms but also allows us to unravel the origin of genetic correlations among the phenotypic traits, that is pleiotropy versus genetically linked genes and facilitates the selection of donors with combinations of traits that are likely to be adaptive and advantageous for breeding in target environments (Zhao *et al*., 2011). Within 4 - 8 Mb region containing the GWAS peak, 17 candidate genes were observed. Protein trichome birefringence-related (TBR) genes are said to be involved in increased levels of crystalline secondary wall cellulose in trichomes and stem development (Bischoff *et al*., 2010). This gene is also known to be involved in many developmental processes including deference of insects, herbivores, microbes, maintenance of leaf temperature and transpiration regulation (Zhao *et al*., 2015). Homeodomain-leucine zipper genes are unique to plant kingdom and participate in organ and vascular development including trichome development (Ariel *et al*., 2007; Zhao *et al*., 2015). MYB- domain transcription factor genes like myb-like HTH are involved in specification of the leaf proximo-distal axis and also regulates trichome differentiation in *Nicotiana tabacum* (tobacco) (Payne *et al*., 1999). AGAMOUS - like 80 (AGL80) is a member of MADS- domain transcription factors, initially identified as floral meristem regulators and play important roles especially in flower and fruit development (Smaczniak *et al*., 2012). Zinc- finger protein 8 (ZFP8) is required for the initiation of inflorence trichomes in response to gibberellin and cytokinin (Gan *et al*., 2007). In *Cucumis sativus*, ethylene treatment increased the number of cells per trichomes (Kazama *et al*., 2004). The pentatricopeptide repeat (PPR) family has been found to function in plant defense (Nzuki *et al*., 2017). No functional resistance genes have been cloned in cassava (Lozano *et al*., 2015); however, genes found in this study have strong homology with previously reported genes from other species.

4.5 CONCLUSION

The current study is the first to apply GWAS to dissecting the genetic basis of cassava green mite resistance on African cassava germplasm. The genome-wide association analysis led to the identification of 17 candidate genes that appear to be associated with cassava green mite resistance, leaf pubescence and leaf retention in *M. esculenta*. This approach provides useful tool to plant breeders to discover valuable genes and alleles from the world cassava germplasm collection for cultivar improvement. The most significant marker (S8_5962253) observed for CGMS, LP and LR was stable across the locations, this provides promising targets for the application of marker-assisted selection. Validation studies involving fine mapping and joint linkage mapping will be required to clearly understand the relationship between these candidate genes and the phenotypes observed in the GWAS hit found in this study. The heritability estimates indicate that selection of these traits should result in significant gains in cassava germplasm improvement. The population structure analysis showed that the accessions occupied similar genetic space, which provides information about the relatedness amongst accessions.

CHAPTER FIVE

5.0 ASSESSMENT OF STABILITY AND ADAPTABILITY EFFECTS ON RESISTANCE TO CASSAVA GREEN MITE AND OTHER AGRONOMIC TRAITS OF CASSAVA USING AMMI ANALYSIS

5.1 INTRODUCTION

Cassava is a starchy root crop, which is widely grown in many parts of sub – sahara Africa, with Nigeria being the highest producer (FAO, 2014). More than 50% of Nigeria's population eats cassava at least once in a day (Njoku *et al.*, 2014) and it serves as famine reserve crop in the country. Cassava serves as a primary food source and income generation for more than 1 billion people (Lebot, 2009; Ospina and Ceballos, 2002) including the poorest on the continent. It is also used for animal feed and industrial raw material such as starch and bio-fuel production (Ceballos *et al.*, 2012).

The yield of cassava has declined due to abiotic and biotic stresses. Among the major pests of cassava, cassava green mite (CGM) has been called the most destructive pest of cassava because it has been reported to cause the greatest yield losses in the Americas and Africa (Bellotti *et al.*, 2012), especially in the seasonally dry regions of the lowland tropics. However, the selection of resistant genotypes to these constraints is complicated because of the complexity of the genotype responses across environments. This differential genotypic expression across environments is referred to as genotype x environment interaction (GEI) (Fox *et al.*, 1997). This type of interaction reduces selection efficiency and the accuracy of genotype recommendation (Farshadfar, 2013). In plant breeding and varietal release programmes, adequate consideration of GEI enables plant breeders to identify superior genotypes with better stability and locations that best represents production environments (Yan *et al*., 2000). Most GEI studies on cassava have focused on yield traits such as fresh root yield (Egesi *et al*., 2007; Ssemakula and Dixon, 2007). Only few experiments have aimed at studying the effects of GEI on CGM (Bellotti *et al*., 2012; Chalwe, 2012). It is often difficult to determine the stability pattern of genotypic responses across environments without the use of appropriate analytical and statistical tools such as additive main effects and multiplicative interactions (AMMI).

The AMMI model integrates analysis of variance (ANOVA) and principal component analysis (PCA) into a unified approach that can be used to analyze multi-locational trials (Crossa *et al*., 1990; Gauch and Zobel, 1996). AMMI uses analysis of variance to study the main effects of genotypes and environments and a principal component analysis for the residual multiplicative interaction among genotypes and environments. AMMI analysis can be used to determine stability of the genotypes across locations and also helping in grouping environments with the best genotypes into mega-environments using the PCA (principal component axis) scores and AMMI stability value (ASV) (Hagos and Abay, 2013). The ASV is based on the AMMI model's IPCA1 and IPCA2 (interaction principal components axes 1 and 2, respectively) scores for each genotype (Purchase *et al*., 2000). Genotypes having the least ASV are considered as widely adapted genotypes. Stability per se does not give much information about the level of yield so Farshadfar *et al*. (2011) and Tumuhimbise *et al*. (2014) used yield stability index (YSI) which combined high yield performance with stability. Both the YSI and the GSI are based on the sum of the ranking due to ASV scores and yield or performance ranking. Lower YSI and GSI values indicate genotypes that combine high yield or performance with stability.

Shoot morphological traits such as high leaf pubescence, leaf retention, stay green, large shoot tip size and compactness have been reported to promote cassava resistance to CGM (Aina *et al.*, 2007), therefore selecting genotypes for stability and enhanced expression of such traits would improve the effectiveness of resistance and yield (Aina *et al.*, 2007). In this study, multi-locational trials were conducted and AMMI was used to study GEI for CGM resistance and other useful agronomic traits. The main objective of the study is to assess the performance and stability of cassava genotypes for CGM resistance and other useful agronomic traits. The specific objectives are to

1. Identify best genotypes that exhibit high stability to CGM resistance within and across environments
2. Identify stable genotypes with enhanced expression of the shoot morphological traits that promote resistance to CGM and increase yield
3. Identify locations that best represent target environment for high expression of the traits

5.2 MATERIALS AND METHODS

5.2.1 Study sites

The study was conducted in 2015 and 2016 at three sites: Umudike (annual rainfall of 2200 mm; altitude 120 m; mean annual temperature of 22 to 31^{o}C; coordinates 7^{o}24’E, 5^{o}29’ N; Dystric Luvisol soils; humid forest); Igbariam (annual rainfall of 1800 mm; altitude 150 m; mean annual temperature of 24 to 32^{o}C; coordinates 7^{o}31’ E, 5^{o}56’ N; Dystric Luvisol soils; forest–savanna transition); and Otobi (annual rainfall of 1500 mm; altitude 319 m; mean annual temperature of 24 to 35^{o}C; coordinates 7^{o}20’ E, 8^{o}41’ N; Ferric Luvisol soils; southern Guinea savanna) in Nigeria. These sites represent the major cassava-growing agro-ecological zones in the country.

5.2.2 Development of the study population

The population was formed by crossing two parents with contrasting responses to CGM; TMEB778 is the female parent and TMEB419 is the male parent. TMEB778 is tolerant to CGM and high yielding. In contrast, TMEB419 is very susceptible to CGM. These genotypes, TMEB419 and TMEB778 were chosen for the evaluation because of their commercial relevance in Nigeria.

Pairwise crossing blocks for TMEB778 and TMEB419 parents were established at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

One hundred and twenty harvested F_1 botanical seeds were allowed a two - month dormancy period before being sowed in nurseries in February 2014 under screen house conditions. The seeds were sowed in trays filled with sterilized soil with a mixture of loamy and sandy soil in a ratio of 2:1, respectively in the screen house. Seeds germinated quickly at optimal soil temperatures (30 to 35^{0}C) and moisture regimes. They were irrigated twice daily, in the morning and evening. Out of the one hundred and twenty seeds sown, only eighty seeds started germinating from 10 to 12 days after planting and were transplanted when they attained 15 to 20 cm height. After two months in the nursery, F_1 seedlings were transplanted to a well-prepared field and only sixty-two seedlings survived the shock of transplanting to the field. Harvesting was done at 12 months after planting in April 2015, after which they were cloned to generate at least 10 stem cuttings per seedling for clonal evaluation.

5.2.3 Field layout and experimental design

The sixty-two F_1 progeny were used in the study. The trial was laid out in a randomized incomplete block design with three replications. Each plot consisted of 5 plants per row on ridges. Spacing between ridges was 1m and also between plants within the row providing a total population of 10 000 plants ha^{-1}. The trial was evaluated in 2015/2016 and 2016/2017 cropping seasons. The parental clones served as the check or control. NPK 15:15:15 fertilizer was applied at the rate of 600kg/ha. Fertilizer was applied at eight weeks after planting using the ring method, where a round small channel is made around the plants to input the fertilizers after planting. The field was weeded three times during the first four months. Weeding was done by hoe.

5.2.4 Data collection

Data were collected on cassava green mite severity, leaf pubescence, leaf retention, stay green, shoot tip size and shoot tip compactness. The traits were evaluated during the peak of dry season (January) and beginning of rainy season (April) at six and nine months after planting respectively (Table 5.1). At harvest (12 months after planting), fresh root yield, biomass and root dry matter content were evaluated (Table 5.1).

Table 5. 1 Description of the studied traits

Trait type	Trait name	Abbreviation	Description of trait
Stress trait (categorical)	Cassava green mite severity	CGMS6 CGMS9	Cassava green mite severity at 6 and 9 months after planting (MAP). Symptom severity is scored on a scale from 1 = no symptoms to 5 = extremely severe
Morphological (categorical)	Leaf pubescence	LP6 LP9	Visual rating of the degree of hairiness on the young leaf with 0 = glabrous, 3 = little pubescence, 5 = moderate pubescence and 7 = high pubescence at 6 and 9 MAP
Morphological (categorical)	Leaf retention	LR6 LR9	Visual rating of leaf longevity using a scale of 1= very poor, 2 = poor, 3 = fair, 4 = good, 5 = outstanding at 6 and 9 MAP
Morphological (categorical)	Stay Green	SG6 SG9	Visual rating based on leaf longevity and stay green ability using a scale of 1 = poor (<50% of the leaves are live and green), 2 = moderately good (50-74% of the leaves are live and green), 3 = very good (≥75% of the leaves are live and green) at 6 and 9 MAP

Table 5.1 continued

Morphological (categorical)	Shoot Tip Size	STS6 STS9	Visual assessment of shoot apices based on how large or small the shoot apices are and categorised on a scale of 1-3 where 1 = small 2= medium and 3 = large at 6 and 9 MAP
Morphological (categorical)	Shoot Tip Compactness	STC6 STC9	Visual assessment of the compactness of shoot apices based on how closely the shoot apices are; with 1 = loose, 2 = moderately compact, 3 = compact at 6 and 9 MAP
Agronomic (continuous)	Fresh Root Yield	FRY	Total fresh weight of storage roots harvested per plot (scale kg)
Agronomic (continuous)	Biomass	Biomass	Total fresh weight of leaves, stem and original planting stake (scale kg)
Agronomic (continuous)	Root Dry Matter Content	RDMC	Determined by the specific gravity method by Kawano *et al*. (1987) (measured in %)

5.2.5 Data analysis

Data were analyzed using Genstat version 12.1 and R statistical software package. The additive main effect and multiplicative interaction (AMMI) analysis was performed using the model suggested by Farshadfar *et al*. (2011) as follows:

$$Y_{ij} = \mu + G_i + E_j + \sum_{k=1}^{n} \lambda_k \alpha_{ik} \gamma_{jk} + e_{ij}$$

where Yij is the yield of the ith genotype in the jth environment, μ is the grand mean, Gi is the ith genotypic effect, Ej is the jth environment effect, λ_k is the square root of the eigenvalue of the PCA axis k, α_{ik} and γ_{jk} are the principal component scores for PCA axis k of the ith genotype and the jth environment, respectively, and eij is the residual.

The AMMI model fits the analysis of variance for genotypes and environments effects as the additive main effects and fits the genotype by environment interaction by principal component analysis.

5.2.5.1 Stability analysis

5.2.5.1.1 AMMI stability value (ASV) analysis

AMMI stability value (ASV) was calculated for each genotype according to the relative contributions of the principal component axis scores (IPCA1 and IPCA2) to the interaction sum of squares.

The AMMI stability value (ASV) as described by Purchase *et al*. (2000) was calculated as follows:

$$ASV = \sqrt{\left[\frac{IPCA1_{Sum\ of\ squares}}{IPCA2_{Sum\ of\ squares}}(IPCA1_{score})\right]^2 + (IPCA2_{score})^2}$$

where IPCA1Sum of squares/IPCA2Sum of squares is the weight given to the IPCA1-value by dividing the IPCA1 sum of squares (from the AMMI analysis of variance table) by the IPCA2 sum of squares. The larger the IPCA score is, either negative or positive, the more adapted a genotype is to a certain environment. Smaller ASV scores indicate a more stable genotype across environments (Farshadfar *et al.*, 2011).

5.2.5.1.2 Yield stability index

A stability index was calculated for each genotype based on summing the ranking of overall mean performances for each trait and the ranking for ASV for each trait.

Yield stability index was also calculated using the sum of the ranking based on yield (traits) and ranking based on the AMMI stability value. The YSI was calculated as follows:

YSI = RASV + RY;

Where: YSI = yield stability index for the genotype across environments for each trait; RASV = rank of the genotype across environments based on ASV; RY = rank of the genotypes based on mean performance across environments. The genotype with the lowest YSI was considered the best for a particular trait across environments. To identify superior genotypes across traits, the YSI ranks of each genotype were summed for all the traits, and the genotype with smallest rank sum (∑rank) was considered the best across traits.

Descriptive statistics and pearson's correlation were also carried out.

5.3 RESULTS

5.3.1 Descriptive statistics

Summary statistics of phenotypic data obtained for two growing seasons 2015/2016 and 2016/2017 in the three locations; Igbariam, Otobi and Umudike are presented in Table 5.2. The mean of CGM severity was 2.59 and 2.16 (on a scale of 1 – 5) at 6 MAP and 9 MAP respectively across all the genotypes evaluated. The CGM severity recorded at 9 MAP is lower than that of the 6 MAP. Otobi had the highest mean CGM severity (3.03 at 6 and 2.34 at 9 MAP), and was significantly ($P \leq 0.05$) different from Igbariam (2.44 at 6 MAP and 2.27 at 9 MAP) and Umudike (2.33 at 6 MAP and 1.83 at 9 MAP). The highest mean LP at 6 and 9 MAP were recorded in Igbariam and was significantly different ($P \leq 0.05$) from Umudike and Otobi. Similar result was observed for LR at 6 and 9 MAP. Igbariam had the highest mean LR (4.15 at 6 MAP and 4.23 at 9 MAP), followed by Umudike (3.61 at 6 MAP and 2.90 at 9MAP) and then Otobi (3.50 at 6 MAP and 2.35 at 9 MAP). Most genotypes showed higher stay green at 9 MAP than at 6 MAP (2.45 vs. 2.16, respectively). SG at 6 MAP was significantly higher at Igariam (2.56), than Umudike (2.18) and Otobi (1.66). Most genotypes at Igbariam had larger and compact shoot tip at 6 and 9 MAP than Umudike and Otobi. The mean of STC was 2.55 at 6 MAP and 2.62 at 9 MAP at Igbariam, 2.36 at 6 MAP and 1.72 at 9 MAP for Umudike and then 1.86 at 6 MAP and 1.31 at 9 MAP at Otobi. Similar trend was observed for STS at 6 and 9 MAP. The highest mean biomass yield was recorded at Igbariam (11.40) which was significantly ($P \leq 0.05$) different from Umudike (8.51) and Otobi (5.08). The fresh root weight was significantly higher at Igbariam (28.40 t/ha) than Umudike (19.42 t/ha) and Otobi (16.56 t/ha). The highest dry matter content was recorded at Igbariam (27.97), followed by Umudike (27.36) and then Otobi (26.65). Additionally, these locations were significantly different at $P \leq 0.05$ for dry matter content. The standard error of mean (SE) ranged from 0.06 to 0.90.

Variabilities for the traits evaluated for the two growing seasons in the three locations were estimated by coefficient of variation, ranged from 22.70% for RDMC to 78.30% for LP at 9 MAP. Broad-sense heritability estimates (h^2_B) ranged from 0.19 to 0.73. Heritability estimates was highest for RDMC (0.73) and least heritable trait was CGMS at 6 MAP (0.19).

Table 5. 2 Descriptive statistics (mean ± standard error of mean (SE), coefficient of variation (CV) and broad-sense heritability estimates (h^2_B) for phenotypic data across locations and years

	Igbariam			Otobi			Umudike			Pooled			
Trait	Mean ± SE	CV (%)	h^2_B	Mean ± SE	CV (%)	h^2_B	Mean ± SE	CV (%)	h^2_B	Mean ± SE	CV (%)	h^2_B	LSD$_{(0.05)}$
CGMS6	2.44 ± 0.04	22.28	0.39	3.03 ± 0.07	30.00	0.28	2.33 ± 0.07	39.73	0.32	2.59 ± 0.08	30.00	0.19	0.16
CGMS9	2.27 ± 0.07	44.06	0.21	2.34 ± 0.05	30.81	0.17	1.83 ± 0.05	38.50	0.23	2.16 ± 0.06	38.60	0.32	0.16
LP6	4.37 ± 0.20	63.66	0.20	2.95 ± 0.23	101.50	0.28	4.21 ± 0.19	59.35	0.47	3.88 ± 0.28	71.00	0.25	0.55
LP9	5.06 ± 0.20	55.53	0.45	0.93 ± 0.14	193.70	0.35	2.83 ± 0.18	86.21	0.42	3.09 ± 0.25	78.30	0.24	0.49
LR6	4.15 ± 0.06	22.58	0.49	3.50 ± 0.08	28.77	0.20	3.61 ± 0.07	26.56	0.30	3.78 ± 0.09	25.60	0.24	0.19
LR9	4.23 ± 0.07	22.25	0.38	2.35 ± 0.07	41.69	0.16	2.90 ± 0.07	33.27	0.37	3.26 ± 0.09	29.60	0.27	0.19
SG6	2.56 ± 0.04	21.19	0.58	1.66 ± 0.05	40.82	0.27	2.18 ± 0.06	34.11	0.42	2.16 ± 0.07	30.00	0.32	0.13
SG9	2.43 ± 0.04	23.65	0.44	2.41 ± 0.04	23.64	0.16	2.51 ± 0.05	23.74	0.38	2.45 ± 0.05	23.40	0.32	0.12
STC6	2.55 ± 0.04	24.22	0.38	1.86 ± 0.06	46.38	0.25	2.36 ± 0.05	28.11	0.40	2.28 ± 0.07	31.40	0.21	0.14
STC9	2.62 ± 0.05	25.19	0.48	1.31 ± 0.05	46.24	0.15	1.72 ± 0.06	45.56	0.53	1.94 ± 0.07	35.50	0.23	0.13
STS6	2.65 ± 0.04	24.71	0.49	1.34 ± 0.05	48.15	0.13	1.85 ± 0.06	45.97	0.41	2.00 ± 0.07	35.90	0.29	0.14
STS9	2.71 ± 0.04	21.56	0.36	1.95 ± 0.07	47.42	0.25	2.46 ± 0.06	31.10	0.51	2.40 ± 0.07	31.70	0.21	0.15
Biomass	11.40 ± 0.49	62.54	0.47	5.08 ± 0.35	71.55	0.33	8.51 ± 0.46	90.45	0.52	8.55 ± 0.47	71.60	0.30	1.23
FRY	28.40 ± 1.16	60.00	0.11	16.56 ± 0.88	66.71	0.12	19.42 ± 0.99	70.00	0.17	21.96 ± 0.90	55.00	0.20	2.87
RDMC	27.97 ± 0.48	21.80	0.72	26.65 ± 0.40	22.70	0.64	27.07 ± 0.43	19.29	0.89	27.37 ± 0.42	22.70	0.73	1.25

5.3.2 Correlation analysis

Table 5.3 presents phenotypic correlation between cassava green mite severity, shoot morphological and yield traits. There was a significant positive correlation between CGM severity at 6 and 9 MAP ($P \leq 0.001$, $r = 0.64$). This result showed that approximately 60% of the plants with severity at 6 MAP also had symptoms at 9 MAP. A significant negative correlation was observed between CGMS at 6 MAP and LP at 6 MAP ($P \leq 0.001$, $r = -0.63$), LP at 9 MAP ($P \leq 0.001$, $r = - 0.54$), LR at 6 MAP ($P \leq 0.001$, $r = -0.50$), LR at 9 MAP ($P \leq 0.001$, $r = -0.54$), SG at 6 MAP ($P \leq 0.001$, $r = -0.25$), SG at 9 MAP ($P \leq 0.001$, $r = -0.22$), STS at 6 MAP ($P \leq 0.001$, $r = -0.39$), STS at 9 MAP ($P \leq 0.001$, $r = -0.34$), STC at 6 MAP ($P \leq 0.05$, $r = -0.11$), STC at 9 MAP ($P \leq 0.01$, $r = -0.15$), biomass ($P \leq 0.001$, $r = -0.28$), FRY ($P \leq 0.001$, $r = -0.46$) and RDMC ($P \leq 0.001$, $r = -0.25$). Similar results were observed between CGMS at 9 MAP and all other traits evaluated. The results also showed that plants with severe CGM had glabrous to little leaf pubescent, poor leaf retention and stay green, small and loose shoot tip, low dry matter content, low root and stem yield .

On the other hand, a significant positive correlation was observed between LP at 6 MAP and LP at 9 MAP ($P \leq 0.001$, $r = 0.61$), LR at 6 MAP ($P \leq 0.001$, $r = 0.46$), LR at 9 MAP ($P \leq 0.001$, $r = 0.40$), SG at 6 MAP ($P \leq 0.001$, $r = 0.40$), SG at 9 MAP ($P \leq 0.001$, $r = 0.42$), STS at 6 MAP ($P \leq 0.001$, $r = 0.64$), STS at 9 MAP ($P \leq 0.001$, $r = 0.64$), STC at 6 MAP ($P \leq 0.001$, $r = 0.23$), STC at 9 MAP ($P \leq 0.001$, $r = 0.20$), biomass ($P \leq 0.001$, $r = 0.27$), FRY ($P \leq 0.001$, $r = 0.26$) and RDMC ($P \leq 0.05$, $r = 0.10$). The results showed that plants with high leaf pubescent had outstanding leaf retention, very good stay green, large and compact shoot tip tend to have high dry matter content and high yield

Table 5. 3 Phenotypic correlation coefficients for cassava green mite severity and other agronomic traits

	CGMS6	CGMS9	LP6	LP9	LR6	LR9	SG6	SG9	STS6	STS9	STC6	STC9	Biomass	FRY	RDMC
CGMS6	1	-0.64***	-0.63***	-0.54***	-0.50***	-0.54***	-0.25***	-0.22***	-0.39***	-0.34***	-0.11*	-0.15**	-0.25***	-0.46***	-0.25***
CGMS9		1	-0.46***	-0.43***	-0.11*	-0.13**	-0.15**	-0.11*	-0.25***	-0.22***	-0.16**	-0.12**	-0.26***	-0.48***	-0.22***
LP6			1	0.61***	0.46***	0.40***	0.40***	0.42***	0.64***	0.64***	0.23***	0.20***	0.27***	0.26***	0.10*
LP9				1	0.40***	0.52***	0.47***	0.53***	0.61***	0.62***	0.45***	0.43***	0.21***	0.25***	0.11*
LR6					1	0.74***	0.61***	0.65***	0.32***	0.40***	0.22***	0.23***	0.46***	0.41***	0.12*
LR9						1	0.52***	0.54***	0.25***	0.30***	0.47***	0.49***	0.72***	0.50***	0.15**
SG6							1	0.83***	0.51***	0.49***	0.21***	0.22***	0.66***	0.68***	0.02^{ns}
SG9								1	0.50***	0.50***	0.25***	0.27***	0.68***	0.68***	0.07^{ns}
STS6									1	0.47***	0.33***	0.12**	0.18**	0.46***	0.01^{ns}
STS9										1	0.40***	0.12**	0.21***	0.50***	0.08^{ns}
STC6											1	0.61***	0.40***	0.35***	0.08^{ns}
STC9												1	0.45***	0.32***	0.05^{ns}
Biomass													1	0.62***	0.15**
FRY														1	0.12**
RDMC															1

***significant at P<0.001; **significant at P<0.01; *significant at P<0.05, ns= not significant

5.3.3 AMMI analysis of variance

Cassava green mite severity (CGMS)

The AMMI ANOVA indicated highly significant (P < 0.001) effects of environment and genotype by environment interaction (P < 0.01) for CGMS6 but were not significant for CGMS9. IPCA1 was significant (P < 0.001 and P < 0.05) for CGMS6 and CGMS9 respectively (Table 5.4).

Genotype effect was highly significant (P < 0.001) for CGMS6 and CGMS9. Genotype effect accounted for 18.50% of the treatment sum of squares for CGMS at 6 MAP and 19.24% for CGMS at 9 MAP whereas the genotype by environment interaction and environment effects explained 74.63% and 6.94% at 6 MAP and 74.63% and 6.10% at 9 MAP. The first two interaction principal component axes (IPCA1 and IPCA2) accounted for 30.24% and 25.47% of the interaction sum of squares at 6 MAP and 29.01% and 24.83% at 9 MAP respectively (Table 5.4).

Table 5. 4 Summary of AMMI analyses for cassava green mite severity at 6 and 9 MAP of 62 cassava genotypes grown in six environments (three locations x two years) in Nigeria

		CGMS6					**CGMS9**				
Source	**df**	**SS**	**MS**	**F**	**% TSS**	**% GEI SS**	**SS**	**MS**	**F**	**% TSS**	**% GEI SS**
Total	1115	965.30	0.87				982.50	0.88			
Treatments	371	331.30	0.89	1.10***			349.20	0.94	1.15***		
Genotypes	61	61.30	1.01	1.23***	18.50		67.20	1.10	1.35***	19.24	
Environments	5	23.00	4.61	1.45***	6.94		21.30	4.27	1.47^{ns}	6.10	
Block	12	38.10	3.17	3.90*			34.70	2.90	3.54***		
GEI	305	247.00	0.81	0.99**	74.55		260.60	0.85	1.04^{ns}	74.63	
IPCA1	65	74.70	1.15	1.41***		30.24	75.60	1.16	1.42^{ns}		29.01
IPCA2	63	62.90	1.00	1.23^{ns}		25.47	64.70	1.03	1.26^{ns}		24.83
Error	732	595.90	0.81				598.60	0.82			

df = degrees of freedom; SS = sums of squares; MS = mean square; %TSS = percentage of treatment SS; %GEI SS = percentage of genotype by environment interaction SS; F = f-value; IPCA = interaction principal component axis; ***significant at P<0.001; **significant at P<0.01; *significant at P<0.05, ns= not significant; GEI = Genotype by environment interaction. Note: the block source of variation refers to blocks within environments

Leaf pubescence (LP)

Genotype by environment interaction was highly significant ($P < 0.001$) for LP6 and LP9. Effect of environment was highly significant ($P < 0.001$) for LP9 but not significant for LP6. Genotype effect and IPCA1 were highly significant ($P < 0.001$) for LP 6 and LP 9 (Table 5.5).

Genotype effect accounted for 18.46% of the treatment sum of squares for LP at 6 MAP and 14.01% for LP at 9 MAP whereas the genotype by environment interaction and environment effects explained 72.68% and 8.86% at 6 MAP and 70.51% and 15.52% at 9 MAP. The first two interaction principal component axes (IPCA1 and IPCA2) accounted for 33.24% and 25.82% of the interaction sum of squares at 6 MAP and 28.46% and 21.69% at 9 MAP respectively (Table 5.5).

Table 5. 5 Summary of AMMI analyses for leaf pubescence at 6 and 9 MAP of 62 cassava genotypes grown in six environments (three locations x two years) in Nigeria

		LP6					**LP9**				
Source	**df**	**SS**	**MS**	**F**	**% TSS**	**% GEI SS**	**SS**	**MS**	**F**	**% TSS**	**% GEI SS**
Total	1115	9273.00	8.32				7008.00	6.28			
Treatments	371	3613.00	9.74	1.31***			2262.00	6.10	1.08***		
Genotypes	61	667.00	10.93	1.47***	18.46		317.00	5.20	0.92***	14.01	
Environments	5	320.00	63.94	3.83^{ns}	8.86		351.00	70.16	1.39***	15.52	
Block	12	200.00	16.69	2.24***			605.00	50.39	8.91**		
GEI	305	2626.00	8.61	1.15***	72.68		1595.00	5.23	0.92***	70.51	
IPCA1	65	873.00	13.43	1.80***		33.24	454.00	6.99	1.24***		28.46
IPCA2	63	678.00	10.75	1.44^{ns}		25.82	346.00	5.49	0.97^{ns}		21.69
Error	732	5460.00	7.46				4141.00	5.66			

df = degrees of freedom; SS = sums of squares; MS = mean square; %TSS = percentage of treatment SS; %GEI SS = percentage of genotype by environment interaction SS; F = f-value; IPCA = interaction principal component axis; ***significant at P<0.001; **significant at P<0.01; *significant at P<0.05, ns= not significant; GEI = Genotype by environment interaction. Note: the block source of variation refers to blocks within environments

Leaf retention (LR)

Genotype by environment interaction was significant ($P < 0.05$) for LR6 and LR9. There was highly significant ($P < 0.001$) effect of environment for LR9 but not significant for LR6. Genotype effect was highly significant ($P < 0.001$) for LR9 and very significant ($P < 0.01$) for LR6. IPCA1 was very significant ($P < 0.05$) for LR6 and LR9 (Table 5.6).

For LR at 6 and 9 MAP, 20.89% and 14.94% of the treatment sum of squares was due to genotype effect respectively whilst environment and interaction effects accounted for 4.61% and 74.50% at 6 MAP and 10.07% and 74.98% at 9 MAP. IPCA1 and IPCA2 captured 39.74% and 19.09% for LR at 6 MAP and 41.61% and 17.78% for LP at 9 MAP, respectively (Table 5.6).

Table 5. 6 Summary of AMMI analyses for leaf retention at 6 and 9 MAP of 62 cassava genotypes grown in six environments (three locations x two years) in Nigeria

		LR6					**LR9**				
Source	**df**	**SS**	**MS**	**F**	**% TSS**	**% GEI SS**	**SS**	**MS**	**F**	**% TSS**	**% GEI SS**
Total	1115	1321.70	1.19				1520.20	1.36			
Treatments	371	535.70	1.44	1.37**			570.80	1.54	1.41***		
Genotypes	61	111.90	1.83	1.74**	20.89		85.30	1.40	1.28***	14.94	
Environments	5	24.70	4.93	3.75^{ns}	4.61		57.50	11.49	0.93***	10.07	
Block	12	15.80	1.32	1.25***			148.70	12.39	11.33^{ns}		
GEI	305	399.10	1.31	1.24*	74.50		428.00	1.40	1.28*	74.98	
IPCA1	65	158.60	2.44	2.32**		39.74	178.10	2.74	2.50**		41.61
IPCA2	63	76.20	1.21	1.15^{ns}		19.09	76.10	1.21	1.10^{ns}		17.78
Error	732	770.20	1.05				800.70	1.09			

df = degrees of freedom; SS = sums of squares; MS = mean square; %TSS = percentage of treatment SS; %GEI SS = percentage of genotype by environment interaction SS; F = f-value; IPCA = interaction principal component axis; ***significant at $P<0.001$; **significant at $P<0.01$; *significant at $P<0.05$, ns= not significant; GEI = Genotype by environment interaction. Note: the block source of variation refers to blocks within environments

Stay green (SG)

The AMMI ANOVA indicated highly significant ($P < 0.001$) effect of environment for SG9 but not significant for SG6. Genotype by environment interaction was significant ($P < 0.05$) for SG6 but not significant for SG9. Genotype effect was highly significant ($P < 0.001$) for SG6 and SG9. IPCA1 was very significant ($P < 0.01$) for SG6 and SG9 (Table 5.7).

SG at 6 and 9 MAP showed that 16.41% and 17.12% of treatment SS was attributable to genotypic effect, 2.90% and 8.52% to environmental effect, 80.69% and 74.32% to G x E interaction effect respectively. IPCA1 and IPCA2 accounted for 30.46% and 21.32% of G x E sum of squares at 6 MAP and 26.84% and 22.46% at 9 MAP, respectively (Table 5.7).

Table 5. 7 Summary of AMMI analyses for stay green at 6 and 9 MAP of 62 cassava genotypes grown in six environments (three locations x two years) in Nigeria.

		SG6					**SG9**				
Source	**df**	**SS**	**MS**	**F**	**% TSS**	**% GEI SS**	**SS**	**MS**	**F**	**% TSS**	**% GEI SS**
Total	1115	372.39	0.33				625.40	0.56			
Treatments	371	131.06	0.35	1.09***			230.10	0.62	1.24***		
Genotypes	61	21.51	0.35	1.08***	16.41		39.40	0.65	1.29***	17.12	
Environments	5	3.80	0.76	3.03^{ns}	2.90		19.60	3.93	1.62***	8.52	
Block	12	3.01	0.25	0.77^{ns}			29.20	2.43	4.86^{ns}		
GEI	305	105.75	0.35	1.06*	80.69		171.00	0.56	1.12^{ns}	74.32	
IPCA1	65	32.21	0.50	1.52**		30.46	45.90	0.71	1.41^{ns}		26.84
IPCA2	63	22.55	0.36	1.10^{ns}		21.32	38.40	0.61	1.22^{ns}		22.46
Error	732	238.32	0.33				366.20	0.50			

df = degrees of freedom; SS = sums of squares; MS = mean square; %TSS = percentage of treatment SS; %GEI SS = percentage of genotype by environment interaction SS; F = f-value; IPCA = interaction principal component axis; ***significant at $P<0.001$; **significant at $P<0.01$; *significant at $P<0.05$, ns= not significant; GEI = Genotype by environment interaction. Note: the block source of variation refers to blocks within environments

Shoot tip compactness (STC)

The AMMI ANOVA indicated highly significant ($P < 0.001$) effect of environment for STC9 but not significant for STC6. Genotype by environment interaction was significant ($P < 0.05$) for STC6 and STC9. Genotype effect was very significant ($P < 0.01$) for STC6 and significant ($P < 0.05$) for STC9. IPCA1 was significant for STC6 and STC9 (Table 5.8).

STC at 6 and 9 MAP captured 18.95% and 13.75% of percentage treatment sum of squares due to genotypic effects respectively. The percentage treatment SS due to environmental effect and G x E interaction effect contributed 12.18% and 68.87% for STC at 6 MAP and 15.78% and 70.43% at 9 MAP, respectively. IPCA1 and IPCA2 based on G x E interaction explained 38.46% and 22.48% at 6 MAP and 33.17% and 23.44% at 9 MAP (Table 5.8).

Table 5. 8 Summary of AMMI analyses for shoot tip compactness at 6 and 9 MAP of 62 cassava genotypes grown in six environments (three locations x two years) in Nigeria.

		STC6					**STC9**				
Source	**df**	**SS**	**MS**	**F**	**% TSS**	**% GEI SS**	**SS**	**MS**	**F**	**% TSS**	**% GEI SS**
Total	1115	708.30	0.64				670.70	0.60			
Treatments	371	239.00	0.64	1.05***			232.00	0.63	1.22***		
Genotypes	61	45.30	0.74	1.21**	18.95		31.90	0.52	1.02*	13.75	
Environments	5	29.10	5.82	3.29^{ns}	12.18		36.60	7.32	1.41***	15.78	
Block	12	21.20	1.77	2.89***			62.50	5.21	10.14**		
GEI	305	164.60	0.54	0.88*	68.87		163.40	0.54	1.04*	70.43	
IPCA1	65	63.30	0.97	1.59*		38.46	54.20	0.83	1.62**		33.17
IPCA2	63	37.00	0.59	0.96^{ns}		22.48	38.30	0.61	1.18^{ns}		23.44
Error	732	448.10	0.61				376.10	0.51			

df = degrees of freedom; SS = sums of squares; MS = mean square; %TSS = percentage of treatment SS; %GEI SS = percentage of genotype by environment interaction SS; F = f-value; IPCA = interaction principal component axis; ***significant at $P<0.001$; **significant at $P<0.01$; *significant at $P<0.05$, ns= not significant; GEI = Genotype by environment interaction. Note: the block source of variation refers to blocks within environments

Shoot tip size (STS)

The AMMI ANOVA indicated highly significant ($P < 0.001$) effect of environment for STS9 and very significant ($P < 0.01$) for STS6. Genotype by environment interaction was significant ($P < 0.05$) for STS6 but not significant for STS9. Genotype effect and IPCA1were significant ($P < 0.05$) for STS6 and STS9 (Table 5.9).

For STS at 6 and 9 MAP, 17.48% and 13.63% of the treatment sum of squares was due to genotype effect respectively whilst environment and genotype by environment interaction effects accounted for 12.54% and 69.99% at 6 MAP and 11.69% and 74.68% at 9 MAP. IPCA1 and IPCA2 captured 37.05% and 25.54% for STS at 6 MAP and 34.10% and 20.45% for STS at 9 MAP, respectively (Table 5.9).

Table 5. 9 Summary of AMMI analyses for shoot tip size at 6 and 9 MAP of 62 cassava genotypes grown in six environments (three locations x two years) in Nigeria.

		STS6					STS9				
Source	**df**	**SS**	**MS**	**F**	**% TSS**	**% GEI SS**	**SS**	**MS**	**F**	**% TSS**	**% GEI SS**
Total	1115	780.50	0.70				767.10	0.69			
Treatments	371	279.20	0.75	1.15***			267.80	0.72	1.21***		
Genotypes	61	48.80	0.80	1.22*	17.48		36.50	0.60	1.00*	13.63	
Environments	5	35.00	7.00	3.65*	12.54		31.30	6.26	1.23***	11.69	
Block	12	23.00	1.92	2.94***			60.90	5.08	8.48*		
GEI	305	195.40	0.64	0.98*	69.99		200.00	0.66	1.09^{ns}	74.68	
IPCA1	65	72.40	1.11	1.70*		37.05	68.20	1.05	1.75*		34.10
IPCA2	63	49.90	0.79	1.21^{ns}		25.54	40.90	0.65	1.08^{ns}		20.45
Error	732	478.30	0.65				438.40	0.60			

df = degrees of freedom; SS = sums of squares; MS = mean square; %TSS = percentage of treatment SS; %GEI SS = percentage of genotype by environment interaction SS; F = f-value; IPCA = interaction principal component axis; ***significant at $P<0.001$; **significant at $P<0.01$; *significant at $P<0.05$, ns= not significant; GEI = Genotype by environment interaction. Note: the block source of variation refers to blocks within environments

Fresh root yield (FRY)

Genotype by environment interaction, genotype effect and IPCA1 were highly significant (P < 0.001) for FRY. Effect of environment for FRY was very significant (P < 0.01) for FRY (Table 5.10).

Genotype by environment interaction effect (76.45%) contributed a greater proportion of the treatment sum of squares compared with genotype effect (18.09%) and environment effect (5.46%). The first two principal component axes (IPCA1 and IPCA2) accounted for 30.79% and 21.79% of the interaction sum of squares respectively (Table 5.10).

Table 5.10 Summary of AMMI analysis for fresh root yield of 62 cassava genotypes grown in six environments (three locations x two years) in Nigeria.

	FRY					
Source	**df**	**SS**	**MS**	**F**	**% TSS**	**% GEI SS**
Total	1115	386139.00	346.30			
Treatments	371	139401.00	375.70	1.15***		
Genotypes	61	25218.00	413.40	1.26***	18.09	
Environments	5	7610.00	1522.00	2.51*	5.46	
Block	12	7267.00	605.60	1.85***		
GEI	305	106573.00	349.40	1.07***	76.45	
IPCA1	65	32812.00	504.80	1.54***		30.79
IPCA2	63	23226.00	368.70	1.13^{ns}		21.79
Error	732	239471.00	327.10			

df = degrees of freedom; SS = sums of squares; MS = mean square; %TSS = percentage of treatment SS; %GEI SS = percentage of genotype by environment interaction SS; F = f-value; IPCA = interaction principal component axis; ***significant at P<0.001; **significant at P<0.01; *significant at P<0.05, ns= not significant; GEI = Genotype by environment interaction. Note: the block source of variation refers to blocks within environments

Root dry matter content (RDMC)

The AMMI ANOVA indicated significant ($P < 0.05$) effects of environment and genotype for RDMC. Genotype by environment interaction and IPCA1 highly significant ($P < 0.001$) for RDMC (Table 5.11).

The influence of genotype by environment interaction (80.00%) on root RDMC was greater than both genotype (18.16%) and environment effects (1.84%). The first two IPCAs (IPCA1 and IPCA2) contributed to 33.59% and 27.15% of the interaction sum of squares respectively (Table 5.11).

Table 5.11 Summary of AMMI analysis for root dry matter content of 62 cassava genotypes grown in six environments (three locations x two years) in Nigeria

		RDMC				
Source	**df**	**SS**	**MS**	**F**	**% TSS**	**% GEI SS**
Total	1115	45748.00	41.03			
Treatments	371	17965.00	48.42	1.33***		
Genotypes	61	3263.00	53.49	1.47*	18.16	
Environments	5	331.00	66.12	0.67^{ns}	1.84	
Block	12	1187.00	98.91	2.72*		
GEI	305	14372.00	47.12	1.30***	80.00	
IPCA1	65	4827.00	74.25	2.04***		33.59
IPCA2	63	3902.00	61.94	1.70*		27.15
Error	732	26596.00	36.33			

df = degrees of freedom; SS = sums of squares; MS = mean square; %TSS = percentage of treatment SS; %GEI SS = percentage of genotype by environment interaction SS; F = f-value; IPCA = interaction principal component axis; ***significant at $P<0.001$; **significant at $P<0.01$; *significant at $P<0.05$, ns= not significant; GEI = Genotype by environment interaction. Note: the block source of variation refers to blocks within environments

Biomass

Genotypes, environment, genotype by environment interaction and IPCA1 were highly significant (P < 0.001) for biomass (Table 5.12).

Genotype by environment interaction effect (77.76%) contributed a greater proportion of the treatment sum of squares compared with genotype effect (17.57%) and environment effect (4.68%). The first two principal component axes (IPCA1 and IPCA2) accounted for 32.39% and 26.58% of the interaction sum of squares respectively (Table 5.12).

Table 5. 12 Summary of AMMI analysis for biomass of 62 cassava genotypes grown in six environments (three locations x two years) in Nigeria.

		Biomass				
Source	**df**	**SS**	**MS**	**F**	**% TSS**	**% GEI SS**
Total	1115	45345.00	40.67			
Treatments	371	15757.00	42.47	1.13***		
Genotypes	61	2768.00	45.38	1.21***	17.57	
Environments	5	737.00	147.40	0.84***	4.68	
Block	12	2099.00	174.94	4.66***		
GEI	305	12252.00	40.17	1.07***	77.76	
IPCA1	65	3968.00	61.04	1.63***		32.39
IPCA2	63	3257.00	51.70	1.38^{ns}		26.58
Error	732	27489.00	37.55			

df = degrees of freedom; SS = sums of squares; MS = mean square; %TSS = percentage of treatment SS; %GEI SS = percentage of genotype by environment interaction SS; F = f-value; IPCA = interaction principal component axis; ***significant at P<0.001; **significant at P<0.01; *significant at P<0.05, ns= not significant; GEI = Genotype by environment interaction. Note: the block source of variation refers to blocks within environments

5.3.4 Stability analysis

Cassava green mite severity (CGMS)

Based on the scoring of CGMS, where the genotype with the least score (1) is considered as the best performer (resistant) and the highest score (5) is the worst performer (susceptible). Unlike the other traits evaluated, where the genotype with the highest value is the best performer and the least value presents the worst performer. Therefore, genotypes with the highest ASV and YSI scores were considered the best stable for cassava green mite resistance only. For other traits, genotypes with lowest ASV and YSI scores were considered the best performers.

According to ASV, the most stable genotype for CGMS at 6 MAP were IBA131866, IBA131746, IBA131749, IBA131741 and IBA131753 (Table 5.13) while for CGMS at 9 MAP, the genotypes IBA131847, IBA131798, IBA131872, IBA131768 and IBA131825 were the most stable genotypes (Table 5.15). The least stable genotypes at 6 MAP are IBA131870, IBA131858, IBA131863, IBA131851 and IBA131788 (Table 5.13). At 9 MAP, genotypes IBA131741, IBA131839, IBA131844, IBA131746 and IBA131742 are considered as the least stable genotypes (Table 5.15).

YSI ranked IBA131753, IBA131794, IBA131738, IBA131768 and IBA131821 at 6 MAP and genotypes IBA131872, IBA131754, IBA131847, IBA131794 and IBA131863 at 9 MAP as the genotypes that combined resistance with stability (Tables 5.13 and 5.15).

Tables 5.14 and 5.16 presents the best four genotypes selected by AMMI per environment for CGMS6 and CGMS9. At 6 MAP, genotypes IBA131866, IBA131798, IBA131749 were the best performers for the most stable environment (Umudike 2016) while the least stable environment (Igbariam 2016), genotypes IBA131749, IBA131812, IBA131856 and IBA131776 were selected

(Table 5.14). At 9 MAP, the most stable environment (Umudike 2015) identified IBA131847, IBA131798, IBA131730 and IBA131734 as the best performers while the least environment (Otobi 2016) selected genotypes IBA131749, IBA131812, IBA131856 and IBA131776 in that order (Table 5.16).

Table 5. 13 Ranking of the cassava genotypes based on cassava green mite severity, AMMI stability value, and yield stability index (YSI) at 6 MAP

Genotype	**ASV**	**YSI**	**RASV**	**RY**	**Mean**	**YSI rank**
IBA131753	0.78	109.50	58	51.50	1.67	62
IBA131794	0.78	108.50	57	51.50	1.67	61
IBA131738	0.78	107.50	56	51.50	1.67	60
IBA131768	0.62	107.00	49	58.00	1.50	59
IBA131821	0.57	105.00	47	58.00	1.50	58
IBA131796	0.57	104.00	46	58.00	1.50	57
IBA131763	0.57	103.00	45	58.00	1.50	56
IBA131847	0.57	102.00	44	58.00	1.50	55
IBA131872	0.69	101.50	50	51.50	1.67	54
IBA131844	0.57	101.00	43	58.00	1.50	53
IBA131839	0.52	100.00	38	62.00	1.33	51
IBA131861	0.57	100.00	42	58.00	1.50	52
IBA131746	1.04	95.00	61	34.00	2.00	50
IBA131752	0.72	86.00	52	34.00	2.00	49
IBA131749	1.03	85.50	60	25.50	2.17	48
IBA131748	0.44	76.50	33	43.50	1.83	47
IBA131762	0.44	75.50	32	43.50	1.83	46
IBA131817	0.62	73.50	48	25.50	2.17	45
IBA131750	0.76	72.50	55	17.50	2.33	44
IBA131809	0.76	71.50	54	17.50	2.33	43
IBA131866	1.19	71.00	62	9.00	2.67	42
IBA131826	0.52	66.50	41	25.50	2.17	41
IBA131741	0.95	65.50	59	6.50	2.83	40
IBA131827	0.52	64.50	39	25.50	2.17	39
IBA131754	0.23	63.50	12	51.50	1.67	38
IBA131784	0.30	58.50	24.5	34.00	2.00	35
IBA131836	0.30	58.50	24.5	34.00	2.00	36

Table 5.13 Continued

IBA131770	0.30	56.50	22.5	34.00	2.00	33
IBA131842	0.30	56.50	22.5	34.00	2.00	34
IBA131833	0.30	55.00	21	34.00	2.00	32
IBA131776	0.70	54.50	51	3.50	3.17	30
IBA131808	0.76	54.50	53	1.50	3.33	31
IBA131801	0.30	54.00	20	34.00	2.00	29
IBA131797	0.30	53.00	19	34.00	2.00	28
IBA131730	0.03	52.50	9	43.50	1.83	27
IBA131759	0.03	51.50	8	43.50	1.83	25
IBA131849	0.46	51.50	34	17.50	2.33	26
IBA131731	0.03	50.50	7	43.50	1.83	24
IBA131825	0.03	49.50	6	43.50	1.83	23
IBA131864	0.50	49.00	37	12.00	2.50	22
IBA131788	0.03	48.50	5	43.50	1.83	20
IBA131782	0.42	48.50	31	17.50	2.33	21
IBA131819	0.50	48.00	36	12.00	2.50	19
IBA131734	0.42	47.50	30	17.50	2.33	18
IBA131863	0.03	47.00	3.5	43.50	1.83	16
IBA131736	0.46	47.00	35	12.00	2.50	17
IBA131800	0.42	46.50	29	17.50	2.33	14
IBA131858	0.03	45.50	2	43.50	1.83	12
IBA131767	0.42	45.50	28	17.50	2.33	13
IBA131742	0.42	44.50	27	17.50	2.33	11
IBA131777	0.30	43.50	18	25.50	2.17	9
IBA131856	0.52	43.50	40	3.50	3.17	10
IBA131869	0.30	42.50	17	25.50	2.17	8
IBA131743	0.30	41.50	16	25.50	2.17	7
IBA131778	0.30	40.50	15	25.50	2.17	6
IBA131812	0.42	27.50	26	1.50	3.33	5
IBA131757	0.23	23.00	14	9.00	2.67	4
IBA131774	0.23	22.00	13	9.00	2.67	3
IBA131798	0.21	15.00	10	5.00	3.00	2
IBA131870	0.03	7.50	1	6.50	2.83	1

ASV = AMMI stability value; YSI = yield stability index for the genotype across environments for each trait; RASV = rank of the genotype across environments based on ASV; RY = rank of the genotypes based on mean performance across environments.

Table 5. 14 First four AMMI selections based on the most resistant genotypes at 6 MAP in each environment

Rank	Environment	Mean	Effect	1	2	3	4
1	Umudike2016	1.65	-1.01	IBA131866	IBA131798	IBA131749	IBA131872
2	Umudike2015	1.97	0.47	IBA131753	IBA131778	IBA131866	IBA131746
3	Igbariam2016	2.24	1.96	IBA131866	IBA131842	IBA131753	IBA131798
3	Igbariam2015	2.24	1.55	IBA131812	IBA131866	IBA131748	IBA131762
5	Otobi2015	2.26	-2.02	IBA131808	IBA131812	IBA131776	IBA131821
6	Otobi2016	2.36	-0.95	IBA131749	IBA131812	IBA131856	IBA131776

Ranking of the environment, 1 = most stable and 6 = least stable

Table 5. 15 Ranking of the cassava genotypes based on cassava green mite severity, AMMI stability value, and yield stability index (YSI) at 9 MAP

Genotypes	ASV	YSI	RASV	RY	Mean	YSI rank
IBA131872	0.72	105.50	60	45.5	2.33	62
IBA131754	0.63	101.50	56	45.5	2.33	61
IBA131847	0.96	97.00	62	35	2.50	60
IBA131863	0.32	93.50	33.5	60	2.00	59
IBA131794	0.32	93.50	33.5	60	2.00	58
IBA131861	0.32	92.00	32	60	2.00	57
IBA131849	0.32	91.00	31	60	2.00	56
IBA131748	0.32	90.00	30	60	2.00	55
IBA131858	0.53	87.00	52	35	2.50	54
IBA131768	0.71	85.50	59	26.5	2.67	53
IBA131827	0.50	85.00	50	35	2.50	52
IBA131825	0.71	84.50	58	26.5	2.67	51
IBA131869	0.50	84.00	49	35	2.50	50
IBA131826	0.50	83.00	48	35	2.50	49
IBA131763	0.45	81.00	46	35	2.50	48
IBA131801	0.45	78.00	43	35	2.50	47
IBA131731	0.63	75.50	57	18.5	2.83	46
IBA131817	0.28	73.50	28	45.5	2.33	45
IBA131864	0.28	72.50	27	45.5	2.33	44
IBA131836	0.58	71.50	53	18.5	2.83	43
IBA131738	0.28	70.50	25	45.5	2.33	42
IBA131757	0.41	68.50	42	26.5	2.67	41
IBA131800	0.41	67.50	41	26.5	2.67	40
IBA131767	0.62	67.00	55	12	3.00	39
IBA131777	0.59	66.00	54	12	3.00	38

Table 5.15 continued

IBA131785	0.46	65.50	47	18.5	2.83	37
IBA131798	0.87	65.00	61	4	3.50	36
IBA131730	0.36	64.50	38	26.5	2.67	35
IBA131762	0.36	63.50	37	26.5	2.67	34
IBA131770	0.36	62.50	36	26.5	2.67	33
IBA131729	0.27	62.50	17	45.5	2.33	32
IBA131784	0.36	61.50	35	26.5	2.67	31
IBA131870	0.07	60.50	6	54.5	2.17	30
IBA131752	0.50	59.50	51	8.5	3.17	29
IBA131742	0.07	59.50	5	54.5	2.17	28
IBA131746	0.07	58.50	4	54.5	2.17	27
IBA131796	0.19	57.50	12	45.5	2.33	26
IBA131844	0.07	57.50	3	54.5	2.17	25
IBA131749	0.45	57.00	45	12	3.00	24
IBA131759	0.19	56.50	11	45.5	2.33	23
IBA131839	0.07	56.50	2	54.5	2.17	22
IBA131833	0.19	55.50	10	45.5	2.33	21
IBA131741	0.07	55.50	1	54.5	2.17	20
IBA131743	0.19	54.50	9	45.5	2.33	19
IBA131782	0.19	53.50	8	45.5	2.33	18
IBA131778	0.19	52.50	7	45.5	2.33	17
IBA131753	0.20	50.00	15	35	2.50	16
IBA131808	0.20	49.00	14	35	2.50	15
IBA131866	0.39	48.50	40	8.5	3.17	14
IBA131819	0.45	48.00	44	4	3.50	13
IBA131812	0.36	41.00	39	2	3.67	12
IBA131842	0.32	41.00	29	12	3.00	11
IBA131809	0.27	40.50	22	18.5	2.83	10
IBA131734	0.27	39.50	21	18.5	2.83	9
IBA131856	0.27	38.00	19.5	18.5	2.83	8
IBA131821	0.27	38.00	19.5	18.5	2.83	7
IBA131750	0.27	36.50	18	18.5	2.83	6
IBA131851	0.28	32.50	26	6.5	3.33	5
IBA131774	0.28	30.50	24	6.5	3.33	4
IBA131776	0.22	28.00	16	12	3.00	3
IBA131797	0.27	24.00	23	1	3.83	2
IBA131736	0.20	17.00	13	4	3.50	1

ASV = AMMI stability value; YSI = yield stability index for the genotype across environments for each trait; RASV = rank of the genotype across environments based on ASV; RY = rank of the genotypes based on mean performance across environments.

Table 5. 16 First four AMMI selections based on the most resistant genotypes at 9 MAP in each environment

Rank	**Environment**	**Mean**	**Effect**	**1**	**2**	**3**	**4**
1	Umudike2015	1.90	1.29	IBA131847	IBA131798	IBA131730	IBA131734
2	Igbariam2015	2.40	0.86	IBA131797	IBA131767	IBA131749	IBA131785
3	Igbariam2016	2.44	1.19	IBA131847	IBA131798	IBA131774	IBA131836
4	Otobi2015	2.94	-2.15	IBA131749	IBA131767	IBA131768	IBA131798
5	Umudike2016	2.97	-1.84	IBA131736	IBA131812	IBA131851	IBA131731
6	Otobi2016	3.18	0.65	IBA131847	IBA131798	IBA131736	IBA131812

Ranking of the environment, 1 = most stable and 6 = least stable

Leaf pubescence

The genotypes IBA131743, IBA131858, IBA131847, IBA131796 and IBA131819 at 6 MAP and at 9 MAP, genotypes IBA131863, IBA131796, IBA131749, IBA131748 and IBA131849 were the best performers in that order since they have the least scores for ASV (Tables 5.17 and 5.19). IBA131729, IBA131849, IBA131757, IBA131774 and IBA131827 were ranked in that order as the least stable genotypes for LP at 6 MAP (Table 5.17). At 9 MAP, IBA131729, IBA131827, IBA131826, IBA131800 and IBA131821 were ranked as the worst stable genotypes (Table 5.19).

YSI ranked IBA131858, IBA131796, IBA131847, IBA131863 and IBA131754 at 6 MAP and at 9 MAP, IBA131863, IBA131796, IBA131742, IBA131849 and IBA131741 as genotypes with highest pubescent and improved stability at 6 MAP and at 9 MAP (Tables 5.17 and 5.19).

AMMI identified four highest pubescent genotypes in each of the six environments (Tables 5.18 and 5.20). At 6 MAP, four genotypes IBA131796, IBA131731, IBA131743 and IBA131754 were selected for the highest stable environment, Umudike 2016. In the least stable environment, Otobi 2015, genotypes IBA131858, IBA131754, IBA131768 and IBA131817 were selected (Table 5.18). At 9 MAP, the highest stable environment, Igbariam 2015, IBA131754, IBA131847, IBA131863 and IBA131796 were identified while the least environment Otobi 2016, selected genotypes IBA131861, IBA131863, IBA131741 and IBA131870 as the best performers (Table 5.20).

Table 5. 17 Ranking of the cassava genotypes based on leaf pubescence, AMMI stability value, and yield stability index (YSI) at 6 MAP

Genotype	ASV	YSI	RASV	RY	Mean	YSI rank
IBA131858	0.20	5.50	2	3.50	6.00	1
IBA131796	0.22	5.50	4	1.50	6.33	2
IBA131847	0.20	6.50	3	3.50	6.00	3
IBA131863	0.37	15.00	9	6.00	5.67	4
IBA131754	0.51	21.00	13	8.00	5.50	5
IBA131741	0.51	22.00	14	8.00	5.50	6
IBA131759	0.29	26.50	7	19.50	4.83	7
IBA131768	0.60	26.50	25	1.50	6.33	8
IBA131743	0.08	27.00	1	26.00	4.50	9
IBA131748	0.57	28.00	23	5.00	5.83	10
IBA131731	0.54	29.50	16	13.50	5.17	11
IBA131833	0.55	30.00	20	10.00	5.33	12
IBA131738	0.54	30.50	17	13.50	5.17	13
IBA131801	0.54	31.50	18	13.50	5.17	14
IBA131794	0.54	32.50	19	13.50	5.17	15
IBA131784	0.48	38.00	12	26.00	4.50	16
IBA131869	0.32	39.50	8	31.50	4.17	17
IBA131870	0.51	41.00	15	26.00	4.50	18
IBA131861	0.69	42.50	29	13.50	5.17	19
IBA131866	0.66	43.00	26	17.00	5.00	20
IBA131839	0.82	43.00	35	8.00	5.50	21
IBA131753	0.69	43.50	30	13.50	5.17	22
IBA131778	0.28	44.00	6	38.00	3.83	23
IBA131763	0.70	50.50	31	19.50	4.83	24
IBA131819	0.23	54.50	5	49.50	2.67	25
IBA131770	0.73	54.50	32	22.50	4.67	26
IBA131777	0.68	59.50	28	31.50	4.17	27
IBA131752	0.98	64.50	42	22.50	4.67	28
IBA131782	0.67	65.00	27	38.00	3.83	29
IBA131800	0.87	65.00	39	26.00	4.50	30
IBA131821	1.12	66.50	47	19.50	4.83	31
IBA131762	0.96	67.00	41	26.00	4.50	32
IBA131785	0.41	68.50	10	58.50	1.50	33
IBA131798	0.41	69.50	11	58.50	1.50	34
IBA131851	0.90	69.50	40	29.50	4.33	35
IBA131864	1.22	69.50	50	19.50	4.83	36
IBA131734	0.77	71.00	33	38.00	3.83	37
IBA131812	0.59	77.00	24	53.00	2.33	38

Table 5.17 continued

IBA131844	0.98	77.50	43	34.50	4.00	39
IBA131808	0.56	82.50	21	61.50	0.83	40
IBA131736	0.56	83.50	22	61.50	0.83	41
IBA131742	0.77	83.50	34	49.50	2.67	42
IBA131797	0.85	84.00	37	47.00	2.83	43
IBA131746	1.31	86.50	52	34.50	4.00	44
IBA131825	1.31	87.50	53	34.50	4.00	45
IBA131730	1.17	90.00	48	42.00	3.33	46
IBA131749	0.99	91.00	44	47.00	2.83	47
IBA131729	1.98	91.50	62	29.50	4.33	48
IBA131856	0.85	92.50	36	56.50	1.67	49
IBA131872	1.24	93.00	51	42.00	3.33	50
IBA131842	1.32	94.00	54	40.00	3.50	51
IBA131849	1.80	95.50	61	34.50	4.00	52
IBA131836	1.34	97.00	55	42.00	3.33	53
IBA131767	0.86	98.00	38	60.00	1.33	54
IBA131750	1.10	100.00	46	54.00	2.00	55
IBA131776	1.09	101.50	45	56.50	1.67	56
IBA131826	1.43	101.50	57	44.50	3.17	57
IBA131827	1.43	102.50	58	44.50	3.17	58
IBA131809	1.21	104.00	49	55.00	1.83	59
IBA131774	1.45	106.00	59	47.00	2.83	60
IBA131817	1.43	107.50	56	51.50	2.50	61
IBA131757	1.69	111.50	60	51.50	2.50	62

ASV = AMMI stability value; YSI = yield stability index for the genotype across environments for each trait; RASV = rank of the genotype across environments based on ASV; RY = rank of the genotypes based on mean performance across environments.

Table 5. 18 First four AMMI selections based on the highest pubescent genotypes at 6 MAP in each environment

Rank	**Environment**	**Mean**	**Effect**	**1**	**2**	**3**	**4**
1	Umudike2016	5.07	-1.06	IBA131796	IBA131731	IBA131743	IBA131754
2	Igbariam2015	4.47	3.49	IBA131796	IBA131858	IBA131768	IBA131839
3	Igbariam2016	4.39	-2.71	IBA131743	IBA131796	IBA131842	IBA131762
4	Otobi2016	4.16	3.77	IBA131743	IBA131796	IBA131847	IBA131821
5	Umudike2015	3.39	-0.47	IBA131730	IBA131858	IBA131839	IBA131754
6	Otobi2015	2.13	-3.02	IBA131858	IBA131754	IBA131768	IBA131817

Ranking of the environment, 1 = most stable and 6 = least stable

Table 5. 19 Ranking of the cassava genotypes based on leaf pubescence, AMMI stability value, and yield stability index (YSI) at 9 MAP

Genotype	ASV	YSI	RASV	RY	Mean	YSI rank
IBA131863	0.11	2.50	1	1.50	5.17	1
IBA131796	0.16	6.50	2	4.50	4.83	2
IBA131742	0.33	10.50	6	4.50	4.83	3
IBA131849	0.29	11.00	5	6.00	4.67	4
IBA131741	0.35	20.00	10	10.00	4.33	5
IBA131858	0.42	20.00	17	3.00	5.00	6
IBA131738	0.41	21.50	14	7.50	4.50	7
IBA131794	0.41	22.00	12	10.00	4.33	8
IBA131746	0.41	22.50	15	7.50	4.50	9
IBA131839	0.41	23.00	13	10.00	4.33	10
IBA131861	0.73	23.50	22	1.50	5.17	11
IBA131748	0.22	29.50	4	25.50	3.50	12
IBA131870	0.68	33.00	21	12.00	4.17	13
IBA131782	0.50	35.50	19	16.50	4.00	14
IBA131833	0.59	41.00	20	21.00	3.83	15
IBA131798	0.34	43.50	7	36.50	2.83	16
IBA131731	0.34	44.50	8	36.50	2.83	17
IBA131759	0.80	44.50	28	16.50	4.00	18
IBA131869	0.34	45.50	9	36.50	2.83	19
IBA131743	0.80	45.50	29	16.50	4.00	20
IBA131778	0.77	46.00	23	23.00	3.67	21
IBA131784	0.77	47.00	24	23.00	3.67	22
IBA131809	0.80	47.00	30.5	16.50	4.00	23
IBA131825	0.80	47.00	30.5	16.50	4.00	24
IBA131801	0.77	48.00	25	23.00	3.67	25
IBA131768	0.80	48.50	32	16.50	4.00	26
IBA131749	0.22	49.00	3	46.00	2.00	27
IBA131753	0.80	49.50	33	16.50	4.00	28
IBA131754	0.80	50.50	34	16.50	4.00	29
IBA131763	0.79	52.50	27	25.50	3.50	30
IBA131797	0.36	59.00	11	48.00	1.83	31
IBA131730	0.83	63.50	36	27.50	3.33	32
IBA131767	0.47	64.00	18	46.00	2.00	33
IBA131844	0.89	69.00	39	30.00	3.17	34
IBA131808	0.89	70.00	40	30.00	3.17	35
IBA131774	0.42	70.50	16	54.50	1.00	36
IBA131757	0.81	71.50	35	36.50	2.83	37
IBA131770	0.96	73.00	41	32.00	3.00	38

Table 5.19 continued

IBA131752	0.78	77.00	26	51.00	1.67	39
IBA131864	1.04	78.50	42	36.50	2.83	40
IBA131851	0.86	79.00	37	42.00	2.67	41
IBA131750	1.04	79.50	43	36.50	2.83	42
IBA131836	0.86	80.00	38	42.00	2.67	43
IBA131777	1.04	80.50	44	36.50	2.83	44
IBA131847	1.33	82.50	55	27.50	3.33	45
IBA131817	1.30	84.00	54	30.00	3.17	46
IBA131872	1.28	89.50	53	36.50	2.83	47
IBA131762	1.23	93.00	49	44.00	2.50	48
IBA131729	1.88	104.00	62	42.00	2.67	49
IBA131819	1.11	106.00	46	60.00	0.50	50
IBA131842	1.08	107.00	45	62.00	0.17	51
IBA131785	1.11	107.00	47	60.00	0.50	52
IBA131736	1.27	107.00	50	57.00	0.83	53
IBA131827	1.81	107.00	61	46.00	2.00	54
IBA131734	1.11	108.00	48	60.00	0.50	55
IBA131856	1.27	108.00	51	57.00	0.83	56
IBA131776	1.64	108.00	57	51.00	1.67	57
IBA131812	1.27	109.00	52	57.00	0.83	58
IBA131821	1.69	109.00	58	51.00	1.67	59
IBA131800	1.77	110.00	59	51.00	1.67	60
IBA131866	1.40	110.50	56	54.50	1.00	61
IBA131826	1.77	111.00	60	51.00	1.67	62

ASV = AMMI stability value; YSI = yield stability index for the genotype across environments for each trait; RASV = rank of the genotype across environments based on ASV; RY = rank of the genotypes based on mean performance across environments.

Table 5. 20 First four AMMI selections based on the highest pubescent genotypes at 9 MAP in each environment

Rank	**Environment**	**Mean**	**Effect**	**1**	**2**	**3**	**4**
1	Igbariam2015	4.95	3.69	IBA131754	IBA131847	IBA131863	IBA131796
2	Igbariam2016	4.82	-3.21	IBA131861	IBA131863	IBA131794	IBA131796
3	Umudike2016	3.71	2.78	IBA131738	IBA131782	IBA131794	IBA131796
4	Umudike2015	2.31	-2.64	IBA131796	IBA131754	IBA131821	IBA131858
5	Otobi2015	1.16	-1.05	IBA131742	IBA131858	IBA131847	IBA131821
6	Otobi2016	0.89	0.44	IBA131861	IBA131863	IBA131741	IBA131870

Ranking of the environment, 1 = most stable and 6 = least stable

Leaf retention

According to the ASV ranking for stability of genotypes, these genotypes IBA131797, IBA131819, IBA131754 and IBA131774 were selected as the best performers at 6 MAP and least performers were genotypes IBA131827, IBA131729, IBA131836, IBA131782 and IBA131812 (Table 5.21). At 9 MAP, the most stable genotypes were IBA131748, IBA131750, IBA131861, IBA131869 and IBA131782 while the genotypes IBA131729, IBA131797, IBA131817, IBA131872 and IBA131784 were the least stable (Table 5.23).

Based on the YSI ranking, genotypes IBA131768, IBA131801, IBA131863, IBA131858 and IBA131748 were identified as genotypes with better response and improved stability at 6 MAP (Table 5.21). At 9 MAP, the best five genotypes, that had better response and improved stability were IBA131861, IBA131748, IBA131782, IBA131753 and IBA131844 in that order (Table 5.23)

Tables 5.22 and 5.24 present AMMI selection per environment. Igbariam 2015, the first ranked environment identified genotypes, IBA131763, IBA131770, IBA131836 and IBA131844 as the top stable genotypes while the least stable environment Umudike 2015 identified genotypes IBA131827, IBA131749, IBA131750 and IBA 131762 at 6 MAP (Table 5.22). At 9 MAP, the first ranked stable environment Igbariam 2015 selected genotypes IBA131794, IBA131808, IBA131817 and IBA131844 as the best performers while the least stable environment Otobi 2016 selected genotypes IBA131861, IBA131863, IBA131794 and IBA131800 as the top genotypes (Table 5.24).

Table 5. 21 Ranking of the cassava genotypes based on leaf retention, AMMI stability value, and yield stability index (YSI) at 6 MAP

Genotype	ASV	YSI	RASV	RY	Mean	YSI rank
IBA131768	0.15	4.00	3	1.00	4.83	1
IBA131801	0.24	18.50	11	7.50	4.33	2
IBA131863	0.24	19.50	12	7.50	4.33	3
IBA131858	0.24	20.50	13	7.50	4.33	4
IBA131748	0.24	21.50	14	7.50	4.33	5
IBA131861	0.24	22.50	15	7.50	4.33	6
IBA131796	0.24	23.50	16	7.50	4.33	7
IBA131794	0.26	24.00	22	2.00	4.50	8
IBA131763	0.24	24.50	17	7.50	4.33	9
IBA131731	0.24	25.50	18	7.50	4.33	10
IBA131849	0.22	33.00	7	26.00	4.00	11
IBA131870	0.22	34.00	8	26.00	4.00	12
IBA131734	0.22	35.00	9	26.00	4.00	13
IBA131759	0.22	36.00	10	26.00	4.00	14
IBA131819	0.15	38.00	2	36.00	3.83	15
IBA131754	0.15	40.00	4	36.00	3.83	16
IBA131847	0.36	43.50	27	16.50	4.17	17
IBA131741	0.39	44.50	28	16.50	4.17	18
IBA131797	0.06	45.50	1	44.50	3.67	19
IBA131833	0.39	45.50	29	16.50	4.17	20
IBA131800	0.39	46.50	30	16.50	4.17	21
IBA131839	0.47	46.50	39	7.50	4.33	22
IBA131825	0.42	48.50	32	16.50	4.17	23
IBA131762	0.42	49.50	33	16.50	4.17	24
IBA131753	0.42	50.50	34	16.50	4.17	25
IBA131826	0.53	50.50	43	7.50	4.33	26
IBA131730	0.24	55.00	19	36.00	3.83	27
IBA131774	0.16	55.50	5	50.50	3.50	28
IBA131784	0.24	56.00	20	36.00	3.83	29
IBA131738	0.24	57.00	21	36.00	3.83	30
IBA131767	0.22	65.50	6	59.50	3.00	31
IBA131844	0.53	66.00	40	26.00	4.00	32
IBA131866	0.53	67.00	41	26.00	4.00	33
IBA131743	0.28	67.50	23	44.50	3.67	34
IBA131851	0.53	68.00	42	26.00	4.00	35
IBA131777	0.46	72.00	36	36.00	3.83	36
IBA131864	0.46	73.00	37	36.00	3.83	37
IBA131770	0.61	75.00	49	26.00	4.00	38

Table 5.21 continued

IBA131742	0.39	75.50	31	44.50	3.67	39
IBA131746	0.61	76.00	50	26.00	4.00	40
IBA131827	1.02	78.50	62	16.50	4.17	41
IBA131821	0.66	79.00	53	26.00	4.00	42
IBA131785	0.36	80.00	25	55.00	3.33	43
IBA131776	0.36	81.00	26	55.00	3.33	44
IBA131736	0.35	82.00	24	58.00	3.17	45
IBA131869	0.58	82.00	46	36.00	3.83	46
IBA131729	0.94	87.00	61	26.00	4.00	47
IBA131809	0.44	90.00	35	55.00	3.33	48
IBA131856	0.47	93.00	38	55.00	3.33	49
IBA131752	0.79	93.00	57	36.00	3.83	50
IBA131750	0.57	94.50	44	50.50	3.50	51
IBA131778	0.57	95.50	45	50.50	3.50	52
IBA131808	0.62	95.50	51	44.50	3.67	53
IBA131817	0.62	96.50	52	44.50	3.67	54
IBA131842	0.58	98.50	48	50.50	3.50	55
IBA131872	0.69	98.50	54	44.50	3.67	56
IBA131757	0.73	99.50	55	44.50	3.67	57
IBA131749	0.76	100.50	56	44.50	3.67	58
IBA131798	0.58	108.00	47	61.00	2.83	59
IBA131836	0.94	115.00	60	55.00	3.33	60
IBA131782	0.89	118.50	59	59.50	3.00	61
IBA131812	0.80	120.00	58	62.00	2.67	62

ASV = AMMI stability value; YSI = yield stability index for the genotype across environments for each trait; RASV = rank of the genotype across environments based on ASV; RY = rank of the genotypes based on mean performance across environments.

Table 5. 22 First four AMMI selections based on genotypes with outstanding leaf retention at 6 MAP in each environment

Rank	**Environment**	**Mean**	**Effect**	**1**	**2**	**3**	**4**
1	Igbariam2015	4.23	1.86	IBA131763	IBA131770	IBA131836	IBA131844
2	Igbariam2016	4.13	-2.20	IBA131753	IBA131763	IBA131825	IBA131839
3	Umudike2016	4.07	0.43	IBA131742	IBA131748	IBA131768	IBA131770
4	Otobi2016	3.95	1.77	IBA131743	IBA131748	IBA131768	IBA131777
5	Otobi2015	3.61	-1.77	IBA131729	IBA131750	IBA131754	IBA131774
6	Umudike2015	3.16	-0.08	IBA131827	IBA131749	IBA131750	IBA131762

Ranking of the environment, 1 = most stable and 6 = least stable

Table 5. 23 Ranking of the cassava genotypes based on leaf retention, AMMI stability value, and yield stability index (YSI) at 9 MAP

Genotype	ASV	YSI	RASV	RY	Mean	YSI rank
IBA131861	0.16	4.00	3	1.00	4.17	1
IBA131748	0.04	8.50	1	7.50	3.83	2
IBA131782	0.20	19.50	5	14.50	3.67	3
IBA131753	0.22	20.50	6	14.50	3.67	4
IBA131844	0.22	22.50	8	14.50	3.67	5
IBA131833	0.22	23.50	9	14.50	3.67	6
IBA131870	0.22	24.50	10	14.50	3.67	7
IBA131858	0.22	26.50	12	14.50	3.67	8
IBA131746	0.32	27.50	20	7.50	3.83	9
IBA131778	0.32	28.50	21	7.50	3.83	10
IBA131759	0.32	29.50	22	7.50	3.83	11
IBA131741	0.33	30.50	23	7.50	3.83	12
IBA131794	0.33	31.50	24	7.50	3.83	13
IBA131750	0.16	35.00	2	33.00	3.17	14
IBA131809	0.24	37.00	14	23.00	3.50	15
IBA131738	0.24	38.00	15	23.00	3.50	16
IBA131863	0.48	40.00	37	3.00	4.00	17
IBA131742	0.48	41.00	38	3.00	4.00	18
IBA131864	0.48	42.00	39	3.00	4.00	19
IBA131869	0.17	43.50	4	39.50	3.00	20
IBA131821	0.30	46.00	17	29.00	3.33	21
IBA131757	0.30	47.00	18	29.00	3.33	22
IBA131743	0.38	52.00	29	23.00	3.50	23
IBA131770	0.38	53.00	30	23.00	3.50	24
IBA131763	0.38	54.00	31	23.00	3.50	25
IBA131801	0.38	56.00	33	23.00	3.50	26
IBA131754	0.36	57.00	28	29.00	3.33	27
IBA131796	0.38	57.00	34	23.00	3.50	28
IBA131856	0.22	58.50	7	51.50	2.67	29
IBA131839	0.49	58.50	44	14.50	3.67	30
IBA131730	0.36	59.00	26	33.00	3.17	31
IBA131808	0.41	59.00	36	23.00	3.50	32
IBA131768	0.49	59.50	45	14.50	3.67	33
IBA131767	0.36	60.00	27	33.00	3.17	34
IBA131836	0.22	62.50	11	51.50	2.67	35
IBA131749	0.31	65.50	19	46.50	2.83	36
IBA131849	0.49	66.00	43	23.00	3.50	37
IBA131851	0.24	68.50	13	55.50	2.50	38

Table 5.23 continued

IBA131731	0.33	71.50	25	46.50	2.83	39
IBA131842	0.30	75.00	16	59.00	2.33	40
IBA131825	0.52	79.00	46	33.00	3.17	41
IBA131847	0.55	83.00	50	33.00	3.17	42
IBA131762	0.55	86.50	47	39.50	3.00	43
IBA131798	0.38	87.50	32	55.50	2.50	44
IBA131736	0.41	90.50	35	55.50	2.50	45
IBA131777	0.56	90.50	51	39.50	3.00	46
IBA131785	0.49	92.00	40.5	51.50	2.67	47
IBA131827	0.49	93.50	42	51.50	2.67	48
IBA131776	0.55	94.50	48	46.50	2.83	49
IBA131826	0.68	94.50	55	39.50	3.00	50
IBA131800	0.68	95.50	56	39.50	3.00	51
IBA131784	0.69	97.50	58	39.50	3.00	52
IBA131734	0.62	99.50	53	46.50	2.83	53
IBA131817	0.74	99.50	60	39.50	3.00	54
IBA131866	0.65	100.50	54	46.50	2.83	55
IBA131729	1.26	101.50	62	39.50	3.00	56
IBA131812	0.49	102.50	40.5	62.00	1.67	57
IBA131872	0.72	105.50	59	46.50	2.83	58
IBA131819	0.55	108.00	49	59.00	2.33	59
IBA131774	0.60	111.00	52	59.00	2.33	60
IBA131752	0.69	112.50	57	55.50	2.50	61
IBA131797	0.80	122.00	61	61.00	2.17	62

ASV = AMMI stability value; YSI = yield stability index for the genotype across environments for each trait; RASV = rank of the genotype across environments based on ASV; RY = rank of the genotypes based on mean performance across environments.

Table 5. 24 First four AMMI selections based on genotypes with outstanding leaf retention at 9 MAP in each environment

Rank	**Environment**	**Mean**	**Effect**	**1**	**2**	**3**	**4**
1	Igbariam2015	4.34	-1.36	IBA131794	IBA131808	IBA131817	IBA131844
2	Igbariam2016	4.26	-1.61	IBA131742	IBA131754	IBA131762	IBA131777
3	Umudike2015	3.00	-0.85	IBA131866	IBA131730	IBA131785	IBA131819
4	Umudike2016	2.81	2.13	IBA131759	IBA131861	IBA131839	IBA131729
5	Otobi2015	2.40	2.22	IBA131742	IBA131729	IBA131741	IBA131797
6	Otobi2016	2.39	-0.52	IBA131861	IBA131863	IBA131794	IBA131800

Ranking of the environment, 1 = most stable and 6 = least stable

Stay green

For SG at 6 MAP, genotypes IBA131819, IBA131768, IBA131861, IBA131785 and IBA131847 were ranked as the best performers according to ASV ranking while the IBA131749, IBA131798, IBA131836, IBA131817 and IBA131826 were the worst performers (Table 5.25). At 9 MAP, ASV ranked genotypes IBA131778, IBA131836, IBA131870, IBA131844 and IBA131833 as the best genotypes. The worst genotypes were IBA131759, IBA131729, IBA131817, IBA131753 and IBA131872 (Table 5.27).

To identify superior genotypes with very good stay green ability and high stability, YSI was used to rank these genotypes IBA131768, IBA131861, IBA131847, IBA131863 and IBA131825 as the superior genotypes at 6 MAP (Table 5.25). At 9 MAP, the genotypes IBA131748, IBA131778, IBA131870, IBA131844 and IBA131833 as the superior genotypes (Table 5.27).

AMMI selection per environment in tables 5.26 and 5.28 identified four highest performing genotypes in each of the six different environments. At 6 MAP, Otobi 2016 was the most stable environment with the genotypes IBA131734, IBA131752, IBA131784 and IBA131800 as the best performers while environment Umudike 2015 scored the least stable environment with the genotypes IBA131742, IBA131743, IBA131778 and IBA131782 were selected (Table 5.26). Four genotypes IBA131729, IBA131742, IBA131743 and IBA131749 were selected for the most stable environment, Igbariam 2015 while the least stable environment, Otobi 2016 selected genotypes IBA131777, IBA131749, IBA131863 and IBA131754 at 9 MAP (Table 5.28).

Table 5. 25 Ranking of the cassava genotypes based on stay green, AMMI stability value, and yield stability index (YSI) at 6 MAP

Genotypes	ASV	YSI	RASV	RY	Mean	YSI rank
IBA131768	0.01	3.50	2	1.5	3.0	1
IBA131861	0.01	4.50	3	1.5	3.0	2
IBA131847	0.25	12.50	5.5	7.0	2.8	3
IBA131863	0.25	12.50	5.5	7.0	2.8	4
IBA131825	0.27	21.00	14	7.0	2.8	5
IBA131731	0.27	22.00	15	7.0	2.8	6
IBA131839	0.27	23.00	16	7.0	2.8	7
IBA131870	0.27	24.00	17	7.0	2.8	8
IBA131794	0.27	25.00	18	7.0	2.8	9
IBA131754	0.27	26.00	19	7.0	2.8	10
IBA131738	0.29	42.00	22	20.0	2.7	11
IBA131753	0.29	43.00	23	20.0	2.7	12
IBA131869	0.29	44.00	24	20.0	2.7	13
IBA131759	0.29	45.00	25	20.0	2.7	14
IBA131858	0.29	46.50	26.5	20.0	2.7	15
IBA131872	0.29	46.50	26.5	20.0	2.7	16
IBA131801	0.29	48.00	28	20.0	2.7	17
IBA131844	0.29	49.00	29	20.0	2.7	18
IBA131763	0.29	50.00	30	20.0	2.7	19
IBA131729	0.29	50.00	43	7.0	2.8	20
IBA131748	0.29	51.00	31	20.0	2.7	21
IBA131796	0.29	52.00	32	20.0	2.7	22
IBA131741	0.29	53.00	33	20.0	2.7	23
IBA131819	0.01	59.50	1	58.5	2.0	24
IBA131757	0.25	61.00	8	53.0	2.2	25
IBA131808	0.25	62.00	9	53.0	2.2	26
IBA131785	0.01	62.50	4	58.5	2.0	27
IBA131776	0.25	63.00	10	53.0	2.2	28
IBA131821	0.25	64.00	11	53.0	2.2	29
IBA131797	0.25	65.00	12	53.0	2.2	30
IBA131750	0.25	66.00	13	53.0	2.2	31
IBA131767	0.25	68.00	7	61.0	1.8	32
IBA131851	0.51	72.00	52	20.0	2.7	33
IBA131736	0.27	74.00	21	53.0	2.2	34
IBA131842	0.53	74.00	54	20.0	2.7	35
IBA131746	0.53	75.00	55	20.0	2.7	36
IBA131730	0.29	76.50	34	42.5	2.3	37
IBA131866	0.48	76.50	44	32.5	2.5	38

Table 5.25 continued

IBA131827	0.58	77.00	57	20.0	2.7	39
IBA131784	0.29	77.50	35	42.5	2.3	40
IBA131752	0.48	78.00	45.5	32.5	2.5	41
IBA131777	0.48	78.00	45.5	32.5	2.5	42
IBA131826	0.58	78.00	58	20.0	2.7	43
IBA131809	0.29	78.50	36	42.5	2.3	44
IBA131782	0.29	79.50	37	42.5	2.3	45
IBA131849	0.48	79.50	47	32.5	2.5	46
IBA131856	0.29	80.50	38	42.5	2.3	47
IBA131762	0.49	80.50	48	32.5	2.5	48
IBA131812	0.27	81.00	20	61.0	1.8	49
IBA131734	0.29	81.50	39	42.5	2.3	50
IBA131770	0.49	81.50	49	32.5	2.5	51
IBA131743	0.29	82.50	40	42.5	2.3	52
IBA131800	0.49	82.50	50	32.5	2.5	53
IBA131778	0.29	83.50	41	42.5	2.3	54
IBA131833	0.49	83.50	51	32.5	2.5	55
IBA131774	0.29	84.50	42	42.5	2.3	56
IBA131864	0.51	95.50	53	42.5	2.3	57
IBA131742	0.53	98.50	56	42.5	2.3	58
IBA131749	0.76	104.50	62	42.5	2.3	59
IBA131836	0.75	113.00	60	53.0	2.2	60
IBA131798	0.75	114.00	61	53.0	2.2	61
IBA131817	0.65	120.00	59	61.0	1.8	62

ASV = AMMI stability value; YSI = yield stability index for the genotype across environments for each trait; RASV = rank of the genotype across environments based on ASV; RY = rank of the genotypes based on mean performance across environments.

Table 5. 26 First four AMMI selections based on genotypes with very good stay green at 6 MAP in each environment

Rank	**Environment**	**Mean**	**Effect**	**1**	**2**	**3**	**4**
1	Otobi2016	2.53	-1.43	IBA131734	IBA131752	IBA131784	IBA131800
2	Umudike2016	2.50	0.00	IBA131730	IBA131738	IBA131741	IBA131748
2	Igbariam2015	2.50	-1.70	IBA131729	IBA131731	IBA131738	IBA131741
4	Igbariam2016	2.47	1.43	IBA131842	IBA131731	IBA131746	IBA131754
5	Otobi2015	2.45	0.92	IBA131763	IBA131847	IBA131851	IBA131863
6	Umudike2015	2.44	0.77	IBA131742	IBA131743	IBA131778	IBA131782

Ranking of the environment, 1 = most stable and 6 = least stable

Table 5. 27 Ranking of the cassava genotypes based on stay green, AMMI stability value, and yield stability index (YSI) at 9 MAP

Genotypes	ASV	YSI	RASV	RY	Mean	YSI rank
IBA131748	0.19	12.00	10	2.00	2.83	1
IBA131778	0.04	13.50	1	12.50	2.50	2
IBA131870	0.04	15.50	3	12.50	2.50	3
IBA131844	0.04	16.50	4	12.50	2.50	4
IBA131833	0.04	17.50	5	12.50	2.50	5
IBA131849	0.22	19.00	14	5.00	2.67	6
IBA131741	0.23	23.00	18	5.00	2.67	7
IBA131794	0.23	26.00	21	5.00	2.67	8
IBA131861	0.23	26.00	21	5.00	2.67	9
IBA131863	0.23	26.00	21	5.00	2.67	10
IBA131746	0.37	37.00	36	1.00	3.00	11
IBA131768	0.26	48.00	26	22.00	2.33	12
IBA131801	0.26	49.00	27	22.00	2.33	13
IBA131782	0.26	50.50	28.5	22.00	2.33	14
IBA131869	0.26	50.50	28.5	22.00	2.33	15
IBA131763	0.26	52.00	30	22.00	2.33	16
IBA131730	0.19	52.50	6	46.50	1.83	17
IBA131738	0.27	53.00	31	22.00	2.33	18
IBA131757	0.19	53.50	7	46.50	1.83	19
IBA131858	0.27	54.00	32	22.00	2.33	20
IBA131866	0.19	54.50	8	46.50	1.83	21
IBA131754	0.43	54.50	42	12.50	2.50	22
IBA131777	0.27	55.00	33	22.00	2.33	23
IBA131776	0.19	55.50	9	46.50	1.83	24
IBA131796	0.43	56.00	43.5	12.50	2.50	25
IBA131825	0.43	56.00	43.5	12.50	2.50	26
IBA131809	0.19	57.50	11	46.50	1.83	27
IBA131750	0.19	58.50	12	46.50	1.83	28
IBA131742	0.43	58.50	46	12.50	2.50	29
IBA131864	0.43	60.50	48	12.50	2.50	30
IBA131836	0.04	63.00	2	61.00	1.50	31
IBA131839	0.28	65.00	34	31.00	2.17	32
IBA131762	0.28	66.00	35	31.00	2.17	33
IBA131798	0.22	68.50	13	55.50	1.67	34
IBA131812	0.22	70.50	15	55.50	1.67	35
IBA131800	0.42	71.00	40	31.00	2.17	36
IBA131819	0.22	71.50	16	55.50	1.67	37
IBA131827	0.42	72.00	41	31.00	2.17	38

Table 5.27 continued

IBA131851	0.22	72.50	17	55.50	1.67	39
IBA131729	0.65	73.50	61	12.50	2.50	40
IBA131797	0.23	74.50	19	55.50	1.67	41
IBA131821	0.37	75.50	37	38.50	2.00	42
IBA131826	0.37	76.50	38	38.50	2.00	43
IBA131749	0.37	77.50	39	38.50	2.00	44
IBA131785	0.23	78.50	23	55.50	1.67	45
IBA131774	0.23	79.50	24	55.50	1.67	46
IBA131734	0.23	80.50	25	55.50	1.67	47
IBA131759	0.66	84.00	62	22.00	2.33	48
IBA131743	0.51	86.00	55	31.00	2.17	49
IBA131770	0.51	87.00	56	31.00	2.17	50
IBA131731	0.45	87.50	49	38.50	2.00	51
IBA131784	0.51	88.00	57	31.00	2.17	52
IBA131808	0.45	88.50	50	38.50	2.00	53
IBA131847	0.45	89.50	51	38.50	2.00	54
IBA131753	0.51	90.00	59	31.00	2.17	55
IBA131817	0.51	91.00	60	31.00	2.17	56
IBA131767	0.46	98.50	52	46.50	1.83	57
IBA131856	0.46	99.50	53	46.50	1.83	58
IBA131842	0.47	100.50	54	46.50	1.83	59
IBA131872	0.51	104.50	58	46.50	1.83	60
IBA131736	0.43	106.00	45	61.00	1.50	61
IBA131752	0.43	108.00	47	61.00	1.50	62

ASV = AMMI stability value; YSI = yield stability index for the genotype across environments for each trait; RASV = rank of the genotype across environments based on ASV; RY = rank of the genotypes based on mean performance across environments.

Table 5. 28 Ranking of the environment and first four AMMI selections based on genotypes with very good stay green at 9 MAP in each environment

Rank	**Environment**	**Mean**	**Effect**	**1**	**2**	**3**	**4**
1	Igbariam2015	2.61	0.36	IBA131729	IBA131742	IBA131743	IBA131749
2	Igbariam2016	2.55	0.71	IBA131731	IBA131753	IBA131762	IBA131817
3	Umudike2015	2.31	1.40	IBA131746	IBA131759	IBA131762	IBA131770
4	Umudike2016	2.05	-1.80	IBA131729	IBA131767	IBA131827	IBA131743
5	Otobi2015	1.68	-1.75	IBA131729	IBA131746	IBA131827	IBA131842
6	Otobi2016	1.66	1.10	IBA131777	IBA131749	IBA131863	IBA131800

Ranking of the environment, 1 = most stable and 6 = least stable

Shoot tip compactness (STC)

ASV ranked these genotypes IBA131796, IBA131839, IBA131768, IBA131734 and IBA131738 as the most stable genotypes while the worst genotypes were IBA131817, IBA131729, IBA131749, IBA131746 and IBA131730 at 6 MAP (Table 5.29). The best genotypes for STC at 9 MAP were IBA131870, IBA131748, IBA131749, IBA131736 and IBA131819 while the genotypes were IBA131827, IBA131866, IBA131797, IBA131812 and IBA131800 (Table 5.31).

Based on the YSI rankings of the superior genotypes, the genotypes IBA131796, IBA131839, IBA131768, IBA131734 and IBA131738 were selected for STC at 6 MAP (Table 5.29) while at 9 MAP, genotypes IBA131782, IBA131809, IBA131858, IBA131870 and IBA131738 were identified as genotypes that combined compacted shoot tip and stability (Table 5.31).

Genotypes exhibited differential responses to the environments in terms of STC at 6 and 9 MAP (Tables 5.30 and 5.32). For STC at 6 MAP, genotypes IBA131870, IBA131734, IBA131738 and IBA131748 were the best performing genotypes for the most stable environment, Igbariam 2015 while the least stable environment, Otobi 2015 selected genotypes IBA131731, IBA131752, IBA131754 and IBA131777 (Table 5.30). For STC at 9 MAP, genotypes IBA131742, IBA131746, IBA131778 and IBA131809 were the top performers for the most stable environment, Igbariam 2015 while the least stable environment, Otobi 2015 identifed IBA131742, IBA131729, IBA131827 and IBA131746 (Table 5.32).

Table 5. 29 Ranking of the cassava genotypes based on shoot tip compactness, AMMI stability value, and yield stability index (YSI) at 6 MAP

Genotypes	ASV	YSI	RASV	RY	Mean	YSI rank
IBA131796	0.10	4.00	1	3.00	2.83	1
IBA131839	0.10	5.00	2	3.00	2.83	2
IBA131768	0.10	6.00	3	3.00	2.83	3
IBA131734	0.13	23.50	4	19.50	2.50	4
IBA131738	0.13	24.50	5	19.50	2.50	5
IBA131809	0.13	25.50	6	19.50	2.50	6
IBA131863	0.14	26.00	18	8.00	2.67	7
IBA131833	0.13	26.50	7	19.50	2.50	8
IBA131856	0.13	27.50	8	19.50	2.50	9
IBA131794	0.22	27.50	19.5	8.00	2.67	10
IBA131826	0.22	27.50	19.5	8.00	2.67	11
IBA131770	0.13	28.50	9	19.50	2.50	12
IBA131858	0.13	29.50	10	19.50	2.50	13
IBA131741	0.22	29.50	21.5	8.00	2.67	14
IBA131800	0.22	29.50	21.5	8.00	2.67	15
IBA131754	0.13	30.50	11	19.50	2.50	16
IBA131731	0.13	31.50	12	19.50	2.50	17
IBA131763	0.13	32.50	13	19.50	2.50	18
IBA131801	0.13	33.50	14	19.50	2.50	19
IBA131748	0.13	34.50	15	19.50	2.50	20
IBA131821	0.33	43.00	40	3.00	2.83	21
IBA131777	0.33	45.00	42	3.00	2.83	22
IBA131866	0.26	59.50	24	35.50	2.33	23
IBA131869	0.26	60.50	25	35.50	2.33	24
IBA131844	0.26	61.50	26	35.50	2.33	25
IBA131870	0.26	62.50	27	35.50	2.33	26
IBA131762	0.26	63.50	28	35.50	2.33	27
IBA131864	0.34	63.50	44	19.50	2.50	28
IBA131767	0.26	64.50	29	35.50	2.33	29
IBA131847	0.34	64.50	45	19.50	2.50	30
IBA131861	0.34	65.50	46	19.50	2.50	31
IBA131753	0.32	70.50	35	35.50	2.33	32
IBA131825	0.32	72.00	36.5	35.50	2.33	33
IBA131842	0.32	72.00	36.5	35.50	2.33	34
IBA131827	0.52	73.50	54	19.50	2.50	35
IBA131774	0.14	75.00	16	59.00	1.67	36
IBA131808	0.14	76.00	17	59.00	1.67	37
IBA131752	0.61	76.50	57	19.50	2.50	38

Table 5.29 continued

IBA131782	0.31	78.50	33	45.50	2.17	39
IBA131750	0.31	79.50	34	45.50	2.17	40
IBA131729	0.84	80.50	61	19.50	2.50	41
IBA131812	0.22	82.00	23	59.00	1.67	42
IBA131757	0.43	82.50	47	35.50	2.33	43
IBA131742	0.30	83.00	31	52.00	2.00	44
IBA131797	0.33	83.50	38	45.50	2.17	45
IBA131778	0.30	84.00	32	52.00	2.00	46
IBA131836	0.33	84.50	39	45.50	2.17	47
IBA131759	0.47	86.00	50.5	35.50	2.33	48
IBA131784	0.47	86.00	50.5	35.50	2.33	49
IBA131798	0.26	91.50	30	61.50	1.33	50
IBA131851	0.54	91.50	56	35.50	2.33	51
IBA131849	0.44	93.50	48	45.50	2.17	52
IBA131746	0.63	94.50	59	35.50	2.33	53
IBA131776	0.33	97.50	41	56.50	1.83	54
IBA131743	0.33	99.50	43	56.50	1.83	55
IBA131819	0.52	100.50	55	45.50	2.17	56
IBA131872	0.45	101.00	49	52.00	2.00	57
IBA131785	0.49	105.00	53	52.00	2.00	58
IBA131730	0.62	110.00	58	52.00	2.00	59
IBA131749	0.65	112.00	60	52.00	2.00	60
IBA131736	0.47	113.50	52	61.50	1.33	61
IBA131817	0.88	114.00	62	52.00	2.00	62

ASV = AMMI stability value; YSI = yield stability index for the genotype across environments for each trait; RASV = rank of the genotype across environments based on ASV; RY = rank of the genotypes based on mean performance across environments.

Table 5. 30 First four AMMI selections based on genotypes with compacted shoot tip at 6 MAP in each environment

Rank	Environment	Mean	Effect	1	2	3	4
1	Igbariam2015	2.65	0.47	IBA131870	IBA131734	IBA131738	IBA131748
2	Igbariam2016	2.61	-1.18	IBA131731	IBA131754	IBA131819	IBA131746
3	Umudike2016	2.39	-0.69	IBA131746	IBA131842	IBA131856	IBA131738
4	Otobi2016	2.36	1.87	IBA131729	IBA131752	IBA131759	IBA131784
5	Umudike2015	2.31	1.38	IBA131750	IBA131729	IBA131731	IBA131754
6	Otobi2015	1.58	-1.85	IBA131731	IBA131752	IBA131754	IBA131777

Ranking of the environment, 1 = most stable and 6 = least stable

Table 5. 31 Ranking of the cassava genotypes based on shoot tip compactness, AMMI stability value, and yield stability index (YSI) at 9 MAP

Genotype	ASV	YSI	RASV	RY	Mean	YSI rank
IBA131782	0.16	14.50	7	7.50	2.33	1
IBA131809	0.16	15.50	8	7.50	2.33	2
IBA131858	0.16	16.50	9	7.50	2.33	3
IBA131870	0.10	16.50	1	15.50	2.17	4
IBA131738	0.16	17.50	10	7.50	2.33	5
IBA131748	0.10	17.50	2	15.50	2.17	6
IBA131768	0.22	18.50	11	7.50	2.33	7
IBA131746	0.22	19.50	12	7.50	2.33	8
IBA131778	0.22	20.50	13	7.50	2.33	9
IBA131849	0.22	21.50	14	7.50	2.33	10
IBA131861	0.37	41.50	40	1.50	2.50	11
IBA131808	0.27	42.50	15	27.50	2.00	12
IBA131794	0.37	42.50	41	1.50	2.50	13
IBA131833	0.27	44.00	16.5	27.50	2.00	14
IBA131864	0.27	44.00	16.5	27.50	2.00	15
IBA131753	0.27	45.50	18	27.50	2.00	16
IBA131730	0.30	46.50	19	27.50	2.00	17
IBA131759	0.30	47.50	20	27.50	2.00	18
IBA131743	0.30	48.50	21	27.50	2.00	19
IBA131767	0.30	49.50	22	27.50	2.00	20
IBA131844	0.30	50.50	23	27.50	2.00	21
IBA131770	0.30	51.50	24	27.50	2.00	22
IBA131798	0.30	52.50	25	27.50	2.00	23
IBA131801	0.30	53.50	26	27.50	2.00	24
IBA131757	0.35	54.00	38.5	15.50	2.17	25
IBA131863	0.35	54.00	38.5	15.50	2.17	26
IBA131869	0.30	54.50	27	27.50	2.00	27
IBA131763	0.30	55.50	28	27.50	2.00	28
IBA131784	0.30	56.50	29	27.50	2.00	29
IBA131825	0.30	57.50	30	27.50	2.00	30
IBA131741	0.52	58.50	51	7.50	2.33	31
IBA131749	0.15	59.00	3	56.00	1.50	32
IBA131736	0.15	60.00	4	56.00	1.50	33
IBA131796	0.41	60.50	45	15.50	2.17	34
IBA131839	0.58	60.50	53	7.50	2.33	35
IBA131819	0.15	61.00	5	56.00	1.50	36
IBA131785	0.15	62.00	6	56.00	1.50	37
IBA131742	0.72	71.50	56	15.50	2.17	38

Table 5.31 continued

IBA131774	0.33	73.50	32	41.50	1.83	39
IBA131856	0.33	74.50	33	41.50	1.83	40
IBA131752	0.33	75.50	34	41.50	1.83	41
IBA131777	0.33	76.50	35	41.50	1.83	42
IBA131754	0.33	77.50	36	41.50	1.83	43
IBA131731	0.33	78.50	37	41.50	1.83	44
IBA131847	0.57	79.50	52	27.50	2.00	45
IBA131836	0.31	81.00	31	50.00	1.67	46
IBA131776	0.39	84.00	42.5	41.50	1.83	47
IBA131826	0.39	84.00	42.5	41.50	1.83	48
IBA131821	0.39	85.50	44	41.50	1.83	49
IBA131800	0.78	85.50	58	27.50	2.00	50
IBA131750	0.48	96.00	46	50.00	1.67	51
IBA131762	0.48	97.00	47	50.00	1.67	52
IBA131817	0.48	98.00	48	50.00	1.67	53
IBA131729	0.76	98.50	57	41.50	1.83	54
IBA131851	0.48	99.00	49	50.00	1.67	55
IBA131872	0.48	100.00	50	50.00	1.67	56
IBA131827	1.00	112.00	62	50.00	1.67	57
IBA131734	0.63	115.50	54	61.50	1.17	58
IBA131842	0.71	116.50	55	61.50	1.17	59
IBA131866	0.87	117.00	61	56.00	1.50	60
IBA131812	0.78	118.50	59	59.50	1.33	61
IBA131797	0.78	119.50	60	59.50	1.33	62

ASV = AMMI stability value; YSI = yield stability index for the genotype across environments for each trait; RASV = rank of the genotype across environments based on ASV; RY = rank of the genotypes based on mean performance across environments.

Table 5. 32 First four AMMI selections based on genotypes with compacted shoot tip at 9 MAP in each environment

Rank	**Environment**	**Mean**	**Effect**	**1**	**2**	**3**	**4**
1	Igbariam2015	2.66	-1.81	IBA131742	IBA131746	IBA131778	IBA131809
2	Igbariam2016	2.61	0.40	IBA131730	IBA131738	IBA131741	IBA131742
3	Umudike2016	2.02	-1.91	IBA131743	IBA131746	IBA131748	IBA131759
4	Umudike2015	1.57	1.72	IBA131809	IBA131839	IBA131800	IBA131821
5	Otobi2016	1.45	1.51	IBA131809	IBA131847	IBA131738	IBA131794
6	Otobi2015	1.29	0.09	IBA131742	IBA131729	IBA131827	IBA131746

Ranking of the environment, 1 = most stable and 6 = least stable

Shoot tip size (STS)

According to the ASV rankings, these genotypes IBA131754, IBA131809, IBA131858, IBA131784 and IBA131763 were the most stable genotypes for STS at 6 MAP (Table 5.33). At 9 MAP, the most stable genotypes are IBA131819, IBA131785, IBA131736, IBA131753 and IBA131782 (Table 5.35). The least stable genotypes at 6 MAP were IBA131729, IBA131817, IBA131736, IBA131749 and IBA131872 (Table 5.33) while at 9 MAP, the least genotypes were IBA131866, IBA131742, IBA131800, IBA131734 and IBA131827 were selected as the least stable genotypes (Table 5.35).

Based on the YSI ranking of the genotypes with better responses and improved stability. At 6 MAP, genotypes IBA131796, IBA131734, IBA131827, IBA131770 and IBA131768 (Table 5.33) while at 9 MAP, IBA131768, IBA131778, IBA131746, IBA131808 and IBA131738 were selected as genotypes that combined large shoot tip size with high stability (Table 5.35).

AMMI selection per environment is presented in tables 5.34 and 5.36. At 6 MAP, the most stable environment (Igbariam 2015) selected the genotypes IBA131730, IBA131731, IBA131734 and IBA131738 were selected as the best performers (Table 5.34). At 9 MAP, the most stable (Igbariam 2015) selected the genotypes IBA131730, IBA131731, IBA131738 and IBA131746 as the top performers (Table 5.36). Otobi 2015 was the least stable environment for STS at 6 and 9 MAP (Table 5.34 and 5.36).

Table 5. 33 Ranking of the cassava genotypes based on shoot tip size, AMMI stability value, and yield stability index (YSI) at 6 MAP

Genotypes	ASV	YSI	RASV	RY	Mean	YSI rank
IBA131796	0.12	12.00	6	6.00	2.83	1
IBA131734	0.12	14.00	8	6.00	2.83	2
IBA131827	0.12	15.00	9	6.00	2.83	3
IBA131770	0.12	16.00	10	6.00	2.83	4
IBA131768	0.25	25.50	24	1.50	3.00	5
IBA131839	0.25	26.50	25	1.50	3.00	6
IBA131754	0.10	28.50	1	27.50	2.50	7
IBA131757	0.15	29.00	12	17.00	2.67	8
IBA131809	0.10	29.50	2	27.50	2.50	9
IBA131863	0.15	30.00	13	17.00	2.67	10
IBA131858	0.10	30.50	3	27.50	2.50	11
IBA131741	0.22	31.00	14	17.00	2.67	12
IBA131784	0.10	31.50	4	27.50	2.50	13
IBA131763	0.10	32.50	5	27.50	2.50	14
IBA131738	0.22	32.50	15.5	17.00	2.67	15
IBA131801	0.22	32.50	15.5	17.00	2.67	16
IBA131826	0.22	34.00	17	17.00	2.67	17
IBA131794	0.22	35.00	18	17.00	2.67	18
IBA131731	0.22	36.50	19.5	17.00	2.67	19
IBA131748	0.22	36.50	19.5	17.00	2.67	20
IBA131762	0.22	38.00	21	17.00	2.67	21
IBA131753	0.22	39.00	22	17.00	2.67	22
IBA131800	0.22	40.00	23	17.00	2.67	23
IBA131752	0.34	45.00	39	6.00	2.83	24
IBA131821	0.34	46.00	40	6.00	2.83	25
IBA131777	0.34	47.00	41	6.00	2.83	26
IBA131808	0.12	66.00	7	59.00	1.83	27
IBA131833	0.26	66.00	27	39.00	2.33	28
IBA131844	0.26	67.00	28	39.00	2.33	29
IBA131847	0.52	67.00	50	17.00	2.67	30
IBA131851	0.52	67.00	50	17.00	2.67	31
IBA131861	0.52	67.00	50	17.00	2.67	32
IBA131856	0.26	68.50	29.5	39.00	2.33	33
IBA131870	0.26	68.50	29.5	39.00	2.33	34
IBA131864	0.36	69.50	42	27.50	2.50	35
IBA131774	0.12	70.00	11	59.00	1.83	36
IBA131866	0.26	70.50	31.5	39.00	2.33	37
IBA131869	0.26	70.50	31.5	39.00	2.33	38

Table 5.33 continued

IBA131836	0.26	72.00	33	39.00	2.33	39
IBA131767	0.26	73.00	34	39.00	2.33	40
IBA131825	0.29	74.00	35	39.00	2.33	41
IBA131743	0.25	81.00	26	55.00	2.00	42
IBA131849	0.44	82.00	43	39.00	2.33	43
IBA131797	0.31	86.00	36	50.00	2.17	44
IBA131759	0.50	86.50	47.5	39.00	2.33	45
IBA131778	0.50	86.50	47.5	39.00	2.33	46
IBA131750	0.33	87.00	37	50.00	2.17	47
IBA131782	0.33	88.00	38	50.00	2.17	48
IBA131746	0.61	93.00	54	39.00	2.33	49
IBA131819	0.61	94.00	55	39.00	2.33	50
IBA131730	0.61	95.00	56	39.00	2.33	51
IBA131776	0.47	96.00	46	50.00	2.17	52
IBA131785	0.46	99.00	44	55.00	2.00	53
IBA131842	0.46	100.00	45	55.00	2.00	54
IBA131817	0.93	100.00	61	39.00	2.33	55
IBA131729	1.06	101.00	62	39.00	2.33	56
IBA131742	0.54	103.00	53	50.00	2.17	57
IBA131812	0.52	111.00	52	59.00	1.83	58
IBA131872	0.69	113.00	58	55.00	2.00	59
IBA131749	0.70	114.00	59	55.00	2.00	60
IBA131798	0.66	118.50	57	61.50	1.67	61
IBA131736	0.90	121.50	60	61.50	1.67	62

ASV = AMMI stability value; YSI = yield stability index for the genotype across environments for each trait; RASV = rank of the genotype across environments based on ASV; RY = rank of the genotypes based on mean performance across environments.

Table 5. 34 First four AMMI selections based on genotypes with large shoot tip size at 6 MAP in each environment

Rank	**Environment**	**Mean**	**Effect**	**1**	**2**	**3**	**4**
1	Igbariam2015	2.77	-0.20	IBA131730	IBA131731	IBA131734	IBA131738
2	Igbariam2016	2.76	-0.65	IBA131730	IBA131731	IBA131746	IBA131819
3	Umudike2016	2.55	-1.22	IBA131730	IBA131731	IBA131746	IBA131819
4	Otobi2016	2.37	1.87	IBA131776	IBA131817	IBA131782	IBA131743
5	Umudike2015	2.36	1.95	IBA131730	IBA131729	IBA131738	IBA131741
6	Otobi2015	1.73	-1.75	IBA131750	IBA131752	IBA131817	IBA131847

Ranking of the environment, 1 = most stable and 6 = least stable

Table 5. 35 Ranking of the cassava genotypes based on shoot tip size, AMMI stability value, and yield stability index (YSI) at 9 MAP

Genotypes	ASV	YSI	RASV	RY	Mean	YSI rank
IBA131768	0.16	17.50	7.5	10.0	2.3	1
IBA131778	0.16	17.50	7.5	10.0	2.3	2
IBA131746	0.16	19.00	9	10.0	2.3	3
IBA131808	0.16	20.00	10	10.0	2.3	4
IBA131738	0.20	21.50	11.5	10.0	2.3	5
IBA131870	0.20	21.50	11.5	10.0	2.3	6
IBA131861	0.20	23.00	13	10.0	2.3	7
IBA131863	0.20	24.00	14	10.0	2.3	8
IBA131753	0.12	25.50	4	21.5	2.2	9
IBA131809	0.20	25.50	15.5	10.0	2.3	10
IBA131849	0.20	25.50	15.5	10.0	2.3	11
IBA131782	0.12	26.50	5	21.5	2.2	12
IBA131833	0.12	27.50	6	21.5	2.2	13
IBA131858	0.29	29.00	28	1.0	2.7	14
IBA131748	0.36	45.00	42	3.0	2.5	15
IBA131741	0.39	46.00	43	3.0	2.5	16
IBA131794	0.39	47.00	44	3.0	2.5	17
IBA131759	0.27	52.00	17	35.0	2.0	18
IBA131767	0.27	53.00	18	35.0	2.0	19
IBA131801	0.27	54.00	19	35.0	2.0	20
IBA131763	0.27	55.00	20	35.0	2.0	21
IBA131784	0.27	56.00	21	35.0	2.0	22
IBA131819	0.11	56.50	1	55.5	1.5	23
IBA131785	0.11	57.50	2	55.5	1.5	24
IBA131844	0.27	57.50	22.5	35.0	2.0	25
IBA131869	0.27	57.50	22.5	35.0	2.0	26
IBA131736	0.11	58.50	3	55.5	1.5	27
IBA131770	0.36	58.50	37	21.5	2.2	28
IBA131856	0.27	59.00	24	35.0	2.0	29
IBA131743	0.36	59.50	38	21.5	2.2	30
IBA131825	0.27	60.00	25	35.0	2.0	31
IBA131798	0.36	60.50	39	21.5	2.2	32
IBA131731	0.27	61.00	26	35.0	2.0	33
IBA131796	0.52	61.00	51	10.0	2.3	34
IBA131839	0.36	61.50	40	21.5	2.2	35
IBA131730	0.36	62.50	41	21.5	2.2	36
IBA131836	0.31	64.00	29	35.0	2.0	37
IBA131864	0.31	65.00	30	35.0	2.0	38

Table 5.35 continued

IBA131757	0.40	66.50	45	21.5	2.2	39
IBA131817	0.40	67.50	46	21.5	2.2	40
IBA131776	0.66	75.50	54	21.5	2.2	41
IBA131826	0.33	77.00	31	46.0	1.8	42
IBA131762	0.34	78.00	32	46.0	1.8	43
IBA131749	0.28	78.50	27	51.5	1.7	44
IBA131752	0.34	79.00	33	46.0	1.8	45
IBA131774	0.34	80.00	34	46.0	1.8	46
IBA131777	0.34	81.00	35	46.0	1.8	47
IBA131754	0.34	82.00	36	46.0	1.8	48
IBA131821	0.48	82.00	47	35.0	2.0	49
IBA131742	0.76	82.50	61	21.5	2.2	50
IBA131847	0.61	88.00	53	35.0	2.0	51
IBA131800	0.70	95.00	60	35.0	2.0	52
IBA131750	0.50	99.50	48	51.5	1.7	53
IBA131851	0.50	101.00	49.5	51.5	1.7	54
IBA131872	0.50	101.00	49.5	51.5	1.7	55
IBA131729	0.69	102.00	56	46.0	1.8	56
IBA131797	0.55	113.50	52	61.5	1.2	57
IBA131812	0.69	116.00	57	59.0	1.3	58
IBA131842	0.66	116.50	55	61.5	1.2	59
IBA131827	0.69	117.00	58	59.0	1.3	60
IBA131866	0.77	117.50	62	55.5	1.5	61
IBA131734	0.69	118.00	59	59.0	1.3	62

ASV = AMMI stability value; YSI = yield stability index for the genotype across environments for each trait; RASV = rank of the genotype across environments based on ASV; RY = rank of the genotypes based on mean performance across environments.

Table 5. 36 First four AMMI selections based on genotypes with large shoot tip size at 9 MAP in each environment

Rank	**Environment**	**Mean**	**Effect**	**1**	**2**	**3**	**4**
1	Igbariam2015	2.68	1.61	IBA131730	IBA131731	IBA131738	IBA131746
2	Igbariam2016	2.63	0.64	IBA131731	IBA131750	IBA131752	IBA131762
3	Umudike2016	2.10	-1.95	IBA131776	IBA131826	IBA131729	IBA131743
4	Umudike2015	1.79	-1.88	IBA131730	IBA131731	IBA131749	IBA131753
5	Otobi2016	1.47	1.32	IBA131738	IBA131741	IBA131757	IBA131794
6	Otobi2015	1.31	0.27	IBA131742	IBA131729	IBA131817	IBA131821

Ranking of the environment, 1 = most stable and 6 = least stable

Biomass

The genotypes IBA131801, IBA131776, IBA131819, IBA131762 and IBA131872 were ranked as the best five genotypes by ASV scores in that order. The five least genotypes were IBA131839, IBA131746, IBA131730, IBA131770 and IBA131826 (Table 5.37).

To identify genotypes with high biomass and stability, YSI scores were used to select the genotypes IBA131863, IBA131866, IBA131731, IBA131743 and IBA131825 (Table 5.37).

Table 5.38 presents the winning genotypes selected by AMMI per environment. Genotype IBA131863 was identified in four environments (Igbariam 2015, Igbariam 2016, Otobi 2016 and Umudike 2016). This shows that the genotype has a wide adaptability.

Table 5. 37 Ranking of the cassava genotypes based on biomass, AMMI stability value, and yield stability index (YSI)

Genotypes	**ASV**	**YSI**	**RASV**	**RY**	**Mean**	**YSI rank**
IBA131863	0.48	23.00	9	14.00	11.67	1
IBA131866	0.55	28.00	10	18.00	11.10	2
IBA131731	0.78	31.50	20	11.50	11.90	3
IBA131743	0.98	32.00	28	4.00	13.97	4
IBA131825	0.46	33.00	8	25.00	10.00	5
IBA131833	0.96	35.00	26	9.00	12.08	6
IBA131819	0.26	40.00	3	37.00	7.45	7
IBA131776	0.18	42.00	2	40.00	7.30	8
IBA131809	0.97	42.00	27	15.00	11.50	9
IBA131844	1.43	45.00	43	2.00	14.38	10
IBA131785	1.25	46.00	36	10.00	12.03	11
IBA131762	0.32	49.00	4	45.00	6.53	12
IBA131861	0.68	49.00	15	34.00	8.07	13
IBA131796	0.82	49.00	21	28.00	9.17	14
IBA131749	0.95	52.00	25	27.00	9.17	15
IBA131754	1.31	52.00	39	13.00	11.80	16
IBA131869	0.77	54.00	19	35.00	7.73	17
IBA131784	1.12	54.00	32	22.00	10.40	18
IBA131794	1.52	54.00	46	8.00	12.30	19
IBA131798	0.36	56.00	6	50.00	5.97	20
IBA131763	1.91	56.00	53	3.00	14.03	21
IBA131801	0.15	57.00	1	56.00	4.62	22
IBA131757	1.37	57.00	40	17.00	11.23	23
IBA131858	1.82	57.00	50	7.00	12.85	24
IBA131750	1.25	57.50	37	20.50	10.47	25
IBA131817	1.40	58.00	42	16.00	11.27	26
IBA131729	0.45	59.00	7	52.00	5.73	27
IBA131821	0.61	59.00	13	46.00	6.43	28
IBA131768	0.98	59.00	29	30.00	8.73	29
IBA131856	0.57	60.00	11	49.00	6.07	30
IBA131767	0.82	60.00	22	38.00	7.37	31
IBA131808	1.08	61.00	30	31.00	8.67	32
IBA131752	1.31	61.50	38	23.50	10.03	33
IBA131872	0.36	62.00	5	57.00	4.20	34
IBA131746	3.32	62.00	61	1.00	16.27	35
IBA131870	1.89	62.50	51	11.50	11.90	36
IBA131748	1.14	63.00	34	29.00	8.90	37
IBA131774	0.68	65.00	14	51.00	5.88	38

Table 5.37 continued

IBA131770	2.30	65.00	59	6.00	12.93	39
IBA131742	1.50	65.50	45	20.50	10.47	40
IBA131839	3.72	67.00	62	5.00	13.00	41
IBA131827	1.10	70.00	31	39.00	7.33	42
IBA131734	0.75	72.00	17	55.00	5.18	43
IBA131777	0.59	72.50	12	60.50	3.00	44
IBA131741	1.92	73.00	54	19.00	10.88	45
IBA131847	0.69	75.00	16	59.00	3.33	46
IBA131836	1.69	75.00	49	26.00	9.38	47
IBA131842	1.48	76.00	44	32.00	8.37	48
IBA131864	0.95	77.00	24	53.00	5.70	49
IBA131778	1.19	78.00	35	43.00	7.00	50
IBA131849	0.77	78.50	18	60.50	3.00	51
IBA131738	1.13	80.00	33	47.00	6.40	52
IBA131851	1.53	80.00	47	33.00	8.25	53
IBA131826	2.16	81.50	58	23.50	10.03	54
IBA131736	0.86	85.00	23	62.00	2.97	55
IBA131797	1.64	90.00	48	42.00	7.08	56
IBA131782	2.12	93.00	57	36.00	7.60	57
IBA131800	1.39	95.00	41	54.00	5.62	58
IBA131812	1.97	96.00	55	41.00	7.18	59
IBA131759	1.99	104.00	56	48.00	6.15	60
IBA131730	2.49	104.00	60	44.00	6.83	61
IBA131753	1.89	110.00	52	58.00	3.67	62

ASV = AMMI stability value; YSI = yield stability index for the genotype across environments for each trait; RASV = rank of the genotype across environments based on ASV; RY = rank of the genotypes based on mean performance across environments.

Table 5. 38 First four AMMI selections based on genotypes with best high yielding biomass in each environment

Rank	**Environment**	**Mean**	**Effect**	**1**	**2**	**3**	**4**
1	Igbariam2015	12.19	-5.51	IBA131863	IBA131746	IBA131763	IBA131839
2	Igbariam2016	12.00	3.46	IBA131839	IBA131746	IBA131757	IBA131863
3	Umudike2016	8.73	2.25	IBA131858	IBA131863	IBA131844	IBA131827
4	Umudike2015	8.29	4.69	IBA131730	IBA131750	IBA131752	IBA131742
5	Otobi2016	7.45	-5.71	IBA131731	IBA131743	IBA131836	IBA131863
6	Otobi2015	3.66	0.82	IBA131741	IBA131844	IBA131738	IBA131794

Ranking of the environment, 1 = most stable and 6 = least stable

Root dry matter content (RDMC)

Based on ASV rankings, the most stable genotypes were IBA131768, IBA131849, IBA131738, IBA131729 and IBA131753 since they had the lowest ASV scores. Genotypes IBA131861, IBA131796, IBA131748, IBA131749 and IBA131776 were ranked the least stable because they had highest ASV scores (Table 5.39).

YSI identify these genotypes IBA131753, IBA131743, IBA131729, IBA131738 and IBA131849 as the superior genotypes (Table 5.39).

Additive main effect and multiplicative interaction analysis identified four highest superior genotypes in each of the environments (Table 5.40). Four genotypes IBA131743, IBA131849, IBA131754 and IBA131784 were selected for the most stable environment (Igbariam 2016) while the least stable environment (Otobi 2015) identified genotypes IBA131743, IBA131748, IBA131800 and IBA131777 as the best performers (Table 5.40).

Table 5. 39 Ranking of the cassava genotypes based on dry matter content, AMMI stability value, and yield stability index (YSI)

Genotype	ASV	YSI	RASV	RY	Mean	YSI rank
IBA131753	0.53	10.00	5	5.00	31.73	1
IBA131743	0.54	10.00	6	4.00	32.10	2
IBA131729	0.35	15.00	4	11.00	29.55	3
IBA131738	0.28	16.00	3	13.00	29.30	4
IBA131849	0.28	19.00	2	17.00	28.92	5
IBA131863	0.72	23.00	9	14.00	29.24	6
IBA131757	1.04	25.00	24	1.00	33.22	7
IBA131870	1.14	33.00	27	6.00	31.27	8
IBA131869	0.81	34.00	13	21.00	28.33	9
IBA131754	1.21	36.00	33	3.00	32.22	10
IBA131797	0.85	38.00	15	23.00	28.17	11
IBA131808	0.94	38.00	20	18.00	28.79	12
IBA131827	0.88	39.00	17	22.00	28.30	13
IBA131730	0.85	40.00	16	24.00	28.16	14
IBA131844	0.90	43.00	18	25.00	28.09	15
IBA131817	1.42	44.00	42	2.00	32.74	16
IBA131856	1.35	45.00	37	8.00	30.65	17
IBA131785	0.56	46.00	7	39.00	26.69	18
IBA131734	0.65	46.00	8	38.00	26.75	19
IBA131872	1.38	50.00	40	10.00	29.71	20
IBA131736	1.26	51.00	35	16.00	29.02	21
IBA131866	0.96	52.00	21	31.00	27.40	22
IBA131742	1.40	53.00	41	12.00	29.43	23
IBA131767	0.78	54.00	12	42.00	26.47	24
IBA131809	1.08	54.00	26	28.00	27.65	25
IBA131851	1.27	55.00	36	19.00	28.56	26
IBA131759	0.78	56.00	11	45.00	26.25	27
IBA131826	0.91	56.00	19	37.00	26.87	28
IBA131858	1.75	56.00	49	7.00	30.85	29
IBA131812	0.72	57.00	10	47.00	26.01	30
IBA131750	1.16	57.00	28	29.00	27.43	31
IBA131819	1.24	60.00	34	26.00	28.08	32
IBA131768	0.26	61.00	1	60.00	22.68	33
IBA131731	1.65	61.00	46	15.00	29.06	34
IBA131784	2.17	61.00	52	9.00	30.36	35
IBA131825	1.20	68.00	32	36.00	26.88	36
IBA131762	1.16	70.00	29	41.00	26.50	37
IBA131821	0.82	71.00	14	57.00	24.09	38

Table 5.39 continued

IBA131794	0.97	73.00	22	51.00	25.29	39
IBA131741	1.07	73.00	25	48.00	26.00	40
IBA131782	1.46	78.00	44	34.00	27.18	41
IBA131777	1.93	78.00	51	27.00	27.97	42
IBA131770	1.02	79.00	23	56.00	24.11	43
IBA131842	1.66	79.00	47	32.00	27.35	44
IBA131749	2.62	79.00	59	20.00	28.47	45
IBA131800	1.37	83.00	39	44.00	26.28	46
IBA131774	1.43	83.00	43	40.00	26.54	47
IBA131746	1.72	83.00	48	35.00	27.05	48
IBA131798	2.20	83.00	53	30.00	27.42	49
IBA131778	2.31	87.00	54	33.00	27.27	50
IBA131833	1.16	88.00	30	58.00	23.58	51
IBA131847	1.18	90.00	31	59.00	23.37	52
IBA131864	1.65	91.00	45	46.00	26.22	53
IBA131763	1.35	92.00	38	54.00	25.05	54
IBA131836	2.40	99.00	56	43.00	26.38	55
IBA131839	2.31	105.00	55	50.00	25.73	56
IBA131801	2.52	109.00	57	52.00	25.22	57
IBA131748	2.86	109.00	60	49.00	25.77	58
IBA131752	1.84	111.00	50	61.00	22.28	59
IBA131776	2.60	113.00	58	55.00	25.03	60
IBA131796	3.19	114.00	61	53.00	25.14	61
IBA131861	5.39	124.00	62	62.00	19.40	62

ASV = AMMI stability value; YSI = yield stability index for the genotype across environments for each trait; RASV = rank of the genotype across environments based on ASV; RY = rank of the genotypes based on mean performance across environments.

Table 5. 40 First four AMMI selections based on genotypes with best high dry matter content in each environment

Rank	**Environment**	**Mean**	**Effect**	**1**	**2**	**3**	**4**
1	Igbariam2016	28.93	5.80	IBA131743	IBA131849	IBA131754	IBA131784
2	Umudike2016	28.02	-1.40	IBA131753	IBA131776	IBA131743	IBA131849
3	Otobi2016	27.57	-4.40	IBA131762	IBA131734	IBA131743	IBA131849
4	Igbariam2015	28.12	5.21	IBA131858	IBA131768	IBA131743	IBA131836
5	Umudike2015	26.27	0.66	IBA131743	IBA131768	IBA131856	IBA131869
6	Otobi2015	25.76	-5.87	IBA131743	IBA131748	IBA131800	IBA131777

Ranking of the environment, 1 = most stable and 6 = least stable

Fresh root yield

The genotypes with the lowest ASV scores were considered the most stable genotypes. Therefore, the genotypes IBA131866, IBA131749, IBA131759, IBA131746 and IBA131785 were the most stable genotypes while genotypes IBA131767, IBA1317825, IBA131752, IBA131856 and IBA131826 were the least stable genotypes (Table 5.41).

Superior genotypes with high yielding and stability across the environments were identified with their low YSI scores. These genotypes were IBA131866, IBA131746, IBA131872, IBA131741 and IBA131770 (Table 5.41).

Table 5.42 shows the first four genotypes selected by AMMI in the six different environments. Genotypes IBA131825, IBA131767, IBA131856 and IBA131870 were identified in the most stable environment (Igbariam 2015) while the genotypes IBA131844, IBA131767, IBA131734 and IBA131812 were found in the least stable environment (Otobi 2016).

Table 5. 41 Ranking of the cassava genotypes based on fresh root yield, AMMI stability value, and yield stability index (YSI)

Genotypes	ASV	YSI	RASV	RY	Mean	YSI rank
IBA131866	0.09	7.00	1	6.00	31.47	1
IBA131746	0.37	18.00	4	14.00	26.45	2
IBA131872	0.79	22.00	10	12.00	27.37	3
IBA131741	1.01	26.00	13	13.00	26.56	4
IBA131770	1.68	29.00	25	4.00	31.92	5
IBA131749	0.25	36.00	2	34.00	20.89	6
IBA131763	0.75	36.00	8	28.00	23.03	7
IBA131863	0.43	38.00	6	32.00	21.36	8
IBA131748	0.63	38.00	7	31.00	21.96	9
IBA131759	0.35	39.00	3	36.00	20.78	10
IBA131851	1.58	39.00	22	17.00	25.72	11
IBA131819	1.56	41.00	20	21.00	24.67	12
IBA131797	1.30	42.00	18	24.00	23.83	13
IBA131796	1.72	42.00	27	15.00	26.33	14
IBA131849	1.71	44.00	26	18.00	25.67	15
IBA131833	2.18	46.00	37	9.00	28.75	16
IBA131842	2.38	47.00	40	7.00	30.81	17
IBA131785	0.38	48.00	5	43.00	17.64	18
IBA131750	2.01	48.00	32	16.00	25.83	19
IBA131836	1.58	50.00	21	29.00	22.77	20
IBA131743	2.31	50.00	39	11.00	28.17	21
IBA131754	2.75	50.00	47	3.00	34.09	22
IBA131774	2.71	51.00	46	5.00	31.56	23
IBA131870	2.89	54.00	52	2.00	34.44	24
IBA131827	0.96	58.00	12	46.00	17.08	25
IBA131768	2.18	58.00	35	23.00	23.97	26
IBA131738	2.77	58.00	48	10.00	28.67	27
IBA131776	0.87	59.00	11	48.00	16.89	28
IBA131839	1.06	62.00	15	47.00	17.06	29
IBA131734	1.85	62.00	29	33.00	21.33	30
IBA131825	5.82	62.00	61	1.00	38.56	31
IBA131729	1.07	66.00	16	50.00	15.89	32
IBA131782	2.57	67.00	45	22.00	24.48	33
IBA131736	0.78	68.00	9	59.00	12.44	34
IBA131869	1.32	68.00	19	49.00	16.50	35
IBA131798	1.04	69.00	14	55.00	13.89	36
IBA131767	5.99	70.00	62	8.00	30.56	37
IBA131800	1.17	71.00	17	54.00	14.53	38

Table 5.41 continued

IBA131844	1.98	72.00	31	41.00	17.86	39
IBA131784	2.04	72.00	33	39.00	18.67	40
IBA131864	1.98	74.00	30	44.00	17.17	41
IBA131858	2.56	74.00	44	30.00	22.32	42
IBA131817	2.23	76.00	38	38.00	19.82	43
IBA131778	1.62	77.00	24	53.00	14.58	44
IBA131731	2.46	77.00	42	35.00	20.83	45
IBA131826	4.29	77.00	58	19.00	25.47	46
IBA131777	1.58	79.00	23	56.00	13.39	47
IBA131847	3.23	79.00	54	25.00	23.81	48
IBA131856	4.79	79.00	59	20.00	25.11	49
IBA131809	2.18	81.00	36	45.00	17.14	50
IBA131821	3.93	82.00	56	26.00	23.72	51
IBA131757	2.55	83.00	43	40.00	18.66	52
IBA131752	5.09	87.00	60	27.00	23.33	53
IBA131742	1.72	88.00	28	60.00	11.93	54
IBA131762	2.88	88.00	51	37.00	20.60	55
IBA131730	2.16	91.00	34	57.00	13.29	56
IBA131808	2.79	91.00	49	42.00	17.83	57
IBA131794	2.43	93.00	41	52.00	15.14	58
IBA131812	2.86	101.00	50	51.00	15.47	59
IBA131801	2.95	111.00	53	58.00	12.64	60
IBA131753	3.47	116.00	55	61.00	9.92	61
IBA131861	3.94	119.00	57	62.00	9.75	62

ASV = AMMI stability value; YSI = yield stability index for the genotype across environments for each trait; RASV = rank of the genotype across environments based on ASV; RY = rank of the genotypes based on mean performance across environments.

Table 5. 42 First four AMMI selections based on genotypes with best root yielding in each environment

Rank	**Environment**	**Mean**	**Effect**	**1**	**2**	**3**	**4**
1	Igbariam2015	30.04	-7.40	IBA131825	IBA131767	IBA131856	IBA131870
2	Igbariam2016	29.98	-8.16	IBA131825	IBA131767	IBA131856	IBA131870
3	Umudike2016	22.35	6.46	IBA131872	IBA131870	IBA131743	IBA131851
4	Otobi2015	21.40	8.18	IBA131754	IBA131752	IBA131750	IBA131774
5	Umudike2015	14.49	1.69	IBA131782	IBA131770	IBA131872	IBA131833
6	Otobi2016	12.62	-0.78	IBA131844	IBA131767	IBA131734	IBA131812

Ranking of the environment, 1 = most stable and 6 = least stable

5.4 DISCUSSION

There were effects of seasonal variations on the performance and stability of cassava genotypes. In this study, CGM attack were more severe during the dry season (6 MAP) but less severe during the rainy season (9 MAP). High pubescent found on leaves, longevity of leaves and amount of leaves found on the apex tip of cassava during the peak of dry season (6 MAP) reduces CGM severity. Active plant growth were observed with concurrent reduction of the CGM attack during heavy rainfall (9 MAP). Agreeing with the observation of Yaninek *et al.* (1989), new plant growth are triggered by rainfall and mites are washed off the leaves during the rainy seasons.

Broad sense heritability estimates for dry matter content were similar to the findings of Adjebeng-Danquah *et al.* (2017) and Ntawuruhunga *et al.* (2001). Heritability estimates influence the progress that can be made in selection for a trait of interest (Sabesan *et al.*, 2009) . However, broad sense heritability alone does not always give a full indication of genetic gain that can be made through the phenotypic recurrent selection used today in cassava since it includes both additive and nonadditive components of the variation (Pradeepkumar *et al.*, 2006; Singh *et al.*, 2013). Traits that show strong dependency on non-additive genetic effects can still be improved through reciprocal recurrent selection or else by shifting to the use of inbred progenitors (Ceballos *et al.*, 2015)

The significant negative correlation observed between CGMS6 and LP6, LP9, LR6, LR9, SG6, SG9, STS6, STS9, STC6, STC9, biomass, FRY and RDMC suggests that genotypes with high pubescent leaves, outstanding leaf retention, very good stay green, compacted and small shoot tip, high biomass, high RDMC and high FRY tend to be resistant to CGM attack. The negative trend of the association between CGMS and LP is attributed to the leaf trichomes limiting the movement of CGM on the leaves, which in turn results in reduced reproductive capacity of these pests and the damage to the leaves. Moreover, LP, especially on immature leaves and shoot apices, has also been reported to provide suitable habitat for *T. aripo*, a phytoseiid predatory mite. In the two experiments carried out by Onzo *et al*. (2012), 480 predatory mites (*T. aripo*) were recorded on pubescent apices of cassava plants as compared to 280 mites on glabrous apices, confirming that *T. aripo* is attracted mainly to apices of pubescent cultivars in presence of the prey. These authors have also reported that pubescent cultivars produce a certain odour that attracts *T. aripo* to the shoot apices of cassava. This predatory mite has proved to be the most successful natural enemy against CGM in Africa (Yaninek and Hanna, 2003) and South America (Onzo *et al*., 2005). LP is a heritable character that helps to reduce CGM populations in cassava (Hahn *et al*., 1989). The negative correlations between CGM and yield traits (biomass, FRY and RDMC) may be explained by the negative impact of the pest on yield.

The significant positive correlation between LP6 and LP9, LR6, LR9, SG6, SG9, STS6, STS9, STC6, STC9, biomass, FRY and RDMC, implies that these traits can be selected concurrently and can be improved through breeding for CGM resistance. Cassava genotypes that have large and compact shoot apices plus prolonged leaf retention and very good stay green ability have been reported to play a significant role in supporting the continuous survival of *T. aripo* (Zundel *et al*., 2009) and other phytoseiid predatory mites (Pratt *et al*., 2003) during both the rainy and dry seasons.

The combined AMMI analysis of variance revealed that there were significant genotypic variations for all the traits indicating opportunity for selection and prospects for the improvement of cassava for the traits. The impact of environment was highly significant for CGMS6, LP9, LR9, SG9, STC9, STS9, FRY, RDMC and biomass justifying the need for multilocational testing to identify good performers for specific locations. IPCA1 was significant for all the traits studied and it explained the interaction pattern better than IPCA2. The percentage of the treatment sum of squares (SS) captured by an AMMI ANOVA in this study is useful for assessing the overall goodness of fit. There were significant responses of genotype by interaction for CGMS6, LP6, LP9, LR6, LR9, SG6, STC6, STC9, STS6, FRY, RDMC and biomass. This implies different adaptation by the different genotypes suggesting the need to identify and select location specific genotypes for different environments. Alternatively, yield stability analysis can be performed to identify genotypes whose performance remains stable over several years and environments (Mutegi *et al.*, 2009). In this study, genotypes performed better in the optimum environments than under stress conditions. The stress environments provided better heritability for CGMS.

Stability analysis methods are often used by breeders to identify genotypes that have stable performance and respond positively to improvements in environmental conditions (Farshadfar *et al.*, 2012). AMMI stability value (ASV) indicates the stability of genotypes. Stability alone for yield performance is not a criterion for selection since a consistently low yielding genotype can still be stable (Yan and Tinker, 2006). In some cases the most stable genotypes do not always have the best trait performance (de Oliveira *et al.*, 2006). For this reason, high yield is considered with stability in the estimation of yield stability index (YSI). The YSI which is similar to genotype stability index (GSI) proposed by (Farshadfar, 2008) integrates both yield and stability across environments into a single index, to select varieties. The YSI sums the rank of mean yield across environments with the rank of the ASV of genotypes (Baraki *et al.*, 2014a; Tumuhimbise *et al.*, 2014). Genotypes with lowest YSI values are desirable since they combine high mean yield with stability (Baraki *et al.*, 2014b; Bose *et al.*, 2014; Mahmodi *et al.*, 2011; Tumuhimbise *et al.*, 2014).

Based on the scoring of CGMS, where the genotype with the least score (1) is considered as the best performer (resistant) and the highest score (5) is the worst performer (susceptible). Therefore, genotypes with the highest YSI values were considered as the most stable and resistant for cassava green mite resistance only. For other traits, genotypes with lowest YSI values were considered the best performers.

In this study, a genotype that ranked among the top four in one or two environments was considered to be specifically adapted to either or both environments, while a genotype ranked among the top four in more than two out of six environments, was considered to be widely adapted.

CGMS was evaluated at 6 and 9 MAP to identify the most stable and resistant genotypes during the growing period of cassava. YSI ranked IBA131753, IBA131794, IBA131738, IBA131768 and IBA131821 at 6 MAP and genotypes IBA131872, IBA131754, IBA131847, IBA131794 and IBA131863 at 9 MAP as the genotypes that combined resistance with stability. They are the most desirable and can be recommended for wider production or as sources of resistance for breeding programme.

In cassava, LP is said to be the primary trait responsible for resistance to CGM (Hahn *et al.*, 1989). These genotypes IBA131858, IBA131796, IBA131847, IBA131863 and IBA131754 at 6 MAP and at 9 MAP, IBA131863, IBA131796, IBA131742, IBA131849 and IBA131741 exhibited the highest stability with combined high level of LP. These genotypes also had stability for CGM resistance and could be used as source of genes for CGM resistance. Genotype IBA131796 at 6 MAP was widely adapted to the three most stable environments (Umudike2016, Igbariam2015 and Igbariam2016). At 9 MAP genotypes, IBA131796, IBA131863 and IBA131794 were widely adapted to the three most stable environments (Igbariam2015, Igbariam2016 and Umudike2016).

These genotypes are considered to be widely adapted and they are recommended for production within those environments.

The current study revealed the presence of genetic variability in the germplasm for LR in Nigeria. These genotypes IBA131768, IBA131801, IBA131863, IBA131858, IBA131748, IBA131782, IBA131861, IBA131753 and IBA131844 combine high stability with outstanding LR. These genotypes have one characteristic in common, the ability to fold their leaves downward away from the sun during the hot/dry seasons. The action of leaf folding may be a mechanism for water stress avoidance (El-Sharkawy, 2003). It is also suggested that genotypes, that exhibit outstanding LR combined with enhanced SG, are likely to be resistant to both CGM and drought (Nukenine *et al.*, 1999). The genotype IBA131763 at 6 MAP was specifically adapted to the two most stable environment (Igbariam2015 and Igbariam2016). None of the genotypes at 9 MAP were found to be widely adapted to the most stable environments.

It is also proposed that the genetic potential of cassava to retain as many green leaves as possible may be a major factor in tolerance to CGM (Nukenine *et al.*, 1999). These genotypes IBA131768, IBA131861, IBA131847, IBA131863 and IBA131825 at 6 MAP and IBA131748, IBA131778, IBA131870, IBA131844 and IBA131833 at 9 MAP combined very good stay green ability and stability. Genotypes IBA137141and IBA131738 were specifically adapted to the second most stable environment (Umudike2016) and third most stable environment (Igbariam2015) at 6 MAP. At 9 MAP, genotypes IBA131762 was specifically adapted to the second most stable environment (Igbariam2016) and third most stable environment (Umudike2015).

Genotypes with large and compact shoot apices are preferred for sustenance of *T. aripo* as they protect the predatory mite from harsh weather conditions. Based on the YSI rankings of the superior genotypes, the genotypes IBA131796, IBA131839, IBA131768, IBA131734 and IBA131738 were selected for STC at 6 MAP while at 9 MAP, genotypes IBA131782, IBA131809, IBA131858, IBA131870 and IBA131738 were identify as genotypes that combined compacted shoot tip and stability. At 6 MAP, genotypes IBA131796, IBA131734, IBA131827, IBA131770 and IBA131768 while at 9 MAP, IBA131768, IBA131778, IBA131746, IBA131808 and IBA131738 were selected as genotypes that combined large shoot tip size with high stability. Genotypes IBA131863, IBA131866, IBA131731, IBA131743 and IBA131825 were selected for high biomass and stability. Genotype IBA131863 was identified in four environments (Igbariam2015, Igbariam2016, Otobi2016 and Umudike2016). This shows that the genotype has a wide adaptability.

For RDMC, YSI identify these genotypes IBA131753, IBA131743, IBA131729, IBA131738 and IBA131849 as the superior genotypes. Genotype IBA131743 showed a wide adaptability since it was found in the six of the tested environments.

The superior genotypes IBA131866, IBA131746, IBA131872, IBA131741 and IBA131770 were selected as combining high FRY with stability. These genotypes are also resistant to CGM. Two genotypes IBA131870 and IBA131767 were widely adapted to three environments for FRY; the former was found in the high and moderately high yielding environments, and the latter was seen in high, moderately high and low yielding environments. This suggests that IBA131767 was insensitive to seasonal effects and can be recommended for production in the environments. IBA131825 and IBA131865 were adapted to the two most stable environment (Igbariam2015 and Igbariam2016). This indicates that the genotypes were better able to exploit their full potential yield in the good environments.

5.5 CONCLUSION

Genotype by environment interaction was significant for most of the traits indicating the need to test the genotypes in multiple environments before effective selection can be made. The high broad sense heritability estimates obtained for RDMC suggest that significant progress can be made through selection for improvement of genotypes for this trait. The study has identified stable high yielding genotypes such as IBA131866, IBA131746, IBA131872, IBA131767 and IBA131770 which also combine high FRY and resistance to CGM, suggesting it is possible to combine these traits in cassava as desired by farmers. These genotypes can be evaluated in more environments to assess their adaptability and possible recommendation for release to farmers for cultivation. Umudike location displayed a low pest pressure followed by Igbariam location which showed a moderately high pest pressure then Otobi location appeared to be the highest pest pressure zone. However, the study contributed to the promotion of food security in Nigeria and elsewhere where cassava is grown through the identification of CGM resistant genotypes that also have high FRY and RDMC. CGM resistance, high FRY and RDMC are farmer-preferred traits. Therefore, enhancement of such traits through plant breeding is likely to increase the adoption of new genotypes by farmers.

CHAPTER SIX

6.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSION

Cassava has a long-growth cycle and its production is hampered with abiotic and biotic stresses. The effect of cassava green mite (CGM) alone can cause as high as 80% yield losses (Janssen and Yaninek, 1993). The control of CGM in Nigeria has mainly been based on chemical and cultural practices. However, lack of information regarding the gene(s) conferring resistance is not well known. Most studies on CGM have focused on conventional breeding with limited work on molecular breeding. Identifying the genetic basis of CGM resistance will provide important insights for breeding resistant varieties for sustainable agriculture. Lack of information on farmers' perception of CGM and preferred varietal attributes also limits success of resistance breeding and adoption of cultivars. This research was therefore undertaken with a view to uncover genomic regions underlying the resistance to CGM.

The study is divided into three main parts. The first part was a participatory rural appraisal (PRA) study which was conducted in three major cassava growing states in Nigeria, in order to obtain information on farmers' preferences, perception and knowledge of CGM and other production constraints and to lay the foundation for the development of CGM resistant cultivars in Nigeria. The second part was to study the genetic resistance of CGM using genome-wide association study (GWAS). The last part was to assess the performance and stability of cassava genotypes for CGM resistance and other relevant plant morphological traits in cassava.

The PRA study, which made use of individual and focus group interviews, was conducted with farmers in Anambra, Benue and Abia states. The main findings of the study were:

- Inadequate provision of farm inputs like agricultural credit facilities and farm implements by the government are the most common constraints of cassava production in the three surveyed states.
- High fresh root yield, resistance to pests and diseases and early bulking cassava cultivars are the important attributes that determine adoption and retention of new cassava cultivars by farmers
- The key insect pests identified by farmers are termites, CGM, cassava mealybug and whiteflies.
- Farmers are familiar with symptoms of damage caused by CGM but most of them mistake CGM damage for cassava mosaic disease and mealybug infestation. Use of photographs and live infested plant can give farmers good guidance on the actual pest responsible for such damage.
- Removal of cassava shoot apices and selective pruning of infested shoots are the most effective traditional cultural practices in reducing the population of CGM. According to the farmers, these practices help the plant to escape CGM damage/attack in cassava fields during the dry season.

The genome-wide association study (GWAS) was used to identify genomic regions and candidate genes linked to CGM resistance and other useful traits. The findings were:

- Successfully, 35 unique genes were identified within SNPs associated with cassava green mite resistance, leaf pubescence and leaf retention on chromosome 8.
- The effect of genotype by environment (G x E) interaction was demonstrated for all the traits over three years across the locations by comparing GWAS results. Here, the GWAS

peak associated with the candidate genes are significant in years 2013 and 2015, but not in year 2014. This could mostly be due to different weather patterns experienced during the growing seasons. The effect of G x E interactions was also demonstrated for all the traits in the three locations (Umudike, Otobi and Kano states) across the years. In this case, there was no effect of G x E interactions for all traits. G x E effects improve the ability to detect moderate strength and rare alleles as well as provide better resolution for the hit found in this study. Co-localization of the most significant SNP associated with CGMS, LP and LR at chromosome 8 is possibly an indication of the presence of pleiotropic effects.

- Pleiotropic effects may be beneficial as genes conferring resistance to CGM is linked to leaf pubescence and leaf retention. These desirable traits (leaf pubescence and leaf retention) may be co-introduced along with the pest resistance into susceptible, glabrous cassava varieties.

- The genome-wide association analysis led to the identification of 17 candidate genes that appear to be associated with cassava green mite resistance, leaf pubescence and leaf retention in *M. esculenta*.
- Heritability was generally low to moderate. The heritability estimates indicate that selection of these traits should result in significant gains in cassava germplasm improvement.

Additive main and multiplicative interactions (AMMI) was used to assess the performance and stability of cassava genotypes for CGM resistance and other useful agronomic traits. The findings were:

- There were seasonal effects on the performance and stability of cassava genotypes. CGM attack were more severe during the dry season (6 MAP) and less severe during the rainy season (9 MAP).
- The significant negative correlation observed between CGMS and LP, LR, SG, STS, STC, biomass, FRY and RDMC means that genotypes with high pubescent leaves, outstanding leaf retention, very good stay green, compact and large shoot tip tend to be resistant/tolerant to CGM attack. Resistant/tolerant genotypes tend to have high biomass, FRY and RDMC.
- The combined AMMI analysis of variance revealed that there were significant genotypic variations for all the traits indicating opportunity for selection and prospects for the improvement of cassava for the traits. The impact of environment was highly significant for CGMS6, LP9, LR9, SG9, STC9, STS9, FRY, RDMC and biomass justifying the need for multilocational testing to identify good performers for specific locations. There were significant responses of genotype by interaction for CGMS6, LP6, LP9, LR6, LR9, SG6, STC6, STC9, STS6, FRY, RDMC and biomass. This implies different adaptation by the different genotypes suggesting the need to identify and select location specific genotypes for different environments. In this study, genotypes performed better in the optimum environments than under stress conditions.
- Stable high yielding genotypes such as IBA131866, IBA131746, IBA131872, IBA131767 and IBA131770 which also combine high FRY and resistance to CGM were identified, suggesting it is possible to combine these traits in cassava as desired by farmers.

6.2 RECOMMENDATIONS

- Participation of farmers in breeding programmes from the early to advanced stages of evaluation trial will facilitate the adoption of new improved cultivars.
- The increasing importance of cassava leaves as a cheap source of protein emphases the need for breeders to improve LR and SG in cassava in association with FRY and RDMC
- AMMI stability value (ASV) indicates the stability of genotypes. Stability alone for trait performance is not a criterion for selection since consistently low yielding genotypes can still be stable. For this reason, AMMI based yield selection index (YSI) is recommended because it integrates both trait mean performance and stability across environments into a single index to select superior genotypes.
- The genotypes IBA131866, IBA131746, IBA131872, IBA131767 and IBA131770 combined high FRY with resistance to CGM. These genotypes should be used as sources of CGM resistance and high FRY in future breeding programmes. These genotypes need to be further tested in the presence of *T.aripo* in different locations, to confirm their stability and adaptability for recommendation for release to farmers for cultivation.
- The current study has identified Umudike location to be the most stable, low pest pressure sites which can be used for the multiplication of planting materials. Moderately high (Igbariam location) and high (Otobi location) pest pressure zones that can be used for screening of germplasm for CGM resistance.
- Validation studies involving fine mapping and joint linkage mapping will be required to clearly understand the relationship between these candidate genes and the phenotypes observed in the GWAS hit found in this study.

BIBLIOGRAPHY

Abate, M., Mekbib, F., Ayana, A., & Nigussie, M. (2015). Genotype x Environment & Stability Analysis of Oil Content in Sesame (*Sesamum indicum* L.) evaluated Across Diverse Agro-ecologies of the Awash Valleys in Ethiopia. *American Journal of Experimental Agriculture*, *9*(2), 1–12. https://doi.org/10.9734/AJEA/2015/18482

Abdurakhmonov, I. Y., & Abdukarimov, A. (2008). Application of association mapping to understanding the genetic diversity of plant germplasm resources. *International Journal of Plant Genomics*, *2008*. https://doi.org/10.1155/2008/574927

Adeniji, T. A., Sanni, L. O., Barimalaa, I. S., & Hart, A. D. (2007). Mineral composition of five improved varieties of cassava. *Nigerian Food Journal*, *25*(2), 39–44.

Adjebeng-Danquah, J., Manu-Aduening, J., Gracen, V. E., Asante, I. K., & Offei, S. K. (2017). AMMI Stability Analysis and Estimation of Genetic Parameters for Growth and Yield Components in Cassava in the Forest and Guinea Savannah Ecologies of Ghana. *International Journal of Agronomy*, *2017*. https://doi.org/10.1155/2017/8075846

Agwu, A. E., & Anyaeche, C. L. (2007). Adoption of improved cassava varieties in six rural communities in Anambra State, Nigeria. *African Journal of Biotechnology*, *6*(2).

Aina, O. O., Dixon, A. G. O., & Akinrinde, E. A. (2007). Additive main effects and multiplicative interaction (AMMI) analysis for yield of cassava in Nigeria. *J. Biol. Sci*, *7*, 796–800.

Akcura, M., & Kaya, Y. (2008). Nonparametric stability methods for interpreting genotype by environment interaction of bread wheat genotypes (Triticum aestivum L.). *Genetics and*

Molecular Biology, *31*(4), 906–913.

Allem, A. C. (2002). The origins & taxonomy of cassava. *Cassava: Biology, Production and Utilization*, *1*, 1–16.

Altshuler, D., Daly, M. J., & Lander, E. S. (2008). Genetic mapping in human disease. *Science*, *322*(5903), 881–888. https://doi.org/10.1126/science

Aranzana, M. J., Kim, S., Zhao, K., Bakker, E., Horton, M., & Jakob, K. (2005). Genome-wide association mapping in Arabidopsis identifies previously known flowering time and pathogen resistance genes. *PLoS Genetics*, *1*(5), e60.

Ariel, F. D., Manavella, P. A., Dezar, C. A., & Chan, R. L. (2007). The true story of the HD-Zip family. *Trends in Plant Science*, *12*(9), 419–426.

Asante, I. K., & Offei, S. K. (2003). RAPD-based genetic diversity study of fifty cassava (Manihot esculenta Crantz) genotypes. *Euphytica*, *131*(1), 113–119.

Atwell, S., Huang, Y. S., Vilhjálmsson, B. J., Willems, G., Li, Y., Meng, D., Horton, M.,Platt, A & Traw, M. B. (2010). Genome-wide association study of 107 phenotypes in a common set of Arabidopsis thaliana inbred lines. *Nature*, *465*(7298), 627–631.

Ayanru, D. K. G., & Sharma, V. C. (1983). Chlorophyll depletion in leaves of field-grown cassava clones infested by cassava green spider mites, *Mononychellus tanajoa* (Bondar)(Acarina: Tetranychidae). *Trop. Agric*, *60*(2), 85–88.

Balasubramanian, S., Schwartz, C., Singh, A., Warthmann, N., Kim, M. C. & Maloof, J. N. (2009). QTL mapping in new Arabidopsis thaliana advanced intercross-recombinant inbred lines.

PLoS One, *4*(2), e4318.

Balyejusa Kizito, E., Rönnberg-Wästljung, A.-C., Egwang, T., Gullberg, U., Fregene, M., & Westerbergh, A. (2007). Quantitative trait loci controlling cyanogenic glucoside and dry matter content in cassava (Manihot esculenta Crantz) roots. *Hereditas*, *144*(4), 129–136.

Barabaschi, D., Tondelli, A., Desiderio, F., Volante, A., Vaccino, P., Valè, G., & Cattivelli, L. (2016). Next generation breeding. *Plant Science*, *242*, 3-13.

Baraki, F., Tsehaye, Y., & Abay, F. (2014a). AMMI Analysis of Genotype by Environment Interaction and Stability of Sesame Genotypes in Northern Ethiopia. *Asian Journal of Plant Sciences*, *13*(4–8), 178.

Baraki, F., Tsehaye, Y., & Abay, F. (2014b). Ammi analysis of genotype x environment interaction and stability of sesame genotypes in northern ethiopia. *Asian Journal of Plant Sciences*. https://doi.org/10.3923/ajps.2014.178183

Bastos, J. A. M., & Flechtmann, C. H. W. (1985). Symptoms and injuriousness of the tanajoa mite in'mani{ç}oba'. *Fitossanidade*, 102–105.

Bates, D., & Maechler, M. (2010). Matrix: sparse and dense matrix classes and methods. *R package version 0.999375-43, URL http://cran. r-project. org/package= Matrix*.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2014). Fitting linear mixed-effects models using lme4. *arXiv preprint arXiv:1406.5823*.

Bellotti, A. C. (2008). Cassava pests and their management. In *Encyclopedia of Entomology* (pp. 764–794). Springer.

Bellotti, A. C., Mesa, N., Serrano, J. M., Guerrero, C. J., & Herrera, C. J. (1987). Taxonomic inventory and survey activity for natural enemies of cassava green mites in the Americas. *International Journal of Tropical Insect Science*, *8*, 845–849.

Bellotti, A. C. (2002). Arthropod pests. *Cassava: Biology, Production and Utilization*, 209–235.

Bellotti, A., Campo, B. V. H., & Hyman, G. (2012). *Cassava Production and Pest Management: Present and Potential Threats in a Changing Environment*. *Tropical Plant Biology* (Vol. 5). https://doi.org/10.1007/s12042-011-9091-4

Bellotti, A. C., Smith, L., & Lapointe, S. L. (1999). Recent advances in cassava pest management. *Annual Review of Entomology*, *44*, 343–370. https://doi.org/10.1146/annurev.ento.44.1.343

Bischoff, V., Selbig, J., & Scheible, W.-R. (2010). Involvement of TBL/DUF231 proteins into cell wall biology. *Plant Signaling and Behavior*, *5*(8), 1057–9. https://doi.org/10.4161/psb.5.8.12414

Boerma, H. R., & Walker, D. R. (2005). Discovery and utilization of QTLs for insect resistance in soybean. *Genetica*, *123*(1–2), 181–189.

Boonchanawiwat, A., Sraphet, S., Boonseng, O., Lightfoot, D. A., & Triwitayakorn, K. (2011). QTL underlying plant and first branch height in cassava (Manihot esculenta Crantz). *Field Crops Research*, *121*(3), 343–349.

Bose, L. K., Jambhulkar, N. N., Pande, K., & Singh, O. N. (2014). Use of AMMI and other stability statistics in the simultaneous selection of rice genotypes for yield & stability under direct-seeded conditions. *Chilean Journal of Agricultural Research*, *74*(1), 3–9.

Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y., & Buckler, E. S. (2007). TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics*, *23*(19), 2633-2635.

Brotman, Y., Riewe, D., Lisec, J., Meyer, R. C., Willmitzer, L., & Altmann, T. (2011). Identification of enzymatic and regulatory genes of plant metabolism through QTL analysis in Arabidopsis. *Journal of Plant Physiology*, *168*(12), 1387–1394.

Brown, J. K. M. (2002). Yield penalties of disease resistance in crops. *Current Opinion in Plant Biology*, *5*(4), 339–344.

Browning, B. L., & Browning, S. R. (2009). A unified approach to genotype imputation and haplotype-phase inference for large data sets of trios and unrelated individuals. *The American Journal of Human Genetics*, *84*(2), 210-223.

Buckler, E. S., & Thornsberry, J. M. (2002). Plant molecular diversity and applications to genomics. *Current Opinion in Plant Biology*, *5*(2), 107–111.

Byrne, D. H., Bellotti, A. C., & Guerrero, J. M. (1983a). The cassava mites. *International Journal of Pest Management*, *29*(4), 378–394.

Byrne, D. H., Bellotti, A. C., & Guerrero, J. M. (1983b). The Cassava Mites. *Tropical Pest Management*, *29*(4), 378–394. https://doi.org/10.1080/09670878309370833

Campbell, B. T., & Jones, M. A. (2005). Assessment of genotype by environment interactions for yield and fiber quality in cotton performance trials. *Euphytica*, *144*(1–2), 69–78.

Carver, R. H., & Nash, J. G. (2009). Data Analysis with SPSS Version 16. *Cencage learning India*

Pvt Limited.

Castillo, D., Matus, I., del Pozo, A., Madariaga, R., & Mellado, M. (2012). Adaptability and genotype by environment interaction of spring wheat cultivars in chile using regression analysis, AMMI, & SREG. *Chilean Journal of Agricultural Research*, *72*(2), 167.

Ceballos, H., Iglesias, C. A., Pérez, J. C., & Dixon, A. G. O. (2004). Cassava breeding: Opportunities and challenges. *Plant Molecular Biology*, *56*(4), 503–516. https://doi.org/10.1007/s11103-004-5010-5

Ceballos, H., Kulakow, P., & Hershey, C. (2012). Cassava Breeding: Current Status, Bottlenecks & the Potential of Biotechnology Tools. *Tropical Plant Biology*, *5*(1), 73–87. https://doi.org/10.1007/s12042-012-9094-9

Ceballos, H., Kawuki, R. S., Gracen, V. E., Yencho, G. C., & Hershey, C. H. (2015). Conventional breeding, marker-assisted selection, genomic selection and inbreeding in clonally propagated crops: a case study for cassava. *Theoretical and Applied Genetics*, *128*(9), 1647-1667.

Ceballos, H., Okogbenin, E., Pérez, J. C., López-Valle, L. A. B., & Debouck, D. (2010). Cassava. In *Root and tuber crops* (pp. 53–96). Springer.

Chalwe, A. (2012). Manipulating morphological traits of cassava to enhance host plant resistance & biological control of cassava green mite in Zambia by Able Chalwe BSc . Agriculture (University of Zambia), MSc . Plant Genetic Manipulation (University of Nottingham) A, (December), 179.

Chalwe, A., Melis, R., Shanahan, P., & Chiona, M. (2015). Inheritance of resistance to cassava green mite & other useful agronomic traits in cassava grown in Zambia. *Euphytica*, *205*(1), 103–119. https://doi.org/10.1007/s10681-015-1404-5

Chan, E. K. F., Rowe, H. C., Corwin, J. A., Joseph, B., & Kliebenstein, D. J. (2011). Combining genome-wide association mapping and transcriptional networks to identify novel genes controlling glucosinolates in *Arabidopsis thaliana*. *PLoS Biology*, *9*(8), e1001125.

Chan, E. K. F., Rowe, H. C., & Kliebenstein, D. J. (2010). Understanding the evolution of defense metabolites in *Arabidopsis thaliana* using genome-wide association mapping. *Genetics*, *185*(3), 991–1007.

Chipeta, M. M., Bokosi, J. M., Saka, V. W., & Benesi, I. R. (2013). Combining ability and mode of gene action in cassava for resistance to cassava green mite and cassava mealy bug in Malawi. *Journal of plant Breeding and crop Science*, *5*(9), 195-202.

Choperena, E. del P. (2007). *Identificaci{ó}n de marcadores microsat{é}lites asociados con la resistencia al {á}caro verde (Mononychellus tanajoa) en yuca (Manihot esculenta)*. Thesis: Universidad del Valle, Facultad de Ciencias, Santiago de Cali, Colombia. 92p.

Choperena, E. del P. M., Ospina, C., Fregene, M., Montoya-Lerma, J., & Bellotti, A. C. (2012). Identificación de microsatélites en yuca asociados con la resistencia al ácaro *Mononychellus tanajoa* (Acari: Tetranychidae)/Microsatellite identification associated with resistance to the mite Mononychellus tanajoa (Acari: Tetranychidae). *Revista Colombiana de Entomolog{'\i}a*, *38*(1), 70.

Clark, M. D., & Watkins, E. (2012). Broad-sense heritability estimates of turfgrass performance characteristics in native prairie Junegrass Germplasm. *HortScience*, *47*(9), 1228–1233.

Cock, J. H. (1982). Cassava: a basic energy source in the tropics. *Science*, *218*(4574), 755–762.

Cock, J. H. (1985). New Potential for a Neglected Crop. *IADS, Development Oriented Literature Series, Westview Press, Colorado, USA p192*.

Cockram, J., White, J., Zuluaga, D. L., Smith, D., Comadran, J., & Macaulay, M. (2010). Genome-wide association mapping to candidate polymorphism resolution in the unsequenced barley genome. *Proceedings of the National Academy of Sciences*, *107*(50), 21611–21616.

Collard, B. C. Y., Jahufer, M. Z. Z., Brouwer, J. B., & Pang, E. C. K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. *Euphytica*, *142*(1–2), 169–196.

Collard, B. C. Y., & Mackill, D. J. (2008). Marker-assisted selection : an approach for precision plant breeding in the twenty-first century Marker-assisted selection : an approach for precision plant breeding in the twenty-first century. *Phil Trans R Soc B*, *363*(8), 557–572. https://doi.org/10.1098/rstb.2007.2170

Crossa, J. (1990). Statistical analyses of multilocation trials. *Advances in Agronomy*, *44*, 55–85.

Crossa, J., Gauch, H. G., & Zobel, R. W. (1990). Additive main effects and multiplicative interaction analysis of two international maize cultivar trials. *Crop Science*, *30*(3), 493–500.

Crowell, S., Korniliev, P., Falcão, A., Ismail, A., Gregorio, G., Mezey, J., & McCouch, S. (2016). Genome-wide association and high-resolution phenotyping link Oryza sativa panicle traits to numerous trait-specific QTL clusters. *Nature communications*, *7*, 10527.

da Silveira, L. C., Kist, V., de Paula, T. O., Pereira Barbosa, M. H., Peternelli, L. A., & Daros, E.

(2013). AMMI analysis to evaluate the adaptability and phenotypic stability of sugarcane genotypes. *Scientia Agricola*, *70*(1), 27–32. https://doi.org/10.1590/S0103-90162013000100005.

De Moraes, G. J., Moreira, A. N., & Delalibera, I. (1995). Growth of the mite Mononychellus tanajoa (Acari: Tetranychidae) on alternative plant hosts in northeastern Brazil. *The Florida Entomologist*, *78*(2), 350–354.

de Oliveira, E. J., & de Godoy, I. J. (2006). Pod yield stability analysis of runner peanut lines using AMMI. *Crop Breeding & Applied Biotechnology*, *6*(4), 310–317.

de Oliveira, E.J., de Resende, M.D.V., da Silva Santos, V., Ferreira, C.F., Oliveira, G.A.F., da Silva, M.S., de Oliveira, L.A. & Aguilar-Vildoso, C.I. (2012). Genome-wide selection in cassava. *Euphytica*, *187*(2), 263–276. https://doi.org/10.1007/s10681-012-0722-0

den Berg, H., & Jiggins, J. (2007). Investing in farmers—the impacts of farmer field schools in relation to integrated pest management. *World Development*, *35*(4), 663–686.

Dobón, A., Canet, J. V., Perales, L., & Tornero, P. (2011). Quantitative genetic analysis of salicylic acid perception in Arabidopsis. *Planta*, *234*(4), 671–684.

Duputié, A., David, P., Debain, C., & McKey, D. (2007). Natural hybridization between a clonally propagated crop, cassava (*Manihot esculenta* Crantz) and a wild relative in French Guiana. *Molecular Ecology*, *16*(14), 3025–3038.

Duputié, A., Salick, J., & McKey, D. (2011). Evolutionary biogeography of Manihot (Euphorbiaceae), a rapidly radiating Neotropical genus restricted to dry environments.

Journal of Biogeography, *38*(6), 1033–1043.

Eberhart, S. A., & Russell, W. A. (1966). Stability parameters for comparing varieties. *Crop Science*, *6*(1), 36–40.

Echebiri, R. N., & Edaba, M. E. I. (2008). Production and utilization of cassava in Nigeria: prospects for food security and infant nutrition. *PAT*, *4*(1), 38-52.

Egesi, C. N., Ilona, P., Ogbe, F. O., Akoroda, M., & Dixon, A. (2007). Genetic variation & genotype X environment interaction for yield and other agronomic traits in cassava in Nigeria. *Agronomy Journal*, *99*(4), 1137–1142. https://doi.org/10.2134/agronj2006.0291

Ekanem, A. P., Eyo, V. O., & Ndome, C. B. (2010). The effect of diet with different inclusion level of cassava leaf meal (CLM) *Manihot utilissima* on the growth performance of Heteroclarias fingerlings. *Journal of Science & Multidisciplinary Research*, *2*, 58–67.

El-Sharkawy, M. A. (2003). Cassava biology and physiology. *Plant Molecular Biology*, *53*(5), 621–641.

Elias, M., McKey, D., Panaud, O., Anstett, M. C., & Robert, T. (2001). Traditional management of cassava morphological and genetic diversity by the Makushi Amerindians (Guyana, South America): perspectives for on-farm conservation of crop genetic resources. *Euphytica*, *120*(1), 143–157.

Elshire, R. J., Glaubitz, J. C., Sun, Q., Pol&, J. A., Kawamoto, K., Buckler, E. S., & Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PloS One*, *6*(5), e19379.

Esuma, W., Herselman, L., Labuschagne, M.T., Ramu, P., Lu, F., Baguma, Y., Buckler, E.S. & Kawuki, R.S. (2016a). Genome-wide association mapping of provitamin A carotenoid content in cassava. *Euphytica*, *212*(1), 97–110. https://doi.org/10.1007/s10681-016-1772-5

Esuma, W., Herselman, L., Labuschagne, M. T., Ramu, P., Lu, F., Baguma, Y., … Kawuki, R. S. (2016b). Genome-wide association mapping of provitamin A carotenoid content in cassava. *Euphytica*, *212*(1), 97–110.

Esuma, W., Kawuki, R. S., Herselman, L., & Labuschagne, M. T. (2016). Stability and genotype by environment interaction of provitamin A carotenoid and dry matter content in cassava in Uganda. *Breeding Science*, *66*(3), 434–443. https://doi.org/10.1270/jsbbs.16004

Ezulike, T. O., & Egwuatu, R. I. (1990). Determination of damage threshold of green spider mite, *Mononychellus tanajoa* (Bondar) on Cassava. *International Journal of Tropical Insect Science*, *11*(1), 43–45. https://doi.org/10.1017/S1742758400019822

Ezulike, T. O., & Egwuatu, R. I. (1993). An investigation into the alternate host plants of green spider mite, *Mononychellus tanajoa* in tropical rainforest of south eastern Nigeria. *Pakistan Journal of Zoology*, *25*(1), 83–85.

Falconer, D. S., Mackay, T. F. C., & Frankham, R. (1996). Introduction to quantitative genetics (4th edn). *Trends in Genetics*, *12*(7), 280.

Famoso, A.N., Zhao, K., Clark, R.T., Tung, C.W., Wright, M.H., Bustamante, C., Kochian, L.V. & McCouch, S.R. (2011). Genetic Architecture of Aluminum Tolerance in Rice (*Oryza sativa*) Determined through Genome-Wide Association Analysis and QTL Mapping. *PLoS Genetics*, *7*(8), e1002221. https://doi.org/10.1371/journal.pgen.1002221

FAO, U. (2014). FAOstat. *Retrieved Feb, 2014*.

FAOSTAT, F. (2017). Statistical data. *Food and Agriculture Organization of the United Nations, Rome*.

Farshadfar, E. (2008). Incorporation of AMMI stability value and grain yield in a single non-parametric index (GSI) in bread wheat. *Pakistan Journal of Biological Sciences*, *11*(14), 1791.

Farshadfar, E. (2013). GGE Biplot analysis of genotype× environment interaction in chickpea genotypes. *European Journal of experimental biology*, *3*(1), 417–423. Retrieved from http://pelagiaresearchlibrary.com/european-journal-of-experimental-biology/vol3-iss1/EJEB-2013-3-1-417-423.pdf

Farshadfar, E., Mahmodi, N., & Yaghotipoor, A. (2011). AMMI stability value & simultaneous estimation of yield & yield stability in bread wheat (Triticum aestivum L.). *Australian Journal of Crop Science*, *5*(13), 1837–1844.

Farshadfar, E., Rafiee, F., & Yghotipoor, A. (2012). Comparison of the efficiency among half diallel methods in the genetic analysis of bread wheat (Triticum aestivum L.) under drought stress condition. *Annals of Biological Research*, *3*(3), 1607–1622.

Finlay, K. W., & Wilkinson, G. N. (1963). The analysis of adaptation in a plant-breeding programme. *Australian Journal of Agricultural Research*, *14*(6), 742–754.

Fischer, G., Shah, M., Tubiello, F. N., & Van Velhuizen, H. (2005). Socio-economic and climate change impacts on agriculture: an integrated assessment, 1990–2080. *Philosophical*

Transactions of the Royal Society B: Biological Sciences, *360*(1463), 2067-2083.

Flint-Garcia, S. A., Thuillet, A.-C., Yu, J., Pressoir, G., Romero, S. M., Mitchell, S. E., … Buckler, E. S. (2005). Maize association population: a high-resolution platform for quantitative trait locus dissection. *The Plant Journal*, *44*(6), 1054–1064.

Fox, P. N., Crossa, J., & Romagosa, I. (1997). Multi-environment testing and genotype by environment interaction. In *Statistical methods for plant variety evaluation* (pp. 117–138). Springer.

Francis, T. R., & Kannenberg, L. W. (1978). Yield stability studies in short-season maize. In *Canadian Journal of Plant Science* (Vol. 58, p. 580).

Gajardo, H. A., Wittkop, B., Soto-Cerda, B., Higgins, E. E., Parkin, I. A. P., Snowdon, R. J., … Iniguez-Luy, F. L. (2015). Association mapping of seed quality traits in *Brassica napus* L. using GWAS and candidate QTL approaches. *Molecular Breeding*, *35*(6), 143.

Gan, Y., Liu, C., Yu, H., & Broun, P. (2007). Integration of cytokinin and gibberellin signalling by Arabidopsis transcription factors GIS, ZFP8 and GIS2 in the regulation of epidermal cell fate. *Development*, *134*(11), 2073–2081.

Gapare, W., Conaty, W., Zhu, Q.-H., Liu, S., Stiller, W., Llewellyn, D., & Wilson, I. (2017a). Genome-wide association study of yield components and fibre quality traits in a cotton germplasm diversity panel. *Euphytica*, *213*(3), 66.

Gapare, W., Conaty, W., Zhu, Q. H., Liu, S., Stiller, W., Llewellyn, D., & Wilson, I. (2017b). Genome-wide association study of yield components and fibre quality traits in a cotton germplasm diversity panel. *Euphytica*, *213*(3), 1–22. https://doi.org/10.1007/s10681-017-1855-y

Gauch, H. G., & Zobel, R. W. (1996). AMMI analysis of yield trials. In "Genotype by environment interaction".(Eds MS Kang, HG Gauch) pp. 85--122. CRC Press: Boca Raton, FL.

Gauch Jr, H. G. (1988). Model selection and validation for yield trials with interaction. *Biometrics*, 705–715.

Gauch Jr, H. G. (1992). AMMI analysis on yield trials. *CIMMYT Wheat Special Report (CIMMYT)*.

Gebhardt, C., Ballvora, A., Walkemeier, B., Oberhagemann, P., & Schüler, K. (2004). Assessing genetic potential in germplasm collections of crop plants by marker-trait association: a case study for potatoes with quantitative variation of resistance to late blight and maturity type. *Molecular Breeding*, *13*(1), 93–102.

Glaubitz, J. C., Casstevens, T. M., Lu, F., Harriman, J., Elshire, R. J., Sun, Q., & Buckler, E. S. (2014). TASSEL-GBS: a high capacity genotyping by sequencing analysis pipeline. *PloS One*, *9*(2), e90346.

Gordon, D., & Finch, S. J. (2005). Factors affecting statistical power in the detection of genetic association. *Journal of Clinical Investigation*, *115*(6), 1408.

Gore, M.A., Chia, J.M., Elshire, R.J., Sun, Q., Ersoz, E.S., Hurwitz, B.L., Peiffer, J.A., McMullen, M.D., Grills, G.S., Ross-Ibarra, J. & Ware, D.H. (2009). A first-generation haplotype map of maize. *Science*, *326*(5956), 1115–1117.

Goyal, A., & Prasad, R. (2010). 27 Some Important Fungal Diseases and Their Impact on Wheat Production. *Management of Fungal Plant Pathogens*, 362.

Gupta, P. K., Langridge, P., & Mir, R. R. (2010). Marker-assisted wheat breeding: present status and future possibilities. *Molecular Breeding*, *26*(2), 145–161.

Gupta, P. K., Rustgi, S., & Mir, R. R. (2008). Array-based high-throughput DNA markers for crop improvement. *Heredity*, *101*(1), 5–18.

Hagos, H. G., & Abay, F. (2013). AMMI and GGE biplot analysis of bread wheat genotypes in the northern part of Ethiopia. *Journal of Plant Breeding & Genetics*, *1*(1), 12–18.

Hahn, S. K., Bai, K. V, & Asiedu, R. (1990). Tetraploids, triploids, and 2n pollen from diploid interspecific crosses with cassava. *TAG Theoretical & Applied Genetics*, *79*(4), 433–439.

Hahn, S. K., Isoba, J. C. G., & Ikotun, T. (1989). Resistance breeding in root and tuber crops at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. *Crop Protection*, *8*(3), 147–168. https://doi.org/10.1016/0261-2194(89)90022-7

Hamblin, M. T., Mitchell, S. E., White, G. M., Gallego, J., Kukatla, R., Wing, R. A., … Kresovich, S. (2004). Comparative population genetics of the panicoid grasses: sequence polymorphism, linkage disequilibrium and selection in a diverse sample of Sorghum bicolor. *Genetics*, *167*(1), 471–483.

Hamblin, M. T., & Rabbi, I. Y. (2014). The Effects of Restriction-Enzyme Choice on Properties of Genotyping-by-Sequencing Libraries: A Study in Cassava .*Crop Science*, *54*(6), 2603–2608.

Hanna, R., Onzo, A., Lingeman, R., Yaninek, J. S., & Sabelis, M. W. (2005). Seasonal cycles and persistence in an acarine predator-prey system on cassava in Africa. *Population Ecology*, *47*(2), 107–117. https://doi.org/10.1007/s10144-005-0215-2

Hao, D., Chao, M., Yin, Z., & Yu, D. (2012). Genome-wide association analysis detecting significant single nucleotide polymorphisms for chlorophyll and chlorophyll fluorescence parameters in soybean (Glycine max) landraces. *Euphytica*, *186*(3), 919-931.

Hedrick, P. W. (1987). Gametic disequilibrium measures: proceed with caution. *Genetics*, *117*(2), 331–341.

Herren, I. H. R. (1989). Dynamics of Mononychellus tanajoa (Acari : Tetranychidae) in Africa : Seasonal Factors Affecting Phenology & Abundance.

Hershey, C. (1987). Cassava Breeding/A Multidisciplinary Review. *CIAT. Cali. Colombia*. Retrieved from a multidisciplinary Review 4.

Hillocks, R. J., & Wydra, K. (2002). Bacterial, fungal and nematode diseases. *Cassava: Biology, Production and Utilization*, 261–280.

Howeler, R. H. (2002). Cassava mineral nutrition & fertilization. *Cassava: Biology, Production and Utilization*, 115–147.

Huang, X., Sang, T., Zhao, Q., Feng, Q., Zhao, Y., Li, C., Zhu, C., Lu, T., Zhang, Z., Li, M. & Fan, D. (2010). Genome-wide association studies of 14 agronomic traits in rice landraces. *Nature Genetics*, *42*(11), 961–967. https://doi.org/10.1038/ng.695.

Huang, X., Zhao, Y., Li, C., Wang, A., Zhao, Q., Li, W., Guo, Y., Deng, L., Zhu, C., Fan, D. &

Lu, Y. (2012). Genome-wide association study of flowering time and grain yield traits in a worldwide collection of rice germplasm. *Nature Genetics*, *44*(1), 32–9. https://doi.org/10.1038/ng.1018

Hunter, K. W., & Crawford, N. P. S. (2008). The future of mouse QTL mapping to diagnose disease in mice in the age of whole-genome association studies. *Annual Review of Genetics*, *42*, 131–141.

Hyten, D. L., Hartman, G. L., Nelson, R. L., Frederick, R. D., Concibido, V. C., Narvel, J. M., & Cregan, P. B. (2007). Map location of the locus that confers resistance to soybean rust in soybean. *Crop Science*, *47*(2), 837–838.

INGRAM, W. R. (1982). Potential for the biocontrol of cassava green mite in Africa. In *Herren, Hans Rudolf; Hennessey, Ronald N.; Bitterli, Rhonda (eds.). Biological control and host plant resistance to control the cassava mealybug and green mite in Africa: Proceedings of an international workshop, December 6-10, 1982.*

International HapMap Consortium. (2007). A second generation human haplotype map of over 3.1 million SNPs. *Nature*, *449*(7164), 851.

Islam, M. R., Man, M. M., Khatun, H., Sharma, N., Islam, M. Z., Akter, A., & Biswas, P. (2014). AMMI Analysis of yield performance and stability of rice genotypes across different Haor areas. *Eco-Friendly Agriculture Journal*, *7*, 20–24.

Janssen, A., & Yaninek, J. S. (1993). Cassava green mites: A challenge for experts in biological control. *Experimental and Applied Acarology*, *17*(1–2), 1–4. https://doi.org/10.1007/BF00156940.

Janssens, M. (2001). Cassava. *Crop Production in Tropical Africa. Raemaekers, RH (Ed). Directorate General for International Coorperation. Brussels*, 195–218.

Jennings, D. L. (1963). Variation in pollen and ovule fertility in varieties of cassava, and the effect of interspecific crossing on fertility. *Euphytica*, *12*(1), 69–76.

Jiang, D.K., Sun, J., Cao, G., Liu, Y., Lin, D., Gao, Y.Z., Ren, W.H., Long, X.D., Zhang, H., Ma, X.P. & Wang, Z. (2013). Genetic variants in STAT4 and HLA-DQ genes confer risk of hepatitis B virus-related hepatocellular carcinoma. *Nature Genetics*, *45*(1), 72–5. https://doi.org/10.1038/ng.2483

Kang, M. S. (2002). Genotype-environment interaction: progress & prospects. *Quantitative Genetics, Genomics & Plant Breeding*, 221–243.

Kang, H. M., Zaitlen, N. A., Wade, C. M., Kirby, A., Heckerman, D., Daly, M. J., & Eskin, E. (2008). Efficient control of population structure in model organism association mapping. *Genetics*, *178*(3), 1709-1723.

Kawano, K. (1980). Cassava. *Hybridization of crop plants*, (hybridizationof), 225-233.

Kawuki, R. S., Ferguson, M., Labuschagne, M., Herselman, L., & Kim, D. (2009). Identification, characterisation and application of single nucleotide polymorphisms for diversity assessment in cassava (*Manihot esculenta* Crantz). *Molecular Breeding*, *23*(4), 669–684.

Kazama, H., Dan, H., Imaseki, H., & Wasteneys, G. O. (2004). Transient exposure to ethylene stimulates cell division and alters the fate and polarity of hypocotyl epidermal cells. *Plant Physiology*, *134*(4), 1614–1623.

Kraakman, A. T. W., Martinez, F., Mussiraliev, B., Van Eeuwijk, F. A., & Niks, R. E. (2006). Linkage disequilibrium mapping of morphological, resistance, and other agronomically relevant traits in modern spring barley cultivars. *Molecular Breeding*, *17*(1), 41–58.

Kraakman, A. T. W., Niks, R. E., den Berg, P. M. M. M., Stam, P., & Van Eeuwijk, F. A. (2004). Linkage disequilibrium mapping of yield and yield stability in modern spring barley cultivars. *Genetics*, *168*(1), 435–446.

Kushwaha, U. K. S., Mangal, V., Bairwa, A. K., Adhikari, S., Ahmed, T., Bhat, P., ... & Tamta, R. (2017). Association Mapping, Principles and Techniques. *Journal of Biological and Environmental Engineering*, *2*(1), 1-9.

Lander, E. S., & Schork, N. J. (1994). Genetic dissection of complex traits. *Science-New York Then Washington*, 2037.

Landjeva, S., Korzun, V., & Börner, A. (2007). Molecular markers: actual & potential contributions to wheat genome characterization and breeding. *Euphytica*, *156*(3), 271–296.

Lebot, V. (2009). *Tropical root & tuber crops: cassava, sweet potato, yams & aroids*. Cabi.

Leuschner, K. (1982). Pest control for cassava & sweet potato. In *Root crops in Eastern Africa: proceedings of a workshop held in Kigali, Rwanda, 23-27 Nov. 1980*.

Lin, C. S., & Binns, M. R. (1988). A method of analyzing cultivar x location x year experiments: a new stability parameter. *TAG Theoretical & Applied Genetics*, *76*(3), 425–430.

Lozano, R., Hamblin, M. T., Prochnik, S., & Jannink, J.-L. (2015). Identification and distribution of the NBS-LRR gene family in the Cassava genome. *BMC Genomics*, *16*(1), 360.

https://doi.org/10.1186/s12864-015-1554-9

Lyon, W. F. (2009). A Plant-feeding Mite *Mononychellus tanajoa* (Bondar) (Acarina : Tetranychidae) New to the African Continent Threatens Cassava (*Manihot esculenta* Crantz) in Uganda , East Africa A Plant-feeding Mite *Mononychellus tanajoa* (Bondar) (Acarina : Tetranyc, *7793*(August 2017), 17–19. https://doi.org/10.1080/09670877309412727

Mackay, I., & Powell, W. (2007). Methods for linkage disequilibrium mapping in crops. *Trends in Plant Science*, *12*(2), 57–63.

Magoon, M. L., Krishnan, R., & Bai, K. V. (1970). Cytogenetics of the F1 hybrid between cassava and ceara rubber, & its backcross. *Genetica*, *41*(1), 425–436.

Mahmodi, N., Yaghotipoor, A., & Farshadfar, E. (2011). AMMI stability value and simultaneous estimation of yield & yield stability in bread wheat (*Triticum aestivum*). *Australian Journal of Crop Science*, *5*(13), 1837.

Manu-Aduening, J. A., Lamboll, R. I., Mensah, G. A., & Gibson, R. W. (2007). Farmers' perceptions and knowledge of cassava pests and diseases and their approach to germplasm selection for resistance in Ghana. *Annals of Applied Biology*, *151*(2), 189–198.

Markham, R. H., Robertson, I. A. D., & Kirkby, R. A. (1987). Cassava green mite in East Africa: a regional approach to research and control. *International Journal of Tropical Insect Science*, *8*(4-5–6), 909–914.

Masinde, E. A., Mkamillo, G., Ogendo, J. O., Hillocks, R., Mulwa, R. M. S., Kimata, B., & Maruthi, M. N. (2018). Genotype by environment interactions in identifying cassava (

Manihot esculenta Crantz) resistant to cassava brown streak disease. *Field Crops Research*, *215*(October 2017), 39–48. https://doi.org/10.1016/j.fcr.2017.10.001

McCouch, S. R., Wright, M. H., Tung, C.-W., Maron, L. G., McNally, K. L., Fitzgerald, M., … Mezey, J. (2016). Open access resources for genome-wide association mapping in rice. *Nat. Commun*, *7*, 10532. https://doi.org/10.1038/ncomms10532

Mcgregor, M. (2016). Phylogenetic diversity of cassava green mite , *Mononychellus progresivus* from different geographical sites in East Africa cassava , *Manihot esculenta* Crantz , is an important staple food for over 800 million people world-wide (Nweke , 1996 ; FAO , 2007), *24*, 63–71.

Mebelo, M., Hanna, R., & Toko, M. (2002). Cassava green mite biocontrol in Zambia: progress through 2001. In *Proceedings of the 3rd international regional meeting of the Africa-wide cassava green mite biocontrol project, International Institute of Tropical Agriculture, Biological Control Center for Africa, Cotonou, Republic of Benin* (pp. 20–22).

Megevand, B., Yaninek, J. S., & Friese, D. D. (1987). Classical biological control of the cassava green mite. *International Journal of Tropical Insect Science*, *8*(4-5–6), 871–874.

Miranda, G. V., Souza, L. V. de, Guimarães, L. J. M., Namorato, H., Oliveira, L. R., & Soares, M. O. (2009). Multivariate analyses of genotype x environment interaction of popcorn. *Pesquisa Agropecuária Brasileira*, *44*(1), 45–50.

Molo, R., Aool, W., Adumo, S., & Mutisya, D. L. (2016). Integrating cassava varieties and *Typhlodramulus aripo* to sustain biological control of cassava green mite. *African Crop Science Journal*, *24*(1), 117-126.

Muengula-Manyi, M., Nkongolo, K. K., Bragard, C., Tshilenge-Djim, P., Winter, S., & Kalonji-Mbuyi, A. (2012). Incidence, severity and gravity of cassava mosaic disease in savannah agro-ecological region of DR-Congo: analysis of agro-environmental factors. *American Journal of Plant Sciences*, *3*(4), 512.

Muojiama, S. O. (2017). *The Influence Of Levels and Modes Of NPK Fertilizer Application On Growth and Yield Of Some Improved Cassava Varieties In Nsukka, South Eastern Nigeria*.

Murphy, S. T. (1984). Biological control of the cassava green mite (Mononychellus spp.) in East Africa. *Integrated Pest Management of Cassava Green Mite.(Edited by AH Greathead, RH Markham, RJ Murphy, ST Murphy & IAD Robertson)*, 55–61.

Mutegi, C. K., Ngugi, H. K., Hendriks, S. L., & Jones, R. B. (2009). Prevalence & factors associated with aflatoxin contamination of peanuts from Western Kenya. *International Journal of Food Microbiology*, *130*(1), 27–34.

Mutisya, D. L., Wambua, J. M., Miano, D. W., & Charles, W. (2015). Farmer perceptions of cassava green mite pest impact in eastern Kenya, *3*(3), 354–358.

Myles, S., Peiffer, J., Brown, P. J., Ersoz, E. S., Zhang, Z., Costich, D. E., & Buckler, E. S. (2009). Association mapping: critical considerations shift from genotyping to experimental design. *The Plant Cell*, *21*(8), 2194–2202.

Nassar, N. M. A., & Ortiz, R. (2009). Cassava Genetic Resources: Manipulation for Crop Improvement. *Plant Breeding Reviews*, *31*, 247.

Naveed, M., Nadeem, M., & Islam, N. (2007). AMMI analysis of some upland cotton genotypes for yield stability in different milieus. *World J. Agric. Sci*, *3*(1), 39–44.

Ndayiragije, P. (1984). Cassava green mite (*Mononychellus tanajoa* (Bondar)) in Burundi. In *Integrated pest management of cassava green mite. Proceedings of a regional training workshop in East Africa, 30 April-4 May 1984* (pp. 67–73).

Njoku, D., Egesi, C., & Gracen, V. (2014). Identification of Pro-vitamin A Cassava (*Manihot esculenta* Crantz) Varieties for Adaptation and Adoption through Participatory Research. *Journal of Crop Improvement*, *28*(3), 361–376. https://doi.org/10.1080/15427528.2014.888694

Nordborg, M., & Weigel, D. (2008). Next-generation genetics in plants. *Nature*, *456*(7223), 720.

Ntawuruhunga, P. H., Rubaihayo, P., Whyte, J. B. A., Dixon, A. G. O., & Osiru, D. S. O. (2001). Additive main effects and multiplicative interaction analysis for storage root yield of cassava genotypes evaluated in Uganda. *African Crop Science Journal*, *9*(4), 591–598.

Nukenine, E. N., Dixon, A. G. O., Hassan, A. T., & Asiwe, J. A. N. (1999). Evaluation of cassava cultivars for canopy retention and its relationship with field resistance to green spider mite. *African Crop. Sci*, *7*(1), 47Y57.

Nweke, F. I., Spencer, D. S. C., & Lynam, J. K. (2002). *The cassava transformation: Africa's best-kept secret*. Michigan State University Press.

Nyiira, Z. M. (1972). Report of investigation of cassava mite, *Mononychellus tanajoa* (Bondar). *Kaw&a Research Station, Kampala, Uganda*, *14*.

Nyiira, Z. M. (1982). Cassava green mite: its distribution and possible control. In *Root crops in*

Eastern Africa: proceedings of a workshop held in Kigali, Rwanda, 23-27 Nov. 1980.

Nyiira, Z. M., & Mutinga, M. J. (1977). Tetranychidae Pests of Cassava, *Manihot esculenta* Crantz, in Uganda and their Natural Enemies. *East African Agricultural and Forestry Journal*, *43*(1), 1–4.

Nzuki, I., Katari, M.S., Bredeson, J.V., Masumba, E., Kapinga, F., Salum, K., Mkamilo, G.S., Shah, T., Lyons, J.B., Rokhsar, D.S. & Rounsley, S. (2017). QTL Mapping for Pest and Disease Resistance in Cassava & Coincidence with some Introgression Regions derived from *M. glaziovii*. *Frontiers in Plant Science*, *8*(July), 1168. https://doi.org/10.3389/fpls.2017.01168

Ojwang, P. P. O., Melis, R., Songa, J. M., Githiri, M., & Bett, C. (2009). Participatory plant breeding approach for host plant resistance to bean fly in common bean under semi-arid Kenya conditions. *Euphytica*, *170*(3), 383.

Okogbenin, E., & Fregene, M. (2003). Genetic mapping of QTLs affecting productivity and plant architecture in a full-sib cross from non-inbred parents in Cassava (*Manihot esculenta* Crantz). *Theoretical & Applied Genetics*, *107*(8), 1452–1462.

Okogbenin, E., Marin, J., & Fregene, M. (2008). QTL analysis for early yield in a pseudo F 2 population of cassava. *African Journal of Biotechnology*, *7*(2).

Onyeka, T. J., Dixon, A. G. O., & Ekpo, E. J. A. (2005). Identification of levels of resistance to cassava root rot disease (Botryodiplodia theobromae) in African landraces and improved germplasm using *in vitro* inoculation method. *Euphytica*, *145*(3), 281–288. https://doi.org/10.1007/s10681-005-1646-8

Onzo, A., Hanna, R., Negloh, K., Toko, M., & Sabelis, M. W. (2005). Biological control of cassava green mite with exotic & indigenous phytoseiid predators—effects of intraguild predation and supplementary food. *Biological Control*, *33*(2), 143–152.

Onzo, A., Hanna, R., & Sabelis, M. W. (2003). Interactions in an acarine predator guild: Impact on *Typhlodromalus aripo* abundance and biological control of cassava green mite in Benin, West Africa. *Experimental and Applied Acarology*, *31*(3–4), 225–241. https://doi.org/10.1023/B:APPA.0000010380.44408.05

Onzo, A., Hanna, R., & Sabelis, M. W. (2012). The predatory mite Typhlodromalus aripo prefers green-mite induced plant odours from pubescent cassava varieties. *Experimental and Applied Acarology*, *58*(4), 359–370.

Oraguzie, N. C., Gardiner, S. E., Rikkerink, E. H. A., & Silva, H. N. (2007). *Association mapping in plants*. Springer.

Ospina, B., & Ceballos, H. (2002). Sistemas modernos de producci{ó}n, procesamiento, utilizaci{ó}n y comercializaci{ó}n CIAT Colombia 586p.

Pasam, R. K., Sharma, R., Malosetti, M., van Eeuwijk, F. A., Haseneyer, G., Kilian, B., & Graner, A. (2012). Genome-wide association studies for agronomical traits in a world wide spring barley collection. *BMC Plant Biology*, *12*(1), 16. https://doi.org/10.1186/1471-2229-12-16

Patterson, N., Price, A. L., & Reich, D. (2006). Population structure and eigenanalysis. *PLoS Genetics*, *2*(12), e190.

Payne, T., Clement, J., Arnold, D., & Lloyd, A. (1999). Heterologous myb genes distinct from

GL1 enhance trichome production when overexpressed in Nicotiana tabacum. *Development*, *126*(4), 671–682.

Pradeepkumar, T., Bastian, D., Radhakrishnan, N. V, & Aipe, K. C. (2006). Genetic variation in tomato for yield and resistance to bacterial wilt. *Journal of Tropical Agriculture*, *39*(2), 157–158.

Pratt, P. D., Coombs, E. M., & Croft, B. A. (2003). Predation by phytoseiid mites on Tetranychus lintearius (Acari: Tetranychidae), an established weed biological control agent of gorse (Ulex europaeus). *Biological Control*, *26*(1), 40–47.

Price, A. L., Patterson, N. J., Plenge, R. M., Weinblatt, M. E., Shadick, N. A., & Reich, D. (2006). Principal components analysis corrects for stratification in genome-wide association studies. *Nature Genetics*, *38*(8), 904–909.

Pritchard, J. K., Stephens, M., Rosenberg, N. A., & Donnelly, P. (2000). Association mapping in structured populations. *The American Journal of Human Genetics*, *67*(1), 170–181.

Prochnik, S., Marri, P. R., Desany, B., Rabinowicz, P. D., Kodira, C., & Mohiuddin, M. (2012). The cassava genome: current progress, future directions. *Tropical Plant Biology*, *5*(1), 88–94.

Pujol, B., Mühlen, G., Garwood, N., Horoszowski, Y., Douzery, E. J. P., & McKey, D. (2005). Evolution under domestication: contrasting functional morphology of seedlings in domesticated cassava and its closest wild relatives. *New Phytologist*, *166*(1), 305–318.

Purchase, J. L. (1997). *Parametric analysis to describe genotype x environment interaction & yield* *stability in winter wheat*. Ph. D. Thesis, Department of Agronomy, Faculty of Agriculture of the University of the Free State, Bloemfontein, South Africa.

Purchase, J. L., Hatting, H., & Van Deventer, C. S. (2000). Genotype by environment interaction of winter wheat (Triticum aestivum L.) in South Africa: II. Stability analysis of yield performance. *South African Journal of Plant & Soil*, *17*(3), 101–107.

Quinlan, A. R., & Hall, I. M. (2010). BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics*, *26*(6), 841-842.

Rafalski, J. A. (2010). Association genetics in crop improvement. *Current Opinion in Plant Biology*, *13*(2), 174–180.

Ravel, C., Praud, S., Murigneux, A., Linossier, L., Dardevet, M., Balfourier, F., … Charmet, G. (2006). Identification of Glu-B1-1 as a candidate gene for the quantity of high-molecular-weight glutenin in bread wheat (Triticum aestivum L.) by means of an association study. *Theoretical and Applied Genetics*, *112*(4), 738.

Ravindran, V. (1993). Cassava leaves as animal feed: Potential and limitations. *Journal of the Science of Food and Agriculture*, *61*(2), 141–150. https://doi.org/10.1002/jsfa.2740610202

Reilly, K., Bernal, D., Cortés, D. F., Gómez-Vásquez, R., Tohme, J., & Beeching, J. R. (2007). Towards identifying the full set of genes expressed during cassava post-harvest physiological deterioration. *Plant Molecular Biology*, *64*(1–2), 187–203.

Roy, M., Brodeur, J., & Cloutier, C. (2003). Temperature and sex allocation in a spider mite. *Oecologia*, *135*(2), 322–326.

Sabesan, T., Suresh, R., & Saravanan, K. (2009). Genetic variability and correlation for yield and grain quality characters of rice grown in coastal saline low land of Tamilnadu. *Electronic Journal of Plant Breeding*, *1*(1), 56–59.

Schulthess, F., Baumgärtner, J. U., & Herren, H. R. (1987). Factors influencing the life table statistics of the cassava mealybug *Phenacoccus manihoti*. *Insect Science and Its Application*, *8*(4–6), 851–856.

Septiningsih, E. M., Sanchez, D. L., Singh, N., Sendon, P. M. D., Pamplona, A. M., Heuer, S., & Mackill, D. J. (2012). Identifying novel QTLs for submergence tolerance in rice cultivars IR72 and Madabaru. *Theoretical and Applied Genetics*, *124*(5), 867–874. https://doi.org/10.1007/s00122-011-1751-0

Shukla, G. K. (1972). Some statistical aspects of partitioning genotype environmental components of variability. *Heredity*, *29*(2), 237–245.

Shukla, P. T. (1976). Preliminary report on the green mite (Mononychellus tanajoa, Bonder) resistance in Tanzanian local cassava varieties. *East African Agricultural and Forestry Journal*, *42*(1), 55–59.

Singh, A. K., Sharma, P., & Singh, P. K. (2013). Studies on genetic characteristic of upland rice (*Oryza sativa* L.). *International Journal of Agriculture, Environment and Biotechnology*, *6*(4), 515.

Sleper, D. A., & Poehlman, J. M. (2006). *Breeding field crops*. (No.Ed. 5). Blackwell publishing.

Smaczniak, C., Immink, R. G. H., Angenent, G. C., & Kaufmann, K. (2012). Developmental and evolutionary diversity of plant MADS-domain factors: insights from recent studies. *Development*, *139*(17), 3081–3098.

Ssemakula, G., & Dixon, A. (2007). Genotype X environment interaction, stability & agronomic performance of carotenoid-rich cassava clones. *Scientific Research & Essays*, *2*(9), 390–399.

Stich, B., & Melchinger, A. E. (2009). Comparison of mixed-model approaches for association mapping in rapeseed, potato, sugar beet, maize and Arabidopsis. *BMC Genomics*, *10*(1), 94.

Todorovska, E., Christov, N., Slavov, S., Christova, P., & Vassilev, D. (2009). Biotic stress resistance in wheat—Breeding & genomic selection implications. *Biotechnology & Biotechnological Equipment*, *23*(4), 1417–1426.

Tomkiewicz, J., Skovgard, H., Nachman, G., & Munsterswendsen, M. (1993). A Rapid and Nondestructive Method to Assess Leaf Injury Caused by the Cassava Green Mite, *Mononychellus tanajoa* (Bondar) (Acarina, Tetranychidae). *Experimental & Applied Acarology*, *17*(1–2), 29–40. https://doi.org/10.1007/BF00156942

Tommasini, L., Schnurbusch, T., Fossati, D., Mascher, F., & Keller, B. (2007). Association mapping of Stagonospora nodorum blotch resistance in modern European winter wheat varieties. *Theoretical & Applied Genetics*, *115*(5), 697–708.

Tumuhimbise, R., Melis, R., Shanahan, P., & Kawuki, R. (2014). Genotype by environment interaction effects on early fresh storage root yield & related traits in cassava. *The Crop Journal*, *2*(5), 329–337.

Turner, S. D. (2014). qqman: an R package for visualizing GWAS results using QQ and manhattan plots. *bioRxiv*, 5165.

Unartngam, K. P. W. A. J. (2012). Assessment of the Severity of Brown Leaf Spot Disease in Cassava using Image Analysis. *The International Conference of the Thai Society of Agricultural Engineering*, 2–8.

Urbany, C., Stich, B., Schmidt, L., Simon, L., Berding, H., & Junghans, H. (2011). Association genetics in Solanum tuberosum provides new insights into potato tuber bruising and enzymatic tissue discoloration. *BMC Genomics*, *12*(1), 7.

Van Eeuwijk, F. A., Bustos-Korts, D. V., & Malosetti, M. (2016). What should students in plant breeding know about the statistical aspects of genotype by environment interactions. *Crop Science*, *56*(5), 2119–2140. https://doi.org/10.2135/cropsci2015.06.0375

Varshney, R. K., Marcel, T. C., Ramsay, L., Russell, J., Röder, M. S., Stein, N., … Graner, A. (2007). A high density barley microsatellite consensus map with 775 SSR loci. *Theoretical & Applied Genetics*, *114*(6), 1091–1103.

Visscher, P. M. (2008). Sizing up human height variation. *Nature Genetics*, *40*(5), 489–490.

Vom Brocke, K., Trouche, G., Weltzien, E., Barro-Kondombo, C. P., Gozé, E., & Chantereau, J. (2010). Participatory variety development for sorghum in Burkina Faso: Farmers' selection & farmers' criteria. *Field Crops Research*, *119*(1), 183–194.

Wang, M., Yan, J., Zhao, J., Song, W., Zhang, X., Xiao, Y., & Zheng, Y. (2012b). Genome-wide association study (GWAS) of resistance to head smut in maize. *Plant science*, *196*, 125-131.

Wellcome Trust Case Control Consortium. (2007). Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature*, *447*(7145), 661.

Welsch, R., Arango, J., Bär, C., Salazar, B., Al-Babili, S., Beltrán, J., … Beyer, P. (2010). Provitamin A accumulation in cassava (*Manihot esculenta*) roots driven by a single nucleotide polymorphism in a phytoene synthase gene. *The Plant Cell*, *22*(10), 3348–3356.

William, M. N. M. (2012). An Outbreak of Anthracnose Caused by *Colletotrichum gloesporioides f.sp. manihotis* in Cassava in North Western Tanzania. *American Journal of Plant Sciences*, *3*(5), 596–598. https://doi.org/10.4236/ajps.2012.35072

Wilson, C., & Tisdell, C. (2001). Why farmers continue to use pesticides despite environmental, health & sustainability costs. *Ecological Economics*, *39*(3), 449–462. https://doi.org/10.1016/S0921-8009(01)00238-5

Wolfe, M.D., Rabbi, I.Y., Egesi, C., Hamblin, M., Kawuki, R., Kulakow, P., Lozano, R., Carpio, D.P.D., Ramu, P. & Jannink, J.L. (2016). Genome-wide association and prediction reveals the genetic architecture of cassava mosaic disease resistance and prospects for rapid genetic improvement. *The Plant Genome*, 1–248. https://doi.org/10.3835/plantgenome2015.11.0118

Wolfe, M. D., Del Carpio, D. P., Alabi, O., Ezenwaka, L. C., Ikeogu, U. N., Kayondo, I. S., ... & Egesi, C. (2017). Prospects for genomic selection in cassava breeding. *The plant genome*, *10*(3).

Yan, J., Shah, T., Warburton, M. L., Buckler, E. S., McMullen, M. D., & Crouch, J. (2009). Genetic characterization and linkage disequilibrium estimation of a global maize collection using SNP markers. *PloS One*, *4*(12), e8451.

Yan, W.-H., Wang, P., Chen, H.-X., Zhou, H.-J., Li, Q.-P., & Wang, C.-R. (2011). A major QTL, Ghd8, plays pleiotropic roles in regulating grain productivity, plant height, & heading date in rice. *Molecular Plant*, *4*(2), 319–330.

Yan, W., Hunt, L. A., Sheng, Q., & Szlavnics, Z. (2000). Cultivar evaluation and mega-environment investigation based on the GGE biplot. *Crop Science*, *40*(3), 597–605.

Yan, W., & Tinker, N. A. (2006). Biplot analysis of multi-environment trial data: Principles and applications. *Canadian Journal of Plant Science*, *86*(3), 623–645.

Yang, X., Yan, J., Shah, T., Warburton, M. L., Li, Q. & Li, L. (2010). Genetic analysis and characterization of a new maize association mapping panel for quantitative trait loci dissection. *Theoretical & Applied Genetics*, *121*(3), 417–431.

Yaninek, J. S. (1994). Cassava green mite prevention technologies_1994.pdf. *African Crop Science Journal*.

Yaninek, J. S., Gutierrez, A. P., & Herren, H. R. (1989). Dynamics of Mononychellus tanajoa (Acari: Tetranychidae) in Africa: Experimental Evidence of Temperature and Host Plant Effects on Population Growth Rates. *Environmental Entomology*, *18*(4), 633–640. https://doi.org/10.1093/ee/18.4.633

Yaninek, J. S., & Herren, H. R. (1988). Introduction and spread of the cassava green mite, Mononychellus tanajoa (Bondar) (Acari: Tetranychidae), an exotic pest in Africa and the search for appropriate control methods: a review. *Bulletin of Entomological Research*, *78*(1), 1. https://doi.org/10.1017/S0007485300016023

Yaninek, J. S., Onzo, A., & Ojo, J. B. (1993). Continent-wide releases of neotropical phytoseiids against the exotic cassava green mite in Africa. *Experimental & Applied Acarology*, *17*(1–2), 145–160. Retrieved from http://link.springer.com/article/10.1007/BF00156950

Yaninek, S., & Hanna, R. (2003). Cassava Green Mite in Africa - a Unique Example of Successful Classical Biological Control of a Mite Pest on a Continental Scale. In C. Peter Neuenschwander & J. L. Borgemeister (Eds.), *Biological Control in IPM Systems in Africa* (pp. 1–101). CAB International.

Yaseen, M., & Bennett, F. D. (1977). Distribution, biology and population dynamics of the green cassava mite in the Neotropics. In *Proceedings of the Fourth Symposium of the International Society for Tropical Root Crops.*

Yu, J., & Buckler, E. S. (2006). Genetic association mapping and genome organization of maize. *Current Opinion in Biotechnology*, *17*(2), 155–160.

Yu, J., Pressoir, G., Briggs, W. H., Vroh Bi, I., Yamasaki, M., Doebley, J. F., … Buckler, E. S. (2006). A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nature Genetics*, *38*(2), 203–8. https://doi.org/10.1038/ng1702

Zhang, Z., Ersoz, E., Lai, C.-Q., Todhunter, R. J., Tiwari, H. K., Gore, M. a, … Buckler, E. S. (2010). Mixed linear model approach adapted for genome-wide association studies. *Nature Genetics*, *42*(4), 355–360. https://doi.org/10.1038/ng.546

Zhao, J. L., Wang, Y. L., Yao, D. Q., Zhu, W. Y., Chen, L., He, H. Le, … Cai, R. (2015). Transcriptome profiling of trichome-less reveals genes associated with multicellular trichome development in Cucumis sativus. *Molecular Genetics & Genomics*, *290*(5), 2007–2018.

Zhao, K., Tung, C. W., Eizenga, G. C., Wright, M. H., Ali, M. L., Price, A. H., … McCouch, S. R. (2011). Genome-wide association mapping reveals a rich genetic architecture of complex traits in *Oryza sativa*. *Nat Commun*, *2*, 467. https://doi.org/10.1038/ncomms1467

Zhao, K., Wright, M., Kimball, J., Eizenga, G., McClung, A., & Kovach, M. (2010). Genomic diversity and introgression in *O. sativa* reveal the impact of domestication and breeding on the rice genome. *PloS One*, *5*(5), e10780.

Zhou, G., Hao, D., Chen, G., Lu, H., Shi, M., Mao, Y., … Xue, L. (2016). Genome-wide association study of the husk number & weight in maize (Zea mays. *Euphytica*, *210*(2), 195–205.

Zhou, X., & Stephens, M. (2012). Genome-wide efficient mixed model analysis for association studies. *Nature Genetics*, *44*(7), 821–824. https://doi.org/10.1038/ng.2310.Genome-wide

Zhu, C., & Yu, J. (2009). Nonmetric multidimensional scaling corrects for population structure in association mapping with different sample types. *Genetics*, *182*(3), 875–888.

Zhu, X., Hill, R. A., & Nishiyama, A. (2008). NG2 cells generate oligodendrocytes and gray matter astrocytes in the spinal cord. *Neuron Glia Biology*, *4*(1), 19–26.

Zobel, R. W., Wright, M. J., & Gauch, H. G. (1988). Statistical analysis of a yield trial. *Agronomy Journal*, *80*(3), 388–393.

Zundel, C., Nagel, P., Hanna, R., Korner, F., & Scheidegger, U. (2009). Environment and host-plant genotype effects on the seasonal dynamics of a predatory mite on cassava in sub-humid tropical Africa. *Agricultural & Forest Entomology*, *11*(3), 321–331.

APPENDICES

Appendix 4.1a Summary of genome-wide association tests passing the Bonferonni significance threshold for Kano location.

Trait	Marker	Chr	Pos	p	MarkerR^2	log
LP	S8_5962253	8	5962253	1.11E-13	0.07509	12.95479
LP	S8_6439483	8	6439483	2.52E-12	0.06697	11.59862
LP	S8_6439519	8	6439519	2.52E-12	0.06697	11.59862
LP	S8_6439891	8	6439891	2.52E-12	0.06697	11.59862
LP	S8_6439935	8	6439935	3.06E-12	0.06647	11.51462
CGMS	S8_5962253	8	5962253	5.48E-12	0.06496	11.261
LP	S8_6125583	8	6125583	6.41E-11	0.05863	10.19347
CGMS	S8_6439935	8	6439935	6.64E-11	0.05853	10.17776
LP	S8_6061415	8	6061415	9.49E-11	0.05762	10.02254
LP	S8_6061421	8	6061421	9.49E-11	0.05762	10.02254
LR	S8_6439483	8	6439483	1.85E-08	0.04417	7.732617
LR	S8_6439519	8	6439519	1.85E-08	0.04417	7.732617
LR	S8_6439891	8	6439891	1.85E-08	0.04417	7.732617
CGMS	S8_5389232	8	5389232	4.08E-08	0.04217	7.389563
STC	S8_6439483	8	6439483	4.82E-08	0.04174	7.316674
STC	S8_6439519	8	6439519	4.82E-08	0.04174	7.316674
STC	S8_6439891	8	6439891	4.82E-08	0.04174	7.316674
LR	S8_6061415	8	6061415	1.36E-07	0.03912	6.865632
LR	S8_6061421	8	6061421	1.36E-07	0.03912	6.865632
LP	S8_5191862	8	5191862	1.52E-07	0.03884	6.817101
SG	S8_5962253	8	5962253	2.08E-07	0.03805	6.682062
LP	S8_5728618	8	5728618	2.14E-07	0.03798	6.670074
LP	S8_5726888	8	5726888	2.27E-07	0.03783	6.643038
STC	S8_6061415	8	6061415	2.64E-07	0.03746	6.579186
STC	S8_6061421	8	6061421	2.64E-07	0.03746	6.579186
LP	S8_5739136	8	5739136	3.04E-07	0.03709	6.516827
LP	S8_5409185	8	5409185	4.26E-07	0.03625	6.370509
LP	S8_6508308	8	6508308	4.72E-07	0.03599	6.325653
CGMS	S8_6939658	8	6939658	4.75E-07	0.03598	6.323672
CGMS	S8_5739136	8	5739136	5.00E-07	0.03584	6.300787
CGMS	S8_5726888	8	5726888	5.14E-07	0.03578	6.289248
LP	S8_5744781	8	5744781	6.08E-07	0.03535	6.216154

Appendix 4.1b Summary of genome-wide association tests passing the Bonferonni significance threshold for Otobi location.

Trait	Marker	Chr	Pos	p	MarkerR^2	log
LP	S8_5962253	8	5962253	7.77E-14	0.07593	13.10936
LP	S8_6439483	8	6439483	9.17E-13	0.06951	12.03783
CGMS	S8_5962253	8	5962253	2.96E-12	0.06648	11.52937
CGMS	S8_6439483	8	6439483	2.73E-11	0.06075	10.56393
CGMS	S8_6439519	8	6439519	2.73E-11	0.06075	10.56393
CGMS	S8_6439891	8	6439891	2.73E-11	0.06075	10.56393
CGMS	S8_6439935	8	6439935	4.38E-11	0.05953	10.35864
LP	S8_6061415	8	6061415	6.48E-11	0.05852	10.18818
LP	S8_6061421	8	6061421	6.48E-11	0.05852	10.18818
CGMS	S8_6125583	8	6125583	1.98E-10	0.05567	9.703927
LP	S8_6494548	8	6494548	6.68E-10	0.05255	9.175087
CGMS	S8_6494548	8	6494548	1.22E-09	0.05103	8.915174
CGMS	S8_6061415	8	6061415	1.58E-09	0.05036	8.80181
CGMS	S8_6061421	8	6061421	1.58E-09	0.05036	8.80181
LP	S8_5389240	8	5389240	5.45E-09	0.04721	8.263412
LP	S8_5962010	8	5962010	3.20E-08	0.04273	7.495013
LP	S8_5962464	8	5962464	3.43E-08	0.04255	7.465111
CGMS	S8_5389240	8	5389240	5.62E-08	0.0413	7.250411
CGMS	S8_6576192	8	6576192	8.79E-08	0.04017	7.055957
LP	S8_5389232	8	5389232	1.03E-07	0.03977	6.985816
CGMS	S8_6508308	8	6508308	1.26E-07	0.03928	6.901183
LR	S8_5962253	8	5962253	1.59E-07	0.03868	6.798658
LR	S8_6125583	8	6125583	2.15E-07	0.03792	6.667683
LR	S8_6061415	8	6061415	2.52E-07	0.03752	6.598651
LR	S8_6061421	8	6061421	2.52E-07	0.03752	6.598651
LP	S8_5388872	8	5388872	2.80E-07	0.03725	6.55233
LP	S8_5389151	8	5389151	2.80E-07	0.03725	6.55233
LR	S8_6439483	8	6439483	2.94E-07	0.03713	6.531224
LR	S8_6439519	8	6439519	2.94E-07	0.03713	6.531224
LR	S8_6439891	8	6439891	2.94E-07	0.03713	6.531224
CGMS	S8_5389232	8	5389232	3.91E-07	0.03642	6.407468
LR	S8_6439935	8	6439935	4.26E-07	0.0362	6.370448
LP	S8_5745904	8	5745904	4.51E-07	0.03606	6.345718
CGMS	S8_5388872	8	5388872	5.18E-07	0.03571	6.285964
CGMS	S8_5389151	8	5389151	5.18E-07	0.03571	6.285964

Appendix 4.1c Summary of genome-wide association tests passing the Bonferonni significance threshold for Umudike location

Trait	Marker	Chr	Pos	p	MarkerR^2	log
LP	S8_5962253	8	5962253	3.05E-14	0.07837	13.5157
LP	S8_6439483	8	6439483	9.12E-13	0.06952	12.04001
LP	S8_6439519	8	6439519	9.12E-13	0.06952	12.04001
LP	S8_6439891	8	6439891	9.12E-13	0.06952	12.04001
CGMS	S8_5962253	8	5962253	1.51E-12	0.06821	11.82102
LP	S8_6439935	8	6439935	1.87E-12	0.06767	11.72816
LP	S8_6125583	8	6125583	4.86E-12	0.0652	11.31336
CGMS	S8_6439519	8	6439519	2.23E-11	0.06127	10.6517
CGMS	S8_6439891	8	6439891	2.23E-11	0.06127	10.6517
CGMS	S8_6439935	8	6439935	3.24E-11	0.0603	10.48945
LP	S8_6061415	8	6061415	8.18E-11	0.05793	10.08725
LP	S8_6061421	8	6061421	8.18E-11	0.05793	10.08725
CGMS	S8_6125583	8	6125583	2.72E-10	0.05485	9.565431
LP	S8_6494548	8	6494548	3.74E-10	0.05403	9.427128
CGMS	S8_6061415	8	6061415	9.70E-10	0.0516	9.013228
CGMS	S8_6061421	8	6061421	9.70E-10	0.0516	9.013228
CGMS	S8_6494548	8	6494548	1.66E-09	0.05023	8.779892
LR	S8_5962253	8	5962253	2.85E-09	0.04886	8.545155
LR	S8_6061415	8	6061415	1.75E-08	0.04425	7.756962
LR	S8_6061421	8	6061421	1.75E-08	0.04425	7.756962
CGMS	S8_6576192	8	6576192	1.86E-08	0.0441	7.730487
LP	S8_5962010	8	5962010	2.76E-08	0.0431	7.559091
LP	S8_5962464	8	5962464	4.26E-08	0.042	7.37059
LP	S8_6576192	8	6576192	4.78E-08	0.04171	7.320572
CGMS	S8_6508308	8	6508308	6.13E-08	0.04108	7.21254
CGMS	S8_5389240	8	5389240	1.09E-07	0.03963	6.962574
LP	S8_6508308	8	6508308	1.51E-07	0.03881	6.821023
LP	S8_5745904	8	5745904	2.77E-07	0.03728	6.55752
LP	S8_6939658	8	6939658	2.83E-07	0.03723	6.548214
CGMS	S8_6939658	8	6939658	3.32E-07	0.03683	6.478862
STC	S8_5962253	8	5962253	5.31E-07	0.03565	6.274905
CGMS	S8_5388872	8	5388872	6.30E-07	0.03522	6.200659
CGMS	S8_5389151	8	5389151	6.30E-07	0.03522	6.200659

Appendix 4.2 Details of 35 genes annotated as candidates for association with CGM resistance.

Genes	Chromosome	Gene description
Manes.08G023800	8	A20/AN1-like-zinc-finger-family-protein
Manes.08G035100	8	AGAMOUS-like-80
Manes.08G024700	8	Basic-leucine-zipper-(bZIP)-transcription-factor-family-protein
Manes.08G046700	8	bZIP-transcription-factor-family-protein
Manes.08G058500	8	C2H2-like-zinc-finger-protein
Manes.08G048200	8	C2H2-type-zinc-finger-family-protein
Manes.08G048800	8	CCCH-type-zinc-finger-protein-with-ARM-repeat-domain
Manes.08G043300	8	Concanavalin-A-like-lectin-protein-kinase-family-protein
Manes.08G043400	8	Cysteine/Histidine-rich-C1-domain-family-protein
Manes.08G041400	8	Disease-resistance-responsive-(dirigent-like-protein)-family-protein
Manes.08G034200	8	Dof-type-zinc-finger-DNA-binding-family-protein
Manes.08G026100	8	F-box-family-protein
Manes.08G043900	8	homeobox-from-Arabidopsis-thaliana
Manes.08G046400	8	KH-domain-containing-protein-/-zinc-finger-(CCCH-type)-family-protein
Manes.08G033400	8	Leucine-rich-repeat-protein-kinase-family-protein
Manes.08G031700	8	Mitochondrial-transcription-termination-factor-family-protein
Manes.08G058000	8	myb-domain-protein-106
Manes.08G045400	8	myb-like-HTH-transcriptional-regulator-family-protein
Manes.08G047800	8	NIMA-related-kinase-5
Manes.08G047500	8	Pectin-lyase-like-superfamily-protein
Manes.08G053900	8	Pentatricopeptide-repeat-(PPR-like)-superfamily-protein
Manes.08G060500	8	PENTATRICOPEPTIDE-REPEAT-596
Manes.08G043700	8	peroxidase-2
Manes.08G035700	8	Plant-specific-transcription-factor-YABBY-family-protein
Manes.08G044700	8	Plastid-lipid-associated-protein-PAP-/-fibrillin-family-protein
Manes.08G044000	8	PROTEIN TRICHOME BIREFRINGENCE-RELATED
Manes.08G044400	8	Protein-phosphatase-2C-family-protein
Manes.08G047700	8	proton-gradient-regulation-3
Manes.08G049300	8	Remorin-family-protein
Manes.08G046500	8	Rhodanese/Cell-cycle-control-phosphatase-superfamily-protein
Manes.08G051000	8	sterol-methyltransferase-1
Manes.08G028400	8	Tetratricopeptide-repeat-(TPR)-like-superfamily-protein
Manes.08G051200	8	villin-2
Manes.08G041900	8	zinc-finger-protein-8
Manes.08G026900	8	SAUR-like-auxin-responsive-protein-family-