GENETIC MAPPING AND EVALUATION OF CASSAVA (*Manihot esculenta* CRANTZ) FOR DROUGHT TOLERANCE AND EARLY BULKING IN MARGINAL SAVANNAH ECOLOGY OF NIGERIA

by

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THESIS

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DECLARATION

I declare that the thesis hereby submitted to the University of Limpopo, for the degree of Doctor of Philosophy in Agriculture has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

Ewa, F (Mrs)	April, 2021
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Surname, Initials (Title)	**Date**

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DEDICATION

‘In memory of my father, late Mr Ewa Udu’ (The great economist!).

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LIST OF ACRONYMS AND ABBREVIATIONS

A	Additive
AFLP	Amplified Frangment Length Polymorphism
ANOVA	Analysis of Variance
BH	First branching height
BIOM	Total plant biomass
BRLEV	Branching level
C1	Second population
CBB	Cassava bacterial blight
CET	Clonal evaluation trial
CGM	Cassava green mite
CIAT	Central Agricultural Research Institute, Columbia
CIM	Composite interval mapping
cM	Centimorgan
CMD	Cassava mosaic disease
CV	Coefficient of variation
D	Dominance
DArT	Diversity arrays technology
DMC	Dry-matter content
DNA	Deoxyribonucleic acid
DRY	Dry root yield
EB	Early bulking
EMS	Expected mean squares

F_1	First filial generation
FRY	Fresh root yield
GCV	Genotypic coefficient of variation
GXE	Genotype by environment
GxHAxY	Genotype by harvest age by year
GxY	Genotype by year
h^2	broad sense heritability
HAxY	Harvest age by year
HFB	Height of first branching
HI	Harvest index
HRL	Height of retained leaves
IITA	International Institute for Tropical Agriculture
KASP	Competitive variance explained
LG	Linkage group
LR	Leaf retention
MAP	Months after planting
MARS	Marker-assisted recurrent selection
MAS	Marker-assisted selection
MSe	Error mean square
Msg	Genotypic mean square
NRCRI	National Root Crops Research Institute
NS	Not significant
NTPs	National performance trials
OD	Over dominance

OPT	OptiMAS
PA	Parent A
PB	Parent B
PCA	Principal Component analysis
PCR	Polymerace chain reaction
PCV	Phenotypic coefficient of variation
PD	Partial dominance
PH	Total plant height
PLPCOL	Pulp colour
PLTHT	Plant height
PVE	Phenotypic variance explained
QTL	Quantitative trait loci
r	Replication
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribo nucleic acid
RTDIAM	Root diameter
RTSH	Root shape
RTWT	Root weight
SCARLEV	Scar Level
SCARNO	Scar number
SI	Selection Index
SNPs	Single nucleotide polymorphism
SSA	Sub-Saharan Africa
SSRs	Simple sequence repeats

Total yld	Total yield
VIG	Plant vigour
Wab	Weight of above ground biomass
WOTL	Length of leafless stem
Wr	Weight of roots
WTLV	Length of stem with leaves
$\bar{Y}$	Grand mean
$\sigma^2 e$	Environmental variance
$\sigma^2 g$	Genotypic variance
σ_p^2	Phenotypic variance

ARTICLES IN PREPARATION FROM THE THESIS

1. Ewa, F., Asiwe, J.A.N., Okogbenin, E., Ogbonna, A., and Egesi, C. SNP mapping in cassava (*Manihot esculenta* Crantz) and QTL discovery for productivity in moderate drought stress environment in Africa.

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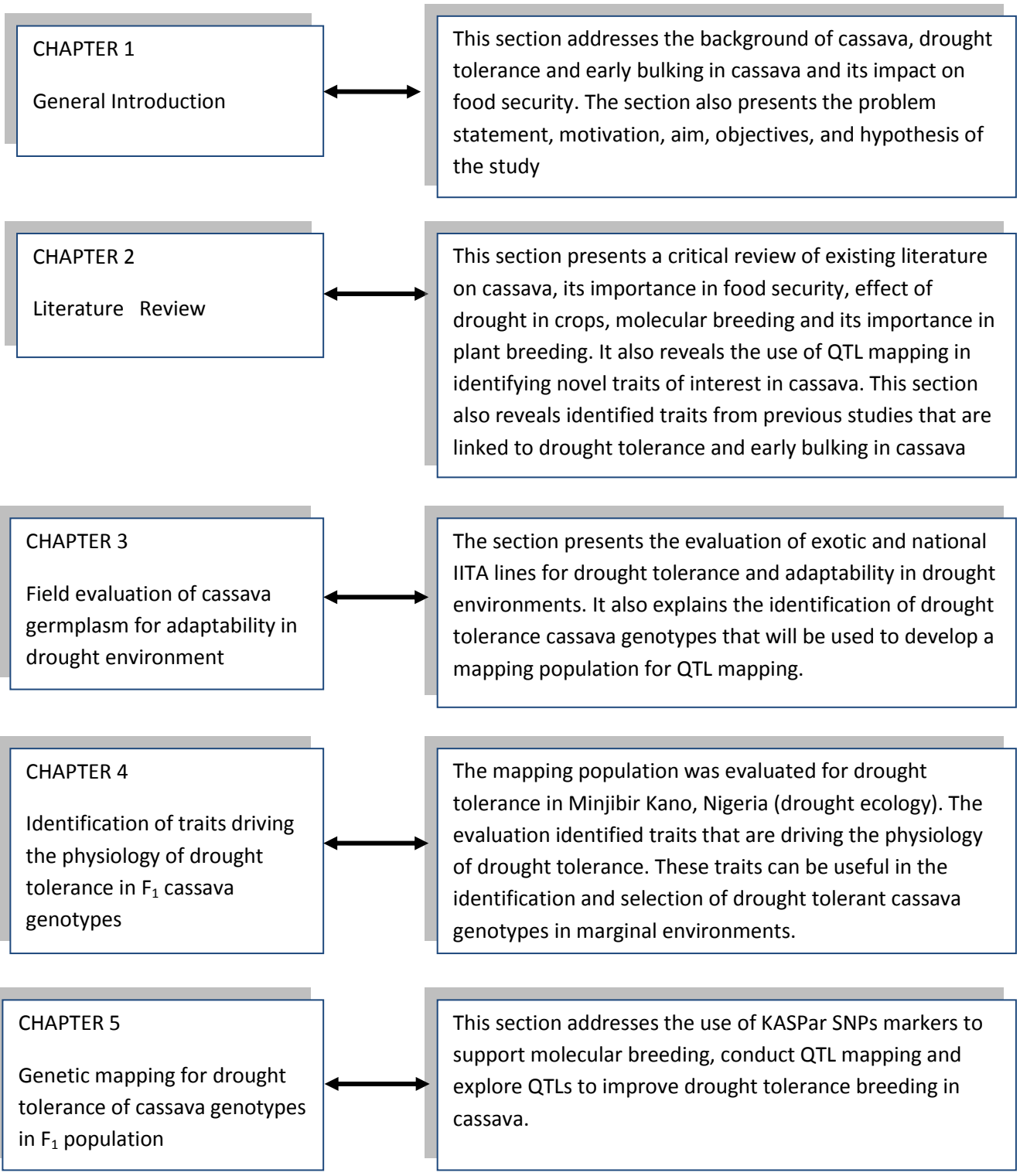
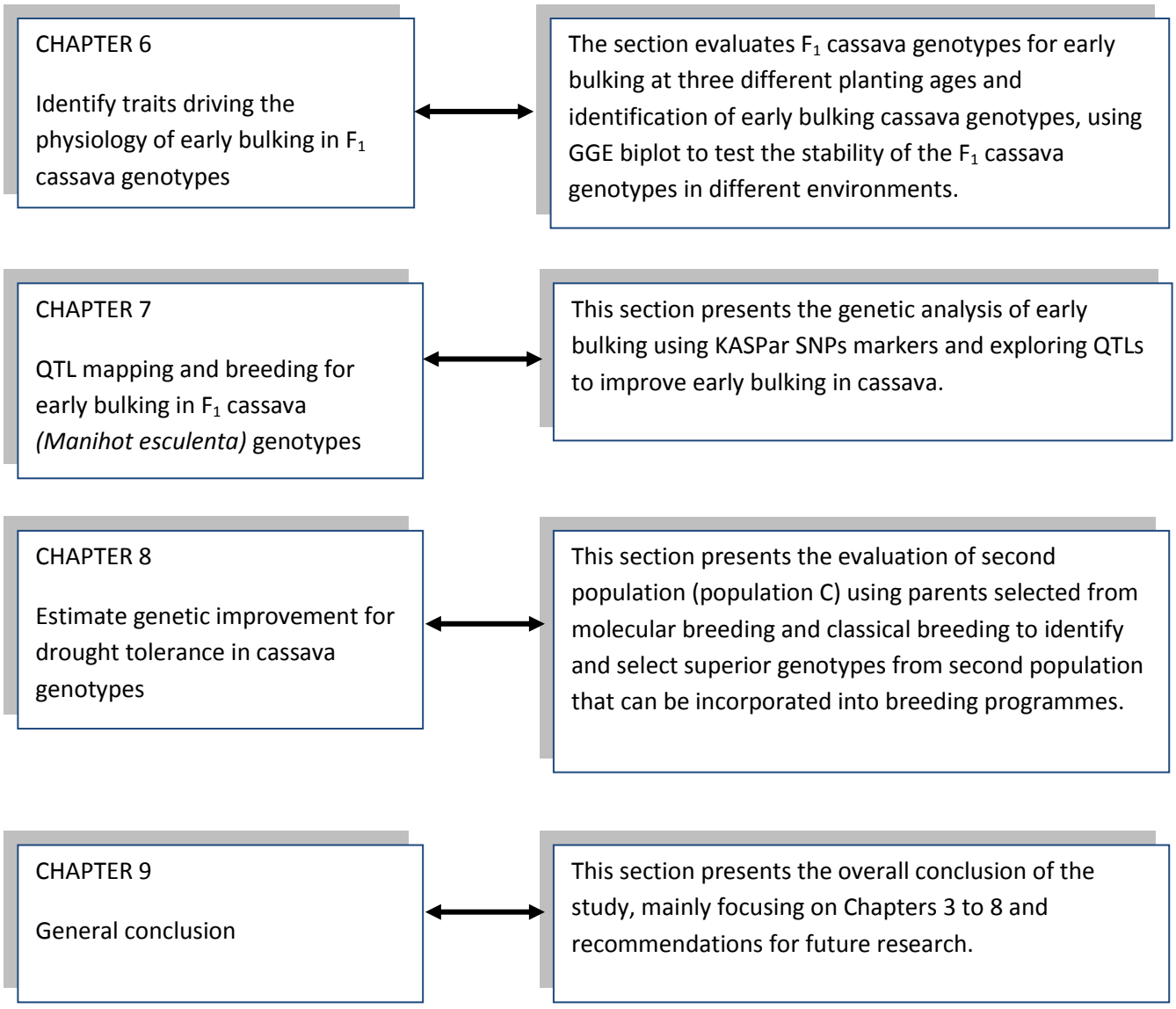
THESIS STRUCTURE

The subsequent chapters in this thesis are presented as follows:




CHAPTER 6
Identify traits driving the physiology of early bulking in F_1 cassava genotypes
The section evaluates F_1 cassava genotypes for early bulking at three different planting ages and identification of early bulking cassava genotypes, using GGE biplot to test the stability of the F_1 cassava genotypes in different environments.
CHAPTER 7
QTL mapping and breeding for early bulking in F_1 cassava *(Manihot esculenta)* genotypes
This section presents the genetic analysis of early bulking using KASPar SNPs markers and exploring QTLs to improve early bulking in cassava.
CHAPTER 8
Estimate genetic improvement for drought tolerance in cassava genotypes
This section presents the evaluation of second population (population C) using parents selected from molecular breeding and classical breeding to identify and select superior genotypes from second population that can be incorporated into breeding programmes.
CHAPTER 9
General conclusion
This section presents the overall conclusion of the study, mainly focusing on Chapters 3 to 8 and recommendations for future research.

ABSTRACT

Cassava (*Manihot esculenta* Crantz) is a widely cultivated crop in many tropical countries in Africa, Latin America, and Asia. Cassava is a staple food security crop for over one billion people worldwide. It is a multi‑purpose and well adapted to different agricultural production systems. Although cassava is adaptable to marginal soils with low fertility, and to irregular rainfall conditions, as it allows a relatively stable productivity and flexibility for harvesting process, the challenges posed by global climate change (both temperature and drought severity increasing) have caused negative impacts on this crop's productivity. Given the increasing demand for higher productivity to improve food security and alleviate poverty in the dry prone regions of Africa, there is a concurrent increasing demand to expand production into marginal ecologies and improve its adaptation in such ecologies. Breeding efforts have resulted in the development of high-yielding varieties, but due to late bulking and long time taken before crop is ready for harvest, the improved varieties were not easily adopted by farmers. The complex nature of yield and other productivity traits, coupled with the biology of cassava, make it more challenging to improve the crop. However, biotechnology has revolutionised breeding with the development of advanced molecular tools that have facilitated breeding-by-design approaches leading to effective manipulation of genes for complex traits. The potential and impact of the new tools are now providing a stronger basis to adopt molecular breeding to genetically improve the crop for key traits. The main objectives of the research were to: (i) Develop a mapping population and identify traits driving the physiological basis of drought tolerance in F_1 cassava genotypes; (ii) Identify traits linked with early bulking in the F_1 population; (iii) Identify quantitative trait *loci* (QTLs) for drought tolerance and early bulking in F_1 cassava genotypes; and (iv) Estimate the genetic improvement for drought tolerance in the F_1 population. Two genotypes (TMS98/0505 and TMS98/0581) with contrasting desirable traits such as high yield in marginal environment, good disease resistance, vigour, and flowering potentials were used in the development of the mapping population used in this study. Results indicate that there was a positive correlation between yield, yield-related traits .and morphological/physiological traits. Principal component analysis identified the scar level, height of stem with leaf, fresh root yield, dry root yield, root number and dry-matter content as traits driving drought tolerance in marginal environment. This study also identified early-bulking cassava varieties in the F_1 population and traits associated with early bulking. Fresh root yield was significantly associated with morphological and productivity traits while principal component analysis identified important traits such as root weight, root number, plant biomass, fresh root yield, plant height, , and stem diameter. Composite interval mapping identified 27QTLs and 30

QTLs in the first and second year, respectively, associated with the traits phenotyped in dry savannah ecology of Nigeria, while 16 and 12 QTLs associated with early bulking at 7 MAP were identified in the first and second year. Identification of these loci will aid breeding for drought tolerance and early root bulking in cassava. There was a better performance among traits such as biomass, root number, dry-matter content, number of scars, number of leaves, and length of stem with leaf in the second population (population C) than in the first population (population B). Twenty superior genotypes were selected from population C, which will be incorporated in the breeding programmes for further evaluation and germplasm enhancement.

KEY WORDS: Composite interval mapping, Fresh root yield, *Manihot esculenta,* morphological traits, Population C, physiological traits.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background of the Study

Cassava (*Manihot esculenta Crantz*) is a staple food for nearly a billion people in 105 countries of the world, especially tropical Africa, South America, and Asia (FAO, 2008). It is better than both maize (*Zea mays*) and sorghum (*Sorghum bicolor*) as an efficient producer of carbohydrate under optimal growing conditions. Cassava is grown in tropical and subtropical areas of the world between latitude 30^{o}N and 30^{o} S of the equator under various ecological and agronomical conditions (Onwueme, 1978; International Institute for Tropical Agriculture [IITA], 2001; El-Sharkawy, 2012). Cassava requires a warm, moist climate with mean temperatures of 24^{o}C to 30^{o}C and does well on light, sandy loamy soil with medium fertility.

Cassava production has continued to expand throughout the lowland tropics mainly on the less fertile, poor quality agricultural lands. In Africa, the capacity of cassava to grow and yield well on low fertility soils, its ability to withstand locust attacks and drought as well as its low production cost provide the economic incentive to use it as a replacement for other traditional root crops such as yam (*Dioscorea* spp.).

Nigeria is the world's largest producer of cassava with annual harvest of 60 million tonnes (FAOSTAT, 2019). Cassava is consumed by about 500 million Aficans everyday.. It is drought tolerant, as it can grow in harsh dry areas where cereals will not thrive with reasonable yield. While cassava can respond to high inputs, it however can produce reasonable yield in poor nutrient marginal soils. The storage roots of cassava are utilised either fresh or after processing into dry products such as flour, starch, garri and animal feed in the case of bitter cultivars' higher cyanogenic glycosides (Balagopalan, 2002; Dufour, 1988; Essers, 1995; Westby, 2002).

Cassava is now cash crop in many countries of the world. Cassava starch is also now being produced competitively. The production and processing of cassava has created many jobs, in addition to increased exports earnings. The crop has attracted foreign investments and contributed to the industrialisation and modernisation of several rural areas in undeveloped and underdeveloped countries.

Cassava is grown in diverse agro-ecologies, ranging from humid to sub-humid tropics and in the savannah, including the regions with low or erratic rainfall. It is mostly grown in regions

where rainfall is abundant to maximise productivity. Given the increasing demand for higher productivity to improve food security and alleviate poverty, there is an increasing demand to expand production into marginal ecologies and improve its adaptation in such ecologies (Varshney *et al*., 2018; Blum, 2017; Blum, 2016). With increasing drought occurrence and climate change threat, this need has been further exacerbated.

High genetic variation for these productivity and drought tolerance traits have been found in cassava germplasm (Aina *et al*., 2007; De Olivera *et al.,* 2017) and breeders are exploring these genetic resources to increase cassava's resilience to drought-prone ecologies. Beyond the challenge of enhancing productivity, cassava is also constrained by its biological limitations. It has a long growth cycle of typically 12 months in humid zones, and between 18 and 24 months in marginal lands. Breeding has resulted in high-yielding varieties, but due to late bulking, the improved varieties were not adopted by farmers, because of the long time taken to attain maturity before they are ready for harvest, especially in the dry marginal environments (Olasanmi *et al.,* 2017; Okechukwu and Dixon, 2009).

The complex nature of yield and other productivity traits, coupled with the biology of cassava, make it more challenging genetically to improve the crop. Biotechnology, through the development of advanced molecular tools, has facilitated breeding-by-design approaches to effectively manipulate genes, and offer solutions to problems associated with complex traits. Several molecular tools, including genetic markers, have been developed for cassava. Molecular markers with their ability to exploit information at the gene level, based on genome mapping and marker-assisted genetic analysis and breeding, offer practical solutions to many breeding problems affecting cassava. Since the development of the first genetic map of cassava, based on restriction-length polymorphism markers (Fregene *et al.,* 1997), other new maps have since followed (Mba *et al.,* 2001; Okogbenin *et al.,* 2006, Kunkeaw *et al.,* 2010; Sraphet *et al.,* 2011), based on other markers (for example, simple sequence repeats, expressed sequence tags).

Single nucleotide polymorphism markers are now available for molecular studies in cassava. SNPs are ideal markers, as they allow the use of genotyping platforms that can assay many individuals for thousands of SNP markers in parallel. The potential and impact of these new tools are now providing a stronger basis to adopt molecular breeding to genetically improve the crop for key traits in crops. Most economic traits were generally quantitatively inherited and required good breeding designs to effectively develop superior high performance varieties that can meet the demands of smallholder farmers, especially in marginal ecologies. This study, therefore, focused on the development of superior drought-tolerant and early-bulking genotypes for higher adaptation in marginal ecologies through marker-

assisted breeding. These desirable traits are difficult to breed and genetically evaluate using conventional methods, and therefore, they are logical targets for molecular breeding through marker-assisted recurrent selection (MARS) (Peleman and van der Voort, 2003). The objectives of this study were to develop a mapping population, and identify traits driving the physiological basis of drought tolerance and those linked with early bulking in the population; to identify quantitative trait loci (QTLs) for drought tolerance and productivity in a marginal environment; estimate the genetic improvement for drought tolerance and early bulking; and evaluate and select the best performing genotypes for inclusion in national performance trials (NPTs) for savannah ecologies.

1.2 Problem Statement

Cassava is a staple food crop in many parts of Africa, where it is a source of calories, vegetable and animal feed. Increased food demands and the high population growth in Africa has increased pressure to expand production into the dry marginal environments, a situation that has been further compounded by the effects of climate change (Westby, 2002). Given the expanding importance of cassava as food, feed, and industrial crop, genotypes with high yield, early bulking and drought tolerance are considered strategic, and highly desired for production in marginal areas in the dry savannahs, where favourable lands exist, but are highly prone to drought stress with average cassava yields below 5 tonnes/ha (Balagopalan, 2002).

Most of the commercial varieties under cultivation were developed for high rainfall ecologies, and these are therefore late bulking and not well adapted to drought-prone ecologies. Drought tolerance and early bulking are complex traits, because they are polygenic, and not easily and efficiently manipulated in breeding. In order to expand the production of cassava into the drought-prone ecologies, strategies involving marker-assisted breeding for the development of drought tolerant and early bulking traits must be developed to fast-track the development of cassava varieties.

1.3 Motivation for the Study

Cassava is a crop that is still dynamically evolving; and farmers in varied and harsh environments need to have access to cassava germplasm that is unique to their production niches. However, no strong effort has been made to deploy drought-tolerant traits more widely in the cassava gene pools, due to the complexity of breeding for these traits and the lack of efficient breeding tools to support such initiative, especially in agro-ecologies with a long, dry season or prone to drought of the major African cassava-producing countries. An IFAD-funded study (Duque, 2012) underscored the importance of breeding for drought tolerance, as perhaps the most effective strategy for improving adaptation, and boosting

cassava productivity in marginal drought-prone environments. Over 70% of cassava produced in Africa falls within the semi-arid agro-ecologies and guinea savannah (FAO, 2006). It is therefore necessary to breed for drought-tolerant cassava varieties to sustain crop production and productivity under limited water resources. Studies have revealed that late bulking is the single most important factor responsible for rejection and abandonment of cassava cultivars in African countries (Okechukwu and Dixon, 2009; Kamau *et al.,* 2011). Early bulking varieties are those that have the capacity to have reasonable yield levels between 7 and 8 months. In order to better harness the potential of cassava, especially in the current changing climatic conditions, there is a need to develop, evaluate, and select early-bulking cultivars that can be harvested at 7-10 months after planting.

1.4 Aim of the Study

The aim of the study was to develop superior drought tolerant and early bulking genotypes for higher adaptation in marginal ecologies through marker-assisted breeding.

1.5 Objectives of the Study

The objectives of the study were to:

a) Develop a mapping population and Identify traits driving the physiological basis of drought tolerance in an F1 mapping population of F_1 cassava genotypes.

b) Identify traits linked with early bulking in the F_1 population of cassava genotypes

c) Identify quantitative trait loci (QTLs) for drought tolerance and early bulking in the F_1 cassava genotypes.

e) Estimate a genetic improvement for drought tolerance in the F_1 population.

1.6 Hypotheses

a) There are no traits significantly associated with drought tolerance in cassava.

b) There are no traits associated with early bulking in cassava.

c) The QTLs linked to drought and early bulking traits cannot be mapped.

d) Genetic improvement for drought tolerance cannot be estimated in the F_1 population

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter will focus on the description of cassava crop and its adaptation in a dry and marginal ecology. It will also review studies relating to the use of marker-assisted selection in breeding for drought tolerance and early bulking in cassava genotypes. It will also address the importance of molecular breeding in fast-tracking the breeding for drought tolerance and early bulking, and thereby shortening the breeding cycle in cassava.

2.2: The Genus Manihot

Cassava is a member of the family *eEuphorbiaceae* (spurge family), subfamily of *Crotonoideae*, tribe of *Manihoteae,* genus *Manihot* and species *esculenta*. The genus Manihot comprises of 98 species (Rogers and Appan, 1973), ranging from sub-shrubs to shrubs and trees, with the majority producing latex and containing cyanogenic glucosides. Rogers and Appan (1973) classified *Manihot* species into 19 sections, varying from trees in the section *Glaziovianne* to sub-shrubs in the section *stipularis*. All *Manihot* species, including cassava (*Manihot esculenta*), have chromosome number of 2n = 36 (Nassar, 1978). Inter-specific hybrids between cassava and its wild relatives show a fair regular meosis, and back-crossed generations exhibit high fertility (Nassar, 2000).

2.3 Centres of Origin and Adaptation

Casssava's centre of origin was first reported to be Central America, including Colombia, Venezuela, Guatemala, and Southern Mexico, due to the large number of varieties present there (Sauer, 1952; Rogers, 1965). Cassava was introduced to Africa by the Portuguese in the 16th century (Cock, 1985). Originally, Allen (1994) proposed that modern cultivated cassava, *M. esculenta ssp. esculenta*, originated directly from the extant wild subspecies *M. esculenta ssp. flabellifolia*. A detailed molecular analysis, based on the single-copy nuclear gene encoding glyceraldehyde 3-phosphate dehydrogenase (Olsen and Schall, 1999), indicated that cassava was domesticated specifically from populations of *M. esculenta ssp. flabellifolia*. The 98 known wild species of the New World genus Manihot are distributed across warm regions of the Americas, from the southern Arizona to Argentina (Rogers and Appan, 1973). About 80 species of the genus occur in the northern part of South America, while the secondary centre of diversity occurs in Mexico and Central America with about 17 species of the genus, plus the related taxon *Manihotoides pauciflora*. Central Brazil has the highest diversity of Manihot species and is a home to about 40 wild species. Ranvoise

(1973) suggested that the sweet cassava varieties may have been domesticated in Meso-America (Central America), while the bitter ones were grown in the northern regions of South America. Cassava is mostly grown in areas where the average rainfall is over 1000 mm / year, even though it can also be found in areas with as little as 700 mm / year. The crop can be found in locations with rainfall as high as 3000 mm / year but will not stand poor drainage. On heavy soils, one day of flooding can kill the crop. Cassava is tremendously tolerant of low soil pH and a high level of aluminium saturation. In other words, it produces moderate yield where other crops fail as a result of low pH and high levels of aluminium (Cock, 1982; 1985; Cock and Howeler, 1979).

2.4 Botany and Morphological Description of the Cassava Plant

Cassava (*Manihot esculenta*) is also called “yuca” (Spanish), “manioc” (French), and “mandioca” (Portuguese) (Lokko *et al.,* 2007). The height of cassava usually ranges from 1-4 m. The roots are the main storage organ. Plants propagated from true seeds develop a primary tap root system from which adventitious roots originate, and the tap root and some of the adventitious roots become storage roots. Plants propagated from stem cuttings develop adventitous roots that develop into a fibrous root system, which increases in diameter within 30 to 60 days and forms tuberous roots, also called ‘storage roots’. The cassava root consists of the bark (outermost layer, 0.5 - 2% of the organ; easily removed by simple scratching), the peel (1 - 2 mm thick; 8 - 15% of the tuber; it contains most of the toxic cyanogenic glucosides), and the fleshy starchy parenchyma (83 - 92% of the tuber), which is the edible part of agricultural importance (Lebot, 2009; Tewe, 1992).

The mature cassava stem is woody and cylindrical. It is formed by alternating nodes and internodes. The branching height can be as low as 20 cm. Cassava leaves are simple, formed by the lamina and petiole. Generally, there is an uneven number of lobes, ranging from 3 - 9 lobes. Cassava is a monoecious species producing both male (pistillate) and female (staminate) flowers. The male flowers are numerous and develop near the tip of the plant, while the female flowers are fewer in number and develop closer to the base of the inflorescence (Ekanayake *et al.,* 1997). Female flowers open 1 to 2 weeks before the male flowers, a process known as protogyny. Developing seeds are viable 2 months after pollination, and the fruit becomes mature about one month after that, or about 3 months after pollination (Ceballos *et al.,* 2002). The fruit is a trilocular schizocarp, and seeds are ovoid-ellipsoidal, approximately 100 mm long, and 4 - 6 mm thick, with six staight prominent longitudinal ridges or aristae (Alves, 2002). Newly harvested seeds exhibit physiological dormancy, and require 3 to 6 months of storage at ambient temperature before they will

germinate (Jennings and Iglesias, 2002). Seeds can remain viable when stored under ambient conditions for 1 year, although germination percentages start to decline after 6 months (Rajendran *et al.,* 2002).

2.5 Cassava Breeding and Genetics

The critical aim of cassava breeding is to integrate into clonal cultivars all the characteristics not yet fully explored, which appear to be associated with high yield expressed both in terms of quantity and quality per unit of area and time. Increased yield, multiple pest and disease resistance, desirable agronomic traits such as appropriate plant architecture, early bulking of storage roots, with high dry-matter content, low cyanide content and consumer preference traits, for example, easy peeling, and early vigour in plant growth (for high foliage yield as leaf vegetable) have been the main breeding objectives (Lokko *et al.,* 2006). Breeding cassava relies on several selection stages (single row trial (SRT), preliminary, advanced, and uniform yield trials, UYT).

Molecular biology has been successful in diagnostics for cassava diseases and their genetic diversity (Restrepo and Verdier, 1997; Hernández Pérez *et al.,* 1999; Monger *et al.,* 2001; Álvarez *et al.,* 2003, 2009; Calvert *et al.,* 2008; Legg *et al.,* 2011); gene expression studies in host-pathogen interactions (Hong and Stanley, 1995; Fregene et al., 2004; Kemp et al., 2004, 2005); introgression of resistance to cassava mosaic disease (CMD) in Latin American germplasm (Egesi *et al.,* 2006; Okogbenin *et al.,* 2007); or dissection of the pathway leading to post-harvest physiological deterioration in cassava roots (Reilly *et al.,* 2007). The first molecular map of cassava was first published two decades ago (Fregene *et al.,* 1997). Yet, the only successful applied experience of marker-assisted selection in cassava breeding to date has been for resistance to CMD (Fregene *et al.,* 2000; Akano *et al.,* 2002; Rabbi *et al.,* 2014).

Recurrent selection, combined with a broad genetic base, has been reported to be the most efficient method for improving cassava base populations. High frequencies of genes for specific desirable characteristics, including yield components, root quality, pest resistance, tolerance to soil and climatic stresses, and stability of production across environments are progressively accumulated through recurrent selection (Hahn *et al.,* 1980). Cassava is highly heterozygous, and most traits are heterozygous at many loci. Segregation usually occurs at the first hybrid generation. Each hybrid seed is potentially a new cultivar (Bueno, 1985). Success in hybridisation depends on the selection methods used as well as the combining ability of the best genotypes. A good selection site should include as many physical and

biological constraits as much as possible. This will allow for the final selection to have a chance of being widely adapted and adopted (Egesi *et al.,* 2007; Akinbo, 2008). Significant progress has been made in breeding for insect pest and disease resistance, improved yield and other agronomic and quality characteristics (Okogbenin *et al.,* 2007; Dixon *et al.,* 2008; Akinbo, 2008). Backcrossing has also been a useful procedure for the transfer of resistance into elite populations by providing resistant lines quickly to prevent the severe infestation of relevant pests. Back-crossing followed by selection (Garzon *et al.,* 2008) has been used extensively to introduce new sources of insect pest and disease resistance from related *Manihot* species.

Knowledge on the inheritance of key traits of agronomic relevance in cassava is highly limited. Several constraints impede the elucidation of the genetics of cassava's key traits; they include a long growth cycle, allogamy, allotetraploidy and inbreeding depression on selfing (Kawuki *et al.,* 2011; Hershey, 2011; Ceballos *et al*., 2004). In many cases, the cassava breeder has had to work without clear understanding of the genetic basis of the traits to be improved. Molecular markers are being integrated into breeding to address some of these constraints. Molecular markers with their ability to exploit information at the gene level, based on genome mapping and marker-assisted genetic analysis and breeding, offered clear practical solutions to many of the problems affecting cassava breeding.

2.6 Genetic Markers

Genetic markers are important developments in the field of plant breeding (Gitishree *et al.,* 2017). The genetic marker is a gene or DNA sequence with a known chromosome location controlling a particular gene or trait. It can also be described as a specific gene or DNA sequence that produces a detectable trait with a known location on the chromosome that can be used to study family and population, identify cells, species, or individuals. Genetic markers are closely related to the target gene, and they act as sign or flags (Collard *et al.,* 2005). To be a useful genetic marker, the marker locus should show an experimentally detectable variation among individuals in the test population (Liu, 1998). Once the variation is identified, and the genotypes of all individuals in the test population are known, the frequency of recombination events between loci can be used to estimate linkage distances between markers (Okogbenin *et al.,* 2011).

During the 1990s, different types of molecular markers (DNA or RNA) were gradually developed and used for these same initial purposes (Asante and Offei 2003; Carmo *et al.,* 2015; Carvalho and Schaal 2001; Kawuki *et al.,* 2009; Maredia *et al.,* 2016; Marmey *et al.,*

1993; Moyib *et al.,* 2007; Rabbi *et al.,* 2015; Zacarias *et al.,* 2004). Markers were also used to distinguish hybrids from self-pollinations in breeding nurseries. These uses for molecular-marker technologies offer clear advantages that become even more evident with their constant reduction in costs and enhancement of discriminating capacity. Diversity studies based on molecular markers have been particularly useful to assess the relationship among different Manihot species and the evolution of this genus (Deputié *et al.,* 2011; Olsen and Schaal 2001; Roa *et al.,* 1997; Second *et al.,* 1997).

Genetic markers are broadly grouped into two categories: classical markers and DNA / molecular markers. Morphological, cytological, and biochemical markers are types of classical markers. Some examples of DNA markers are restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs), single nucleotide polymorphism (SNP), and diversity arrays technology (DArT) markers (Jiang GL 2013).

2.7 Classical markers

2.7.1 Morphological markers

These are referred to as phenotypic traits or characters. They are also called visible or classical markers. Morphological markers can visually differentiate qualities such as seed structure, flower colour, growth habit, and other important agronomic traits. They do not require any specialised biochemical and molecular technique. The main disadvantages of morphological markers are that they are limited in number, influenced by the plant growth stages and various environmental factors (Eagles *et al.,* 2001).

2.7.2 Biochemical markers

They are allelic variants of enzymes, referred to as isozymes, multi-molecular forms of enzymes that are coded by various genes, but have the same functions. As they are allelic variations of enzymes, gene and genotypic frequencies can be estimated with biochemical markers. Biochemical markers have been successfully applied in the detection of genetic diversity, population structure, gene flow, and population subdivision (Mateu-andrés *et al.,* 2005). They are co-dominant, easy to use, and cost effective. However, being less in number, they detect less polymorphism, and they are affected by various extraction methodologies, plant tissues, and different plant growth stages (Mondini *et al.,* 2009). They were the first molecular markers used in cassava. Typically, the first applications focused on analysing genetic diversity or they were used for identification purposes (Ramírez *et al.,* 1987; Hussain *et al.,* 1987; Lefèvre and Charrier 1993).

2.7.3 Cytological markers

These markers are related to the variations present in the numbers, banding patterns, size, shape, order, and position of chromosomes. These variations reveal differences in the distributions of euchromatin and heterochromatin. For example, G bands are produced by the Giemsa stain; Q bands are produced by quinacrine hydrochloride; and R bands are the reversed G bands. These chromosome landmarks can be used in the differentiation of normal and mutated chromosomes. Such markers can also be used in the identification of linkage groups and in physical mapping (Jiang, 2013).

2.8 Molecular/DNA Markers

Molecular markers are nucleotide sequences and can be investigated through the polymorphism existing between the nucleotide sequences of different individuals. A perfect DNA marker should be co-dominant (determination of homozygous and heterozygous states of diploid organisms); evenly distributed throughout genome; highly reproducible; easy and fast to assay; available; allow for an easy exchange of data between laboratories; display a selective neutral behaviour (the DNA sequences of any organism are neutral to environmental conditions or management practices); and have the ability to detect a higher level of polymorphism (Mondini *et al.,* 2009).

The numerous types of molecular markers that are commonly applied to cassava to date include the following.

2.8.1 Restriction Fragment Length Polymorphism (RFLPs)

RFLPs were the first markers to be used in human genome mapping (Bostein *et al.,* 1980), and were later adopted for plant genome mapping (Weber and Helentjaris, 1989). They are co-dominant and can identify a unique locus (Tanksley *et al.,* 1989). Base-pair deletions, mutations, inversions, translocations, and transpositions are the main causes for the variation resulting in the RFLP pattern. These variations lead to the gain or loss of recognition sites, resulting in fragments of various length and polymorphism. The restriction enzymes will not cut the fragment if a single base-pair variation occurs in the recognition site. However, if this point mutation occurs in one chromosome but not the other, it is called heterozygous for the marker, as both bands are present.

2.8.2 Amplified Fragment Length Polymorphism (AFLP)

This is a polymerase chain reaction (PCR) based tool. It is based on PCR amplification of restriction enzymes and oligonucleotide primers (Vos *et al.,* 1995). It is used in DNA fingerprinting whereby individuals are identified by their respective DNA profiles. AFLP

markers are cost effective, and there is no need for prior sequence information. In AFLP, both good-quality and partly degraded DNA can be used; however, this DNA should not contain any restriction enzymes or PCR inhibitors. In AFLP, two restriction enzymes (a frequent cutter and a rare cutter) are used for the cutting of DNA. Each end of the resulting fragments is ligated with the oligonucleotides. Oligonucleotides are short nucleic acid fragments used for the ligation in PCR (Madhumati *et al.,* 2014). One end is specific for the rare cutter (6-bp recognition site), and the other one for the frequent cutter (3-bp recognition site). This will lead to the amplification of only those fragments that have been cut by these cutters. For the development of primers, known sequences of adapters are used. Adapters are short, enzyme-specific DNA sequences, generally used for fishing out an unknown DNA sequence (Vos *et al.,* 1995). After performing PCR, visualisation is done in either agarose gel or polyacrylamide gel stained with $AgNO_3$ or by autoradiography (Madhumati *et al.,* 2014). This approach is very useful in saturation mapping, discrimination between varieties and it is also a useful tool for genetic engineering.

2.8.3 Micro-satellites *or SSRs*

Microsatellites, also called SSRs, are short tandem repeats and simple sequence length polymorphisms (Litt *et al.,* 1989; Tautz*.,* 1989). Microsatellites can be mononucleotide (A), dinucleotide (GT), trinucleotide (ATT), tetranucleotide (ATCG), pentanucleotide (TAATC), or hexanucleotide (TGTGCA) (Weber *et al.,* 1990).

Microsatellites are distributed in the genome; however, they are also present in the chloroplast (Provan *et al.,* 2001) and mitochondria (Rajendrakumar *et al.,* 2007). SSRs represent the lesser repetition per locus with higher polymorphism level (Zane *et al.,* 2002). This high polymorphism level is due to the occurrence of various numbers of repeats in microsatellite regions and can be detected with ease by PCR (Kalia *et al.,* 2011). Occurrence of SSRs may be due to slippage of single-strand DNA, a recombination of double-strand DNA, transfer of mobile elements (retrotransposons), and mismatches. Common motifs present in SSRs are mono: A, T; Di: AT, GA; Tri: AGG; Tetra: AAAC. The sequences that are flanking the SSRs are mostly conserved and used in the development of primers. Development of a genomic library and sequencing a segment of the studied genome will result in the development of these primers. The development of SSR markers involves the development of an SSR library and thereafter the detection of specific microsatellites. Following this, the detection of favourable regions for primer designing is done, and then PCR is performed. Interpretation and evaluation of banding patterns are performed, and assessment of PCR products is performed for investigation of polymorphism.

SSR markers are considered as markers of choice, as they are co-dominant, with high reproducibility, and greater genome abundance; and they can be used efficiently in plant mapping studies (Tautz*.,* 1989; Kalia *et al.,* 2011).

2.8.4 Single nucleotide polymorphisms (SNPs)

They are a new set of molecular markers used in genetic studies. SNPs are an abundant source of sequence variants, and of all the molecular-marker technologies available today, they provide the highest marker density (Thompson, 2012). SNPs have become the markers of choice. They are extremely useful for creating a high-density genetic map, which cannot be achieved with other genetic marker classes. A single nucleotide polymorphism (SNP) is a DNA sequence variation occurring when a single nucleotide (A, T, C, or G) in the genome differs between members of a biological species or paired chromosomes in an individual. Within a population, SNPs can be assigned a minor allele frequency (lowest allele frequency) at a locus that is observed in a population (Okogbenin *et al.,* 2011). The benefits of SNP assays include increased speed of genotyping, lower cost, and the parallel assays of multiple SNPs (Thompson, 2014). SNPs are necessary in such applications for association mapping and construction of high-density genetic maps, which usually require genotyping of thousands of SNPs in many individuals (Akhunov *et al.,* 2009). They are most suited in the construction of high-resolution genetic maps, studying evolutionary history, and performing genome-wide association mapping experiments. Two main advantages of SNPs over other molecular markers are in terms of their abundance (Zhu *et al.,* 2003) and availability of a wide array of technologies for high throughput SNP analysis (Fan *et al.,* 2006). Due to their abundance in nature, SNPs have the potential to provide the basis for a superior and highly informative genotyping assay. SNPs markers are largely biallelic in nature. The biallelic nature of SNPs makes them less informative per locus when compared to multiallelic markers such as RFLPs and microsatellites, but they overcome this exertion through their abundance, which allows the use of a greater number of loci. They are less mutable when compared to other markers, particularly microsatellites. This low rate of recurrent mutation makes them evolutionarily stable. They are excellent markers for studying complex genetic traits and for understanding the genomic evolution.

2.9 Marker Assisted Selection (MAS)

Molecular breeding has been applied in the genetic improvement of crops. Marker-assisted selection (MAS) is the selection of individuals with specific alleles for traits controlled by a limited number of loci (6-8). It is a process whereby a marker (morphological, biochemical or

one based on DNA/RNA variation) is used for indirect selection of the genetic determinant(s) of a trait of interest (for example, productivity, disease resistance, abiotic stress tolerance, and quality). MAS is desirable when phenotypic screening is particularly expensive and laborious; and it is very useful for pyramiding multiple resistance genes. MAS has been successfully performed for many oligogenic traits (Garland *et al.,* 2000; Murai *et al.,* 2001; Jia *et al.,* 2002; Komori *et al.,* 2003). Marker-assisted selection enables a precise identification of genotypes without environmental influence. Marker-assisted selection also aids in the reduction of the breeding cycle. It saves a lot of time in the breeding process. As a result of selection, it aids in the reduction of large breeding populations at the seedling stage. This is important in cassava breeding because of the length of the growing cycle and the cost involved in the evaluation process. Pre-selection made at the F_1 stage using molecular markers will help reduce the large population, thereby enhancing the efficiency of a clonal evaluation trial (CET). The selection of progenies based on genetic values derived from molecular-marker data substantially increases the rate of genetic gain, especially if the number of cycles of evaluation or generations can be reduced (Meuwissen *et al.,* 2001).

Wild relatives are important sources of genes for pest resistance in cassava (Hahn *et al.,* 1980; Chavarriaga *et al.,* 2004), but there is a need to reduce or eliminate undesirable donor genome content. Linkage drag can lengthen the process for this to be achieved, thereby making it unrealistic for breeders (Fregene *et al.,* 2007). Stam and Zeven (1981) indicated that markers could reduce the linkage drag, thereby reducing the number of generations required on the back-cross scheme. MAS in cassava helps to reduce the length of time required for the introgression of traits from wild relatives.

Cassava mosaic disease (CMD) is the most widespread disease of economic importance in Africa (Okogbenin *et al.,* 2007). Yield losses caused by CMD were as high as 100% (Thresh *et al.,* 1994) in some years. MAS has helped to develop *CMD2*, a gene that allowed resistance to CMD (Akano *et al.,* 2002). Okogbenin *et al.* (2007) showed that the *CMD2* gene introgressed into elite varieties using markers, which showed 65% resistance to CMD with a severity of 1 or 2. Another application of MAS was in the development of inbred lines. Cassava genotypes are heterozygous. The speed of inbreeding depends on the average heterozygosity of the original parental lines; the homozygosity level of the selected genotypes at the end of the self-pollinating phase; and the process of selection of progenies to be self pollinated (Scotti *et al.,* 2000). In the inbreeding process, phenotypically there is decrease in vigour, which is correlated with an increased level of homozygosity, while the aim is to select vigourous plants tolerant to inbreeding. Molecular markers can be used to estimate the level of homozygosity of a given plant, enabling the selection of plants with true tolerance to inbreeding. Molecular markers can help identify regions in the genome that are

particularly related to the expression of heterosis, and suitable for mearsuring genetic distances among inbred lines to direct crosses with higher probabilities of high heterosis. However, most agronomic traits are quantitative in nature, and are the result of the joint action of several loci on chromosomes (QTL). To efficiently combine the best haplotypes for effective development of superior genotypes, marker-assisted recurrent selection (MARS) will best be suited to increase the frequency of favourable alleles, based on a multi-parental strategy, and using a 'breeding-by-design' approach to recombine favourable alleles to build ideal haplotypes for target traits that are complex in nature. Breeding-by-design allows breeders to exploit known allelic variation to design superior genotypes by combining multiple favourable alleles (Peleman and van der Voort, 2003). MARS involves several cycles of the marker-based selection and is effective in increasing the frequencies of favourable QTL or marker alleles. MARS is the identification and selection of several genomic regions (up to 20 or even more) for complex traits within a population.

2.10 Genetic Mapping and Gene Discovery

Genetic mapping employs different methods for identification of a gene's locus as well as for determination of the distance between two genes. Gene mapping is considered the major area of research in which molecular markers are used today. The principle of genetic mapping is based on chromosomal recombination that occur during meiosis, which results in the segregation of genes. The first steps of building a genetic map are the development of genetic markers and a mapping population. The closer the two markers are on the chromosome; the more likely they are to be passed on to the next generation together. Therefore, the 'co-segregation' patterns of all markers can be used to reconstruct their order.

Markers have been used to generate several molecular-genetic maps for cassava. Since the first development of the first genetic map of cassava (Fregene *et al.*, 1997), other new maps have followed (Mba *et al.,* 2001; Okogbenin *et al.,* 2006; Kunkeaw *et al.,* 2010; Sraphet et al., 2011). Over 1700 cassava SNPs are now available for molecular studies (Pablo *et al.,* unpublished; Morag *et al.,* unpublished). SNPs are ideal markers, as they allow the use of genotyping platforms that can assay many individuals for thousands of SNP markers in parallel. The strategy for utilising markers is primarily driven by their availability and cost of genotyping platforms. One of the primary objectives of genetic mapping and gene tagging efforts in cassava is to provide tools that can increase the cost effectiveness and efficiency of cassava breeding. Desirable characters that are difficult to evaluate when using conventional methods are logical targets for molecular breeding of cassava. They include insect pest and diseases, traits expressed only at the end of the crop's growing cycle, and those for which phenotype is difficult to measure. Various markers have been used to tag

several traits in cassava. Molecular markers have been used to tag three different sources of CMD resistance (*M. glaziovii*, *TME3* and TMS97/2205) (Fregene *et al.,* 2000; Akano *et al.,* 2002). About six markers were found to be associated with CBB, explaining 9-27% of the phenotypic variance of response to five Xanthomonas strains (Jorge *et al.,* 2000). Early bulking is another trait evaluated in cassava; and results from the analysis of this trait showed that it was mostly affected by harvest index and dry foliage. Three QTLs, explaining 25-33% of phenotypic variance, were found for dry foliage, while five other QTLs associated with harvest index with phenotypic variance in the range of 18-27% were identified (Okogbenin and Fregene, 2002).

2.11 Genetic Linkage Map

Genetic linkage is the tendency of alleles that are close together on a chromosome to be inherited together during the meiosis phase of sexual reproduction. Genes, whose loci are nearer to each other and less likely to be separated into different chromatids during chromosomal crossover, are said to be genetically linked. A linkage map is a genetic map of a species or experimental population that shows the position of its known genes or genetic markers relative to each other in terms of recombination frequency. The percentage of segregating progeny that are recombinants for a pair of linked loci is the recombination frequency. The recombination frequency gives an estimate of the distance between two loci in a chromosome, on the assumption that the probability of crossover is proportional to the distance between loci (Liu, 1998). Many statistical procedures have been used to detect linkage and estimate the recombination fraction at two-point or multi-point levels (Ott, 1991). These procedures are the fundamentals of linkage map construction. The recombination fraction is not additive along a chromosome, and the departure from additivity increases with distance between loci (Liu, 1998). Additivity assumes that the average number of crossovers per chromatid occurring between two loci is directly proportional to the distance between the two loci. Estimates of the frequency of crossing over are most reliable when genes are closely linked, for example, 1 to 10 map units (centi-Morgans or cM), where a cM is the chance of a 1% recombination.

Genetic linkage mapping is used for localising and isolating both simple and complex traits. Molecular markers placed on genetic maps allow the development and efficient use of indirect selection schemes for germplasm improvement, thereby increasing precision in the manipulation of both qualitative and quantitative traits. This, in turn, is the basis for MAS, where markers closely associated with a trait(s) of interest can be used to introgress a specific gene(s) of interest into a desired background (Taylor *et al.,* 2004).

Genetic linkage maps can provide a more direct method for selecting desirable genes via their linkage to easily detectable molecular markers (Tanksley *et al.,* 1989). Once a trait is identified and mapped, MAS could be used to introduce the trait into a wide variety of populations. MAS can reduce breeding population sizes, continuous recurrent testing, and the time required to develop a superior line. In cassava, the application of MAS has been developed more recently compared to other major crops, with the construction of genetic linkage maps using RFLP, isoenzymes and SSR markers at CIAT (Fregene *et al.,* 1997; Mba *et al.,* 2001). Despite the low saturation of loci in the genetic maps of cassava, marker loci are randomly distributed over linkage groups, and the information from these maps has been utilised in cassava genetics. Genes for resistance to CMD have been mapped including a major one (*CMD2*) (Akano *et al.,* 2002). MAS for breeding CMD resistance has been applied successfully for introducing resistance into elite gene pools at CIAT (CIAT, 2003; Fregene and Mba 2004), and to introgress resistance to cassava green mite (CGM), and CMD into local Tanzanian varieties.

2.12 Quantitative Trait Loci Mapping (QTL Mapping)

QTL mapping is a process of locating genes with effects on quantitative traits by using molecular markers. Molecular markers are ideal to study and map QTLs, which can be efficiently used in MAS as well (Angaji *et al.,* 2009). It can be defined as the marker-facilitated genetic dissection of complex phenotypes' variation through appropriate experimental design and statistical analysis of segregating materials (Angaji, 2009). It is based on measuring the mean difference between lines with contrasting marker alleles. QTL mapping is basic research activity that requires careful planning of crosses and high precision phenotyping. QTL mapping is important in determining the number of genes influencing the trait, to find the location of the gene, and establish the effect of dosage of these genes on the variation of the trait.

Most agricultural traits of economic interest are polygenic and quantitative in nature, and are controlled by many genes on the same/different chromosome. The chromosomal regions having genes for these quantitative traits are referred to as QTL. QTL mapping is a method in which molecular markers are utilised to locate the genes that affect the traits of interest. Such traits are divided into two groups: one is quantitative and the second one refers to qualitative traits. Discontinuous variations can be shown by qualitative traits, while continuous variation occurs in quantitative traits.

2.13 QTL mapping methodology

For QTL mapping, two diverse parents should be selected; they should be diverse enough to exhibit an adequate level of polymorphism. The diverse parents must have allelic variations that affect the trait of interest. Near-isogenic lines (NILs), DHs, backcrosses (BCs), recombinant inbred lines (RILs), heterozygous F_1 populations and F_2 populations can be used as the mapping population (Paterson *et al.,* 1996). Practically 50–250 individuals are selected in a mapping population; however, for high-resolution and fine mapping, a larger size of the mapping population is required (Mohan et al*.,* 1997 and Dhingani *et al.,* 2015).

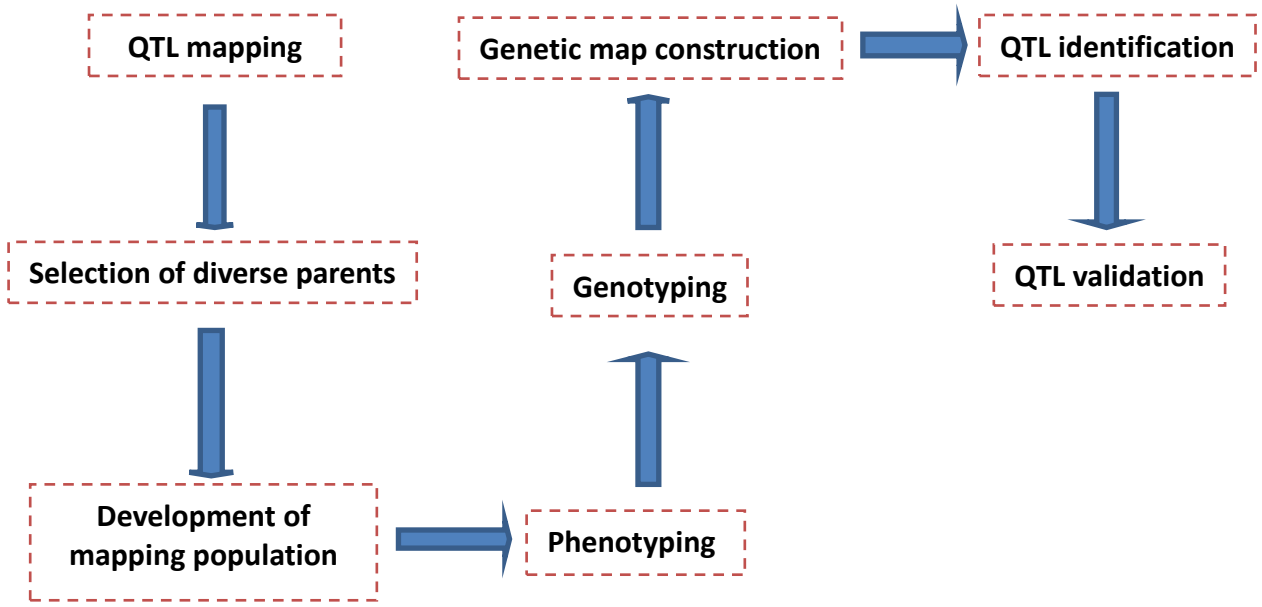


Fig. 2.1: QTL mapping methodology

2.13 Selection of Markers for QTL Mapping

Different types of markers such as RFLP, AFLP, ISSR, SSR, ESTs, DArT and SNPs have been commonly used for the construction of linkage maps in several crops (Semagn *et al.,* 2006). Normally, for genetic mapping studies, 100–200 markers have been used for the construction of linkage maps (Mohan *et al.,* 1997). The marker number, however, varies according to the studies and directly depends on the species genome size, as larger genome-sized species require a larger number of markers. However, with the advent of New generation Sequencing (NGS), several thousands of DNA markers are now utilised for high-resolution genetic mapping (Bernardo, 2015).

2.14 Genetic/Linkage Map Construction

The linkage map is a road map that describes the position and relative genetic distance between markers (Paterson *et al.,* 1996). QTL mapping is based on marker segregation via chromosome recombination during meiosis, in which those markers that are tightly linked

with each other will be transferred together, more commonly during recombination as compared to those that are away from each other. This recombination frequency is used to calculate the recombination fractions. Through the segregation analysis, the actual distance and relative order of markers can be calculated (Collard *et al.,* 2005). Odds ratios (the ratio of linkage versus no linkage) are used for the calculation of the linkage between markers. This value is called LoD, or logarithm of odds (Risch *et al.,* 1993). For the construction of linkage maps, LoD values of > 3 are considered ideal (Collard *et al.,* 2005). Different marker systems may be included in the analysis of populations. Computer packages such as Mapmaker (Lander and Botstein, 1986; Lander *et al.,* 1987), MapManager (Manly *et al.,* 2001), and JoinMap (Van Ooijen and Voorrips, 2001) have been developed to aid in the analysis of genetic data for map construction. These computer programs use data obtained from segregating populations to estimate recombination frequencies that are then used to determine the linear arrangement of genetic markers by minimising recombination events (Tuberosa *et al.,* 2002).

2.15 QTL Validation

After the QTL detection, it is necessary to validate the identified putative QTL. For this purpose, diverse populations will be developed by crossing different parents to check the presence of a putative QTL in other populations with different genetic background. NILs are commonly used for the confirmation and validation of QTL (Collard *et al.,* 2005). NILs have been used to precisely evaluate the effect of different pollen sterility loci in rice (Wu *et al.,* 2015). Confirmation of QTL provides the information about the marker to be used or not for MAS (Ogbonnaya *et al.,* 2001).

2.16 Drought Stress

Drought is a meteorological term and is commonly defined as a period without significant rainfall. It could also be a moderate loss of water, which leads to stomatal closure and limitation of gas exchange. Mostly, drought stress occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation. Drought (water stress) is one of the most important environmental stresses and occurs for several reasons, including low rainfall, salinity, high and low temperatures, and high intensity of light, among others. Drought stress is a multidimensional stress and causes changes in the physiological, morphological, biochemical, and molecular traits in plants. It is the major abiotic stress limiting crop productivity worldwide (Sani and Westgate, 2000). Water deficit and salt stresses are global challenges to ensure survival of agricultural crops and sustainable food production (Jaleel *et al.,* 2009; Nakayama *et al.,* 2007). In plants,

a better understanding of the morpho-anatomical and physiological basis of changes in water stress resistance could be used to select or create new varieties of crops to obtain a better productivity under water stress conditions (Nam *et al.,* 2001; Martínez *et al.,* 2007).

2.17 Mechanism of Drought Tolerance in Plants

Plants respond, adapt to, and survive under drought stress by the stimulation of various morphological, biochemical, and physiological responses. Plant drought tolerance involves changes at whole-plant, tissue, physiological, and molecular levels. Manifestation of a single or a combination of inherent changes determines the ability of the plant to sustain itself under limited moisture supply. To cope with the drought, tolerant plants initiate defence mechanisms against water deficit (Chaves and Oliveira, 2004). Some of the mechanisms of drought tolerance are reviewed below.

2.18 Morphological Mechanisms

2.18.1 Drought escape

Escape from drought is attained through a shortened life cycle or growing season, allowing plants to reproduce before the environment becomes dry. Drought escape occurs when the phenological development is successfully matched with periods of soil moisture availability, where the growing season is shorter and terminal drought stress predominates (Araus *et al.,* 2002). Matching growth duration of plants to soil moisture availability is critical to realise high seed yield (Siddique *et al.,* 2003). Studies showed that in field-grown clones of robusta coffee, leaf shedding in response to drought stress occurred sequentially from older to younger leaves, suggesting that the more drought-sensitive the clone, the greater the extent of leaf shedding (DaMatta, 2004). Developing short-duration varieties has been an effective strategy for minimising yield loss from terminal drought, as early maturity helps the crop to avoid the period of stress (Kumar and Abbo, 2001).

2.18.2 Drought avoidance

Drought avoidance consists of mechanisms that reduce water loss from plants, due to stomatal control of transpiration, and maintaining water uptake through an extensive and prolific root system (Turner *et al.,* 2001; Kavar *et al.,* 2007). The root characters such as biomass, length, density, and depth are the main drought avoidance traits that contribute to final yield under terminal drought environments (Subbarao *et al.,* 1995; Turner *et al.,* 2001). A deep and thick root system is helpful for extracting water from considerable depths (Kavar *et al.,* 2007). Glaucousness or waxy bloom on leaves helps with maintenance of high tissue water potential and is therefore considered as a desirable trait for drought tolerance (Richards *et al.,* 1986; Ludlow and Muchow, 1990). Dehydration avoidance is desirable in

modern agriculture, where drought resistance needs the maintenance of economically viable plant production under dehydration stress. The role of dehydration avoidance is maintaining water supply, and sustaining leaf hydration and turgidity with the purpose of maintaining stomatal opening and transpiration if possible under water deficit conditions. This is important for leaf gas exchange, photosynthesis, and plant production through carbon assimilation.

2.18.3 Phenotypic flexibility

Plant growth is greatly affected by water deficit. At a morphological level, the shoot and root are the most affected, and both are the key components of plant adaptation to drought. Plants generally limit the number and area of leaves in response to drought stress to cut down the water budget at the cost of yield loss (Schuppler *et al.,* 1998). Since roots are the only source to acquire water from soil, the root growth, its density, proliferation, and size are key responses of plants to drought stress (Kavar *et al.,* 2007). It has long been established that plants bearing small leaves are typical of xeric environments. Such plants withstand drought very well, albeit their growth rate and biomass are relatively low (Ball *et al.,* 1994). Leaf pubescence is a xeromorphic trait that helps protect the leaves from excessive heat load. Hairy leaves have reduced leaf temperatures and transpiration (Sandquist and Ehleringer, 2003), while inter- and intra-specific variations exist for the presence of this trait. Under high temperature and radiation stress, hairiness increases the light reflectance, and minimises water loss by increasing the boundary layer resistance to water vapour movement away from the leaf surface.

The possession of a deep and thick root system allows access to water deep in the soil, which was considered important in determining drought resistance in upland rice (Kavar *et al.,* 2007).

2.19 Physiological Mechanisms

2.19.1 Cell and tissue water conservation

Improved tissue water status may be achieved through osmotic adjustment and/or changes in cell wall elasticity. This is essential for maintaining physiological activity for extended periods of drought (Kramer and Boyer, 1995). Osmotic adjustment allows the cell to decrease the osmotic potential and, therefore, increases the gradient for water influx and maintenance of turgor. It had been reported that wild melon plants survived drought by maintaining their water content without wilting of leaves even under severe drought. It had

been identified that among various mechanisms, osmotic adjustment, abscisic acid, and induction of dehydrins may confer tolerance against drought injuries by maintaining high tissue water potential (Turner *et al.,* 2001). With the accumulation of solutes, the osmotic potential of the cell is lowered, which attracts water into the cell and helps with turgor maintenance. The maintenance of turgor is consistent with other studies of species with elastic cell walls, despite a decrease in leaf water volume. Osmotic adjustment helps to maintain the cell water balance with the active accumulation of solutes in the cytoplasm, thereby minimising the harmful effects of drought (Morgan, 1990). Osmotic adjustment is an important trait in delaying dehydrative damage in water-limited environments by continued maintenance of cell turgor and physiological processes (Taiz and Zeiger, 2006). The osmotic adjustment also facilitates a better translocation of pre-anthesis carbohydrate partitioning during grain filling (Subbarao *et al.,* 2000), while high turgor maintenance leads to higher photosynthetic rate and growth (Ludlow and Muchow, 1990; Subbarao *et al.,* 2000).

2.19.2 Plant growth regulators

Plant growth regulators, when applied externally, and phytohormones, when produced internally, are substances that influence physiological processes of plants at very low concentrations (Morgan, 1990). Both these terms have been used interchangeably, particularly when referring to auxins, gibberellins, cytokinins, ethylene, and abscisic acid (Taiz and Zeiger, 2006). Under drought conditions, endogenous contents of auxins, gibberellins, and cytokinin usually decrease, while those of abscisic acid and ethylene increase (Nilsen and Orcutte, 1996).

However, phytohormones play vital roles in the drought tolerance of plants. Auxins induce new root formation by breaking root apical dominance induced by cytokinins. As a prolific root system is vital for drought tolerance, auxins have an indirect but key role in this regard. Drought stress limits the production of endogenous auxins, usually when contents of abscisic acid and ethylene increase (Nilsen and Orcutte, 1996). Abscisic acid is abundant in all flowering plants and is generally recognised as a stress hormone that regulates gene expression and acts as a signal for the initiation of processes involved in the adaptation to drought and other environmental stresses. It had been proposed that abscisic acid and cytokinin have opposite roles in drought stress. Increase in abscisic acid and decline in cytokinins levels favour stomatal closure, and limit water loss through transpiration under water stress (Morgan, 1990). Increased abscisic acid concentration leads to many changes in plant development, physiology, and growth. Abscisic acid alters the relative growth rates of various plant parts such as the increase in the root-to-shoot dry weight ratio, inhibition of leaf area development, and production of prolific and deeper roots (Sharp *et al.,* 1994). It

triggers the complex series of events leading to stomatal closure, which is an important water conservation response (Turner *et al.,* 2001). With its effect in closing stomata, abscisic acid can control the rate of transpiration and may be involved in the mechanism conferring drought tolerance in plants.

2.19.3 Compatible solutes and osmotic adjustment

One of the most common stress tolerance strategies in plants is the over-production of different types of compatible organic solutes (Serraj and Sinclair, 2002). Compatible solutes are of low molecular weight; they are highly soluble compounds that are usually nontoxic even at high cytosolic concentrations. In general, they protect plants from stress by contributing towards osmotic adjustment, detoxification of reactive oxygen species, stabilisation of membranes, and native structures of enzymes and proteins.

Osmotic adjustment is a mechanism of maintaining water relations under osmotic stress. It involves the accumulation of series of osmotically active molecules/ions, including soluble sugars, sugar alcohols, proline, glycinebetaine, organic acids, calcium, potassium, chloride ions, and so forth. In a water deficient condition and as a result of solute accumulation, the osmotic potential of the cell is lowered, which attracts water into the cell, and helps with the maintenance of turgor. Osmotic adjustment helps plants perform better in terms of their growth, photosynthesis, and assimilated partitioning to grain filling as organelles and cytoplasmic activities take place at about a normal pace (Ludlow and Muchow, 1990; Subbarao *et al.,* 2000). As a mechanism, osmotic adjustment had been suggested to be an important trait in postponing the dehydration stress in water-scarce environments (Morgan, 1990).

2.20 Effect of Drought on Cassava

In Africa, the cassava growth cycle is typically interrupted by 3-6 months of drought, influencing various plant physiological processes that give rise to depressed growth, development, and economic yield (Pardales *et al.,* 2001; Bakayoke *et al.,* 2009). Connor *et al.* (1981) reported that when rainfall was withheld from cassava for 10 weeks, commencing 12 weeks after planting, tuber yield was reduced by 32% compared to the control. Porto *et al.* (1989) evaluated cassava grown in a lysimeter with water stress conditions imposed over a 100-day period with no water, starting from 3-6 months after planting. They reported that the accumulation of dry-matter as well as root dry weight reduced more by water stress beginning at 3 rather than at 6 months after planting. Thus, the effect of stress was severe

during the period of rapid leaf growth and tuberisation rather than the later period of tuber bulking.

2.21 Breeding and Selection for Early Bulking in Cassava

Bulking in cassava refers to the swelling or thickening of the storage roots as they are filled with excess assimilates after the plant has satisfied its needs for vegetative growth. Wholey and Cock (1974) stated that bulking commences in cassava at 2 months after planting, whereas Okogbenin and Fregene (2002) reported starch initiation as early as 6 weeks after planting (WAP), and rapid differentiation in root size at 9 weeks after planting.

Studies have revealed that late bulking is the single most important factor responsible for rejection and abandonment of cassava cultivars in African countries (Okechukwu and Dixon, 2009; Kamau *et al.,* 2011). Early-bulking varieties have the capacity to deliver reasonable yield levels after between 7 and 8 months. In order to better harness the potential of cassava, especially in the current changing climatic conditions, it is necessary to develop, evaluate, and select early-bulking cultivars that can be harvested at 7-10 months after planting.

There is a growing trend among farmers towards developing early-bulking cassava in response to increased early-bulking cassava cultivars (Kamau, 2006; Tumuhimbise *et al.,* 2012; Bassey and Gamaliel, 2013). The study conducted by Kawano (2003) acclaimed the use of fresh storage root yield (FSRY) as the criterion for evaluating early bulking. Hershey (2012) reported on a research done on early bulking in CIAT that genotypes with the highest FSRY at an early harvest time tended to be the highest yielding genotypes at later stages, and that therefore, high yield is co-selected for early bulking.

In a study conducted by Okogbenin and Fregene (2002) to investigate the traits associated with early bulking, they found that starch initiation time, storage root diameter, plant height, harvest index, dry foliage mass, number of storage roots, and plant vigour were all significantly correlated with dry storage root yield. Thus, they suggested that those factors were components triggering early yield as a complex trait. Regression analysis of their study showed that storage root diameter, dry foliage mass, and harvest index were the most important factors for early bulking in cassava. Storage root diameter was the most important factor at the initial phase of root bulking, whereas harvest index and foliage appeared as the most important factors at the late phase of storage root development during the evaluation period. They then concluded that one should select for high harvest index (HI), dry foliage mass or both when breeding for early bulking.

CHAPTER 3

FIELD EVALUATION OF CASSAVA GERMPLASM FOR ADAPTABILITY IN DROUGHT – PRONE ENVIRONMENTS AND DEVELOPMENT OF A MAPPING POPULATION

ABSTRACT

Cassava accounts for approximately one-third of the staple food production and provides over 50% of the energy for more than 300 million people in sub-Saharan Africa. It is particularly important in areas where food supply is constantly threatened by environmental constraints such as moisture/water stress. Given the expanding importance of cassava as food, feed, and industrial crop, genotypes that are drought tolerant are considered strategic. They are highly desirable for production in marginal areas in the dry savannahs, where favourable lands exist, but which are highly prone to drought stress with average cassava yields below 5 tonnes/ha. Although cassava can survive in a drought-prone environment, its production is limited by moisture stress. However, a suitable solution to improve cassava production in marginal environments is to develop and deploy drought-tolerant varieties. Field trial in three replications was conducted at Minjibir to evaluate Latin American (CIAT) and some African cultivars for drought tolerance. The trials consisted of the national IITA lines that comprised eight African elite cassava varieties, while the 36 exotic lines were the CIAT germplasm. The objective of this study was to screen these genotypes for yield, productivity and identify the best line for adaptation in marginal environments. Plant characteristics measured included fresh root yield, dry root yield, harvest index, and number of leaves. Results from the study identified two genotypes, TMS98/0505 with high dry root yield of 15 t/ha, and TMS98/0581 with moderate dry root yield of 7 t/ha. Analysis of variance for the exotic lines showed significant differences among the genotypes for fresh root yield, dry root yield, and harvest index. Genotypes such as CR14B-218 and CR14B-67 showed a high recovery rate of leaves on resumption of rain. Fresh root yield ranged from 9.69 - 48.0 t / ha, of which genotypes with fresh root yield above 10 t / ha (twice the existing average yield) were regarded as having good adaptation to drought in a marginal environment. The selection index indicated 20 genotypes from the population, which will further be tested for yield stability and adaptation in different drought environments.

Key words: Drought tolerance; dry savannahs; *Manihot esculenta*; marginal environment; root yield

3.1 Introduction

Cassava is cultivated as the most important staple food crop for over 900 million people in the world (Beyene, 2012). It is the most important staple crop in Africa after maize, and the 6th most often consumed crop in the world (Benesi *et al.,* 2004). The storage roots can be boiled and eaten as in the case of sweet cassava cultivars, which are low in cyanogenic glycosides, processed into dry products such as garri, flour, starch, and animal feed in the case of bitter cultivars that are high in cyanogenic glycosides (Balagopalan, 2002; Westby, 2002). The leaves can also be consumed as vegetables, and they serve as an important source of proteins, minerals, and vitamins in the human and animal diet in many African and Asian countries (Okogbenin, 2013). A study conducted by the International Food Policy Research Institute (IFPRI) predicted an overall 2.44% annual growth rate in the use of cassava as food in sub-Savannah Africa (SSA), and a growth of 1.53% per annum in cassava for animal feed (Scott *et al.,* 2000).

The rapidly increasing global population growth rate has put a lot of pressure on the available arable land, extending agricultural production to unfavourable semi-arid zones (Challinor *et al.,* 2007; Baulcombe *et al.,* 2009). In order to keep pace with the increase in population numbers, it is necessary to develop drought-tolerant varieties in the marginal environments, because many of the world's poorest and most food insecure households living in these areas depend on cassava as their principal source of food, nutrition, and family income (Bergantin *et al.,* 2004).

In Africa, the cultivation of cassava is common mostly in humid environments where there is adequate rainfall, while in the marginal environments, its production is mainly in the hands of poorly-resourced farmers who cannot afford the cost of irrigation. Therefore, there is dire need to extend the breeding activities to the marginal ecology to enhance productivity.

As advancement to this approach, the National Root Crops Research Institute (NRCRI) undertook steps to test some lines from Central Agricultural Research Institute Columbia (CIAT) and the International Institute for Tropical Agriculture (IITA) to address this initiative. This study was intended to screen cassava lines from Nigeria and Latin America for yield and productivity, identify the best lines for adaptation that can be utilised to develop new germplasm with better agronomic traits and generate mapping population.

3.2 Materials and Methods

3.2.1 Description of field experimental site

The genotypes were evaluated at Minjibir, Kano (dry location). Kano is situated in the Sudan savannah ecology with three months of rainfall period in a year. The long rains are received between July and August, and short rains in September and October. Kano is located at latitude 12°3'N and Longitude 8°32'E. The altitude is 473 m above sea level. The local temperature ranges between 18.7°C to 66.5°C, while the average annual temperature is 41.9°C. Relative humidity ranges between 13% and 68%, while the annual relative humidity is 31.1%. The rainfall range is from 0 to 320 mm, while the average annual rainfall is 270 mm, which is low for plant growth, and therefore poses a high risk (25-75%) of crop failure (Sombroek et al., 1982; Jaetzold et al., 2006). The soils are a sandy ferruginous type of the latosols group, which is highly weathered, markedly laterised, and slightly acidic in reaction to low organic matter content, and phosphorous, where its total nitrogen rarely exceeds 0.2%.

3.2.2 National IITA lines

These are elite Nigerian lines developed by IITA Ibadan. They were selected for evaluation in this study because they are cultivars commonly grown by farmers.

3.2.3 Exotic Lines

These are Latin American germplasm with the CMD2 resistance gene. They were introduced into NRCRI as *in vitro* plantlets. The plantlets were inspected and those found contaminated, broken, or malformed were eliminated, while clean plantlets were kept in a culture room. They were sub-cultured, hardened, and planted for field evaluation. Based on their performance in NRCRI, they were planted in Kano for further evaluation against drought tolerance. They were evaluated together with some national IITA and NRCRI germplasm.

Table 3.1: Cassava lines used in the trial

S/NO	CIAT (Exotic lines)	CIAT (Exotic lines)	IITA/NRCRI Lines (National)
1	AR38-8 (CIAT)	24 CW450-46 (CIAT)	1 TMS 419 (IITA)
2	CR100-15 (CIAT)	25 AR9-45 (CIAT)	2 TMS92/0326 (IITA)
3	CR100-8 (CIAT)	26 CW451-13 (CIAT)	3 TMS95/0289 (IITA)
4	CR14B-218 (CIAT)	27 CW450-46 (CIAT)	4 TMS98/0505 (IITA)
5	CR15B-3 (CIAT)	28 CW450-36 (CIAT)	5 TMS98/0510 (IITA)
6	CR15B-6 (CIAT)	29 AR9-17 (CIAT)	6 TMS98/0581(IITA)
7	CR15B-9 (CIAT)	30 CR15B-7 (CIAT)	7 NR/0004 (NRCRI)
8	CR20A-2 (CIAT)	31 CW82-16 (CIAT)	8 TMS97/0057 (NRCRI)
9	CR24B-9 (CIAT)	32 CW482-3 (CIAT)	
10	CR39-13 (CIAT)	33 CW495-9 (CIAT)	
11	CR42-4 (CIAT)	34 CW444-30 (CIAT)	
12	CR52A-1 (CIAT)	35 CW452-1 (CIAT)	
13	CR60B-10 (CIAT)	36 CW495-1 (CIAT)	
14	CW450-12 (CIAT)		
15	CW450-46 (CIAT)		
16	CW451-80 (CIAT)		
17	CW452-9 (CIAT)		
18	CW482-16 (CIAT)		
19	CW485-15 (CIAT)		
20	CW450-46 (CIAT)		
21	CW482-3 (CIAT)		
22	CW525-1 (CIAT)		
23	CW536-11 (CIAT)		

3.2.4 Experimental design

The experimental design used was an alpha triple lattice. The trial consisted of 8 elite national cassava genotypes including a commercial variety 419 and 36 genotypes of Latin American germplasm (CIAT lines). The plot size was 20 m^2 with 20 plants per plot.

3.2.5 Data collection

To assess the response of the genotypes to drought, the following traits were measured; plant architecture, plant vigour, number of scars, plant height, length of stems with scars, number of leaves, branching levels, harvest index, dry root yield, and fresh root yield. Data

were taken randomly from the net plants of each genotype. Harvest index, dry root yield, and fresh root yield were measured at harvest.

3.2.5.1 Plant height

Plant height was determined on the primary stem and was measured from the ground to the apex using a metre rule calibrated in meters.

3.2.5.2 Length of stems with scars

Length of stem with scars was determined by taking the length of the stem where the scar was present from the ground to the top measured in metres.

3.2.5.3 Branching levels

This was determined by recording the number of divisions or joints of branching.

3.2.5.4 Plant vigour

Plant vigour was assessed by visual rating of the plants on a scale of 1 - 5, where score 1 is very weak (plants stunted with very thin stems), while 5 is extra vigorous (very fast growing, strong, and no bending).

3.2.5.5 Plant architecture

Plant architecture was determined by visual rating of the plants on a scale of 1 - 5, where score 1 is very good (erect, no branching), while 5 is very poor (highly profusely branched).

3.2.5.6 Number of leaves

The number of leaves was assessed by counting leaves on the stems of net plants and the values recorded.

3.2.5.7 Harvest index

This was determined by finding the ratio of the storage root weight to total biomass:

> HI = Wr/Wr+Wab. Where Wr = weight of roots, Wab = weight of above ground biomass.

3.2.5.8 Fresh root yield

Fresh root yield was derived from the equation: FRY = (root weight (kg)*10)/plot size.

3.2.5.9 Dry storage root yield

This was derived by multiplying the fresh root yield with dry-matter content.

3.2.5.10 Cassava mosaic disease and cassava bacterial blight

Cassava mosaic disease (CMD) and cassava bacterial blight (CBB) were assessed during the crop growth at 3, 6 and 9 months after planting on a scale of 1-5, where score 1 is highly resistant, while score 5 is highly susceptible.

3.2.6 Data analysis

The data collected were subjected to statistical analysis using Genstat software Ver. 14.1 (Genstat, 2015). Analysis of variance was used to determine variation among traits.

The key traits that represent useful parameters for assessing breeding values were considered as variables for the selection index equation. The selection index method was calculated based on the most important variables. The variables were weighted based on the importance criteria in breeding objectives. The selection index was constructed using the standardised deviation units (Steel and Torrie, 1960) to avoid the problems related to the magnitudes used to measure different variables. The selection index is calculated using the formula:

SI = (X_1*W_1) + (X_2*W_2) + (X_3*W_3) + (X_n*W_n), where W_1. W_2, W_3….W_n are the respective weights for each variable. X_1, X_2, X_3…..X_n = different variables, while each of the variables were standardised using the classical statistical formula (Steel and Tome, 1960: X‘ = (Xi-μ) / St.Dev. where X‘ is the standard value; Xi is the original value; μ is the mean of the population; St.Dev. is standard deviation for the variables analysed. The calculation was done as follows:

SI = SI = (FRY*10) + (DRY *8) + (DMC*8) + (HI *8) - (Arch*8) + (Vig. *7) - (CMD*7) - (CBB * 7)

3.3 Results

3.3.1 National IITA lines

These sets of materials were screened, and selection was made basically on yield (Fig. 3.1). Genotypes with dry root yield of 5 t / ha and above were selected as drought-tolerant genotypes, while the ones with dry root yield of less than 5 t / ha were not selected (Fig. 3.1). The results showed that all the genotypes were tolerant to drought except for TME419 and TMS98/0510. Based on the result obtained, the genotypes TMS98/0505 and TMS98/0581 were used as the drought-tolerant parents, while TMS98/0510 was used as susceptible parent for the cross. Seeds developed from crosses were 866 seeds (TMS98/0505 x

TMS98/0581) and 1133 seeds (TMS9/0505 x TMS98/0510) (Table 3.2). The mapping populations were planted in the screen house, and later transplanted into the field. The survival rate of cross combination between TMS98/0505 x TMS98/0581 was 36% in the field, while that of the other cross combination was 18% (Table 3.2). As a result of high population size in the field, cross combination TMS98/0505 x TMS98/0581 was selected to be the genetic mapping population.

The field evaluation for CMD resistance showed that most of the genotypes showed no symptom of cassava mosaic disease, while very few were susceptible to the disease (Fig. 3.2).

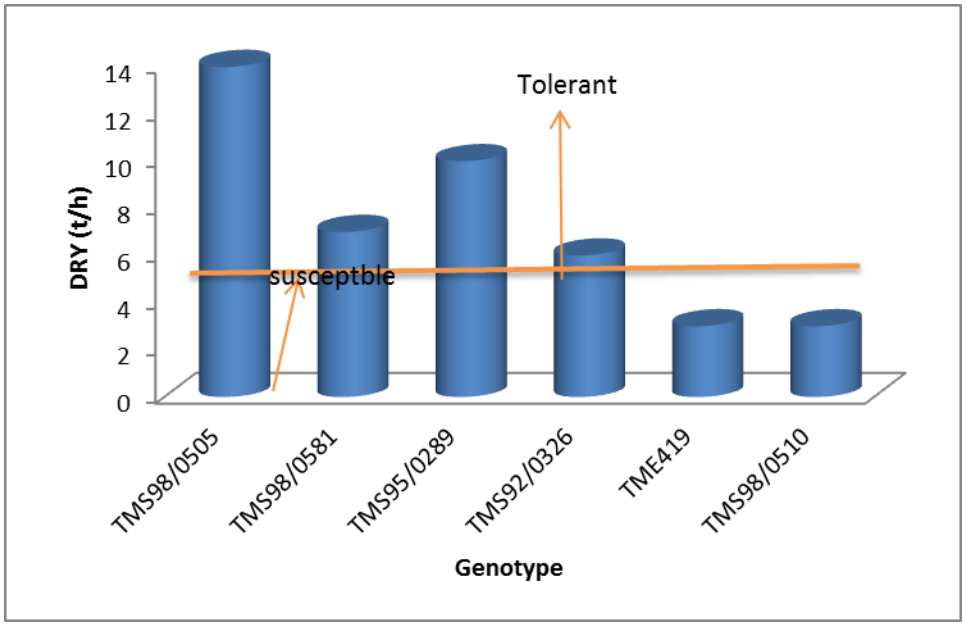


Fig. 3.1: Dry root yield of IITA varieties

DRY = dry root yield.

Table 3.2: The number of seeds planted in screen house and population size in the field

Cross	Seeds planted	Population size in field	% survival in field
TMS 98/0505 x TMS 98/0510	1133	204	18
TMS98/0505 x TMS 98/0581	866	310	36

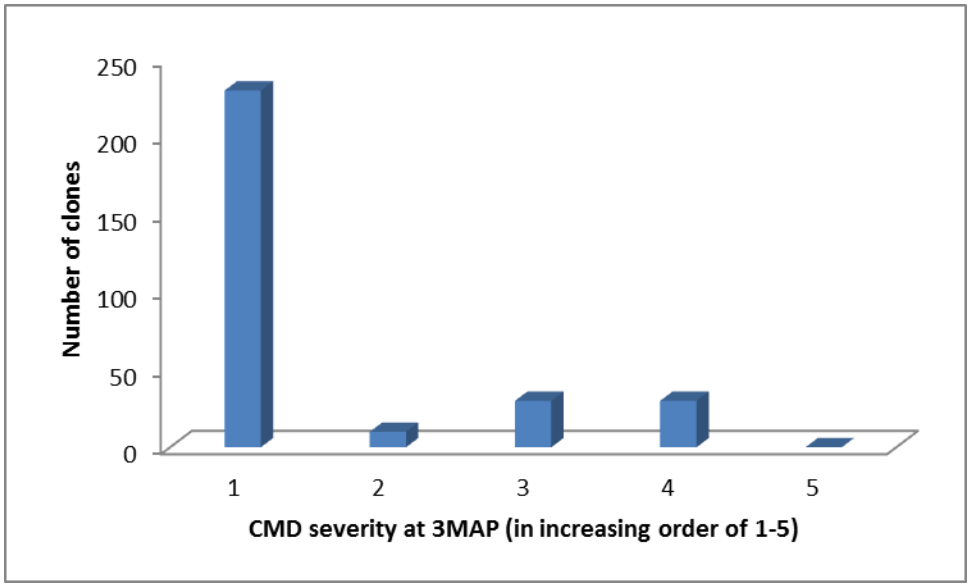


Fig. 3.2: Distribution of cassava genotypes to cassava mosaic disease resistance in mapping population B (TMS98/0505 x TMS98/0581)

3.3.2 Exotic lines

3.3.2.1 Morphological and physiological traits

Plant physiology related to leaf drop during stress, and leaf growth when the rain resumed, are shown in Fig. 3.3. The leaf growth was measured as the total number of leaves per genotype between 8 MAP and 11 MAP. Results indicate that there was a very slow growth during the dry season. The growth rate of leaves at the peak of dry season did not change significantly from 8 MAP to 9 MAP (Fig. 3.3). At 9 MAP, rains resumed, and the plants began to recover from stress for the next three months when the plants were ready for harvest. Resumption of rains resulted in a rapid response of leaf growth generally (Fig. 3.3). Genotypes such as CR14B-218 and CR14B-67 were found to show a high recovery rate. Results of other morphological traits are shown in Table 3.3. There was a wide variation in the response of the clones to the traits evaluated (Table 3.3). Plant height and number of leaves increased at harvest, while length of stem with scars was found to reduce during harvest (Table 3.3).

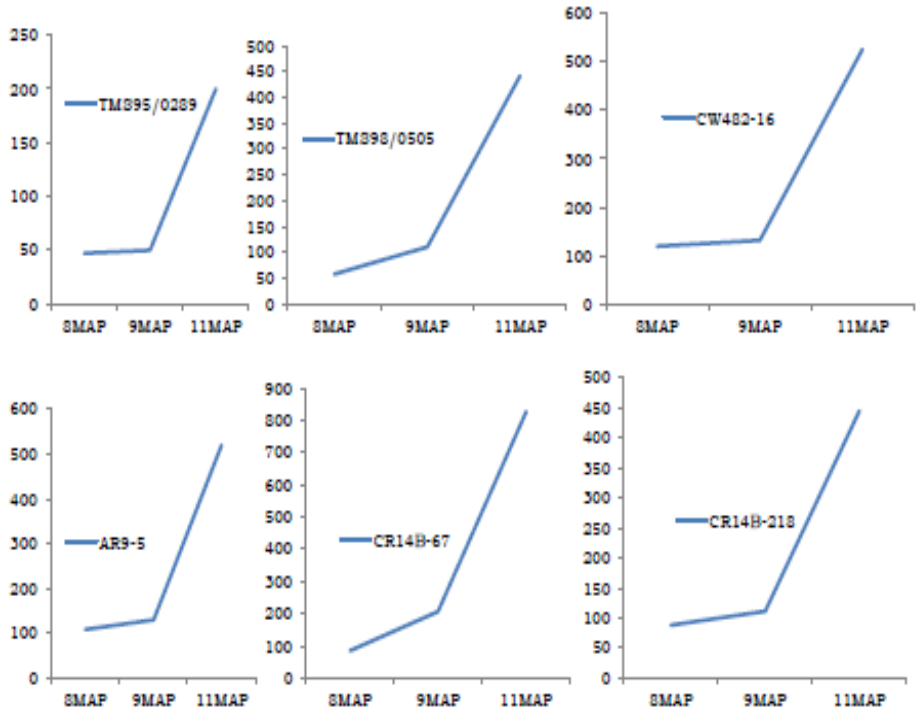


Fig. 3.3: Growth curve of number of leaves of some genotypes during moisture stress (8-9 MAP) and recovery rate on resumption of rains

Table 3.3: Summary of morphology data measured for 59 varieties evaluated at Minjibir, Kano

Traits	Minimum	Maximum	Std Deviation
Architecture	1	5	0.81
Vigour	2	5	0.82
Number of scars during drought	2	102.4	13.80
Plant height during drought (m)	0.27	2.12	0.29
Plant height at harvest (m)	0.64	2.6	0.29
Length of stems with scars(m)	0.33	1.519	0.20
Length of stems with scars at harvest (m)	0.24	1.7	0.27
Number of leaves during drought	10	380.75	71.81
Number of leaves at harvest	26	993	166.22
Branching levels (m)	1	3.6	0.56
Branching height (m)	0.05	10.37	0.99

3.3.2.2 Disease evaluation

Disease evaluation showed that most of the genotypes (30 and 20) were resistant to cassava mosaic disease and cassava bacterial blight, respectively, while few of the genotypes were susceptible (Table 3.4). There was a wide range of variation of the genotypes in response to the diseases evaluated.

Table 3.4: Summary of disease rating of elite local lines screened at Minjibir, Kano

Trait	Minimum	Maximum	Average	Standard deviation	Number. selected
CMD	1	3	1.05	0.25	30
CBB	1	4	2.36	0.93	20

3.3.2.3 Yield harvest

Analysis of variance revealed significant ($P < 0.05$) difference among genotypes in the fresh root yield, dry root yield, and harvest index (Table 3.5). This variation among genotypes will help in the selection of genotypes for drought tolerance in marginal environments. The summary of yield and yield-related traits also showed a wide range of variation among the genotypes (Table 3.6). The local check, Dankata, resulted in low yield when compared to some of the genotypes, but with high dry-matter content. Over 30 Good promising and potential genotypes such as AR9-45, CR15B-7, AR9-5, CW451-80, CW595-9, CR14-4, CW420-75, CR14B-218, TMS419, CW482-3 were found with fresh root yield above 10 t/ha (Fig. 3.4).

Table 3.5: Analysis of variance for yield and yield related traits of cassava genotypes in Minjibir, Kano

Source	DF	Sum of squares	Mean square	Fval	Pr > F
Fresh root yield (t/ha)	58	4013.53	69.19	1.87	0.005
Dry root yield (t/ha)	58	299.01	5.19	1.51	0.04
Harvest index	58	0.96	0.01	2.45	0.0001

Table 3.6: Summary of yield and yield related traits at harvest and check

Trait	Minimum	Maximum	SD	Dakata (Local check)
FRY	9.69	48.80	7.20	9.88
DRY	3.22	26.07	3.84	3.22
HI	0.24	0.59	0.08	0.38
DMC	19.68	37.34	3.81	30.62

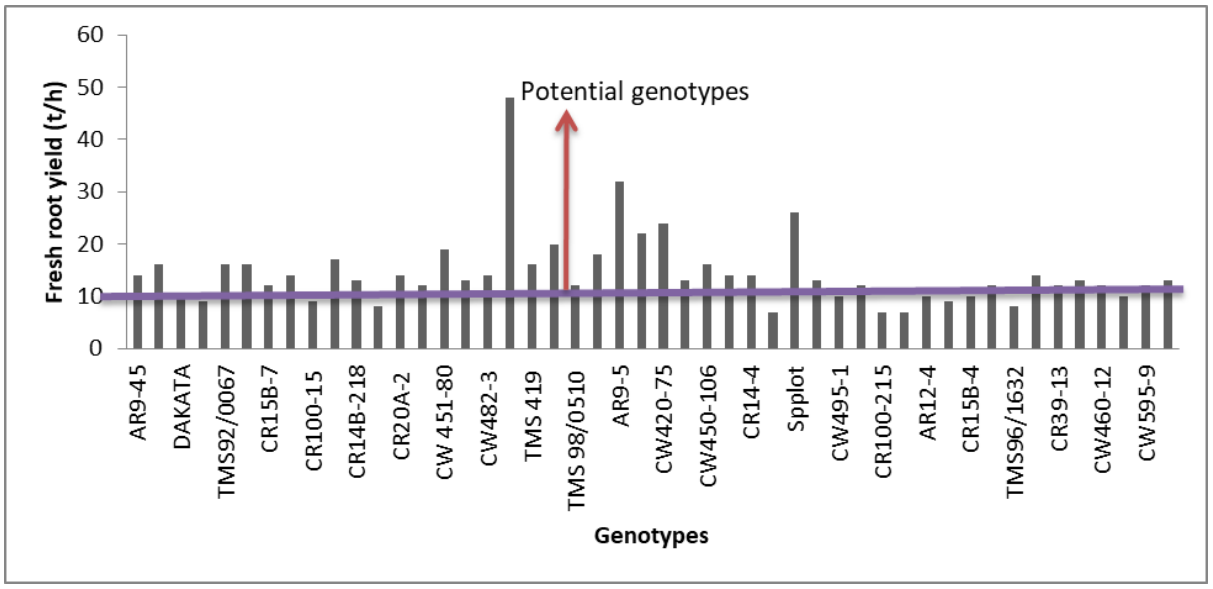


Fig. 3.4: Fresh root yield of exotic cassava lines

3.3.2.4 Selection of good performance of best genotypes

The selection index was used to estimate the genetic potential and good performance of genotypes. Important traits that represent useful determinants for assessing breeding values were considered as variables for the selection index equation. Results of the 20 best genotypes are shown in Table 3.7.

Table 3.7: Best 20 clones by selection index

Ranking	Variety	Selection index
1	NR87/184	121.3
2	CW450-106	113.27
3	CR52A-1	105.92
4	CR100-15	104.91
5	DAKATA	103.47
6	CW444-30	102.46
7	CW525-1	96.84
8	CW495-1	96.56
9	CW525-11	90.86
12	CR100-8	86.96
11	CW460-12	78.57
12	CR100-215	71.44
13	CW536-11	69.41
14	CW495-9	66.90
15	AR9-5	36.92
16	TMS92/02324	30.06
17	TMS92/0067	15.95
18	CW450-75	10.37
19	TMS98/0581	8.88
20	AR14-4	8.51

3.4 Discussion

Cassava is an essential crop for increasing food security in sub-Saharan Africa (SSA) and other food-insecure regions. Despite being a drought-tolerant crop, low water availability is still among the most significant limitations to cassava production in low rain-fed areas.

The result of the national evaluation revealed that two genotypes (TMS98/0505 and TMS98/0581) were selected basically as a result of their yield. Selection of parental materials for tolerance to water stress and poor soils has resulted in the breeding of improved germplasm adapted to stress environments (Okogbenin *et al.,* 2013). Cassava genotypes that exhibited high yield in drought-prone environments showed that they were better adapted to the environment, and so can be tagged as being drought tolerant. The genotypes TMS98/0505 and TMS98/0581 had contrasting desirable traits such as good yield under marginal environment, good disease resistance, vigour and flowering potentials.

These genotypes were selected and used in the development of the genetic mapping population that will be further evaluated in drought-prone environments for QTL mapping.

The high resistance in most of the genotypes to CMD showed that the use of resistant parents effectively maximised the probability of obtaining high F_1 progeny size with resistance to CMD disease. Cassava is highly heterozygous and is expected to segregate. The use of resistant parents to CMD is expected to lead to progenies that minimally segregate for CMD disease resistance to improve mapping for drought tolerance without any confounding effect of the disease. Phenotyping is very critical to the success in QTL mapping, and it is extremely important that performance of the F_1 mapping population is not confounded by CMD disease.

One of the modern breeding strategies for drought tolerance is the characterisation of germplasm in response to extended water shortages (Okogbenin *et al.,* 2013). In the exotic evaluation, genotypic differences in leaf drop were used to identify genotypes that were severely affected by drought. Some genotypes showed heavy leaf loss, which is a mechanism by the plant to reduce physiological stress due to low moisture. Such genotypes are thus highly sensitive to stress. Some genotypes, for example, CR15B-62, completely defoliated their leaves, showing it is not well adapted to stress. However, a good number of genotypes (over 80%) were able to maintain good foliage with different degrees during the peak of drought, indicating tolerance to drought stress. Cassava reduces water loss through closing its stomata (Setter and Fregene, 2007) and decreasing its leaf area through leaf shedding (Alves and Setter, 2000; Burns *et al.,* 2010). Hence, leaf shedding is an effective adaptation mechanism as an effect of moisture stress (Okogbenin *et al.,* 2013). The high recovery rate of shaded leaves tells that cassava is quick to resume growth when conditions are favourable. Such rapid recovery in leaf growth has been reported by many researchers (Connor *et al.,* 1981; Baker, 1986; El-Sharkawy, 2002), which increases light interception and canopy photosynthesis, thus compensating for previous losses in biomass, particularly root yield. The high recovery rate of cassava from stress at the onset of rainfall also explains the increase in other morphological traits such as plant height and number of leaves at harvest. The reduction in the length of stem with scars is the result of the rapid recovery in leaf growth at onset of rain at 9 MAP.

There was high resistance to cassava mosaic disease and cassava bacterial blight among the exotic lines. This can be explained by the fact that they were initially screened and selected for cassava mosaic disease in humid environments before taking them to marginal environments for proper field evaluation for drought tolerance.

The significant difference among the genotypes for yield traits and range of values of traits suggests that the evaluated cassava genotypes had adequate genetic variability. Turyagyenda *et al.* (2015) reported similar results when they subjected different cassava genotypes to water stress.

Potential genotypes exhibited fresh root yield of above 10 t/ha. The average annual cassava yield worldwide is 10 t/ha, ranging from 6 t/ha in Mozambique to 26 t/ha in India (Okogbenin *et al.,* 2013). The overall performance of the genotypes was determined by several traits using the selection index method. This method was calculated based on the most appropriate variables and was constructed using standardised deviation units (Steel and Torrie, 1960) to avoid problems related to the magnitudes used to measure different variables. The local check (Dankata) was one of the leading clones based on the selection index. This highlighted that it has some good attributes such as good dry matter content, good vigour, and low cyanide content, which may have aided its ease of adaptation, and its acceptability by farmers. It has good resistance for CMD in dry ecology where disease pressure is low but does not survive in a high-disease pressure zone in southern Nigeria. Early evaluations of the Latin American lines (CR, AR, CW) were resistant to cassava mosaic disease, as they were developed with markers and carried the CMD2 gene in them. They will continue to be evaluated to test their stability to cassava mosaic disease in different locations.

3.5 Conclusion

From the national evaluation, two genotypes were selected, TMS98/0505 and TMS98/0581. They were used to develop the mapping population for molecular studies. The mapping population will be evaluated for drought tolerance in a marginal environment; the potential genotypes selected will be used further for MARs studies (marker-assisted recurrent selection). Based on the result obtained using exotic lines, genotype by environment (G X E) studies will be done to test the yield stability of the genotypes.

CHAPTER 4

IDENTIFICATION OF TRAITS DRIVING THE PHYSIOLOGICAL BASIS OF DROUGHT TOLERANCEIN F_1 CASSAVA GENOTYPES

ABSTRACT

Cassava has great importance for food security. It is worldwide considered as the staple food for over one billion people. It is highly adaptable to marginal soils with low fertility and irregular rainfall conditions. However, the challenges posed by global climate change have caused a negative impact on the crop's productivity. Breeding strategies to develop drought-resistant genotypes and gaining an understanding of the basis for drought resistance to cope with limited rainfall and semi-arid conditions are critical. The experiment was carried out in Minjibir, Kano. F_1 cassava genotypes developed from two elite cassava varieties, TMS980505 and TMS980581, were evaluated for their morphological / physiological trait responses, and adaptability to drought. Results showed significant ($P \leq 0.05$) differences among the genotypes for most of the parameters evaluated. The phenotypic coefficient of variation estimate was higher than the genotypic variation. There was a positive correlation between yield and other yield-related traits such as fresh root yield and root number, fresh root yield and dry root yield, fresh root yield and harvest index; yield and morphological/physiological traits such as plant height, scar number, leaf number; among physiological/morphological traits such as the number of leaves and scar level, and length of leafless stem and branching level. Principle component analysis identified 99% and 97% variation for productivity traits during the first and the second year; and 74% and 84% variation for physiological traits for the two years, respectively. The analysis identified scar level, height of stem with leaf, fresh root yield, dry root yield, root number, and dry-matter content as traits driving drought tolerance in a marginal environment, and these were found common in PC1 and PC2 in both years for the physiological and productivity traits. Results from this study can aid in the identification and selection of drought-tolerant cassava varieties under stress conditions, which can be exploited to develop high-yielding cassava varieties for drought-prone areas to ensure food security.

Key words: Cassava; climate change; drought tolerance; food security; physiological traits

4.1 Introduction

Crop growth and development are constantly influenced by environmental conditions such as stresses, which are the most important yield-reducing factors in the world (Dennis, 2000). Drought stress is considered as one of the crop performance's limiting factors and a threat to successful crop production. Drought tolerance is an important trait related to yield. To

improve this trait, breeding requires fundamental changes in the set of relevant attributes, where the final output will be drought-tolerant genotypes (Maleki *et al.,* 2010). Cassava is a crop that is still dynamically evolving, and farmers in varied and harsh environments need to have access to cassava germplasm that is unique to their production niches. No strong effort has been made to deploy drought-tolerant traits more widely in cassava gene pools, due to the complexity of breeding for these traits and a lack of efficient breeding tools to support such initiative, especially in agro-ecologies with a long dry season or the major African countries that are prone to drought. In an IFAD-funded study (Duque, 2012), results revealed that root yield of drought-tolerant varieties was more stable under drought conditions compared to that of other elite varieties. The drought-tolerant varieties yielded 70-80% of potential fresh root yield when watered for the first three months, followed by no water until harvest (12 months after planting), compared to 10-40% yield in other elite varieties. This result underscores the importance of breeding for drought tolerance as perhaps the most effective strategy for improving adaptation and boosting cassava productivity in marginal drought-prone environments. Therefore, it is important to widely deploy traits that confer tolerance to drought in African cassava gene pools, particularly those of semi-arid agro-ecologies (< 500mm per year) or Guinea savannah agro-ecologies, where rainfall, although adequate (> 1200mm per year), is concentrated in 6-7 months, followed by a long dry season of 5-6 months every year. Over 70% of cassava produced in Africa falls within the afore-mentioned agro-ecologies (FAO, 2006). The most pronounced effects of the long dry season are that optimal planting date is limited to a narrow window of two months, after the rains have established, and a lengthened time (from 10-12 months to 16-18 months) without rain until harvest. The immediate economic effects are a period of limited food, the 'hunger months' or the last two months of the dry season, and the absence of processing industries (critical for enhanced income for rural communities) due to the lack of a year-round supply of roots caused by a narrow planting window. Therefore, it is necessary to breed for drought-tolerant cassava varieties to sustain crop production and productivity under limited water resources, as many of the world's poorest and most food-insecure households live in these areas and are highly dependent on this crop as their principal source of food, nutrition, and family income (Bergantin *et al.,* 2004). In dry ecology, drought imposes slow crop development that extends the harvest of cassava beyond 12 months after planting (MAP). This is a long time to harvest, and much more precarious as famine, which happens under drought conditions, sets in. This threat can only be reduced if drought-tolerant cassava varieties are bred to sustain crop production under limited water resources.

4.2 Materials and Methods

4.2.1 Description of field experimental site

The genotypes were evaluated at Minjibir, Kano (dry location). Kano is situated in the Sudan savannah ecology, which experiences three months of rainfall in a year. The long rains are received between July and August, and short rains in September and October. The site is located at latitude 12°3'N and Longitude 8°32'E. The altitude is 473 m above sea level. The temperature ranges between 18.7°C and 66.5°C, while the annual average temperature is 41.9°C. The relative humidity ranges between 13% and 68%, while the annual relative humidity is 31.1%. The rainfall range is between 0 and 320 mm, while the average annual rainfall is 270 mm, which provides low potential for plant growth and a high risk (25-75%) for crop failure (Sombroek et al., 1982; Jaetzold et al*.,* 2006). The soils are a sandy ferruginous type of the latosols group, which is highly weathered, markedly laterised, and slightly acidic in reaction to low organic matter content and phosphorous; its total nitrogen rarely exceed 0.2%.

4.2.2 Plant materials and field experimental design

A total of 253 F_1 progenies developed from intra-specific crosses between two elite cassava genotypes (TMS98/0505 and TMS98/0581) were used in this study. The parents (TMS98/0505 and TMS98/0581) and three other elite cassava genotypes (TMS91/02324, TMS30572, and TME419) were used as checks. Cassava cuttings of uniform length (20-30 cm) were planted in an augmented design and lattice design, replicated twice for first and second year, respectively. For the augmented design, a single row planting of five plant stands per plot was planted, while the 20 plant stands per plot was planted using a lattice design. The stakes were horizontally placed in the soil at the recommended 1 m spacing within plants per row, and 1 m separation between the rows. For homogenous germination and plant establishment, irrigation was applied in all the plots through overhead and drip irrigation systems. Ninety days after planting (DAP), water stress was imposed by withholding all irrigation. Cassava should be established for three months under rainfall or irrigation before tolerance to drought can be measured effectively. The materials were evaluated during two cropping seasons; the first season in April 2016, and the second season in April 2017.

4.2.3 Data collection

For phenotyping activities, several data were collected. There are several traits thought to be associated with drought tolerance in cassava (El-Sharkawy and Cadavid, 2002; Setter and

Fregene, 2007), but it is not known to what extent these traits confer improved root yield under drought, which is the principal trait of interest to farmers. The current approach is to obtain precise phenotypic measurement of root yield under drought conditions as the primary measurement for drought tolerance. The list of agro-morphological descriptors used to phenotype cassava, their method, and schedule of measurement have been described (Okgbenin et al., 2013). To evaluate cassava response to drought, the following phenotypic traits were measured.

4.2.3.1 Morphological characters

4.2.3.1.1 Plant height

Plant height was determined on the primary stem and was measured from the ground level to the apex using a metre rule calibrated in meters.

4.2.3.1.2 Length of stems with scars

Length of stem with scars was determined by taking the length of the stem where the scar was present from the ground to the top measured in metres.

4.2.3.1.3 Branching levels

This was determined by recording the number of divisions or joints of branching.

4.2.3.1.4 Plant Vigour

Plant vigour was assessed by a visual rating of the plants on a scale of 1 - 5; where a score of 1 is very weak (plants stunted with very thin stems), while 5 is extra vigorous (very fast growing, strong and no bending).

4.2.3.1.5 Plant architecture

Plant architecture was determined by visual rating of the plants on a scale of 1-5; where a score of 1 is very good (erect, no branching), while 5 is very poor (highly profusely branched).

4.2.3.1.6 Number of leaves

The number of leaves was assessed by counting the leaves on the stems of net plants, and the values were recorded.

4.2.3.1.7 First branching height (BH)

This was determined by measuring the primary stem from the ground level to the first branch using a metre rule.

4.2.3.1.8 Height of leafless stem

The height of the leafless stem region was measured with a metre rule from the ground to the first (top-most) leaf scar (vacant node)

4.2.3.1.9 Number of scars

They are the nodes without a leaf. The number of vacant nodes was numerically counted and recorded.

4.2.3.1.10 Leaf retention

Leaf retention was scored on a percentage basis by measuring the total plant height from soil surface upwards and the length from the first intact leaf-petiole to the uppermost apical meristem on the of main stem containing retained leaves HRL.

LR (%) = HRL/PH *100

Where LR = leaf retention

HRL = height retained leaves

PH = total plant height.

4.2.3.2 Productivity traits (root yield and related characters)

4.2.3.2.1 Number of roots

The harvested roots were counted and recorded.

4.2.3.2.2 Root weight

This was determined by weighing the total number of roots harvested from each plot as weighed on a standard weighing balance.

4.2.3.2.3 Biomass

The stem and foliage of all harvested plants were bulked and weighed on a weighing balance.

4.2.3.2.4 Harvest index (HI)

This was measured as the ratio of storage root weight to total biomass.

HI = Wr/Wr + Wab

Where Wr = weight of roots

Wab = weight of above ground biomass.

4.2.3.2.5 Fresh storage root yield

(FRY) = (root weight (kg) *10)/stands harvested.

4.2.3.2.6 Percentage dry-matter content

Estimation of dry-matter content (DMC) (measured as a percentage) was determined using the specific gravity methodology (Kawano *et al.,* 1987). Approximately 1 to 3 kg of roots were weighed in a hanging scale (W_A), and then the same sample was weighed with the roots submerged in water (W_W).

Specific gravity = weight in air/weight in air-wight in water

Dry-matter content was estimated using the formula:

$$\% \text{ DMC} = [158.3] * [W_A/W_A - W_W] - 142.0]$$

4.2.3.2.7 Starch content

Starch content was determined using the formular:

Starch content = [210.8 * Specific gravity] – 213.40 (Kawano *et al.,* 1987)

4.2.3.2.8 Dry root yield (DRY)

This was derived by multiplying FRY with percentage DMC.

4.2.3.2.9 Cassava mosaic disease (CMDs)

Cassava mosaic severity was assessed during the crop growth at 3, 6 and 9 months after planting on a scale of 1-5.

Table 4.1: Cassava mosaic disease (CMDs) score index

Cassava mosaic disease (CMD) score index	Definition
1	Highly resistant (no symptom)
2	Resistant (mild distortion at the base of leaflets with the rest of leaflet appearing green and healthy
3	Moderately resistant (strong mosaic pattern on entire leaf, narrowing and distortion of lower than 30% of leaflets
4	Susceptible (severe mosaic, distortion of 60% of leaflets
5	Highly susceptible (severe mosaic, distortion of more than 80% of leaflets

IITA, 1990

Cassava bacterial blight (CBB) severity was scored on a scale of 1-5 at 3, 6, and 9 MAP and at harvest as shown in Table 3.3.

Table 4.2: Cassava bacterial blight disease severity score index

Cassava bacterial blight (CBB) score Index	Definition
1	Highly resistant (no symptom)
2	Resistant (angular leaf spotting)
3	Moderately resistant (exclusive leaf blight, leaf wilting and defoliation, gum exudation on stems and petiole)
4	Susceptible (extensive leaf blight, wilt, defoliation and stem die back)
5	Highly susceptible (complete defoliation and stem die back)

IITA, 1990

Cassava green mite (CGM) data was collected during the dry spell of the growing season. The scoring scale is 1-5 as shown in Table 4.

Table 4.3: Cassava green mite disease severity score index

Score keys	Definition
1	Highly resistant (no symptoms)
2	Resistant (scattered chlorotic on young leaves with no reduction in leaf size)
3	Moderately resistant (severe chlorotic symptoms with slight reduction in leaf size)
4	Susceptible (severe chlorotic symptoms with reduced leaf size of young shoots)
5	Highly susceptible (very severe chlorosis with significant reduction in leaf size of extensive defoliation and candle stick appearance)

IITA, 1990

4.3 Data Analysis

All phenotypic data was analysed with Genstat application, using the analysis of variance procedures to determine variation among the genotypes. Correlation analysis was used to determine the traits that strongly and significantly influenced drought tolerance. Principal component analysis was used to determine the traits that were the main contributors to drought tolerance and productivity traits. Estimates of variance components were obtained by equating the observed mean squares from ANOVA with their expected mean squares (EMS). Phenotypic and genotypic coefficients of variation were computed using the Excel package.

Genotypic variance component:

$$\sigma^2 g = MSg - {}^{MSe}/_{r}$$

Where *MSg* is the genotypic mean square, MSe is the error mean square and r is replication.

Environmental variance component:

$$\sigma^2 e = {}^{MSe}/_{r}$$

Phenotypic variance component:

$$\sigma_p^2 = \sigma_g^2 + \sigma_e$$

Genotypic and phenotypic coefficients of variation were calculated according to the method suggested by Burton and Dewane (1953) as:

Genotypic coefficient of variation (GCV)

$$GCV = \frac{\sqrt{\sigma_g^2}}{X^-} \times 100$$

Phenotypic coefficient of variation (PCV)

$$PCV = \frac{\sqrt{\sigma_p^2}}{\bar{x}} \times 100$$

Where X^- *is the grand mean value of the trait*

Broad sense heritability (h^2)

$$h^2 = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

4.4 Results

Edapho-climatic conditions in Minjibir Kano State during drought trials:

Rainfall (mm): The rainfall pattern was incremental during the period of evaluation with the highest amount of rainfall received in the month of August (Fig. 4.1). Rainfall data during the experimental period (2016-2018) clearly confirmed the bi-modal trend in the rainfall distribution between the 2016 to 2018 planting season. There was a long season of drought from January to April in the years 2016 and 2017, and limited rainfall in April 2017 before the onset of the rains in May, June, July, and August. The amount of rainfall started declining from September, and then entered the drought season in October to December.

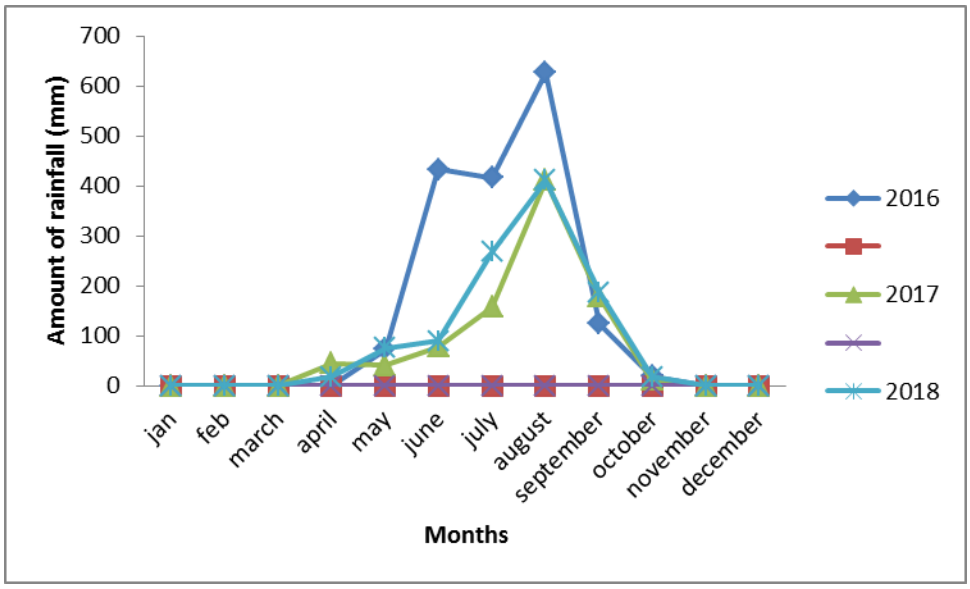


Fig. 4.1: Total monthly distribution of rainfall during the experimentation period (2016-2018)

Temperature (°C) and rainfall distribution:

The mean monthly maximum and minimum temperatures were comparatively constant in the two planting seasons (Fig. 4.2). They were observed to follow the same trend. The highest amount of temperature was recorded in April and May, which then declined from September to December, but started rising again from February. The reduction in temperature in the months of September till December could be attributed to the peak of the rainy season and commencement of harmattern, a very dry, dusty easterly or north-easterly wind on the West African coast, occurring from December to February. The same was applicable for rainfall distribution, where the highest amount of rainfall was recorded in the month of August for the two planting seasons. The very month the trial was started, there was no rainfall, and drought also set in from October to March when the trial was harvested in both planting seasons (Figures 4.2 and 4.3).

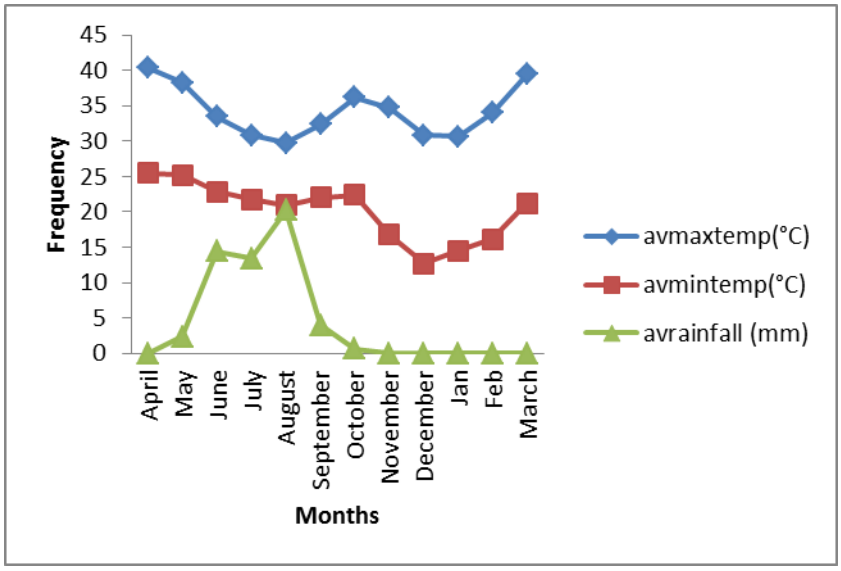


Fig. 4.2: Distribution of maximum temperature, minimum temperature and rainfall in the 2016/2017 planting season

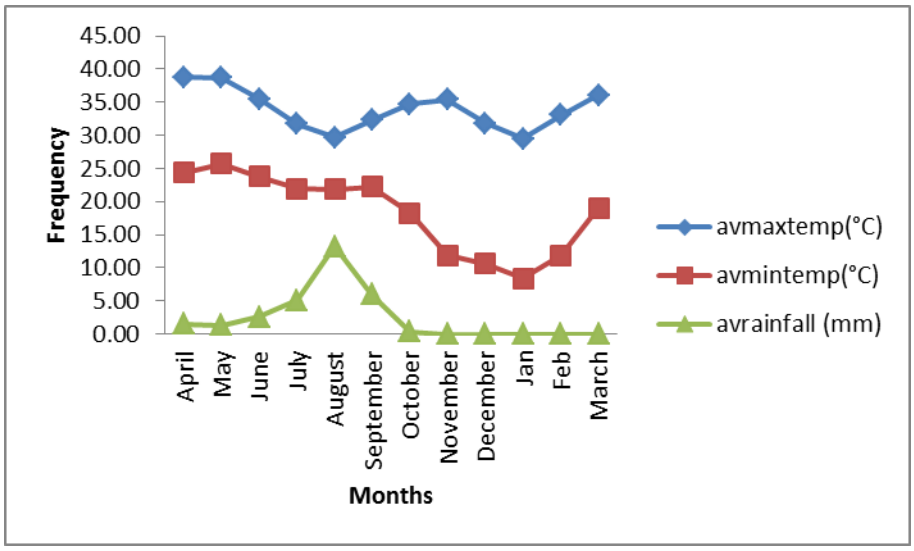


Fig. 4.3: Distribution of maximum temperature, minimum temperature, and average rainfall distribution in the 2017/2018 planting season, Minjibir, Kano

4.4.1 Analysis of variance

The results of the analysis of variance are shown in Tables 4.4 and 4.5. The mean squares indicated that genotype effects were significant at $P \leq 0.05$ for branch level, cassava mosaic disease, leaf number, root number, and scar level in the first year; and they were significant for most of the parameters in the second year, except for branch level, dry-matter content, and cassava bacterial blight.

Table 4.4: Year 1 analysis of variance of productivity, morphological traits, and diseases in mapping population B (TMS98/0505 x TMS98/0581) in Minjibir, Kano

	Mean Square		
Attributes	Genotype	Error	Variance ratio
Biomass (kg)	7.87	23.21	0.34ns
Branch height (kg)	201.3	261.1	0.77ns
Branch level	1.80	0.59	3.07*
CMDs	0.095	0.009	10.12***
DMC (%)	22.31	55.67	0.40ns
DYLD (t/h)	23.40	18.07	1.29ns
FYLD (t/h)	223.0	190.1	1.17ns
HI	0.017	0.04	0.38ns
Leaf number	5711.1	671.5	8.05***
Leaf retention	99.3	108.2	0.92ns
Plant architecture	0.922	0.994	0.93ns
Plant height (cm)	911.1	538.8	1.69ns
Root number	60.68	22.44	2.70*
Root weight (kg)	13.63	57.66	0.23ns
Scar level (cm)	1204.6	420.9	2.86*
Scar no	263.4	209.9	1.25ns
Plant vigour	0.72	0.44	1.62ns
With leaf (cm)	79.0	128.0	0.62ns
CBBs	0.19	0.11	1.86ns
CGMs	0.35	0.41	0.84ns

NS is not significant, *, ** & *** is significant at 5, 1 & 0.1%, CMDs = cassava mosaic disease severity, DMC= dry matter content, DYLD= dry root yield, FYLD= fresh root yield, HI= harvest index, CBBs= cassava bacterial blight secerity, CGMs= cassava green mite disease severity

Table 4.5: Year 2 analysis of variance of productivity, morphological traits and diseases in mapping population B (TMS98/0505 x TMS98/0581) in Minjibir, Kano

	Mean square		
Attributes	Genotype	Error	Variance ratio
Biomass (kg)	104.71	54.64	1.92***
Branch height (cm)	558.1	184.3	3.03***
Branch level	23.23	30.88	0.75ns
CMDs	0.013	0.006	2.17***
DMC (%)	29.20	24.54	1.19ns
DYLD (t/h)	32.36	13.80	2.34***
FYLD (t/h)	398.4	179.0	2.23***
HI	0.022	0.011	1.89***
Leaf number	3141	1410	2.23**
Leaf retention	200.7	125.8	1.60***
Plant arch	0.57	0.45	1.28*
Plant height (cm)	1229.0	815.0	1.51**
Root number	548.2	289.3	1.90***
Root weight (kg)	183.74	13767.28	2.04***
Scar level (cm)	962.2	621.5	1.55***
Plant vigour	1.11	0.66	1.68***
Scar number	426.4	232.8	1.83***
With leaf (cm)	355.8	217.5	1.64***
CBBs	0.34	0.31	1.10ns

4.4.2 Mean values of traits

In the first year's evaluation, results from simple statistics (Table 4.6) show that the percentage dry-matter ranged between 16.30% (TMS98/0581) and 45.34% (002B), while the mean dry-matter content across the genotypes was 31.51%. This shows that most of the genotypes had good and high dry-matter content. As Kano is a dry ecology, the cultivars are expected to have high dry-matter content. The coefficient of variation for dry-matter content was 15.47%. The highest fresh root yield (FYLD) (80 t/ha) was recorded for genotype 159B, while the lowest (0.5 t/ha) was recorded for genotype 230B. The mean value of fresh root yield across the genotype was 24.09 t/ha, while the coefficient of variation was high (61.64%). For plant biomass, the minimum value was 0.20, and the maximum value was 24.20, while the mean across the genotypes was 4.59. Standard deviation for plant biomass

was 2.98 with a high coefficient of variation of 64.98%. Traits with low standard deviation include plant biomass, dry-matter content, dry root yield, harvest index, leaf retention, root number, root weight, and plant vigour. This shows that the mean values of these traits are very close to each other; they are not dispersed, while traits such as branch height, fresh root yield, leaf number, plant height, and scar level had moderate and high standard deviation, signifying that the mean values of these traits are very much dispersed. The coefficient of variation ranged between 15.47% and 75.32% for all traits evaluated in year 1. The lowest coefficient of variation was observed in dry-matter content, while the highest coefficient of variation was observed in root weight.

In the second year evaluation, simple statistics showed that mean values for yield and other yield-related traits such as plant biomass, dry-matter content, dry root yield, fresh root yield, harvest index, root number, and root weight were 13.75%, 25.73%, 9.71%, 37.43%, 0.23%, 31.09%, and16.53% (Table 4.7). Their mean values were not very far from their maximum values, showing that most of the genotypes performed very well. For other traits measured such as length at first branching, leaf number, leaf retention, plant height, scar level, plant vigour, and scar number, their mean values were 52.64, 97.59, 37.41, 123.29, 76.47, 3.36, and 64.52. For all the traits evaluated in the second year, their standard deviation ranged between 0.10 and 40.21. The traits with very low standard deviation were biomass, dry-matter content, dry root yield, harvest index, root weight, and plant vigour. The coefficient of variation across all traits ranged between 14.82% dry-matter content up to 57.89% root weight. A wide range of variation was observed in traits such as plant biomass, root number, and root weight.

Table 4.6: Simple statistics of drought and productivity traits during year 1

Traits	Min	Max	mean	SD	CV (%)
Biomass (kg)	0.20	24.20	4.59	2.98	64.98
Branch height (cm)	1.53	118.00	36.26	14.69	40.52
DMC (%)	16.30	45.34	31.51	4.87	15.47
DRY (t/h)	0.15	27.59	7.67	4.79	62.50
FRY (t/h)	0.50	80.00	24.09	14.85	61.64
HI	0.06	0.86	0.32	0.13	25.07
Leaf number	10.17	219.08	80.29	45.48	40.52
Leaf retention	0.40	63.82	20.81	10.22	49.12
Plant height (cm)	35.38	211.00	123.98	29.72	23.97
Root number	1.00	61.00	12.82	7.74	60.35
Root weight (kg)	0.10	32.80	5.46	4.11	75.32

Scar level (cm)	19.88	155.00	87.34	26.57	30.42
Plant vigour	0.00	5.00	3.35	0.84	25.19
Scar number	14.40	123.50	70.86	16.13	22.76

*DMC = dry-matter content; FRY = fresh root yield; DRY = dry root yield; HI = harvest index

Table 4.7: Simple statistics of drought and productivity traits during year 2

Traits	Min	Max	Mean	SD	CV (%)
Biomass (kg)	1.20	40.65	13.75	7.22	52.56
Branch height (cm)	0.00	134.83	52.64	17.13	32.54
DMC (%)	16.30	41.86	25.73	3.81	14.82
DYLD (t/h)	1.57	27.80	9.71	4.01	41.36
FYLD (t/h)	6.67	68.33	37.43	14.11	37.69
HI	0.07	0.63	0.23	0.10	44.62
Leaf number	0.00	202.50	97.59	40.21	41.20
Leaf retention	0.00	78.07	37.41	10.93	29.21
Plant height (cm)	0.00	198.17	123.29	25.99	21.08
Root number	2.00	86.00	31.09	16.90	54.34
Root weight (kg)	1.50	48.40	16.53	9.57	57.89
Scar level	0.00	145.67	76.47	22.59	29.55
Plant vigour	2.00	5.00	3.36	0.75	22.18
Scar number	0.00	115.25	64.52	15.24	23.62

4.4.3 Trait correlations

Pearson's correlation coefficient analysis was used to study the relationships among the cassava traits (Kawano, 1990; Asogwa et al., 2006). Correlation analysis for the first and second year is shown in Tables 4.8 and 4.9. In the first-year analysis, root yield, which is the ultimate aim of cassava breeding, was positively correlated with all the traits, except for scar level, length of leafless stem (WOT), cassava mosaic disease, and cassava bacterial blight. Some of the stronger correlations ($r > 0.5$) among these traits include < 0.0001*** storage root weight, whose correlation coefficient was $r = 0.71$ and < 0.0001*** dry root yield, whose correlation coefficient was $r = 0.96$. Other traits with moderate values include plant biomass (0.37), root number ($r = 0.31$), harvest index ($r = 0.46$), branching level ($r = 0.20$), plant vigour ($r = 0.18$), scar number ($r = 0.16$) and number of leaves ($r = 0.35$). Some traits such as dry-matter content and plant height were positively correlated with fresh root yield, although not significant. Harvest index correlated positively and significantly with dry-matter content, dry root yield, and root weight at $r = 0.25$, 0.51, and 0.15, respectively. It also had a

positive correlation with leaf number and WTLV, although not significant. On the other hand, dry-matter content positively correlated with dry root yield, plant height, length of leafless stem, plant vigour, scar number, branching level, and root number; and all were significant, except for branching level. Dry-matter content also negatively correlated with leave number, length of stem with leaf, root weight, plant biomass, and cassava bacterial blight. Dry root yield positively and significantly correlated with some yield and yield-related traits such as root weight, total plant biomass, plant vigour, and root number.

Other agronomic traits that correlated positively with dry root yield included leaf number, length of stem with leaves, and branching level. Plant height had a very strong correlation with agronomic traits such as scar level ($r = 0.85$), length of leafless stem ($r = 0.86$), plant vigour ($r = 0.69$), and scar level ($r = 0.22$). Plant height also correlated positively with yield traits such as root weight, total plant biomass, and root number. Scar number correlated positively and significantly with leaf number, scar level, length of leafless stem, root weight, total plant biomass, plant vigour, root number, and branching level, but negatively with cassava bacterial blight. Leaf number correlated positively with yield-related traits such as plant vigour, total plant biomass, root weight, and root number. It also correlated positively with other agronomic traits such as scar level, length of leafless stem, length of stem with leaves, and branching level; and correlated negatively with cassava mosaic disease, and cassava bacterial blight. Scar level had a positive correlation with most of the yield-related traits. Root weight, total pant biomass, plant vigour, and root number significantly and positively correlated with each other. In the second year, it was also observed that fresh root yield highly and positively correlated with yield-related traits and other agronomic traits. It was also observed that most of the morphological traits correlated positively with yield-related traits such as root weight, plant vigour, plant biomass, and root number.

Table 4.8: First year Trait Pearson correlation coefficient in mapping population B

Traits	FRY	HI	DMC	DRY	PLTHT	SCARNO	LEVNO	SCARLEV	WTLV	WOTL	RTWT	BIOM	VIG	RTNO	CMD	CBB	BRLEV
FRY	1																
HI	0.46***	1															
DMC	0.03ns	0.25***	1														
DYLD	0.96***	0.51***	0.25***	1													
PLTHT	0.05ns	-0.30***	0.15*	-.07ns	1												
SCARNO	0.16**	-0.04ns	0.10ns	0.16ns	0.55***	1											
LEVNO	0.35**	0.07ns	-0.01ns	0.31***	0.26***	0.26***	1										
SCARLEV	-0.02ns	-0.36***	0.15*	0.01ns	0.85***	0.58***	0.18**	1									
WTLV	0.30***	0.03ns	-0.18**	0.22***	-0.01ns	-0.09ns	0.06ns	0.89***	1								
WOTL	-0.04ns	-0.28***	0.27***	0.01ns	0.86***	0.56***	0.19**	-0.17**	-0.24***	1							
RTWT	0.71***	0.15*	-0.03ns	0.64***	0.24***	0.24***	0.21***	0.15*	0.30***	0.10ns	1						
BIOM	0.37***	-0.36***	-0.08ns	0.30***	0.48***	0.30***	0.22***	0.41***	0.24***	0.39***	0.74***	1					
VIG	0.18**	-0.20**	0.20**	0.22***	0.69***	0.49***	0.31***	0.66***	-0.02ns	0.28***	0.30***	0.54***	1				
RTNO	0.31***	0.03ns	0.12*	0.31***	0.35***	0.26***	0.20***	0.31***	0.06ns	0.73***	0.65***	0.64***	0.43***	1			
CMD	-0.09ns	-0.02ns	0.061ns	-0.08ns	0.02ns	0.011ns	-0.01ns	0.05ns	-0.05ns	0.04ns	-0.11ns	-0.07ns	0.02ns	-0.02ns	1		
CBB	-0.22ns	-0.17ns	-0.08ns	-0.22ns	-0.14*	-0.17**	-0.27***	-0.03ns	-0.09ns	-0.08ns	-0.23***	-0.17**	-0.16**	-0.18**	0.09ns	1	
BRLEV	0.20**	-0.02ns	0.06ns	0.20**	0.22***	0.14*	0.33***	0.17**	0.03ns	0.20**	0.15*	0.37***	0.37***	0.15*	0.09ns	-0.01ns	1

FRY = fresh root weight (t/h), HI = harvest index, DMC = dry matter content (%), DYLD = dry root yield (%), SCARNO = scar number, SCARLEV= scar level, WTLV = length of stem with leaves (cm), WOTL = length of leafless stem (cm), RTWT = root weight (kg), BIOM = total plant biomass (kg), VIG = plant vigour, CMD = cassava mosaic disease, CBB = cassava bacterial blight, BRLEV = branching level

Table 4.9: Second year Trait Pearson correlation coefficient in mapping population B

Traits	FRY	HI	DMC	DYLD	PLTHT	SCARNO	LEVNO	SCARLEV	WTLV	WOTL	RTWT	BIOM	VIG	RTNO	CMD	CBB	BRLEV
FRY	1																
HI	-0.41***	1															
DMC	-0.52ns	0.03ns	1														
DYLD	0.90***	-0.37***	0.30***	1													
PLTHT	0.12*	-0.48***	0.001ns	0.12*	1												
SCARNO	0.07ns	-0.27***	-0.05ns	0.05ns	0.50***	1											
LEVNO	0.10ns	0.13ns	0.14*s	0.16*	0.08ns	0.02ns	1										
SCARLEV	0.03ns	-0.39***	-0.05ns	0.02ns	0.79***	0.62***	-0.20**	1									
WTLV	0.12ns	-0.21***	0.06ns	0.13*	0.41***	-0.13**	0.47***	-0.14**	1								
WOTL	0.05ns	0.05ns	-0.03	0.04ns	0.82***	0.63***	-0.20**	0.94***	-0.16**	1							
RTWT	0.58***	-0.66***	-0.01ns	0.53***	0.30***	0.18**	-0.09ns	0.22***	0.11**	0.25***	1						
BIOM	0.36***	-0.83***	0.04ns	0.35***	0.48***	0.23***	-0.16*	0.40***	0.12*	0.39***	0.70***	1					
VIG	0.22***	-0.36***	-0.02ns	0.21***	0.24***	0.13*	-0.12ns	0.22***	0.07ns	0.21***	0.30***	0.31***	1				
RTNO	0.28***	-0.60***	0.03ns	0.26***	0.33***	0.14**	-0.006ns	0.20**	0.20**	0.19**	0.71***	0.64***	0.19***	1			
CMD	0.012ns	0.02ns	0.01ns	0.009ns	-0.07ns	-0.05ns	0.10ns	-0.04ns	-0.05ns	-0.05ns	-0.02ns	-0.04ns	-0.04ns	-0.04ns	1		
CBB	0.001ns	-0.05ns	0.03ns	0.004ns	0.04ns	0.04ns	0.05ns	-0.03ns	0.04ns	0.06ns	0.05ns	0.04ns	0.04ns	0.05ns	0.06ns	1	
BRLEV	-0.02	0.22***	0.11*	0.02ns	0.02ns	-0.11*	0.30***	-0.11*	0.28***	0.08ns	-0.05ns	-0.06ns	-0.09ns	-0.01ns	-0.009ns	-0.03ns	1

4.4.4 Principal component analysis (PCA)

Principal component analysis was used to determine traits that influenced drought tolerance in marginal environments. For the morphological traits, the first five principal component axes (PCs) accounted for 74% and 84% variation in both years (Tables 4.10 and 4.11). In year 1, PC1 accounted for 30% of the variation, and indicated that scar level, scar number, height at first branching, and length of leafless part of stem were the main contributors. PC2 accounted for 17% of the variation and had length of leafy part of stem and leaf retention as the major contributors, which were positively correlated, while PC3 accounted for 11% variation identified leave number and branching level as the main contributors. PC4 contributed 8% of variation, which indicated plant height as the main factor, while PC5's 8% variation indicated plant vigour, scar number, branch level, and leave number as the main contributors. In productivity traits in year 1 (4.12), the first five PCs accounted for 99% of total variation. The results showed that PC1 accounted for 54% of the variation, and identified root weight, fresh root yield, and dry root yield as the main contributors. PC2 had root number and dry-matter content as the main contributors, with 20% variation. PC3 recorded root number, harvest index, and dry-matter content, with 14% variation. PC4 contributed 8% of variation with harvest index as the main factor, while PC5 had root weight as the only contributor, with 3% variation. Some of the physiological traits such as leave number occurred in PC3 and PC5, while in the productivity traits, some of the traits such as root weight occurred in PC1 and PC5; harvest index occurred in PC3 and PC4, while dry-matter content occurred in PC2 and PC3.

In the second year, PC1 accounted for 37% variation for morphological/physiological traits, and identified plant height, scar number, height at first branching, scar level, length of stem without leaves, and leaf retention as the main contributors (Table 4.11). PC2 with 19% variation had plant height, number of leaves, length of stem with leaves, and leaf retention as the main contributors. PC3 identified branching level, height at first branching, and plant vigour as contributors, while PC4 and PC5 had branching level and plant vigour as main contributors, respectively (Table 4.11). For the productivity traits, the total variation for the five PCs accounted for 97% of total variation (4.13). PC1 accounted for 53% of the total variation, and identified root weight, fresh root yield, number of roots, harvest index and dry root yield as the main contributors. PC2, with 22% variation, had number of roots, dry root yield, dry-matter content, and fresh root yield as the main contributors. PC3 identified only dry-matter content, while PC4 had harvest index, root weight, and number of roots as contributors, while PC5 had root weight, root number, and harvest index as contributors (Table 4.13).

Table 4.10: Year 1 principal component analysis of morphological traits in population B and checks

Traits	PC1	PC2	PC3	PC4	PC5
VIG	-0.28	0.14	-0.04	0.22	0.50
PLTHT (cm)	-0.19	-0.12	0.21	-0.90	0.18
SCARNO	-0.39	0.04	0.13	-0.01	-0.36
NLVS	-0.11	0.19	-0.53	-0.20	-0.63
HFB (cm)	-0.32	0.14	0.30	0.15	-0.22
BRLEV	-0.19	0.26	-0.53	-0.10	0.31
SCARLEV (cm)	-0.42	0.00	0.22	0.15	-0.08
WTLV (cm)	-0.02	0.67	0.25	-0.18	0.04
LVRET	0.26	0.62	0.12	0.04	0.00
Eigen value	1.82	1.37	1.10	0.96	0.96
Eigen values as proportion of total variance	0.30	0.17	0.11	0.08	0.08
Cummulative % total variance	0.30	0.47	0.58	0.66	0.74

*VIG = Plant vigour; PLTHT = Plant height; SCARNO = Scar number; NLVS = Number of leaves; HFB = height at first branching; BRLEV = branching level; SCARLEV = scar level; WTL = length of stem with leaf; WOT = length of leafless stem; LVRET = leaf retention

Table 4.11: Year 2 Principal component analysis of morphological traits in population B and checks

TRAITS	PC1	PC2	PC3	PC4	PC5
VIG	-0.14	-0.14	-0.48	-0.07	0.79
PLTHT (cm)	-0.39	-0.38	0.10	0.09	0.04
SCARNO	-0.38	0.02	0.10	0.00	-0.16
LEVNO	0.04	-0.47	0.14	0.01	-0.28
HFB (cm)	-0.31	-0.11	-0.32	-0.06	-0.29
BRNLEV	0.01	-0.12	0.38	-0.88	0.16
SCARLEV (cm)	-0.47	-0.02	0.07	0.14	0.03
WTLV (cm)	0.07	-0.64	-0.01	0.02	0.02
WOTL (cm)	-0.48	-0.03	0.14	0.00	0.06
LVRET	0.36	-0.41	-0.17	0.11	-0.03
Eigen value	2.01	1.46	1.11	1.00	0.92
Eigen value as Proportion of Variance	0.37	0.19	0.11	0.09	0.08
Cumulative % total variance	0.37	0.56	0.67	0.76	0.84

Table 4.12: Year 1 principal component analysis of productivity traits in population B and checks

Traits	PC1	PC2	PC3	PC4	PC5
RTNO	-0.28	-0.59	0.53	0.30	-0.44
FRY (t/h)	-0.52	0.23	-0.03	-0.31	-0.22
HI	-0.38	0.12	-0.52	0.76	-0.03
DMC (%)	-0.12	-0.67	-0.62	-0.38	-0.03
DYLD (t/h)	-0.50	0.33	0.03	-0.31	-0.30
Eigen value	1.79	1.10	0.93	0.71	0.42
Eigen values as proportion of total variance	0.54	0.20	0.14	0.08	0.03
Cummulative % total variance	0.54	0.74	0.88	0.96	0.99

*RTWT = root weight; RTNO = root number; FRY = fresh root yield; HI = harvest index; DMC = dry-matter content; DYLD = dry root yield

Table 4.13: Year 2 principal component analysis of productivity traits in population B and checks

Traits	PC1	PC2	PC3	PC4	PC5
RTWT (kg)	-0.48	0.01	0.06	0.38	-0.35
RTNO	-0.39	0.38	-0.07	0.63	0.37
FRY (t/h)	-0.36	-0.52	0.25	-0.04	0.10
HI	0.43	-0.25	0.15	0.57	-0.56
DMC (%)	0.03	0.31	0.94	-0.08	0.05
DRY (t/h)	-0.34	-0.58	0.12	-0.03	0.08
Eigen value	1.93	1.25	0.98	0.65	0.39
Eigen value as Proportion Variance	0.53	0.22	0.14	0.06	0.02
Cumulative % total variance	0.53	0.75	0.89	0.95	0.97

4.4.5 Mean performance of traits in parents, progenies, and checks

Mean performance of parents, progenies, and checks in the first and second year are shown in Tables 4.14 and 4.15. Results showed that the progenies performed better than their parents (TMS980505 and TMS980581) in most of the traits, except for harvest index, dry-matter content, and leaf number in the first year, while in second year, the progenies performed better than their parents in dry-matter content, dry root yield, number of leaves, and leaf retention. A better performance also occurred in some traits of the progeny compared to the entire checks for both years' mean values of traits, which were high in harvest index, dry-matter content, leaf number, height at first branching, and leaf retention in the checks when compared to mean values of the same traits in the progenies in the first year. In the second year, the checks

displayed better performance in most of the traits, except for dry-matter content, dry root yield, number of leaves, and leaf retention.

Table 4.14: Mean performance of traits of parents, progeny and checks in population B for first year in Minjibir, Kano

Traits	Parents	Progeny	Checks
Plant vigour	3.38	3.46	3.37
Root weight (kg)	5.20	15.05	10.80
Total biomass (kg)	4.46	10.85	9.10
Root number	12.42	27.63	21.58
Fresh root yield (t/h)	23.99	33.96	30.70
Harvest index	0.53	0.37	0.38
Dry-matter content (%)	31.33	27.30	28.55
Dry root yield (t/h)	7.63	8.77	8.68
Plant height (cm)	123.67	141.82	123.61
Scar number	70.83	74.95	67.74
Leaf number	81.98	75.18	90.48
Height at first branching (cm)	35.72	51.66	67.35
Height of leafless stem (cm)	87.04	94.93	81.85
Leaf retention	20.67	26.35	29.13

Table 4.15: Mean performance of traits of parents, progeny and checks in population B for second year in Minjibir, Kano

Traits	Parents	Progeny	Checks
Plant vigour	3.75	3.35	3.80
Root weight (kg)	22.50	16.68	22.88
Total biomass (kg)	15.67	13.74	13.83
Root number	32.50	30.73	46.90
Fresh root yield(t/h)	41.42	37.41	38.57
Harvest index	0.17	0.22	0.21
Dry-matter content (%)	19.91	25.77	23.60
Dry root yield (t/h)	8.28	9.72	9.12
Plant height (cm)	143.75	123.56	136.93
Scar number	74.87	64.65	71.18
Leaf number	60.94	98.97	70.57
Height at first branching (cm)	71.06	52.37	75.14
Height of leafless stem (cm)	90.00	77.00	83.83
Leaf retention	36.25	37.59	36.90

4.4.6 Estimates of variance components

Estimates of phenotypic, genotypic, and environmental variance components are shown in Table 4.16. The environmental variance was higher in traits such as plant height, total biomass, dry-matter content, leaf retention, root number, scar level, scar number, plant vigour, length of leafless stem, and length of leafy stem. The genotypic variance was high in traits such as branch height, dry root yield, fresh root yield, leaf number, and root weight.

The genetic variances for branch height, dry rot yield, fresh root yield, harvest index, leaf number, root weight, and scar level were higher than their corresponding environmental variances. The environmental variances of plant height, total plant biomass, dry-matter content, leaf retention, root number, scar level, scar number, plant vigour, length of leafless stem, and length of leafy stem had lower genetic variances than their corresponding environmental variances. The phenotypic coefficient variation (PCV) was higher than the genotypic coefficient of all the traits' variation. The lowest PCV (14.87%) was attained by dry-matter content, with root weight showing the highest PCV (58.20%). For the genotypic coefficient of variation, the lowest (15.96%) was seen in dry-matter content with the highest (41.57%) genotypic coefficient of variation observed in root weight.

Broad sense heritability estimates ranged between 16.03% for dry-matter content and 66.97% for branch height. High broad sense heritability estimates were obtained for branch height (66.97%), dry root yield (57.43%), fresh root yield (55.07%), harvest index (50.85%), leaf number (55.08%), and root weight (51.03%).

Table 4.16: Estimates of genetic variance in mapping population B and checks

Traits	GV	EV	PV (%)	PCV (%)	GCV (%)	H_2b (%)
Plant height (cm)	207	407.5	614	20.04	11.64	33.71
Branch height (cm)	186.85	92.15	279	31.27	25.57	66.97
Total biomass (kg)	25.04	27.32	52.36	52.70	36.45	47.82
Dry-matter content (%)	2.34	12.26	14.60	14.87	5.96	16.03
Dry root yield (t/h)	9.31	6.90	16.21	41.68	31.59	57.43
Fresh root yield (t/h)	109.70	89.50	199.20	37.74	27.99	55.07
Harvest index	0.00526	0.00508	0.010335	44.78	31.93	50.85
Leaf number	865	705.50	1570.5	40.40	29.92	55.08
Leaf retention	37.45	62.90	100.35	25.86	15.79	37.32
Root number	129.45	144.65	274.1	52.24	35.90	47.22
Root weight (kg)	46.88	44.99	91.87	58.20	41.57	51.03
Scar level	170.45	310.75	481.20	28.97	17.24	35.42
Scar number	96.60	116.40	213.00	22.93	15.44	45.35
Plant vigour	0.2258	0.33205	0.55785	22.30	14.18	40.48
Length of leafless stem (cm)	136.85	170.075	306.925	22.72	15.17	44.59
Length of leafy stem (cm)	69.10	108.75	177.85	28.54	17.79	38.85

*GV = genotypic variance; PV = phynotypic variance; EV = environmental variance; PCV = Phenotypic coefficient of variation; GCV = genotypic coefficient of variation; H_2b = heritability broad sense

4.5 Discussion

The Edapho-climatic conditions (described in Fig. 4.1) during the two planting seasons were typical of a dry ecology. Therefore, the site was suitable for field-based drought experiments. The relatively constant temperature across the years was considered conducive for cassava growth and establishment. Drought stress treatment induced by withholding total irrigation, the drought period during the experiment (April to June, November to March) interspersed between the high and low rainfall, could have contributed to trait variation observed among the genotypes. This supports the literature (de Tafur *et al.,* 1997; Pellet and El-Sharkawy, 1997), which reported cultivation of cassava in marginal lands of low fertility soils, with annual rainfall

ranging between less than 600 mm in the semi-arid tropics and more than 1,500 mm in the sub-humid and humid tropics. A temperatures range between 24°C and 30°C is optimal for cassava growth and production, although the crop can manage a temperature range of between 16°C and 38°C (Cock, 1984; Alves, 2002.

Based on ANOVA, the mean squares (MS) indicated that genotype effects were highly significant ($P \leq 0.05$) for most of the parameters evaluated. This suggests that this population had adequate genetic variability. El-sharkawy (2007) reported similar results when he subjected a range of cassava genotypes to water stress. He established that some genotypes had a high level of drought tolerance, while some were susceptible to drought. He also found out that genotypic effects were more dominant compared to location, and genotype x location effects signifying a strong genetic basis for the phenotypic differences observed among the genotypes. This further suggested that the selection of desirable traits among these genotypes would lead to a significant progress in cassava improvement schemes, since genetic effects are dominant. Cassava response to drought is known to vary with cultivar, duration and severity of water deficit, and stage of development (Burns *et al.,* 2010; Alves, 2002; Lokko *et al.,* 2007; El-Sharkawy, 2002).

The positive correlation of these traits with fresh root yield showed that all the traits were important and were positively associated with each other. This also showed that these traits can make important contributions towards economic yield (Ojulong, 2008). The positive correlation between plant height and fresh root yield showed that increment in vegetative parts had a significant effect on the cassava root yield as has been reported by Tewodros *et al.* (2012). Therefore, an indirect selection for higher fresh root yield may be effective for improving plant height. Varieties with a good top weight tend to produce a good top yield (Okogbenin *et al.,* 2013). Previous study reported by Conor *et al.* (1981) suggested that vigorous genotypes produce better under stress than less vigorous genotypes. Fresh root yield was found to negatively correlate with cassava mosaic disease severity and cassava bacterial blight. Although not significant, this could be because these genotypes were screened for pest at seedling stage before clonal evaluation, and Kano, being a dry ecology may not be the optimal location for expression of cassava mosaic disease. This also suggested that cassava mosaic disease and cassava bacterial blight can lead to a reduction in yield. In Africa, the most important disease affecting cassava is cassava mosaic disease. It had been reported to cause a 100% yield loss in cassava (Thresh *et al.,* 2005). Cassava bacterial blight is also a contributing factor to yield loss in cassava. Studies had shown that yield loss in cassava as a result of bacterial blight disease could be over 20% (Lozano, 1992; Dixon *et al.,* 2002; Ogbe *et al.,* 2003). Farmers selecting crops for increased yield potential and improved adaptation to stress-prone environments often target yield as the principal trait for selection (Araus *et al.,* 2008).

The results of this study revealed that the phenotypic coefficient of variation (PCV) estimates were higher than the genotypic coefficient of variation (GCV) estimates, indicating that the variations in the clones were not only genotypic, but were also due to environmental influence. This observation agreed with earlier findings of Favour *et al.* (2015), Cock (1985) and Akinwale *et al.* (2010). Plant height, total plant biomass, dry-matter content, leaf retention, root number, scar level, scar number, plant vigour, length of leafless stem, and length of leafy stem were more influenced by the environmental factors, as their observed genetic variances were lower than the environmental variances. Bhateria *et al.* (2006) classified heritability estimates as high (> 50%), medium (30% to 50%), and low (< 30%). Broad sense heritability was high and medium in most of the traits, except for the dry-matter content, which had a very low (16%) broad sense heritability. This means that a reliable selection for these traits can be achieved. The importance of this result is that with high broad sense heritability, rapid progress in selection can be achieved, even with simple selection procedures such as a recurrent selection or direct selection, based on their phenotypic performance.

Principal component analysis was used to explain the relative contribution of the various traits to the genotypes' performance and the variation among traits. The wide variations in PC1 and PC2 for both morphological and productivity traits signify variation among traits, which is also good for selection. Combining the important traits, as revealed by PCA, can be used in the selection stage by breeders to select for drought-tolerant cassava genotypes and enhance yield productivity.in marginal environment.

4.6 Conclusion

Traits driving drought tolerance and productivity in marginal environments were identified and these can be used to select genotypes that are tolerant to drought in dry ecology.

CHAPTER 5

GENETIC MAPPING FOR DROUGHT TOLERANCE OF CASSAVA GENOTYPES IN THE F_1 POPULATION

ABSTRACT

Cassava is an important root crop for food and feed in sub-Saharan Africa. As cassava production is rapidly extending into dry areas, genetic improvement for drought tolerance is considered a top priority for the crop's transformation in Africa. Conventional or classical breeding for drought-tolerant cassava varieties is slow, due to its long breeding scheme and the quantitative nature of its inheritance. Identification of genes (QTLs)/markers associated with drought-tolerant traits, due to increasing molecular tools, offers the opportunity to fast-track breeding and theselection for high performance drought tolerant cassava genotypes. This study initiated an action plan, using elite parental lines commonly utilised in African breeding programmes, to develop F_1 populations for drought tolerance breeding, exploring a new set of 2000 SNPs made available under the CGIAR Generation Challenge Programme. This study identified QTLs for drought tolerance and productivity traits in an F_1 population (Population B) developed from a cross between two elite cassava varieties, TMS980505 and TMS980581, widely disseminated in Africa and used in breeding. Principal component analysis identified 99% variation for productivity traits, and 74% variation for physiological traits. The analysis identified scar level, height of leafless stem, height of stem with leaf, leaf retention, fresh root yield, dry root yield, root number, and dry-matter content as traits driving drought tolerance in marginal environments. There were significant positive correlations among several traits. To aid the molecular-genetic analysis of traits, a linkage map covering 1582.8 cM with an average resolution of 3.69 cM was constructed, using 505 polymorphic SNP markers distributed over 21 linkage groups. Composite interval mapping (CIM), using 267 F_1 progeny, identified 27 QTLs associated with the traits phenotyped in the dry savannah ecology of Nigeria in the first year of the trials, while 30 QTLs were identified in the second year. There was co-localisation of multiple QTLs, representing various traits such as dry root yield, root number, and fresh root yield, branching level, and leaf retention; dry root yield, and fresh root yield; harvest index, plant height, and height in first branching during the first and second year, respectively. The phenotypic variance explained by the detected QTLs ranged between 2.30% and 20.42% in year 1, and between 2.31% and 9.48% in the second year. Two major QTLs were also identified with PVE > 19%. QTL mapping for digenic interactions indicated that LoD score values and interaction effects were significant for only five digenic pairs (loci). QTL analysis identified additive and dominance gene effects among the detected QTLs. The identification of QTLs for productivity traits in marginal environments, based on elite parental lines commonly utilised for

genetic improvement of cassava, is anticipated to boost molecular breeding under a recurrent selection programme towards enhancing drought tolerance in cassava for better productivity in Africa.

Key words: Cassava varieties; Composite interval mapping; Marginal environment; QTL analysis; SNP markers

5.1 Introduction

Cassava is an important food and starch crop, with excellent adaptability to multiple environments. Along with maize, and rice, the crop constitutes the most important source of energy in the diet of people living in the tropical countries, where it is a staple for over 800 million people (Burns *et al.,* 2010; Pérez *et al.,* 2012). After maize, cassava is a competitive source of starch worldwide (Strapleton, 2012; Norton, 2014). Traditionally, cassava has been grown in the humid and sub-humid zones of Africa, where the amount of rainfall distribution is between 1000 and 2200 mm (Onwuneme and Sinha, 1991; Olasanmi *et al.,* 2017) In recent years, cassava cultivation has expanded to transitional belts/savannahs with the increasing need for more food due to population pressures and rural economy transformation in Africa. Cassava production has several challenges, especially diseases, insect pests, and abiotic constraints. The cassava mealy bug, cassava bacterial blight, cassava mosaic disease (CMD) and cassava brown streak are the major diseases affecting cassava in Africa. Because of its huge devastating affect and widespread occurrence, CMD was the most prominent disease for several years, until the outbreak of cassava brown streak on the continent. Classical breeding in national and international research programmes resulted in moderate improvements in the resistance to these pests leading up to the late 90s. The integration of early developed molecular markers for cassava fast-tracked discovery of novel genes such *CMD1*, *CMD2*, *CMD3* (Fregene *et al.,* 2000; Akano *et al.,* 2002; Blair *et al.,* 2007; Okogbenin *et al.,* 2012) that facilitated resistance breeding for CMD, and which resulted in the development of CMD resistant varieties. Molecular markers tightly associated with the CMD resistant gene (*CMD2*) are currently being explored with marker-assisted breeding for CMD resistance (Akano *et al.,* 2002). Apart from insect pests and diseases, the rapid expansion of cassava into non-traditional ecologies of Guinea, Sudan, and Sahel savannahs in the last two or more decades have resulted in increased stress conditions for less adapted varieties due to the limited rainfall. This is further aggravated by climate change, and therefore, the most limiting abiotic constraint of cassava is drought (Pérez *et al.,* 2012; Bakayoko *et al.,* 2009). Developing varieties that can withstand moderate stress or severe stress conditions is critical to enhance higher productivity in marginal environments. The development of drought-tolerant cultivars is essential for

maintaining yields under climate change conditions, and for the extension of sub-optimal cropping areas (Tubiello *et al.,* 2006). Plants suffer drought stress when water supply to the roots is less than at optimal level; and as a result of this, it negatively affects the growth, development, and yield of the plant. Breeding for drought tolerance is being prioritised globally through the use of biotechnology tools as an intervention for mitigating the threat to food security caused by climate change (Oliveira *et al.,* 2015; El-Sharkawy., 2007; Okogbenin *et al.,* 2003) and assist in effectively manipulating complex traits to assemble the best genetic background for desired genotypes or varieties to improve food security has been demonstrated in several crops (Chavarriaga *et al.,* 2016). Drought tolerance is a complex phenomenon, as it is driven by several morphological, physiological, and yield component traits (Blum, 2011; Collins *et al.,* 2008). Given that multiple genes are involved that must be combined in good haplotypes through a recurrent scheme robust selection, and advanced support tools to the decision selection is required. The long breeding time needed to develop a new variety of cassava, which usually takes 10 years to develop due to its long growth cycle (12 to 18 months), further makes it critical to explore a fast approach to expedite genetic improvement process of this crop. Therefore, biotechnology offers the best approach in achieving this goal. Molecular markers are now increasingly being integrated into cassava breeding, especially for difficult-to-breed traits, following significant investment in molecular tools for the crops. Since the development of the first molecular map of cassava, which was based mainly on RFLPs (Fregene *et al.,* 1997), several maps based on different molecular markers (AFLP, RAPD, ESTs, and SSR) have been developed (Fregene *et al.,* 1997; Okogbenin *et al.,* 2006; Chen *et al.,* 2010; Kunkeaw *et al.,* 2010, 2011; Sraphet *et al.,* 2011). These maps have been used for QTL mapping for several traits in cassava, including the CMD genes (Akano 2012, Okogbenin *et al.,* 2002, 2003; Lopez *et al.,* 2008, Rabbi *et al.,* 2014). These are now used for breeding. The easy amenability under both low and high throughput systems for genetic studies and breeding made SSR preferable at its advent. However, while it offered good polymorphism, its abundance level was still highly limited to allow for good genome coverage, detect enough genes and predict crop performance. Advances in biological sciences resulted in the development of SNP markers. The probability to find high polymorphisms around target genes have increased with SNPs, given that they are highly abundant in the genomes, and provide the highest map resolution when compared with other marker systems (Jones *et al.,* 2007). In sub-Saharan Africa, there are limited genetic tools and information to support molecular breeding for drought tolerance. The need for the development of highly polymorphic SNP markers that support wide genome coverage and are amenable to applied molecular breeding was considered very critical to improve the understanding of cassava genetics and accelerate genetic improvement of the cassava. The inability to effectively identify key QTLs, driving specific traits imply that genetic variances in trait performance, has not been fully captured or explained. The shift to a genome-wide-assisted

selection to enhance molecular breeding for impact implies a need to switch to SNPs. The CGIAR Generation Challenge Programme (GCP) facilitated the development of KASP SNPs for cassava, which resulted in access to 2000 KASP SNPs. KASPar is apparently suitable for molecular breeding applications such as MAS or MARs. This is crucial in cases where the capacity and resources to undertake development of molecular breeding are limited. This set of SNPs represented the first major and abundant size of SNPs available in the public domain for use by national and international programmes. The cassava genome is estimated to be about 772 Mb (Awoleye *et al.,* 1994), and the need to have a high number of SNPs on the genome to support breeding for target traits in Africa were considered paramount to heighten the genetic improvement of complex traits for prediction and selection accuracy, among which is drought tolerance, have been difficult to manipulate in cassava. The objectives of this paper were therefore to (1) explore SNPs to support molecular breeding for drought tolerance by developing a genetic map of KASP (Competitive allele-Specific PCR) SNPs, based on elite parental lines commonly used for breeding, and identified for adaptability under marginal environment; (2) identify drought tolerance traits in elite African parental lines under moderate stress conditions (at least 6 months without rainfall under a 12-month growth cycle); and (3) conduct QTL mapping to detect QTLs adaptation and drought tolerance, and define pathways to explore QTLs to improving drought tolerance breeding in national breeding programmes.

5.2 Materials and Methods

5.2.1 Development of mapping populations

Based on earlier existing data on evaluated germplasm, the mapping population was developed by crossing two parents TMS 98/0505 (female) and TMS98/0581 (male). TMS98/0505 (with 97 DTP REP2 as a pedigree) is an elite cassava genotype that was officially released in Nigeria in 2005. It has high dry-matter content, high CMD resistance, high yielding and starch content, moderate flowering ability, and early bulking. The female parent TMS98/0581, whose pedigree is MPR POP REP 1, is a released cassava variety with high dry-matter content, high yielding and CMD resistance, staying green, being drought tolerant, and a profuse flowering ability. Cassava has no inbred lines, so both parents are heterozygous. They have been selected for variation in morphological characters and other attributes such as staying green, and adaptation (with TMS98/0505 being more adapted) in the dry savannahs of Nigeria. The parents have also been selected for key target traits (good yield, high dry-matter, and starch content) of breeding importance, as these traits are intended to be co-selected with drought tolerance, because the best F_1 genotypes are planned for further use in the breeding programme. Given that the crop is highly heterozygous and shows multi-allelism, it is expected to segregate even for these key

breeding target traits, whose QTLs would be useful. The other trait of immense consideration was CMD for which both parents are resistant. Both parents are believed to explore the CMD2 dominant gene as part of their genetic resistance to the disease. The development of a large population is expected to at least yield a high number of individuals with good resistance, which is a critical criterion for good phenotyping required for QTL mapping, as disease could confound the genotypic expression of drought tolerance, which is undesirable. This could conveniently permit the exclusion of susceptible lines in the population without significantly affecting results. This approach is necessary to reduce cassava mosaic incidence in the mapping population, given that CMD occurrence is high in cassava growing regions in Africa.

5.2.2 Mapping population nursery

Seedlings were planted in jiffy pots in the screen house at Umudike. One month after planting, they were transplanted to the field as field nursery to evaluate for diseases and generate enough stem cuttings to start the field trial for QTL mapping. Umudike is a high-disease pressure zone for cassava. It is located on the latitude 5°29'N, longitude 7°24'N, altitude 120 m, annual rainfall 2200 mm, annual average temperature of 26°C, relative humidity at 50-95%, and a dystric luvisol as soil type. The seedling nursery was intensively managed to control weeds in the trial at Umudike to ensure good establishment of the population. It was critical to generate enough planting materials, as cassava is clonally propagated. The vigorous genotypes in the nursery generated an average of 10 cuttings, with some genotypes producing a much higher number of cuttings. This step was very important, as it generated the cuttings that were used for the successful conduct of QTL mapping at Minjibir, Kano State, Nigeria. Minjibir is a low-disease pressure zone for cassava; and it was not deemed suitable to assess the genetic response of the mapping population to diseases there. The disease evaluation data from Umudike was to complement other agronomic traits evaluated in Minjibir under moderate-stress conditions.

5.2.3 Description of field experimental site

The genotypes were evaluated at Minjibir, Kano (sub-optimal soil conditions). Kano is in the Sudan savannah ecology, and experiences two to three months of rainfall in a year. This makes it suitable for drought studies. It is located at latitude 12°3'N and longitude 8°32'E. The altitude is 473 m above sea level. The temperature at the location ranges between 18.7°C and 66.5°C, while the annual average temperature is 41.9°C. Relative humidity ranges between 13% and 68%, while annual relative humidity is 31.1%. The rainfall range is between 0 and 320 mm, while the average annual rainfall is 270 mm, which is sub-optimal for good plant growth, and therefore, posing a high risk (25-75%) of crop failure (Sombroek et al., 1982; Jaetzold et al.,

2006). The soils are the sandy ferruginous type of the latosols group, which is highly weathered, markedly laterised, and slightly acidic in reaction to low organic matter content and phosphorous. Its total nitrogen rarely exceeds 0.2% (Abubakar, 2006).

5.2.4 Experimental design and trial

The experiment was for a moderate stress conditions for at least six months of stress (period without rainfall). The 267 F_1 progeny was evaluated for drought tolerance and productivity traits at Minjibir, Kano. The parents (TMS98/0505 and TMS98/0581) and three other elite cassava genotypes were selected for different adaptation levels for dry ecology (TMS91/02324 with low adaptation, TMS30572 with moderate adaptation, and TME419 with high adaptation) and used as check to assess adaptation and drought tolerance in the trial. Cassava cuttings of uniform length (20-30 cm) were planted in an augmented design. The augmented design was used due to the limited supply of planting materials and to permit replication. A single row planting of five plant stands per plot was done. The stakes were placed horizontally in the soil at the recommended 1 m spacing within each row, and 1 m apart between the rows (Ng and Ng, 2002). The trial was established in the dry season and had to be supported with irrigation for the first three months to support homogenous germination, sprouting, and plant establishment. It was then subjected to natural weather conditions (with two months of rainfall) in the ecology during a 12-month growth cycle.

5.2.5 Phenotyping

The mapping population (population B) was established for phenotyping studies, for key traits linked to drought tolerance and high productivity in the dry ecologies. For phenotyping activities, data for several traits was collected on the field. There are several traits thought to be associated with drought tolerance in cassava (El-Sharkawy and Cadavid, 2002; Setter and Fregene, 2007), although it is not known to what extent these traits vary in different genetic backgrounds in driving adaptation and drought tolerance. The following traits were evaluated, based on standard procedures used for phenotyping in cassava:

5.2.5.1 Morphological characters

(As described in Chapter 4)

5.2.5.2 Productivity traits (root yield and related characters)

(As described in Chapter 4)

5.2.6 Genotyping

KASPar SNP markers developed for cassava in another GCP-linked project at the University of Maryland and IITA were validated and subsequently converted to KASPar-based platform. KASPar is flexible and based on competitive allele-specific PCR. KASPar is designed to query one SNP at a time. A total of 2000 SNPs were made available, and 94% (1845) were successfully converted to the LGC system. DNA was extracted from freshly harvested leaves of 256 genotypes of the B population and parents, using the LGC extraction kits. The parents were surveyed for polymorphic SNP markers, and the informative markers were then used to genotype mapping populations at the LGC Genomics Laboratory. Complete details on the principle and procedure of the assay on LGC genotyping high-throughput platforms can be assessed using the link (www.lgcgenomics.com). FlapJack software was used for interactive visualisation of the high-throughput genotype data to check the data quality and marker information across loci for each F_1 genotype and across population per marker. Segregation of the KASPar SNP markers were viewed graphically using the SNP viewer. The markers were tested for goodness of fit to the expected Mendelian ratio following the Chi square test (i.e. test for deviation from expected Mendelian segregation for each marker), and the best segregating and informative markers were used in mapping. The genetic linkage map was calculated with SNP markers using 'CP option' of JoinMap Version 4.1 (Van Ooijen, 2006). JoinMap 4.1 was used to find the order of the markers in the linkage groups. Following the calculation of pairwise recombination frequencies, linkage groups were identified using LoD values ranging from 3 to 7. The Kosambi mapping function was used to calculate centimorgan (cM) distances.

5.2.7 Statistical analysis

The phenotypic data collected was analysed using SAS software (version 9.0). Pearson's phenotypic correlation analysis was used to determine traits that are significantly associated and may aid better understanding of plant performance for drought tolerance and adaptation. Principal component analysis was used to determine the traits that were the main contributors to drought tolerance and productivity traits. Simple statistics using the Excel method were used to assess standard deviation, mean of the traits across the genotypes, and coefficient of variation. Frequency distribution was used to assess variation among the traits and determine the transgressives in the population

5.2.8 Marker-trait analysis and QTL mapping

QTL analysis was initiated with the phenotypic data of population B, starting with a single marker regression analysis using R/QTL (Arends et al., 2010), based on a LoD threshold of ≥ 3.0. Interval mapping (IM) and composite interval mapping with the Bayesian model were used for QTL detection through R/qtl V1.37-11 (Broman 2015). The Haley-Knott regression approach

in R/QTL was also explored for multiple QTL analysis. The peak, map position, confidence interval, estimated effects of the QTLs, interactions among detected QTLs, phenotypic variation (PVE), additive (A) and dominant components for each QTL were determined during the analysis. The D/A ratio of 0-0.20, 0.21-0.80, 0.81-1.20, or > 1.20 was used to determine additive (A), partial dominance (PD), dominance (D) and an over-dominant (OD) mode of gene action, respectively, as described by Stuber et al. (1987). Analysis of the QTLs was carried out using R/qtl software package (Broman *et al.,* 2003).

5.3 Results

5.3.1 Development of the mapping population

The cross between TMS98/0505 and TMS98/0581 (population B parental lines) exhibited good flowering to generate seeds. There was good genetic variation in the plant formation at the nursery. While a high number of the seedlings showed good development, many of the genotypes produced an extremely stunted growth. Once genotypes could support some cutting development (irrespective of their vigour), they were included as part of the population to support the wide genetic variation required for good QTL mapping. The extremely weak genotypes in the nursery, which could not generate enough cuttings to provide an adequate number of plants for QTL mapping, were dropped. Results indicate that over 200 genotypes in population B showed plant development that could have at least 8-10 cuttings, irrespective of its vigour; and they constituted the mapping population used for this study. These genotypes had very good variation in plant development and vigour to support mapping for positive and negative alleles.

5.3.2 Phenotypic data analysis

5.3.2.1 Cassava mosaic disease (CMD)

The mapping population that was evaluated for disease infestation at Umudike at several time intervals (3, 6, 9 MAP) showed that the distribution for CMD resistance was very good for most of the genotypes (above 90%) in the population (Fig. 5.1). The parental lines used for the mapping population were resistant, as typically characterised for the genotypes. A major proportion of the population either showed high resistance or resistance to the disease. Only very few genotypes (less than 5%) were susceptible to CMD, and they were dropped to avoid confounding effects of the disease on plant performance and QTL mapping.

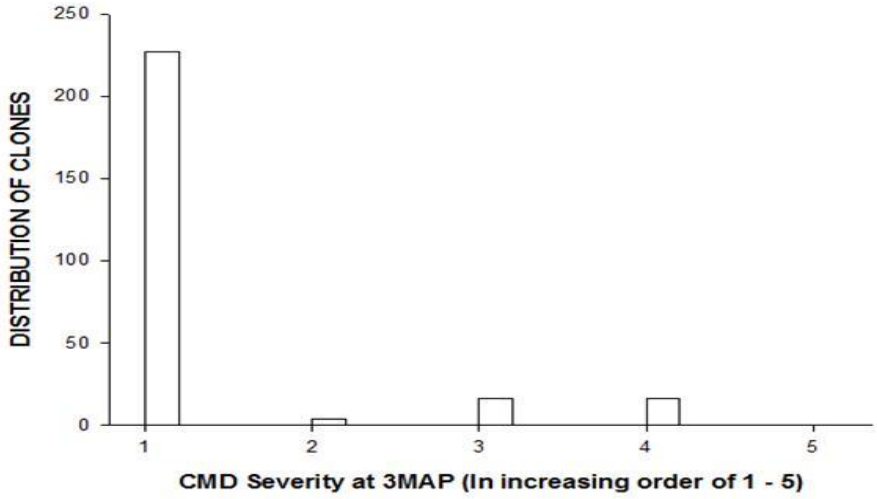


Figure 5.1: Cassava mosaic severity in mapping population B

5.3.2.2 Quantitative variation

The result of the phenotypic statistics, including mean, skewness, minimum, and maximum are shown in Table 5.1. The data suggests that there was good phenotypic variation for the 13 physiological, morphological, and productivity traits evaluated in the population. All ranges of vigour were found in the population (scores from 1 to 5). There was also high variation in plant development as captured, for example, by plant height range showing a height difference of over 200 cm among some genotypes. With respect to productivity traits such as dry-matter content, a big range was also observed (with 24% difference between the lowest and the highest values). Similarly, for dry root yield, differences were as high as 26 t/ha. Results indicated good genetic variation across traits to support QTL mapping. Frequency distribution indicated that some of the traits segregated continuously (Fig. 5.2); and skewness values suggested that some of the traits in the present study were normally distributed and thus suitable for QTL mapping. The standard deviation observed indicated the good degree of dispersion of the measurements for most of the traits. It was also observed that mean values of progeny for most of the traits were higher than those of the parents, except for scar level (Table 5.2).

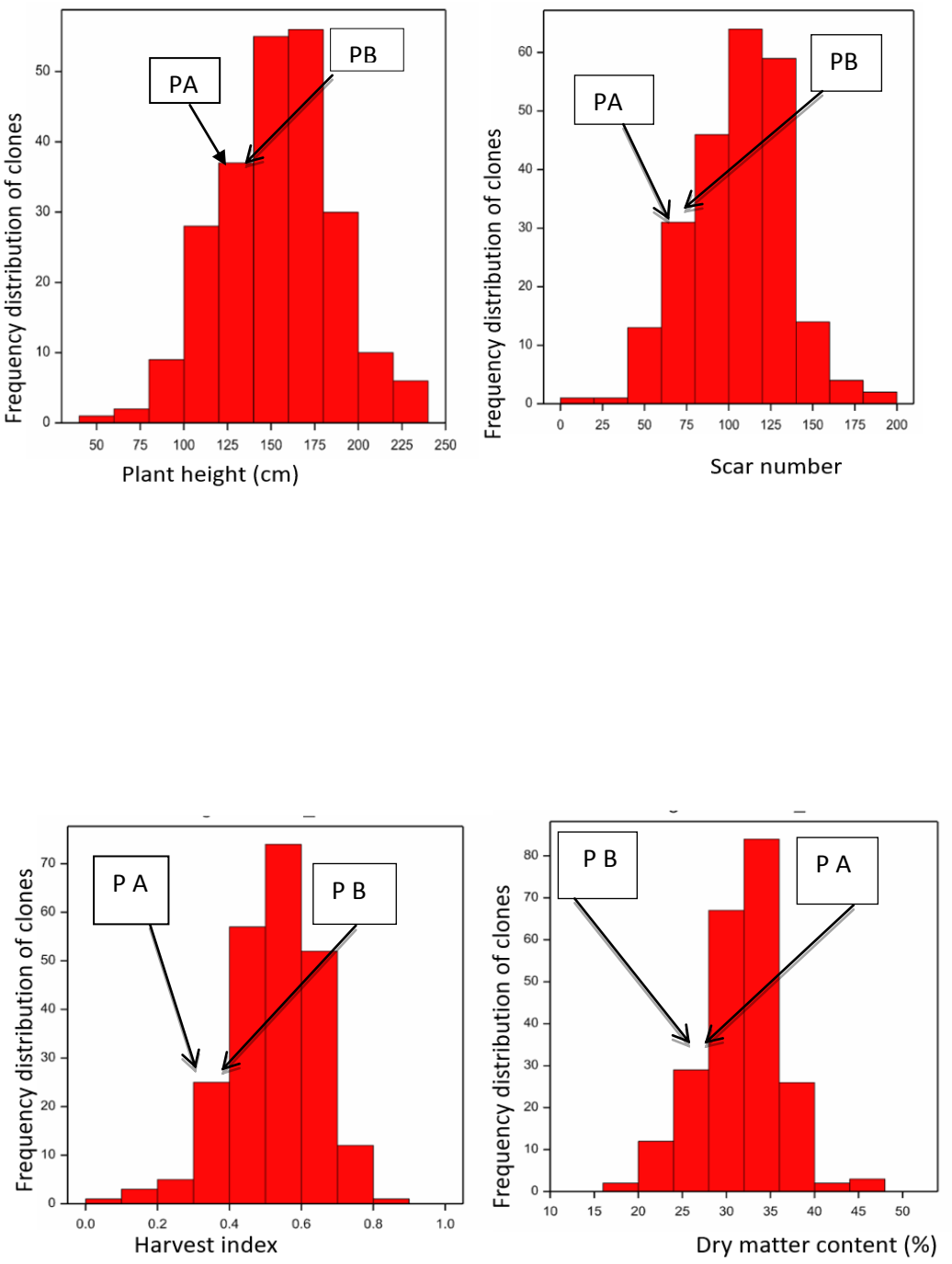


Figure 5.2: Frequency distribution of productivity and morphological traits. PA (parent A) = TMS98/0505; PB (parent B) = TMS98/0581

Table 5.1: Descriptive statistics of physiological and productivity traits population and checks

Traits	Skew	Min	Max	Mean	SD
VIG (1-5)	-0.04	1.00	5.00	3.27	0.91
RTWT (kg)	0.88	0.10	16.00	5.18	3.33
BIOM (kg)	0.70	0.20	14.80	4.43	2.59
RTNO	1.53	1.00	61.00	12.36	7.41
FRY (t/ha)	1.14	0.50	80.00	23.67	14.65
HI	-0.62	0.06	0.86	0.53	0.13
DMC (%)	-0.21	17.47	45.34	31.93	4.55
DYLD (%)	1.25	0.17	26.51	7.67	4.73
SCARNO	-0.38	9.25	186.00	106.41	28.57
NLVS	1.19	0.00	176.67	47.92	33.46
HFB (cm)	2.16	0.00	190.40	47.27	28.21
SCARLEV (cm)	-0.68	0.00	242.40	122.56	60.46
LVRET (%)	1.29	0.40	63.82	20.79	10.22
PLTHT (cm)	-0.02	42.00	255.00	154.01	36.49
WTLV (cm)	1.72	0.00	38.00	9.85	5.82
BLEV	2.35	0.00	79.00	3.14	5.38

*VIG = plant vigour, RTWT = root weight, BIOM = plant total biomass, RTNO = root number. FRY = fresh root yield, HI = harvest index, DMC = dry-matter content, DYLD = dry root yield, SCARNO = number of scars, NLVS = number of leaves, HFB = height at first branching, LVRET = leaf retention.

Table 5.2: Mean values of traits in parents and progeny

Traits	Parents	Progeny
VIG	3.38	3.46
BLEV	1.60	2.10
SCARLEV (cm	112.6	87.99
RTWT (cm)	5.20	15.05
BIOM (kg)	4.46	10.85
RTNO	12.42	27.63
FRY (t/h)	23.99	33.96
HI	0.37	0.53
DMC (%)	27.30	31.33
DYLD (t/h)	7.63	8.77
PLTHT (cm)	123.67	141.82
SCARNO	70.83	74.95
NLVS	75.18	81.98
HFB (cm)	35.72	51.66
LVRET	20.67	26.35

5.3.2.3 Trait correlations

Pearson's correlation coefficient analysis is shown in Table 5.3. Fresh root yield (FRY), which reflects economic productivity, was positively correlated with many traits. It was highly and significantly correlated with dry root yield < 0.0001; r = 0.96). FRY was also moderately and significantly correlated with the harvest index (r = 0.46). The harvest index correlated positively and significantly with productivity trait such as dry-matter content. Dry-matter content, which is a measure of the active utilisable component of productivity, was positively correlated with dry root yield, plant height, length of leafless stem, plant vigour, scar number, branching level, and root number; and all these correlations were significant. Dry root yield, which is the final target of production systems, was positively and significantly correlated with key traits such as total plant biomass, plant vigour, and root number, which may signify breeding selection parameters. Other agronomic traits that were found to be positively correlated with dry root yield include leaf number, length of stem with leaves, and branching level. For morphological expression, correlation analysis identified the traits that were strongly connected. Plant height was strongly and significantly associated with length of leafless stem (r = 0.86), and plant vigour (r = 0.69). Leaf number, which is a measure of the photosynthate generation capacity, was correlated positively with plant vigour, total plant biomass, root weight, and root number. Scar level, which could be a measure of a stress avoidance mechanism (from leaf drop), had a positive correlation with most of the yield-related traits. Root weight, total plant biomass, plant vigour, and root number significantly and positively correlated with each other.

Table 5.3: Trait correlation of physiological and productivity traits in marginal environment

Traits	FRY	HI	DMC	DYLD	PLTHT	SCARNO	LEVNO	SCARLEV	WTLV	WOTL	RTWT	BIOM	VIG	RTNO	CMD	CBB	BRLEV
FRY	1																
HI	0.46***	1															
DMC	0.03ns	0.25***	1														
DYLD	0.96***	0.51***	0.25***	1													
PLTHT	0.05ns	-0.30***	0.15*	-.07ns	1												
SCARNO	0.16**	-0.04ns	0.10ns	0.16ns	0.55***	1											
LEVNO	0.35**	0.07ns	-0.01ns	0.31***	0.26***	0.26***	1										
SCARLEV	-0.02ns	-0.36***	0.15*	0.01ns	0.85***	0.58***	0.18**	1									
WTLV	0.30***	0.03ns	-0.18**	0.22***	-0.01ns	-0.09ns	0.06ns	0.89***	1								
WOTL	-0.04ns	-0.28***	0.27***	0.01ns	0.86***	0.56***	0.19**	-0.17**	-0.24***	1							
RTWT	0.71***	0.15*	-0.03ns	0.64***	0.24***	0.24***	0.21***	0.15*	0.30***	0.10ns	1						
BIOM	0.37***	-0.36***	-0.08ns	0.30***	0.48***	0.30***	0.22***	0.41***	0.24***	0.39***	0.74***	1					
VIG	0.18**	-0.20**	0.20**	0.22***	0.69***	0.49***	0.31***	0.66***	-0.02ns	0.28***	0.30***	0.54***	1				
RTNO	0.31***	0.03ns	0.12*	0.31***	0.35***	0.26***	0.20***	0.31***	0.06ns	0.73***	0.65***	0.64***	0.43***	1			
CMD	-0.09ns	-0.02ns	0.061ns	-0.08ns	0.02ns	0.011ns	-0.01ns	0.05ns	-0.05ns	0.04ns	-0.11ns	-0.07ns	0.02ns	-0.02ns	1		
CBB	-0.22ns	-0.17ns	-0.08ns	-0.22ns	-0.14*	-0.17**	-0.27***	-0.03ns	-0.09ns	-0.08ns	-0.23***	-0.17**	-0.16**	-0.18**	0.09ns	1	
BRLEV	0.20**	-0.02ns	0.06ns	0.20**	0.22***	0.14*	0.33***	0.17**	0.03ns	0.20**	0.15*	0.37***	0.37***	0.15*	0.09ns	-0.01ns	1

*FRY = fresh root weight (t/ha), HI = harvest index, DMC = dry natter content (%), DYLD = dry root yield (%), SCARNO = scar number, SCARLEV = scar level, WTLV = length of stem with leaves (cm), WOTL = length of leafless stem (cm), RTWT = root weight (kg), BIOM = total plant biomass (kg), VIG = plant vigour, CMD = cassava mosaic disease, CBB = cassava bacterial blight, BRLEV = branching level; NS is not significance, *, **, *** is significant at 5, 1 & 0.1%.

5.3.2.4 Principal component analysis

The principal component analysis for measured traits is shown in Tables 5.4 and 5.5. Results based on five PCAs accounted for at least 74% of the variation observed for physiological and 100% for productivity traits (Table 5.4 and 5.5). The PC1 for physiological traits accounted for 30% of the variation, with scar level length of the portion of the stem without leaves being the main contributor. PC2 accounted for 17% of the variation with leaf retention and stem portion with leaves as the major contributors, while PC3 accounted for 11% variation with leaf number and branching level as the main contributors. PC4 contributed 8% of variation with plant height being the main factor, while PC5 similarly explained 8% variation of plant vigour and leaf number being the main contributors. PCA results for the productivity traits are shown in Table 5.5. Results indicated that the first five PCs adequately accounted for 99% of the variation. PC1 in productivity traits accounted for 54% of the variation and identified fresh root yield and dry root yield as the main contributors, while PC2 had root number and dry-matter content as the main contributors, accounting for 20% variation. Root number, harvest index, and dry-matter content were the major contributors with 14% variation for PC3. PC4 contributed 8% of variation, with the harvest index being the main factor, while PC5 had root number contributing with 3% variation.

Table 5.4: Principal component analysis of physiological traits in population B and checks

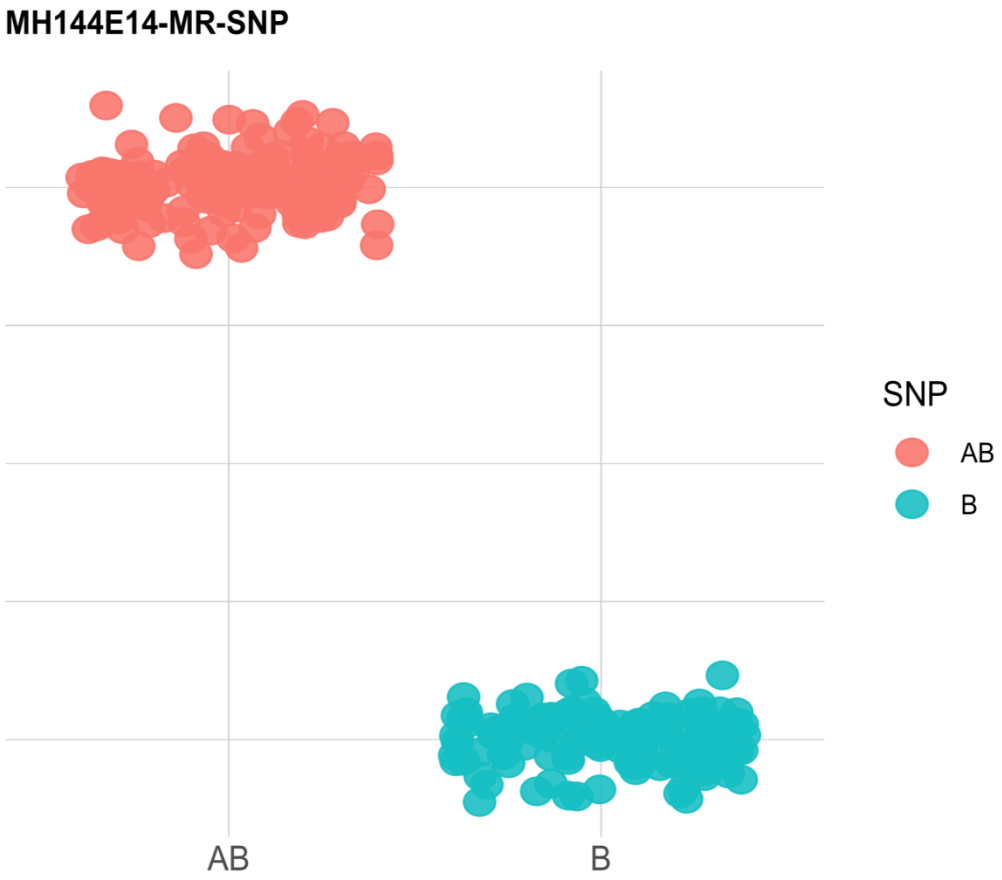
Traits	PC1	PC2	PC3	PC4	PC5
VIG	-0.28	0.14	-0.04	0.22	0.50
PLTHT (cm)	-0.19	-0.12	0.21	-0.90	0.18
SCARNO	-0.39	0.04	0.13	-0.01	-0.36
NLVS	-0.11	0.19	-0.53	-0.20	-0.63
HFB (cm)	-0.32	0.14	0.30	0.15	-0.22
BRLEV	-0.19	0.26	-0.53	-0.10	0.31
SCARLEV (cm)	-0.42	0.00	0.22	0.15	-0.08
WTLV (cm)	-0.02	0.67	0.25	-0.18	0.04
LVRET	0.26	0.62	0.12	0.04	0.00
Eigen value	1.82	1.37	1.10	0.96	0.96
Eigen values as proportion of total variance	0.30	0.17	0.11	0.08	0.08
Cummulative % total variance	0.30	0.47	0.58	0.66	0.74

Table 5.5: Principal component analysis of productivity traits in population B and checks

Traits	PC1	PC2	PC3	PC4	PC5
RTNO	-0.28	-0.59	0.53	0.30	-0.44
FRY (t/h)	-0.52	0.23	-0.03	-0.31	-0.22
HI	-0.38	0.12	-0.52	0.76	-0.03
DMC (%)	-0.12	-0.67	-0.62	-0.38	-0.03
DRY (t/h)	-0.50	0.33	0.03	-0.31	-0.30
Eigen value	1.79	1.10	0.93	0.71	0.42
Eigen values as proportion of total variance	0.54	0.20	0.14	0.08	0.03
Cummulative % total variance	0.54	0.74	0.88	0.96	0.99

5.3.2.5 Genotyping and construction of genetic linkage map

Genotyping of the mapping population with KASPar SNP array resulted in polymorphic markers showing either 1:1 or 3:1 segregation (Fig. 5.3). The polymorphic markers for the cross combination (parental pairs) selected for the development of mapping populations were also estimated to determine the total number of informative markers to be used for QTL mapping in the family B mapping population. Results of the parental survey with SNPs indicated that the number of polymorphic markers in each parent was between 522 and 576 markers, while that of the cross was 856 markers (Table 5.6). After removing ambiguous and unlinked markers, the genetic linkage map was constructed with 505 markers, with fairly good distribution and coverage across all 21 linkage groups (synonymously used as chromosome in this paper) spanning 1582.8 cM in length, with an average marker density of 3.69 cM (Fig. 5.4). Linkage group (LG) 1 was the longest (143.7 cM), while LG 20 was the shortest (22.5 cM). The number of markers per linkage groups ranged between 6 and 48 markers, while the length of the linkage groups ranged between 25.6 cM and 143.7 cM, with inter-marker distance ranging between 1.92 cM and 6.69 cM.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B
AB
B

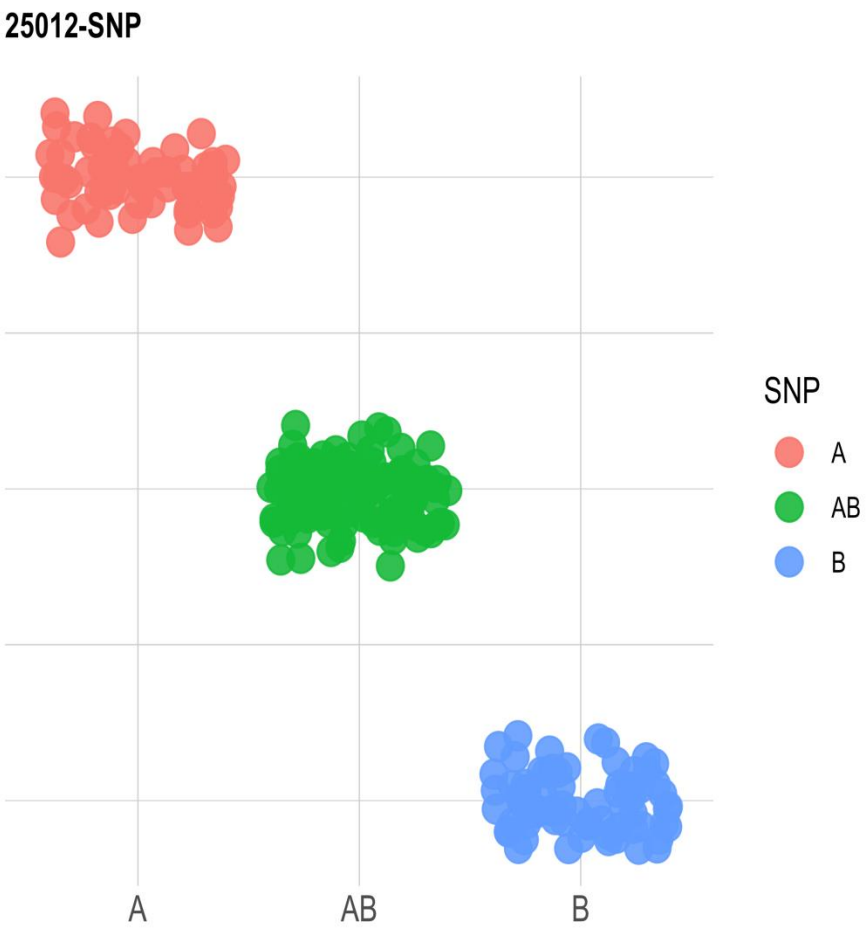
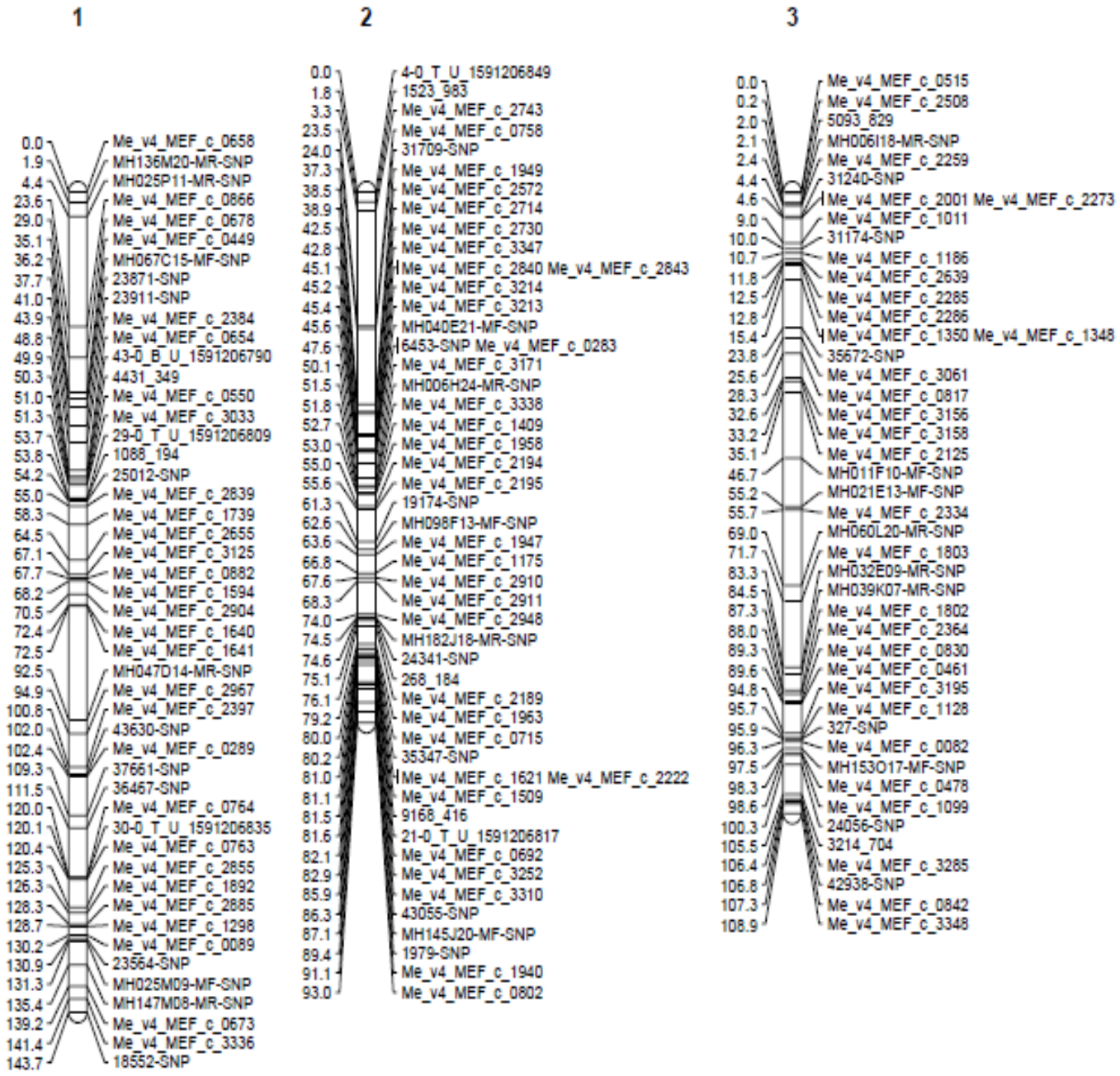


Figure 5.3: Marker segregation types in mapping population B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23.6 Me_v4_MEF_c_0866
29.0 Me_v4_MEF_c_0678
35.1 Me_v4_MEF_c_0449
36.2 MH067C15-MF-SNP
37.7 23871-SNP
41.0 23911-SNP
43.9 Me_v4_MEF_c_2384
48.8 Me_v4_MEF_c_0654
49.9 43-0_B_U_1591206790
50.3 4431_349
51.0 Me_v4_MEF_c_0550
51.3 Me_v4_MEF_c_3033
53.7 29-0_T_U_1591206809
53.8 1088_194
54.2 25012-SNP
55.0 Me_v4_MEF_c_2839
58.3 Me_v4_MEF_c_1739
64.5 Me_v4_MEF_c_2655
67.1 Me_v4_MEF_c_3125
67.7 Me_v4_MEF_c_0882
68.2 Me_v4_MEF_c_1594
70.5 Me_v4_MEF_c_2904
72.4 Me_v4_MEF_c_1640
72.5 Me_v4_MEF_c_1641
92.5 MH047D14-MR-SNP
94.9 Me_v4_MEF_c_2967
100.8 Me_v4_MEF_c_2397
102.0 43630-SNP
102.4 Me_v4_MEF_c_0289
109.3 37661-SNP
111.5 36467-SNP
120.0 Me_v4_MEF_c_0764
120.1 30-0_T_U_1591206835
120.4 Me_v4_MEF_c_0763
125.3 Me_v4_MEF_c_2855
126.3 Me_v4_MEF_c_1892
128.3 Me_v4_MEF_c_2885
128.7 Me_v4_MEF_c_1298
130.2 Me_v4_MEF_c_0089
130.9 23564-SNP
131.3 MH025M09-MF-SNP
135.4 MH147M08-MR-SNP
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141.4 Me_v4_MEF_c_3336
143.7 18552-SNP
2
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24.0 31709-SNP
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38.5 Me_v4_MEF_c_2572
38.9 Me_v4_MEF_c_2714
42.5 Me_v4_MEF_c_2730
42.8 Me_v4_MEF_c_3347
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45.2 Me_v4_MEF_c_3214
45.4 Me_v4_MEF_c_3213
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52.7 Me_v4_MEF_c_1409
53.0 Me_v4_MEF_c_1958
55.0 Me_v4_MEF_c_2194
55.6 Me_v4_MEF_c_2195
61.3 19174-SNP
62.6 MH098F13-MF-SNP
63.6 Me_v4_MEF_c_1947
66.8 Me_v4_MEF_c_1175
67.6 Me_v4_MEF_c_2910
68.3 Me_v4_MEF_c_2911
74.0 Me_v4_MEF_c_2948
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74.6 24341-SNP
75.1 268_184
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79.2 Me_v4_MEF_c_1963
80.0 Me_v4_MEF_c_0715
80.2 35347-SNP
81.0 Me_v4_MEF_c_1621 Me_v4_MEF_c_2222
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82.9 Me_v4_MEF_c_3252
85.9 Me_v4_MEF_c_3310
86.3 43055-SNP
87.1 MH145J20-MF-SNP
89.4 1979-SNP
91.1 Me_v4_MEF_c_1940
93.0 Me_v4_MEF_c_0802
3
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10.0 31174-SNP
10.7 Me_v4_MEF_c_1186
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12.5 Me_v4_MEF_c_2285
12.8 Me_v4_MEF_c_2286
15.4 Me_v4_MEF_c_1350 Me_v4_MEF_c_1348
23.8 35672-SNP
25.6 Me_v4_MEF_c_3061
28.3 Me_v4_MEF_c_0817
32.6 Me_v4_MEF_c_3156
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46.7 MH011F10-MF-SNP
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71.7 Me_v4_MEF_c_1803
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95.9 327-SNP
96.3 Me_v4_MEF_c_0082
97.5 MH153O17-MF-SNP
98.3 Me_v4_MEF_c_0478
98.6 Me_v4_MEF_c_1099
100.3 24056-SNP
105.5 3214_704
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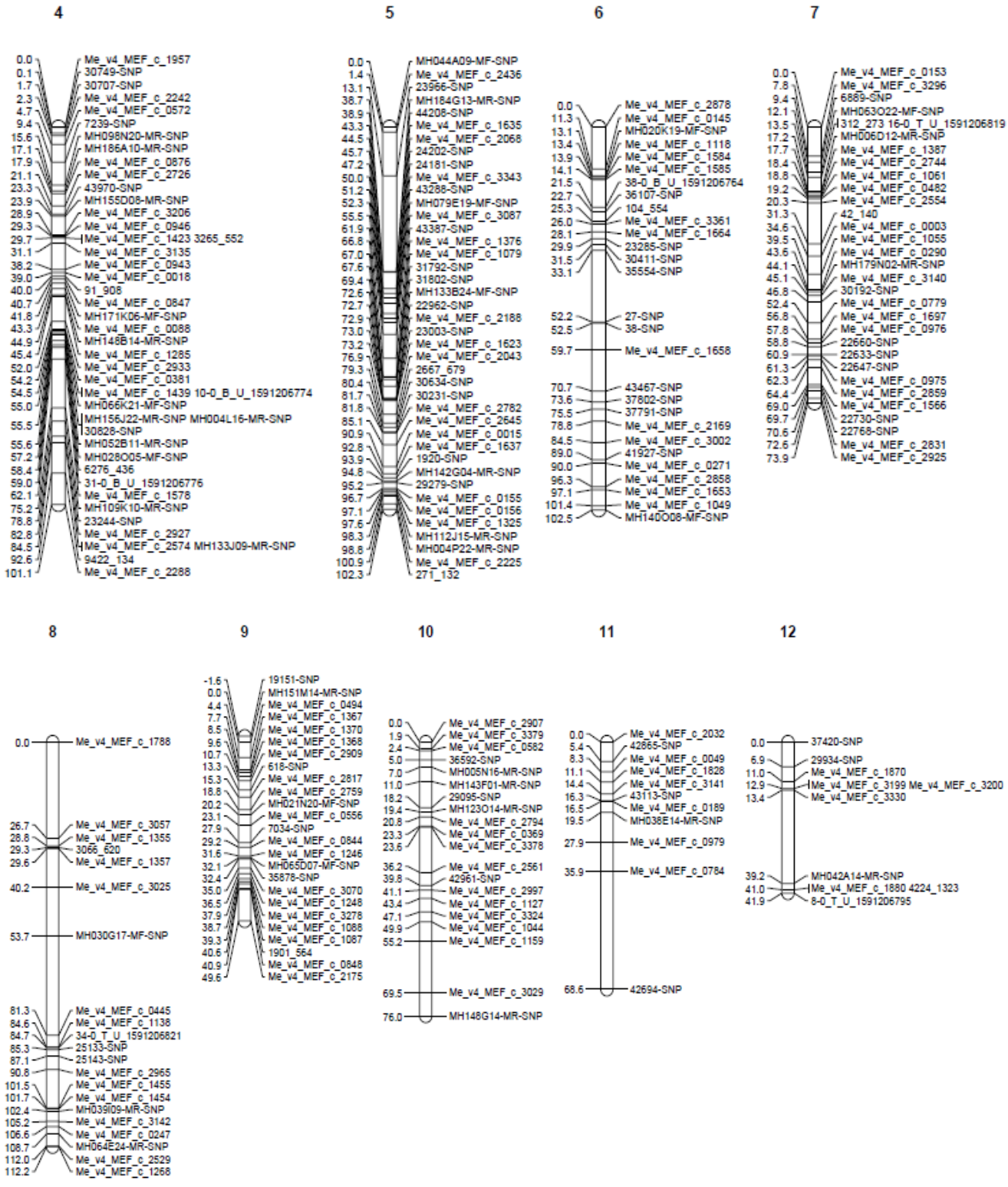
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9.4 7239-SNP
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17.1 MH186A10-MR-SNP
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21.1 Me_v4_MEF_c_2726
23.3 43970-SNP
23.9 MH155D08-MR-SNP
28.9 Me_v4_MEF_c_3206
29.3 Me_v4_MEF_c_0946
29.7 Me_v4_MEF_c_1423 3265_552
31.1 Me_v4_MEF_c_3135
38.2 Me_v4_MEF_c_0943
39.0 Me_v4_MEF_c_0018
40.0 91_908
40.7 Me_v4_MEF_c_0847
41.8 MH171K06-MF-SNP
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44.9 MH148B14-MR-SNP
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52.0 Me_v4_MEF_c_2933
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54.5 Me_v4_MEF_c_1439 10-0_B_U_1591206774
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130828-SNP
55.6 MH052B11-MR-SNP
57.2 MH028O05-MF-SNP
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75.2 MH109K10-MR-SNP
78.8 23244-SNP
82.8 Me_v4_MEF_c_2927
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5
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31.5 30411-SNP
33.1 35554-SNP
52.2 27-SNP
52.5 38-SNP
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70.7 43467-SNP
73.6 37802-SNP
75.5 37791-SNP
78.8 Me_v4_MEF_c_2169
84.5 Me_v4_MEF_c_3002
89.0 41927-SNP
90.0 Me_v4_MEF_c_0271
96.3 Me_v4_MEF_c_2858
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101.4 Me_v4_MEF_c_1049
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13.5 312_273 16-0_T_U_1591206819
17.2 MH006D12-MR-SNP
17.7 Me_v4_MEF_c_1387
18.4 Me_v4_MEF_c_2744
18.8 Me_v4_MEF_c_1061
19.2 Me_v4_MEF_c_0482
20.3 Me_v4_MEF_c_2554
31.3 42_140
34.6 Me_v4_MEF_c_0003
39.5 Me_v4_MEF_c_1055
43.6 Me_v4_MEF_c_0290
44.1 MH179N02-MR-SNP
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56.8 Me_v4_MEF_c_1697
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10.7 Me_v4_MEF_c_2909
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38.7 Me_v4_MEF_c_1088
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11.0 MH143F01-MR-SNP
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23.3 Me_v4_MEF_c_0369
23.6 Me_v4_MEF_c_3378
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39.8 42961-SNP
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55.2 Me_v4_MEF_c_1159
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11
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16.3 43113-SNP
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27.9 Me_v4_MEF_c_0979
35.9 Me_v4_MEF_c_0784
68.6 42694-SNP
12
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6.9 29934-SNP
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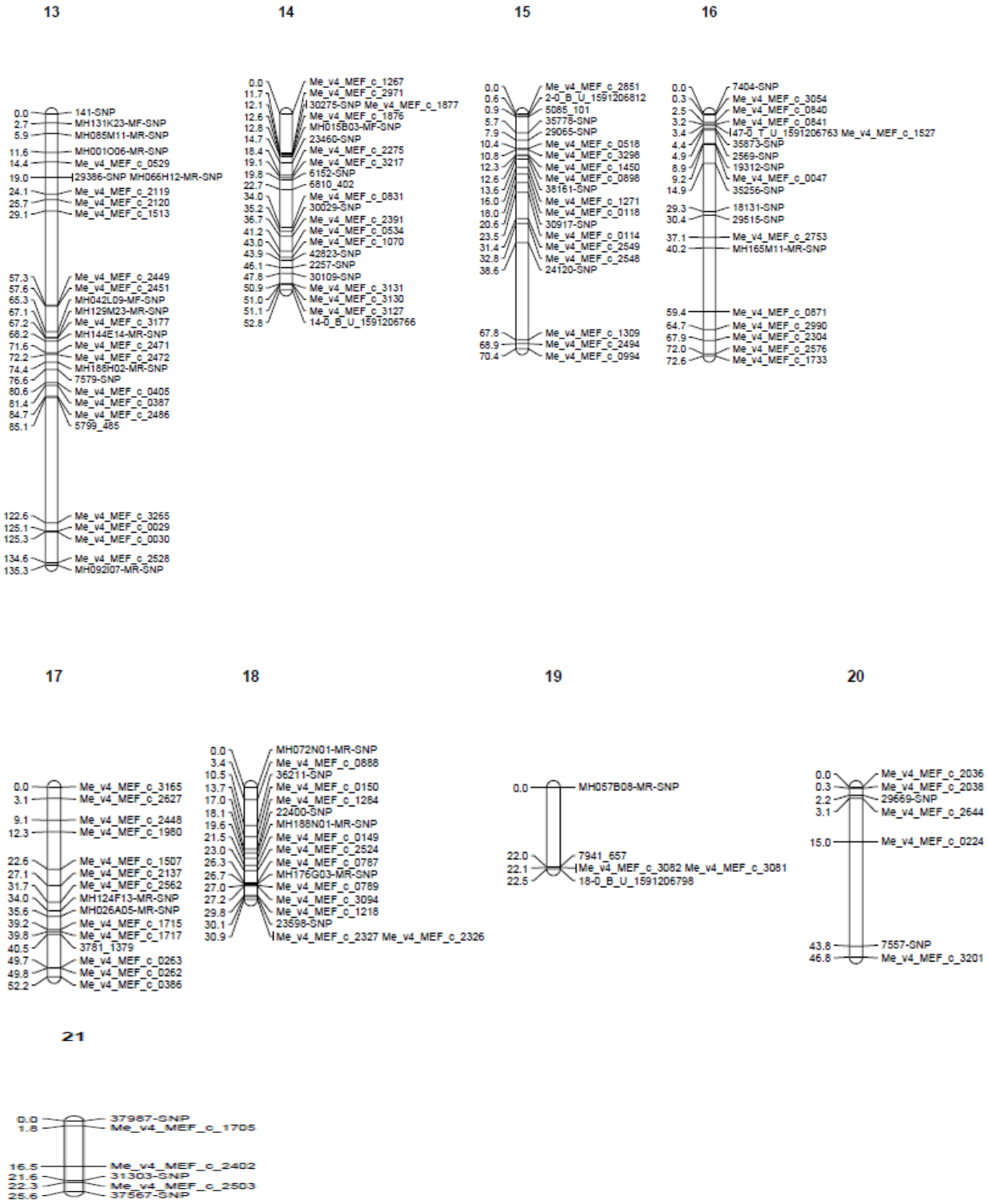


Figure 5.4: Genetic Linkage Map of cassava for mapping population B

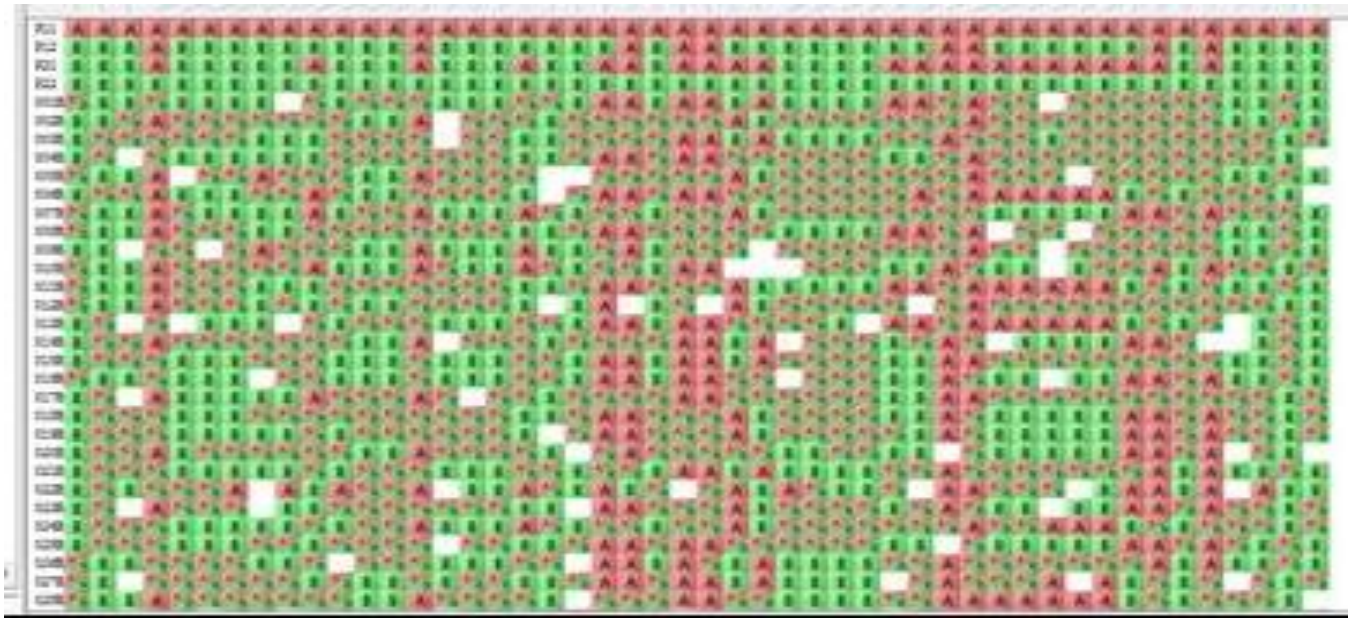


Figure 5.5: A Flapjack representation of marker data for SNP genotyping in Population B

Table 5.6: Number of polymorphic markers per parent and cross

Parents/Cross	Polymorphic markers
TMS98/0505	567
TMS98/0581	522
TMS98/0505 x TMS98/0581 (POP B)	856

5.3.2.6 QTL identification in year 1

Marker-trait linkage and composite interval analysis of population B resulted in the identification of evaluated traits associated with adaptation for drought stress and productivity for the marginal environment targeted for this study. In total, 20 QTLs were identified in 11 linkage groups (LGs 1, 2, 3, 4, 5, 6,7,10, 13, 18, and 21). Significant peak values of LoD scores (LoD ≥ 3), the position of these peaks, the percentage phenotypic variance explained, and the estimated gene actions are shown in Table 5.7. The number of QTLs identified per linkage group ranged between 1 and 5. The identified QTLs were found in11 traits. Results indicated that dry root yield had the highest number of detected QTLs in this analysis.

5.3.2.6.1 Physiologically implied traits

5.3.2.6.1.1 Plant height

A trait that is often linked to growth was found in this study, and analysis resulted in the identification of one QTL (*c18.loc19*), implying that the QTL was located on chromosome 18 at 19 cM. The QTL accounted for 7.298% of the phenotypic variation. The allele influencing this trait (positive effect and significant at P ≤ 0.01) stemmed from TMS98/0505. The QTL showed an over-dominance gene action.

5.3.2.6.1.2 Height at first branching (HFB)

Height at first branching principally defines the architectural outlook of the plant and could condition the amount of foliage associated with plant development. Results indicated HFB was under the control of three QTLs, with additive effects found on three different chromosomes (1, 3, and 7). The phenotypic variation explained by these QTLs ranged between 4.5% and 8%. The alleles significantly influencing HFB originated from the parent TMS98/0505. The gene action for this trait was consistently additive, but with a negative effect showing that these QTLs reduced height at first branching. TMS98/0505 is typically a low to medium HFB variety.

5.3.2.6.1.3 Branching level (BRLEV)

Analysis identified two QTLs (*C13.loc11.6* and *C21.loc0 cM*) located on LG 13 and 21. The two QTL alleles responsible for the increased phenotypic values for this trait in the population stemmed from both parents (i.e. one QTL from either parent). The allele for *C13.loc11.6* originated from TMS98/0581, and was mapped in the interval mk381-mk383, which explained 19.19% of phenotypic variance, thus making it a major QTL. The allele in respect of *c21.loc0* originated from TMS98/0505, and similarly explained 20.42% of phenotypic variance observed, making it a second major QTL for this trait. The two QTLs detected for branching level

expressed different gene actions. QTL *C13.loc11.6* showed over-dominance gene action, while *C21.loc0.0* showed additive gene action.

5.3.2.6.1.4 Leaf retention (LR)

Two QTLs located on chromosome 6 and 21 were significantly associated with LR at highly significant P-values (Table 5.7). The phenotypic variations explained by the QTLs were similar (6% for *c6.loc31*) and 6.8% for *c21.loc0.0*). The QTL *c6.loc31* expressed additive effects, while the second QTL (*C21.loc0.0*) showed dominance gene action.

5.3.2.6.1.5 Scar level (SCARLEV)

This trait revealed several QTLs in the population. Three regions representing different linkage groups were found to be significantly linked to this trait. The LGs include 2, 5, and 6. The LoD scores ranged between 3.25 and 3.61. The PVE was small ranging between 2.3% and 3.07%. Except for QTL *c6.loc0.0,* which had a dominant gene action, the other 2 QTLs (*c2.loc.86* and *c5.loc93.9*) were additive in gene action. The three QTL alleles, influencing trait expression, originated from TMS98/0505. The three QTLs increased scar levels.

5.3.2.6.1.6 Plant vigour (VIG)

One QTL significantly associated with plant vigour was found on LG 3. The phenotypic variation accounted for by the QTL was 7.16%. The QTL allele influencing plant vigour was from parent plant TMS98/0505. The QTL detected for plant vigour showed additive gene action.

5.3.2.6.2 Productivity traits

5.3.2.6.2.1 Dry-matter content (DMC)

This trait revealed one QTL in the mapping population. The QTL (*c3.loc67*) controlling DMC was located in LG 3, and accounted for 7.67% of phenotypic variation. The QTL exhibited additive gene action and increased DMC. The favourable allele was derived from genotype TMS98/0505.

5.3.2.6.2.2 Dry root yield (DRY)

Five different regions of the genome were found to be significantly associated with this trait. The QTLs were found on LGs 1, 3, 4, 7, and 10. The phenotypic variance explained (PVE) by these QTLs ranged between 3.3 and 5.9%. Three QTLs (*c3.loc68, c4.loc57*, and *c7.loc13*) decreased dry root yield; and the alleles involved were derived from TMS98/0581. Two other QTLs (*c1.loc54* and *c10.loc2.37*) increased dry root yield, with the favourably alleles coming from parent line TMS98/0505. Except for QTL *c7.loc13*, which had additive gene action, the other

QTLs exhibited either dominance or over-dominance gene action (Table 5.7). Although results indicate dominance gene action for *c3.loc68*, the possibility of additive gene action for this QTL could also not be excluded.

5.3.2.6.2.3 Fresh root yield (FRY)

Three significant QTLs were found for this trait and mapped to the same LGs (1, 4, and 7) as with DRY. The phenotypic variation explained by the QTLs ranged from 2.83 to 6.795%. The alleles driving yield were from TMS98/0505. All three (loci) QTLs had decreasing effects. As with dry root yield, three gene actions were associated with this trait. Two QTLs had an additive effect, while one QTL each was found having the dominant gene effect (Table 5.7). The QTL *c1.loc54* tended to show additivity, and thus, one could not rule out the possibility of a dominant gene effect at this locus. Although *c4.loc57.17* tended to show dominance gene effects, additive gene effects could not be excluded in the QTL. Results also showed that QTL *c7.loc13* was additive, but dominance could not be excluded.

5.3.2.6.2.4 Harvest Index

Four QTLs significantly associated with the harvest index were identified in LGs 2, 3, 4, and 5. The phenotypic variance explained by these QTLs ranged between 3.8 and 5.42%. TMS 98/0505 contributed QTL alleles that increased the harvest index at two loci (*c3.loc84* and *c5.loc64*), with both having additive effects. The other two loci (*c2.loc84* and *c4.loc57*) had alleles (coming from TMS98/0581) that decreased the harvest index. QTL *c4.loc57* influenced this trait through its additive gene action, while *c4.loc57* expressed over-dominance effects. Thus, three of the four QTLs identified for the harvest index possessed additive gene action. Results showed that QTL *c2.loc84* was indicative of additive gene action, while a dominant gene action cannot be excluded. Although QTL *c4.loc57* was identified for an over-dominance gene action, additive gene action could not be excluded.

5.3.2.6.2.5 Root number

Two QTLs located in LGs 3 (c3.loc70) and 7 (*c7.loc13*) were identified for root number. The phenotypic variance explained by *c3.loc70* was 7%, while *c7.loc13* has a PVE of 5.5%. The two loci increased the root number, with the QTL on LG3 having an additive effect, while the QTL on LG 7 had an over-dominance effect. The QTL alleles influencing this trait were from parent genotype TMS98/0505.

Table 5.7: Composite interval analysis year 1

Trts[a]	QTL[b]	Chro[c]	Flanking markers	position	LoD	Add[d]	Dom[e]	Gene action	%PVE[f]	P-value[g]
Plant height	*c18.loc19*	18	mk491-mk496	19.00	3.85	6.27	36.48	OD	7.296	0.0002***
Height at first branching	*c1.loc68*	1	mk005-mk026	68.0	3.06	1.72	-19.50	A	4.495	0.002**
	c3.loc15	3	mk113-mk116	15.0	3.44	7.99	-14.67	A	4.865	0.001**
	c7.loc35	7	mk272-mk274	35	4.77	83.49	-77.66	A	7.802	2.88e-05***
Branching level	*c13.loc11.6*	13	mk381-mk383	11.6	25.85	-0.10	-20.08	OD	19.19	< 2e-16***
	c21.loc0.0	21	mk515-mk516	0.0	26.91	829.48	-829.83	A	20.42	< 2e-16***
Dry-matter cont.	*c3.loc67*	3	mk122-mk126	67	3.89	1.9452	-1.5500	A	7.67	0.0001***
Dry root yield	*c1.loc54*	1	mk015-mk035	54	3.01	1.62	1.00	PD	3.320	0.007678**
	c3.loc68	3	mk124-mk128	68	4.21	-1.16	-0.94	D	3.703	0.004440**
	c4.loc57.2	4	mk175-mk181	57.16	4.48	-1.27	-2.42	OD	5.940	0.0002***
	c7.loc13	7	mk263-mk264	13.00	4.15	-1.59	7.37	A	4.390	0.002**
	c10.loc2.4	10	mk339-mk341	2.4	3.63	0.31	18.88	OD	3.964	0.003**
Fresh root yield	*c1.loc54*	1	mk001-mk035	54	3.09	-3.85	-1.06	A	2.829	0.02 *
	c4.loc57.2	4	mk175-mk181	57.2	4.87	-4.32	-4.64	D	6.795	6.7e-05***
	c7.loc13	7	mk263-mk264	13.0	4.56	-4.31	23.48	A	4.619	0.0013**
Harvest index	*c2.loc84*	2	mk083-mk095	84.0	3.38	-0.01	0.079	A	3.909	0.004**
	c3.loc84	3	mk124-mk145	84.0	3.84	0.01	-0.074	A	3.805	0.0046**
	c4.loc57	4	mk179-mk181	57.0	4.83	-0.02	-0.14	OD	5.392	0.0005***
	c5.loc64	5	mk204-mk205	64	4.35	0.016	-0.170	A	3.946	0.0037**
Leaf retention	*c6.loc31*	6	mk243-mk245	31	3.52	-0.003	0.033	A	6.044	0.0004***
	c21.loc0	21	mk515-mk516	0	3.95	2.814	2.806	D	6.838	0.0002***
Root number	*c3.loc7.0*	3	mk124-mk129	7	3.87	0.189	-6.390	A	7.044	0.00108**
	c7.loc13	7	mk263-mk264	13.0	3.21	2.638	9.841	OD	5.468	0.00480**
Scarlevel	*c2.loc86*	2	mk093-mk096	86.0	3.61	19.99	-39.42	A	2.77	0.017*
	C5.loc93.9	5	mk219-mk223	93.9	3.31	20.48	-37.30	A	3.07	0.016*
	C6.loc0.0	6	mk232-mk254	0.0	3.25	198.36	185.89	D	2.30	0.035*
Plant vigour	*c3.loc63*	3	mk124-mk141	63.0	3.78	0.05	-0.97	A	7.16	0.00018***

*a = Traits, b = individual QTLs, c = chromosome where the markers qtls are located, d = Additive gene effects, e = dominant gene effects, f = marker significantly associated with trait variation, g= probability of the association between a QTL and marker. When a QTL-marker association is significant at more than one trial the most significant P-value is declared and corresponding PVE and phenotypic effects of QTLs are given. Gene action was estimated by (d)/(a), OD = over-dominance, D = dominance, A = additive, PD = partial dominance.

5.3.2.7 QTLs for multiple traits

Results indicated that several QTLs identified were for more than one trait. Most of the traits identified with this phenomenon were dry-matter content, fresh root yield, and harvest index. For example, QTL *c4.loc57.16* was found to be associated with dry root yield, and fresh root yield. Similarly, *c7.loc.13* was also observed for fresh root yield, root number, and dry root yield. QTL *c21.loc0.0* involved branching level and leaf retention.

Table 5.8: Intervals in which one or more QTLs controlling more than one trait were detected in year 1

Interval	QTL	Linkage group	Traits
mk515-mk516	*C21.loc0.0*	21	BRLEV, LVRET
mk263-mk264	*C7.loc13*	7	RN, FRY, DRY
mk175-mk181	*C4.loc57.2*	4	DRY, FRY

BRLEV = branching level; LVRET = leaf retention; RN = root number; FRY = fresh root yield; DRY = dry root yield

5.3.2.8 Digenic interactions among detected QTL

Five significant digenic interactions were detected with the LoD values and the interaction effect of each pair of interactions being significant. The digenic interactions included additive x additive interaction effect, over-dominance x additive effect, additive x dominance interactive effect, dominant and over-dominant interactive effect, and over-dominance and over-dominance interactive effects (Table 5.9). The phenotypic variance explained by each digenic interaction, ranging between 3.98% and 35.15%, with branching level had the biggest effect.

Table 5.9: Digenic interactions among detected QTL

Trait	QTL interactions	PVE%	F value	Gene action
Branch level	13@11.6:21@0.0	35.15	435.3***	OD*A
Height at first branching	1@68.0:3@15.0	5.88	4.375***	A*A
	3@15.0:7@34.6	4.019	2.916*	A*A
Dry root yield	3@68.0:10@2.4	5.23	4.16**	D*OD
	4@57.2:10@2.4	4.23	3.31*	OD*OD
Leaf retention	6@31.0:21@0.0	3.98	2.72*	A*D
Scar level	3@97.5:21@18.0	4.45	3.44**	A*A

5.3.2.9 QTL identification in year 2

In the second year, marker-trait linkage and composite interval analysis of population B identified 30 QTLs in 12 linkage groups (LGs 1, 2, 3, 4, 5, 6, 7,9,10, 14, 16, and 20). Significant peak values of LoD scores (LoD ≥ 3), the position of these peaks, the percentage phenotypic variance explained, and the estimated gene actions are shown in Table 5.8. The number of QTLs identified per linkage group ranged between 1 and 4. The identified QTLs were found in 13 traits.

5.3.2.9.1 Physiologically implied traits

5.3.2.9.1.1 Plant height

Analysis identified two QTLs (*c2loc45.2* and *c3loc98.0*) linked to plant height. The two QTLs were located in chromosomes 2 and 3 at 45.2 and 98.0 cM, respectively. The QTLs accounted for 4.65% and 5.58%, respectively, of the phenotypic variation. The allele influencing this trait (positive effect and significant at P ≤ 0.01) was from TMS98/0505. The QTL showed an over-dominance gene action.

5.3.2.9.1.2 Height at first branching (HFB)

Results indicated that four QTLs were found on four chromosomes (2, 3, 6, and 9). The phenotypic variation explained by these QTLs ranged between 2.35% and 6.05%. The alleles significantly influencing HFB originated from the parent TMS98/0505. The gene actions for this

trait were additive, dominance, and over-dominance. The QTL *c6.loc 0.00* had a dominant gene effect, but an additive effect may not be excluded.

5.3.2.9.1.3 Branching level (BRLEV)

Analysis identified three significant QTLs (*c3.loc100.3*, *c4.loc54.2,* and *c10.loc4.0*) located on LG 3, 4, and 10. The two QTL alleles, *c3.loc100.3* and *c4.loc54.2*, responsible for increased phenotypic values for this trait in the population were from TMS980505, while the allele c10.loc4.0, which reduced the branching level, originated from TMS980581. The two QTLs (*c3.loc100.3* and *c4.loc54.2*) detected for branching level expressed an over-dominant gene action, while *c10.loc4.0* showed an additive gene action. Although results indicated additive gene action for *c10.loc4.0*, the possibility of dominance gene action for this QTL could also not be excluded.

5.3.2.9.1.4 Scar level (SCARLEV)

One QTL significantly associated with scar level was found on LG 2. The phenotypic variation accounted for by the QTL was 4.54. The QTL allele influencing the scar level was from parent plant TMS98/0505. The QTL detected for scar level showed an additive gene action.

5.3.2.9.1.5 Plant vigour

One QTL significantly associated with plant vigour was found on LG 3. The phenotypic variation accounted for by the QTL was 7.16%. The QTL allele influencing plant vigour was from parent plant TMS98/0505. The QTL detected for plant vigour showed an additive gene action.

5.3.2.9.1.6 Number of scars

Three significant QTLs were found for this trait and mapped to LGs 5, 10, and 14. The phenotypic variation, explained by the QTLs, ranged between 4.9% and 5.39%. The alleles driving yield were from parent genotype TMS98/0505. Two (loci) QTLs *c5.loc73.2* and *c14.loc33.0* had increasing effects from parent TMS980505, while the allele *c10.loc0.0* had a decreasing effect from the parent TMS980581. Two gene actions were associated with this trait. Two QTLs had an over-dominant effect, while one QTL each was found for the additive gene effect (Table 5.8).

5.3.2.9.1.7 Width leaf

This defines the length of the leafy part of cassava stem. Four QTLs significantly associated with the length of leafy part of the stem were identified in LGs 2, 3, 7, and 10. The phenotypic

variance explained by these QTLs ranged from 2.31 to 9.08%. Parent genotype TMS 98/0505 contributed QTL alleles, which increased the length of the leafy part of the stem at two loci *(c3.loc68* and *c3.loc84.5).* The other two loci *(c7.loc13.0* and *c4.loc57*) had alleles (coming from TMS98/0581), which decreased the length of stem with leaf. All the QTLs that influenced this trait expressed additive gene effects. The results showed that QTL *c2.loc84* was indicative of additive gene action, while dominant gene action cannot be excluded. Although QTLs *c7.loc13* and *c10.loc22* were identified for additive gene action, a dominance gene action could not be excluded.

5.3.2.9.2 Productivity traits

5.3.2.9.2.1 Dry root yield (DRY)

The analysis resulted in the identification of one QTL (*c20.loc46.8*), implying that the QTL was located on chromosome 20 at 46.8 cM. The QTL accounted for 6.74% of the phenotypic variation. The allele influencing this trait (positive effect and significant at $P \leq 0.01$) was from TMS98/0505. The QTL showed an over-dominance gene action.

5.3.2.9.2.2 Fresh root yield (FRY)

One significant QTL was found for this trait and mapped to the same LG (20) as with DRY. The phenotypic variation explained by the QTL was 8.1%. The alleles driving yield were from parent genotype TMS98/0505. The QTL showed an over-dominance gene action.

5.3.2.9.2.3 Harvest index

A significant QTL associated with the harvest index was identified in LG3. The phenotypic variance explained by the QTL was 7.39% parent genotype. The QTL (*c3.loc98*) had alleles (coming from TMS98/0581), which decreased the harvest index. Results showed that QTL *c3.loc98* was indicative of over-dominance gene action.

5.3.2.9.2.4 Root number

Two QTLs located in LGs 3 (*c3.loc83.3*) and 6 (*c6.loc29.9*) were identified for root number. The phenotypic variance explained by *c3.loc83.3* was 7.08%, while *c6.loc29.9* had a PVE of 9.48%. The two loci increased the root number with the two QTLs having an additive gene action. The QTL alleles influencing this trait were from TMS98/0505.

5.3.2.9.2.5 Biomass

This trait revealed several QTLs in the population. Four regions representing different linkage groups were found to be significantly linked to this trait. The LGs include 3, 5, 7, and 14. The LoD scores ranged between 3.1 and 3.82. The PVE ranged between 5.06% and 7.83%. Except for QTL *c5.loc73,* which had an over-dominant gene action, the other three QTLs (*c3.loc.84, c7.loc64.4* and *c14.loc43.90*) were additive in gene action. The three QTL alleles (*c3.loc84,* c7.loc64.4, c14.loc43.9) influencing trait expression originated from TMS98/0505, and increased biomass, while *c7.loc64.4* reduced biomass.

Table 5.10: Composite interval analysis year 2

Trts[a]	QTL[b]	Chr[c]	Flanking Markers	Position	LoD	Add[d]	Dom[e]	Gene Action	%PVE[f]	P-value[g]
Biomass(kg)	*c3.Loc84*	3	mk117-mk138	84	3.67	1.67	-3.18	A	6.9	7.78e-05***
	c5.Loc73	5	mk206-mk214	73	3.1	0.54	10.45	OD	5.06	0.00089***
	c7.Loc64.40	7	mk285-mk287	64.4	3.82	-0.14	14.65	A	7.83	2.25e-05***
	c14.Loc43.90	14	mk423-mk425	43.9	3.67	0.89	-6.6	A	5.4	0.0006***
Height at first branching	*c2.Loc82.94*	2	mk061-mk094	82.94	4.36	2.6	-5.33	A	3.065	0.0167*
	c3.Loc98	3	mk126-mk140	98	3.45	0.88	7.56	OD	4.382	0.0030**
	c6.Loc0.00	6	mk232-mk245	0	5.72	3.76	3.5	D	6.05	0.00037***
	c9.Loc7.4	9	mk315-mk325	7.4	3.04	13.5	-13.94	A	2.35	0.043*
Branching level	*c3.Loc100.3*	3	mk139-mk141	100.3	4.84	0.00478	0.369	OD	6.44	0.00016***
	c4.Loc54.2	4	mk171-mk173	54.2	5.35	0.0087	1.267	OD	8.25	1.59e-05***
	c10.Loc4.0	10	mk340-mk341	4	4.57	-0.0075	0.5348	A	7.07	7.24e-05***
Dry root yield (t/ha)	*c20.Loc46.8*	20	mk513-mk514	46.8	3.31	0.74	6.09	OD	6.74	0.00054***
Fresh root yield (t/ha)	*c20.Loc46.8*	20	mk513-mk514	46.8	4.01	1.97	33.05	OD	8.1	0.0001***
Plant vigour	*c1.Loc55*	1	mk017-mk044	55	3.78	0.032	-0.193	A	7.41	0.0001***
	c3.Loc79	3	mk126-mk134	79	4.1	0.009003	-0.131516	A	8.04	5.85e-05***
Harvest index	*c3.Loc98*	3	mk110-mk138	98	3.66	-0.00634	-0.015639	OD	7.39	0.0024***
Number of leaves	*c5.Loc86*	5	mk193-mk220	86	3.05	-0.2533	3.8984	A	4.44	0.015*
	c16.Loc0.32	16	mk451-mk453	0.32	4.32	0.4219	11.1699	OD	7.32	0.0009***
Plant height	*c2.Loc45.2*	2	mk052-mk082	45.2	3.24	1.056	73.64	OD	4.65	0.0041**
	c3.Loc98.0	3	mk126-mk138	98	3.71	1.32	4.72	OD	5.58	0.0014**
Root number	*c3.Loc83.3*	3	mk101-mk138	83.3	3.32	2.53	-5.8	A	7.08	0.00017***
	c6.Loc29.9	6	mk239-mk244	29.9	4.55	1.8	-7.48	A	9.48	1.05e-05***
Scar level (cm)	*c2.Loc85*	2	mk092-mk095	85	4.42	5.02	-6.62	A	4.54	0.005**
Number of scars	*c5.Loc73.2*	5	mk197-mk214	73.2	3.14	0.54	203.27	OD	5.39	0.0007***
	c10.Loc0.0	10	mk338-mk339	0	3.93	-0.85	-86.27	OD	5.28	0.0008***
	c14.Loc33.0	14	mk418-mk422	33	3.36	0.99	-1.97	A	4.9	0.00132***
with leaf (cm)	*c2.Loc68*	2	mk052-mk079	68	3.33	0.62	-18.42	A	4.2	0.00302**
	c3.Loc84.5	3	mk125-mk129	84.5	4.44	4.24	-5.83	A	9.08	5.5e-06***
	c7.Loc13.0	7	mk263-mk264	13	3.96	-0.83	5.82	A	3.8	0.00507**
	c10.Loc22.0	10	mk345-mk347	22	3.39	-0.27	5.93	A	2.31	0.03912*

5.3.2.10 QTLs for multiple traits

Results showed that a number of the identified QTLs were for more than one trait. The traits identified with this phenomenon were dry-matter content, fresh root yield, and harvest index, plant height, and height at first branching. For example, QTL *c20.loc46.8* was found to be associated with dry root yield and fresh root yield, while the QTL c3.loc.98 was found for harvest index, plant height, and height at first branching (Table 5.11).

Table 5.11: QTLs controlling more than one trait in year 2

QTL	Linkage group	Traits
C20.loc46.8	20	DRY, FRY
C3.loc98.0	3	HI, PLTHT, HFB

5.3.2.11 Common and consistent QTLS identified in first and second year

Three common QTLs were common in both years, and their PVE ranged between 2.30% and 6.90% (Table 11). These were *c3.loc84.0, c6.loc0.0,* and *c7.loc13.0* identified at chromosomes 3, 6, and 7, respectively. The common QTL, *c7.loc13.0*, was identified for root number, fresh root yield, dry root yield, and length of stem with the same marker interval mk263-mk264 being identified for both years (Table 5.12).

Table 5.12: Common QTLs with traits in the first and second year analysis

Year	Trait	QTL	Marker Interval	PVE
1st Year	Harvest index	*c3.Loc84.0*	mk124-mk145	3.80
2nd Year	Biomass	*C3.loc84.0*	mk117-mk138	6.90
1st Year	Scar level	*C6.loc0.0*	mk232-mk254	2.30
2nd Year	Height at first branching	*C6.loc0.0*	mk232-mk245	6.05
1st Year	Root number	*C7.loc13.0*	mk263-mk264	5.47
2nd Year	Fresh root yield	*C7.loc13.0*	mk263-mk264	4.62
1st Year	Dry root yield	*C7.loc13.0*	mk263-mk264	4.39
2nd Year	Length of stem with leaf	*C7.loc13.0*	mk263-mk264	3.80

5.4: Discussion

Cassava is one of the traditionally grown resilient crops in the marginal environments of Africa. Cassava has become of paramount importance to the African continent because of its rapidly increasing population and urbanisation which have put immense pressure to expand agricultural production into the savannah regions that are noted for cereal cultivation. While research efforts have been targeted at cassava production under optimal conditions of humid and sub-humid ecologies, genetic improvement for cassava production targeted at dry ecologies has been limited. This is possibly so since cassava had been an 'orphan crop' for a long time before its recent metamorphosis into a food security crop, and even a cash and industrial crop in Africa. The rapid evolution of the crop value chain has further made it imperative to enhance breeding initiatives to make the crop responsive to the new demand and the agribusiness opportunities. Such opportunities hold the key for improving food and nutrition security and contribute to poverty alleviation for the smallholder farmers growing the crop.

While cassava has been reported to produce reasonable yield in comparison to other crops in drought-prone regions, its yield potential in such stressed ecologies requires genetic improvement to sustain the aimed-for yield levels that are needed to guarantee food security. Several studies on drought tolerance for improved productivity had been conducted mainly in Latin America, but with very few studies conducted in Africa to explore the genetic knowledge in African germplasm aimed at driving the advanced breeding for cassava's drought-tolerant varieties. The lack of molecular breeding tools for the crop was seen as serious constraints, especially in Africa. This study was facilitated to strengthen the breeding capacity for drought ecology adapted cassava; exploring practical approaches that could provide cassava breeders in national systems in Africa with the ability and opportunity to identify QTLs in African germplasm that could routinely be utilised to improve breeding for superior cassava varieties; and help to boost crop productivity in marginal environments.

This study utilised improved non-inbred parental materials to develop the F_1 segregating mapping population. The parents (TMS98/0505 as female and TMS98/0581 as male) are popularly grown varieties in Africa, which were tested and found to have the desirable traits for disease tolerance/resistance, and the performance that could be highly complementary to enhance a better productivity in the F_1 'hybrids', thus permitting the mapping of useful genes. The choice of the female parent was based on its better flowering capacity to support the generation of a reasonable amount of seeds for population development. The parents were pre-tested prior to the QTL mapping population development at Minjibir, and they were found to be

among the best in performance among local improved germplasm accessed in Nigeria, with yields above 20 tonnes/ha. Yields in this ecology are generally between 5-7 tonnes/ha. Both parents have been used in several breeding programmes for population development, and QTLs identified that using these parents could have a major advantage for utilisation in 'breeding-by-design' programme, using the knowledge of these QTLs to fast-track varietal improvement.

Both parental lines were selected for CMD resistance to enhance a better F_1 population reaction to the disease to avoid confounding performance, and QTL mapping. CMD is cassava's most limiting disease (Herrera-Campo *et al.,* 2011) in Nigeria, and could affect genetic dissection for traits of interest. Both parents are likely to have the *CMD2* gene-mediated resistance, and this may explain why the F_1 progeny had good CMD resistance (over 90%). The *CMD2* gene is a dominant gene that is frequently utilised in the control of this disease. The good resistance of the population to CMD eliminated the confounding effect the disease would have had on other traits of interest and their expression in the population for QTL mapping. Therefore, CMD distribution in the population was highly skewed towards resistance (Fig. 5.1). The number of plants developed for F_1 were above 200, which was adequate for QTL mapping.

Assessment of the evaluated F_1 population showed good segregation with the identification of transgressives for most of the traits as expected, considering that cassava is a highly heterozygous crop, and the use of non-inbred parents typically allows for good variation and genetic mapping for QTLs. There was high dispersion for the highly variable traits (plant height, number of leaf scars, number of leaves, and yields) as captured by standard deviation (SD) (Table 5.1). There was superior mean performance for the population compared to the parents (for most traits), indicating overall better trait expression in the mapping population, which should support a good selection for candidate genotypes for inclusion in yield test trials, or for continuous advancement in the breeding pipeline. Principal component analysis accounted for 99% variation for productivity traits, and 74% for physiological traits.

Correlation results showed significant association among a number of productivity traits (for example, between root number and fresh root yield (FRY); dry root yield (DRY) and harvest index; DRY and dry-matter content, and so forth) as well as among morphological/physiological traits (plant height vs. number of leaves; leaf number, and plant vigour). Similarly, significant correlations were observed among both sets of traits (DRY and plant biomass; DRY and plant vigour, and so forth). The results observed were similar to those garnered form previous studies

(Okogbenin *et al.,* 2003; Lenis *et al.,* 2006; Fukuda *et al.,* 2010). The F_1 population reflected the expected association among these traits, and its influence in driving plant performance and productivity in the field. Therefore, for the purpose of this study, it reflected the genetic dissection of elite African germplasm for these traits towards drought tolerance development in cassava. Breeding crops for increased yield potential and improved adaptation to stress-prone environments often targets yield as the principal trait for selection (Araus *et al.,* 2008). The positive correlation of these traits with fresh root yield shows that all the traits were important and were positively associated with each other. This also supports previous findings that these traits make important contributions towards economic yield (Ojulong 2008) and can be used in direct selection for yield in marginal environments.

The development of about 2000 KASPar SNP markers under the CGIAR Generation Challenge programme provided an opportunity to build a new molecular breeding platform to utilise these tools to accelerate the molecular breeding in National Agricultural research System (NARs) in Africa for cassava genetic improvement, exploring commonly used elite breeding germplasm. Elite germplasm was used to facilitate the transferability of tools on a regional basis to support breeding programmes. These KASPar SNP markers were the first sets of SNP markers availed to NARs in Africa and were used to drive this study for QTL mapping for traits under a drought-stressed ecology.

Results of the parental survey with SNPs indicated that the number of polymorphic markers in each parent was between 522-567 markers, while polymorphic markers for cross combination (parental pairs) selected for the development of mapping population were 856 SNPs markers. This shows that the SNP markers were highly polymorphic and abundant in the parents. The level of polymorphism found with SNPs in this study was much higher than with other markers such as RFLPs, AFLPs, ESTs, SSRs reported earlier in cassava. It confirmed the suitability of SNPs for genetic studies, and identified them as the preferred markers of choice. With advancement in molecular biology and the increasing power of Nextgen sequencing technologies, coupled with high throughput systems, several thousands of SNPs are now available to support genetic studies (Spindel *et al.,* 2013; Rabbi *et al.,* 2014; Bertioli *et al.,* 2014; Pandey *et al.,* 2017).

The polymorphic markers found in the parents were used to genotype the mapping population towards the construction of genetic linkage and mapping for drought-tolerance related QTLs. The study originally set out to develop a genetic map with at least one marker in a 10 cM

interval to aid QTL mapping and enable improved application of QTLs for breeding. The generated map resulted in a relatively good density SNP-based genetic map of 21 linkage groups (LGs) with good marker distribution and genome coverage. The number of linkage groups was more than 18 pairs expected for cassava (2n = 36), indicating that the map was not fully saturated. The average marker interval for the map was 3.69 cM, relatively comparable to the results reported by Rabbi *et al.* (2012). However, the aim of having a marker at an average of 10 cM interval was therefore achieved. The relatively dense map resulted in a lower marker interval than previously identified with other SSR, RFLP, EST markers. Thus, the marker distribution for this map indicated that it had good genome coverage, suitable for QTL mapping and possible application for breeding purposes. The utilisation of elite breeding parents (for diseases and high yields) for the construction of the genetic map and gene mapping offered great practical opportunities to improve the selection in the breeding pipeline, and track gene flow through generations in the development of advanced lines.

This study identified and mapped 27 and 30 QTLs for morphological/physiological and productivity traits related to drought tolerance under moderate stress field conditions in both years. QTLs were identified for 11 and 13 traits for year 1 and year 2, respectively (Tables 5.7 and 5.8). The phenotypic variance varied from small to moderate and major effects. Not many genetic mapping studies conducted for stress ecologies had been reported in sub-Saharan Africa, which makes this an important study for the development of strategies for the savannah ecologies where increasing expansion of cassava for production is a major focus, particularly given the limitations for other crops. Since agriculture is highly rain-fed reliant, the ability to use the land in this ecology all year round makes cassava more attractive. Two QTLs for branching levels accounted for a relatively large amount (> 19%), and they were thus major QTLs (Collard *et al.,* 2005). Branching level in cassava is a trait that essentially determines the architecture of the crop. Thus, it has implications for assimilate partitioning in the crop, and the source-sink relationship. High branching levels tend to be associated with low BH, while lower branching levels often have high BH. This affects the source-sink relationship, and how well cassava can avoid superfluous growth, and enables efficient utilisation of assimilates for productivity. It has also been observed that plants with low branching height flower early, and often produce more branches than the high branching level varieties. The detection of major QTLs for this trait thus signifies high likelihood to alter this trait beneficially for better performance under drought-stress conditions.

The identified QTLs expressed different gene actions (additive, dominance, and over-dominance). Almost 40% of the QTLs had dominant or over-dominant gene actions, indicating that these modes of gene actions are critical to crop performance. This finding agreed with broadly held views that superiority in field performance is strongly linked to its heterozygosity (Fukuda *et al.,2010;* Collins *et al.,* 2008; Cebellos *et al.,* 2004) A great number of additive gene actions were also found, which showed that for trait development, breeding needs to explore these genes through selfing and a recurrent selection scheme to increase allele frequencies for such loci. For years, there had been the tendency for intercrosses to be explored for heterozygosity, because of the high inbreeding depression found in cassava. However, in recent times, selfing had gradually been strengthened in the cassava breeding pipeline to explore additive genes. These results thus confirm the basis for a molecular breeding approach to effectively combine both additive and dominant gene variance for articulate breeding. Thus, the utilisation of both additive and dominant variances through identified QTLs can be exploited fully for rapid genetic gain for yield improvement in cassava. Being a clonally propagated crop, the dominant gene effects (heterotic loci) can easily be fixed in cassava.

Some QTLs were found to control more than one trait, for example, QTL *c4.loc57.2* for fresh root yield and dry root yield, both on chromosome 4. Similarly, c7.loc13 was involved in the control for both fresh root yield and dry root yield. *c21.loc0.0* was also involved for branching level and leaf retention control (Table 5.9). The localisation of QTL or gene pleiotropic effects have been reported in other QTL mapping studies (Okogbenin and Fregene., 2003; Zhang *et al.,* 2006;, Quarrie *et al.,* 2006; Wei et al., 2009, Athipong *et al.,* 2011). They are part of the key factors underlying the significant correlation observed among yield-related traits as well as the morphological and physiological traits found in this study. Genetic markers co-associated with multiple traits that correlate with each other can be used effectively to select all those traits needed to improve breeding efficiency (Agrama *et al.,* 2007).

This study identified useful QTL alleles from both parents, which may help define useful genetic backgrounds for improved drought-tolerant varieties if combined efficiently in good haplotypes in a good recurrent selection programme. The F_1 mapping population identified some transgressives that could constitute new parental lines for advanced population development for drought tolerance, including the possible inclusion of these F_1 genotypes in a breeding pipeline in Africa for possible release as varieties.

Comparing the QTLs in year 1 and year 2, we found common QTLs in both years, but they were linked with different traits (Table 5.12). For example, *c3.loc84.0* was common in the first and second year, but was linked with different traits (harvest index and biomass). The QTL *c6.loc0.0* was common in the two different years, but linked with scar level in year 1, and height at first branching level in the second year. The same was applicable to *c7.loc13.0*, which was linked to root number, fresh root yield, and dry root yield in year 1, but linked with length of stem with leaf in year 2. There could be several reasons for this observation. The rainfall pattern was not consistent within the period of the study (Chapter 4; Fig. 4.1), and this may have contributed to differences in the phenotypes and traits. The reduction of the population size of the mapping population as a result of the selection in the second year could also be a factor for the differences.

Single nucleotide polymorphic markers (SNP) emerged as powerful genetic tools for genetic studies (Jones *et al.,* 2007). This study was partly driven by the need to build molecular tools to effectively drive an efficient breeding programme for the dry ecologies in Africa. The quantitative inheritance associated with complex traits requires that both minor and major QTLs would have to be harnessed to increase productivity in a recurrent selection scheme driven with the aid of markers for resource-limited national programmes in Africa. This study reflects the first set of analysis done to identify QTLs under drought-stress conditions in Nigeria and Africa. More QTL analysis will be initiated by the authors to support identification of more QTLs for additional different environments under different stress conditions (including for severe stress situations). The aim is to capture yet unidentified QTLs and other interactive effects to develop more tools (QTLs) and support the recurrent selection, combing several sets of QTLs per traits in a marker-assisted recurrent selection as opposed to a few in MAS. The KASPar SNPs, developed under the GCP, are public goods readily available for use by NARs in Africa. The SNP markers already tagged to key traits of drought tolerance can easily be used for genotyping to drive traits in the NAR's breeding programme via high-throughput commercial genotyping platforms. Although genotyping by sequencing (GBS) is rapidly evolving, the capacity to utilise the massive data and access to such technologies could still be complicated and would require more expert support given to national programmes. A great part of future molecular breeding in Africa would be driven by highly informative QTLs requiring successive recombinations. This would be guided by support tools needed to accumulate the desirable alleles for the development of

superior genotypes with good genetic gain that can help to improve food security in drought-prone environments.

5.5 Conclusion

The study identified 27 and 30 QTLs associated with traits phenotyped in the dry savannah ecology of Nigeria in the first and second year using KASPar SNPs. Two major QTLs with PVE > 19 were identified for the trait branching level. The identification of these QTLs will enhance the genetic improvement of cassava, boost molecular breeding and productivity in marginal environments.

CHAPTER 6

TO IDENTIFY TRAITS DRIVING THE PHYSIOLOGICAL BASIS OF EARLY BULKING IN THE F_1 CASSAVA GENOTYPES

ABSTRACT

Late root bulking is a major factor leading to the rejection and abandoning of improved cassava genotypes in sub-Saharan Africa. Worldwide, cassava feeds an estimated population of 800 million people directly or indirectly. Given the expanding importance of cassava as food, feed, and industrial crop, genotypes with a high root yield and early bulking are considered to be of strategic importance, and highly desired for crop production in the marginal areas of the dry savannahs, where favourable lands exist, but they are highly prone to drought stress with average cassava yields of below 5 tonnes/ha. Therefore, the objective of this study was to identify early-bulking cassava genotypes at different harvesting ages, the stability of these genotypes in different environments, and to identify traits significantly associated with early storage root bulking. For this purpose, 135 cassava genotypes were evaluated at Umudike for early root bulking during two planting seasons at three harvesting stages of 7, 10, and 12 months after planting (MAP). Analysis of variance indicated a significant effect on genotype, harvest time, genotype and harvest time, genotype and year, harvest time and year, genotype, harvest time and year with yield and other yield related traits. Pearson's correlation analysis showed a positive correlation between fresh root yield and other morphological and yield-related traits. Principal component analysis indicated root weight, root number, biomass, fresh root yield, plant height, dry root yield, and dry-matter content as main contributors. Phenotypic coefficient of variation was higher than the corresponding genotypic coefficient of variation. The genotype by environment bi plot (GGE) biplot identified genotypes with high mean values for fresh root yield, dry-matter content, starch content, and high root number that are stable in various environments. Cassava genotypes with more than a 100% increase in fresh root yield at 12 MAP over the yield at 7 MAP were regarded as late bulkers, while those with less than a 100% increase in fresh root yield at 12 MAP over yield at 7 MAP were regarded as early bulkers. On this basis, 59 cassava genotypes representing 44% were identified as early bulkers at 12 months over 7 MAP while 90 genotypes at 10 months over 7 MAP.

Key words: Cassava; Drought stress; Early bulking; Late root bulking; Productivity

6.1 Introduction

Cassava (*Manihot esculenta Crantz*) is the third most important source of calories in the tropics after rice and maize (Huang et al., 2001; Food and Agriculture Organization of the United Nations (FAO), 2010) and the sixth most important crop in terms of global annual production (FAO, 2010). Cassava is widely grown in tropical Africa, Asia, and Latin America, and is gaining in importance in developing nations as the fourth most important crop, with production estimated at 226 million tones (FAO, 2009, 2012). It is the staple food of nearly a billion people in 105 countries, where the root provides as much as a third of daily calories (FAO, 2009) and the annual cassava consumption is greatest in Africa. It is also a major staple food for about half of the Nigerian population; and nearly 50 million tonnes of fresh cassava is produced annually in the country (FAO, 2008). According to Nweke *et al.* (1994), cassava's long growth cycle makes it relatively difficult for the crop to be readily available in a reasonably short time to farmers and consumers. Also, the rainfall pattern in some parts of the tropics where rainfall duration is about 6 months or less, creates a long dry season, which make cassava cultivation in such regions difficult. Nweke *et al.* (1994) reported that late bulking (LB) is a major factor leading to poor adoption of improved cassava genotypes in sub-Saharan Africa.

Early bulking (EB) is one of the most important traits of interest to farmers in cassava production areas. It has been found that early bulking in cassava is highly influenced by harvest index, foliage, root diameter, and root number (Okogbenin *et al.,* 2006). Early-bulking varieties are characterised by short growth periods (from planting to harvesting), and a better fit into a short rainy season, with a reduced risk to biotic and abiotic stresses, thereby increasing productivity (Nweke *et al.,* 1994). In dry ecology, drought imposes slow crop development that leads to the harvest of cassava being extended beyond 12 months. Early bulking is therefore considered to be an important measurable trait in marginal environment. Early bulking is believed to identify good bulkers under stress rather than identifying early maturing varieties (Okogbenin *et al.,* 2013). Thus, evaluating early bulking in a drought-prone environment is used when selecting good varieties with potential good yield at 12 MAP.

Early bulking is currently considered a key requirement for cassava to make the transition from being a traditional crop to an industrial one (Okogbenin and Fregene, 2002). It is also important in situations where there is mounting pressure on farmers to intensify their production, and in semi-arid regions, where early bulking cultivars can be harvested after a one year growing cycle. Therefore, to harness the potential of cassava better, particularly in the face of climate change conditions, there is need to identify, develop, evaluate, and select early-bulking cultivars

that can be harvested at 7-9 MAP in a humid environment, and after 12 months in marginal environments. This should go a long way towards increasing the cassava yield per unit time, reducing food shortages, and increasing the income of smallholder farmers in such areas.

6.2 Materials and Methods

A set of 129 F_1 cassava genotypes developed from hybridisation between two improved released cassava genotypes, IITA-TMS 98/0505 (early-bulking variety) and IITA-TMS 98/0581 with five checks (IITA-TMS 30572, TME 419, TMS 91/02324, IITA-TMS 98/0505, and IITA-TMS 98/0581) were evaluated for storage root yield at 7, 10, and 12 months after planting (MAP) during two growing seasons (2015/2016 and 2016/2017) at Umudike (humid forest Umudike), while 126 F_1 genotypes were evaluated for storage root bulking in Sudan's Savannah-Minjibir, Kano at 12 MAP. A simple alpha lattice design was used. Twenty stakes of each genotype were planted in the first and second seasons, respectively, in a plot of 20 m^2 (5 m x 4 m) at a spacing of 1 m x 1 m, giving a plant density of 10,000 plants/ha. The plots were weeded when necessary to minimise competition between weeds and the cassava plants. Three sequential harvests were done at 7, 10, and 12 MAP at Umudike, while harvesting was done at 12 MAP at Kano.

6.2.1 Data collection

To evaluate the F_1 progenies for early bulking, the following traits were measured; fresh root yield (FRY), harvest index, root starch content, number of roots, leaf retention (LR), root dry-matter content (DMC), plant height, vigour, biomass, root diameter, and stem diameter. Some of the cassava diseases and pests evaluated included cassava mosaic disease (CMD) and cassava bacterial blight (CBB). Most of the parameters have been described in Chapters 3 and 4. Genotypes in Umudike were evaluated at 7, 10, and 12 MAP, while the genotypes in Kano were evaluated at 12 MAP.

To further evaluate the cassava response to early bulking, other traits considered were the following:

1) Stem diameter: This was measured with the Vernier caliper in centimeters.
2) Root diameter: The diameter of two harvested roots was estimated using a Vernier caliper.

6.2.3 Data analysis

The collected data were subjected to descriptive analysis using Microsoft Excel software, and analysis of variance (ANOVA) using SAS software (version 9.0). Pearson's phenotypic correlations between traits were performed for the genotypes at 7 MAP averaged across three harvest times, and at 12 MAP for the Kano location. Principal component analysis (PCA) was performed to identify the traits that contributed to the total variation of genotypes. For PCA, the data for the genotypes were averaged across the three harvest times.

Estimates of variance components were obtained by equating the observed mean squares from ANOVA with their expected mean squares (EMS). Phenotypic and genotypic coefficients of variation were computed using the Excel package.
Genotypic variance component:

$$\sigma^2 g = MSg - {}^{MSe}/_{r}$$

Where M S g is the genotypic mean square, MSe is the error mean square, and r is replication.

Environmental variance component:

$$\sigma^2 e = {}^{MSe}/_{r}$$

Phenotypic variance component:

$$\sigma_p^2 = \sigma_g^2 + \sigma_e$$

Genotypic and phenotypic coefficients of variation were calculated according to the method suggested by Burton and Dewane (1953) as:

Genotypic coefficient of variation (GCV)

$$GCV = \frac{\sqrt{\sigma_g^2}}{X^-} \times 100$$

Phenotypic coefficient of variation (PCV)

$$PCV = \frac{\sqrt{\sigma_p^2}}{\bar{x}} \times 100$$

Where X^- *is the grand mean value of the trait*

Broad sense heritability (h^2)

$$h^2 = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

The model for a GGE biplot (Yan, 2002), based on singular value decomposition of the first two principal components, is:

Yij – ì – âj = ël îil çjl + ë2 îi2 çj2 + εij

where Yij is the measured mean (dbh) of genotype i in environment j; ì is the grand mean; âj is the main effect of environment j; ì + âj is the mean yield across all genotypes in environment j; ë1 and ë2 are the singular values for the first and second principal components, respectively; îi1 and îi2 are eigenvectors of genotype i for the first and second principal components, respectively; ç1j and ç2j are eigenvectors of environment j for the first and second principal components, respectively; and åij is the residual associated with genotype i in environment j.

Table 6.1: Ecological factors and study conditions of locations used for the evaluation of cassava genotypes for early root bulking at Umudike and Kano, Nigeria in the 2016/2017 and 2017/2018 growing seasons

Ecological factors and study conditions	Umudike↖	Kano↗
Latitude	5° 29'N	12° 3'N
Longitude	7° 24'N	8° 32'E
Altitude (above sea level)	120 m	473 m
Agroecology	Humid forest	Sudan Savannah
Annual rainfall (amount or range)	2200 mm	270 mm
Temperature range (mean)	26°C	18.7°c to 66.5°c
Relative humidity range	50-95%.	13%-68%
Soil classification	Dystric Luvisol	Sandy loam

↖Agro-meteorological station, National Root Crops Research Institute (NRCRI), Umudike

↗Agro-meteorological station Institute for Agricultural Research (IAR) Kano, Kano State

6.3: Results

Analysis of variance for storage root yield and other agronomic traits at three harvest times

There were significant differences among the genotypes for fresh storage root yield (FRY) at Umudike location at 7, 10, and 12 MAP (Table 6.2). It was also observed that harvest time had a significant effect on yield and other agronomic traits at ($P \leq 0.001$). There was a significant difference in the year for all other traits evaluated, except for fresh root yield. For the interaction genotype and harvest time, there was a significant difference for all the traits evaluated. Interactions genotype x year, and harvest time x year were found to be highly significant at ($P \leq 0.001$), except for root weight for G x Y, and vigour for T x Y. All traits were significant at ($P \leq 0.001$) for the interaction G x T x Y. Coefficient of variation ranged from 15.4% dry-matter content to 80% root weight. Other traits that had high levels of variation were plant biomass (78.6%), and root number (73.1%) (Table 6.2).

Table 6.2: Combined analysis of variance of mean squares for yield and other agronomic traits of 129 F_1 cassava genotypes and five checks evaluated for early root bulking at Umudike, Nigeria in the 2016/17 and 2017/18 growing seasons

Source of variation	DF	FRY	HI	HFB	PLTHT	DMC	STARCH
Genotype (g)	133	638.5***	0.06***	3266.3***	4535.2***	56.36***	99.86***
Harvest time (t)	2	24684.7***	0.61***	223967***	19018***	2794.1***	4918***
year (y)	1	385.9ns	1.71***	29774***	100617***	1046***	1855.4***
g x t	247	301.5***	0.03***	1437.2***	1461.2***	34.93***	62.24***
g x y	133	335.4***	0.04***	2247.9***	2676.7***	39.54***	69.98***
t x y	2	2764..4***	2.58***	22137.4***	303395***	133.57***	228.93***
g x t x y	151	239***	0.02*	1562.2***	903.8ns	27.5***	48.74***
Cv		41.6	21.3	36.8	19.7	15.4	49.3

Cont. Table 6.2: Combined analysis of variance of mean squares for yield and other agronomic traits of 129 F1 cassava genotypes and five checks evaluated for early root bulking at Umudike, Nigeria in the 2016/17 and 2017/18 growing seasons

Source of variation	DF	BIOMASS	DRY	RTNO	RTWT	RTDIM	STDIAM	VIG
Genotype (g)	133	67.41***	48.73***	515.6***	142.59***	6.77***	1.09***	2.43***
Harvest time (t)	2	1566.04***	2853.5***	11755.82***	6697.38***	55.68***	7.8***	65.25***
YEAR (y)	1	1019.34***	159.75***	23134.52***	808.92***	1805.5***	1.11ns	21.35***
g x t	247	31.98***	25.81***	301.58***	85.66***	2.42***	0.72***	0.72***
g x y	133	32.15***	23.48***	125.41***	34.8ns	4.37***	0.93***	1.53***
t x y	2	90.34***	144.17***	21137.28***	3049.58***	353.14***	1.81**	0.57ns
g x t x y	151	23.09***	18.39***	132.3***	30.7ns	2.03***	0.5**	0.7***
cv		78.6	42.8	73.1	80	18.4	29.5	21.2

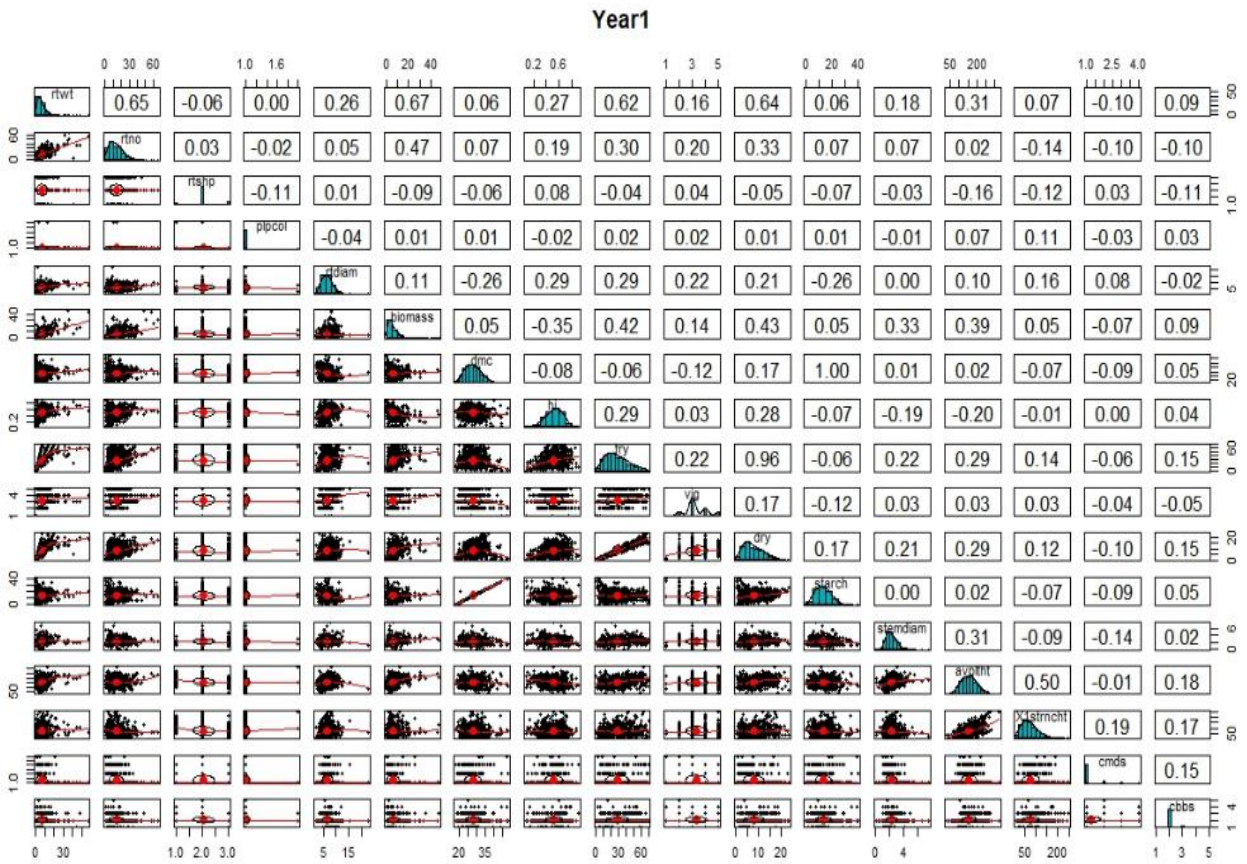
*FRY = fresh root yield; HI = harvest index; HFB = height at first branching; PLTHT = plant height; DMC = dry-matter content; DRY = dry root yield; RTNO = number of roots; RTWT = root weight; RTDIM = root diameter; STDIAM = stem diameter; VIG = plant vigour. NS is not significance, *, **, *** is significant at 5, 1, & 0.1%.

6.3.1: Relationships among traits in Umudike

Pearson's correlation analysis indicated a positive association between most of the traits (morphological and productivity) and fresh storage root yield for first- and second-year analysis (Figs. 6.1 and 6.2). Most of the productivity traits were significantly correlated with each other. Fresh storage root yield correlated positively with harvest index, dry root yield, stem diameter, plant height, height at first branching, root diameter, plant biomass, root number, and root weight, except for dry-matter content, and starch content, which were negatively correlated with fresh storage root yield for both years. The strongest correlation variable was dry root yield (r = 0.96), which was followed by storage root weight (r = 0.62), biomass (r = 0.41), harvest index (r = 0.30), and root diameter (r = 0.31) (Fig. 6.1), while during the second year, the strongest correlation variable was dry root yield (r = 0.92), followed by root weight (r = 0.57), harvest index (r = 0.47), and root number (r = 0.40) (Fig. 6.2). Similarly, dry storage root yield (DRY) positively correlated with root weight (r = 0.64), root number (r = 0.32), root diameter (r = 0.24), plant biomass (r = 0.42), dry-matter content (r = 0.24), harvest index (r = 0.29), starch content (0.24), stem diameter (0.19), and plant height (r = 0.29). Plant biomass was also found to correlate positively with root weight (r = 0.65), root number (r = 0.44), root diameter (r = 0.13), stem diameter (r = 0.31), and plant height (r = 0.38). Root diameter correlated positively with root weight (r = 0.27), root number (r = 0.02), biomass (r = 0.13), fresh root yield (0.31), vigour (r = 0.22), plant height (0.12), and height at first branching (r = 0.17). Stem diameter correlated positively with root weight, root number, plant biomass, fresh root yield, dry root yield, starch content, and plant height (r = 0.14, 0.05, 0.31, 0.21, 0.19, 0.02, and 0.29). Plant vigour associated positively with root weight, root number, root diameter, plant biomass, fresh root yield, dry root yield, dry root yield, starch content, stem diameter, plant height, and had a negative association with cassava bacterial blight (Fig. 6.1). None of the correlations was a strong correlation; all correlations were moderate or weak relationships. In the second year analysis (Fig. 6.2), dry storage root yield significantly and positively correlated with root weight (r = 0.59), root number (r = 0.43), root diameter (r = 0.43), biomass (r = 0.41), harvest index (r = 0.47), fresh root yield (r = 0.92), starch content (r = 0.24), stem diameter (r = 0.29), and plant height (r = 0.42). A very strong correlation was observed in the traits fresh root yield and root weight, while moderate correlation was seen in root number, root diameter, biomass, harvest index, and plant height. Plant biomass had a strong and positive relationship with root weight (r = 0.65), root number (r = 0.63), moderate positive relationship with fresh root yield (r = 0.40), vigour (r = 0.22), dry root yield (r = 0.41) root diameter (r = 0.25), stem diameter (r = 0.16), plant height (r = 0.15), height at first branching (r = 0.13) and a very weak relationship with starch

content (0.05) (Fig. 6.1)Root diameter correlated positively, but showed a moderate correlation with root weight (r = 0.30), biomass (r = 0.25), harvest index (r = 0.31), fresh root yield (r = 0.39), dry root yield (r = 0.43), and stem diameter (r = 0.25). Root diameter also correlated positively with root number, plant vigour, and starch content, but it was a weak relationship with these traits. Similarly, stem diameter had a strong and positive correlation with root weight, root diameter, biomass, fresh storage root yield, plant vigour, dry root yield, starch content, and plant height, but it correlated negatively with CMD and CBB. It was observed that stem diameter had moderate and weak correlations with most of these traits. Plant vigour correlated positively but only moderately with plant biomass, dry-matter content, starch content, stem diameter, and plant height (r = 0.22, 0.24, 0.29, 0.42, -0.16, and -0.36), but negatively correlated with CMD and CBB.

Correlation coefficient analysis was also used to determine the relationship between fresh storage yield and other agronomic traits at 7 MAP (Fig. 6.3). Fresh root yield was strongly and positively correlated with root weight and dry root yield, with r values of 0.76 and 0.97, respectively at 7 MAP. Other traits that were positively correlated with fresh root yield at 7 MAP included root number (r = 0.43), biomass (r = 0.42), harvest index (r = 0.31), vigour (r = 0.37), stem diameter (r = 0.17), and height at first branching (r = 0.15). Fresh root yield was also found to correlate negatively with starch content, dry-matter content, plant height, CMD and CBB.



*Rtwt = root weight, rtno = root number, rtshp = root shape, plpcol = pulp colour, rtdiam = root diameter, dmc = dry-matter content, hi = harvest index, fry = fresh root yield, vig = plant vigour, dry = dry storage root yield, avplht = plant height, X1strancht = first branching height, cmds = cassava mosaic disease severity, cbbs = cassava bacterial blight

Figure 6.1: Pearson's Correlation coefficient among fresh storage root yield, other yield components for the three harvesting stages in year 1

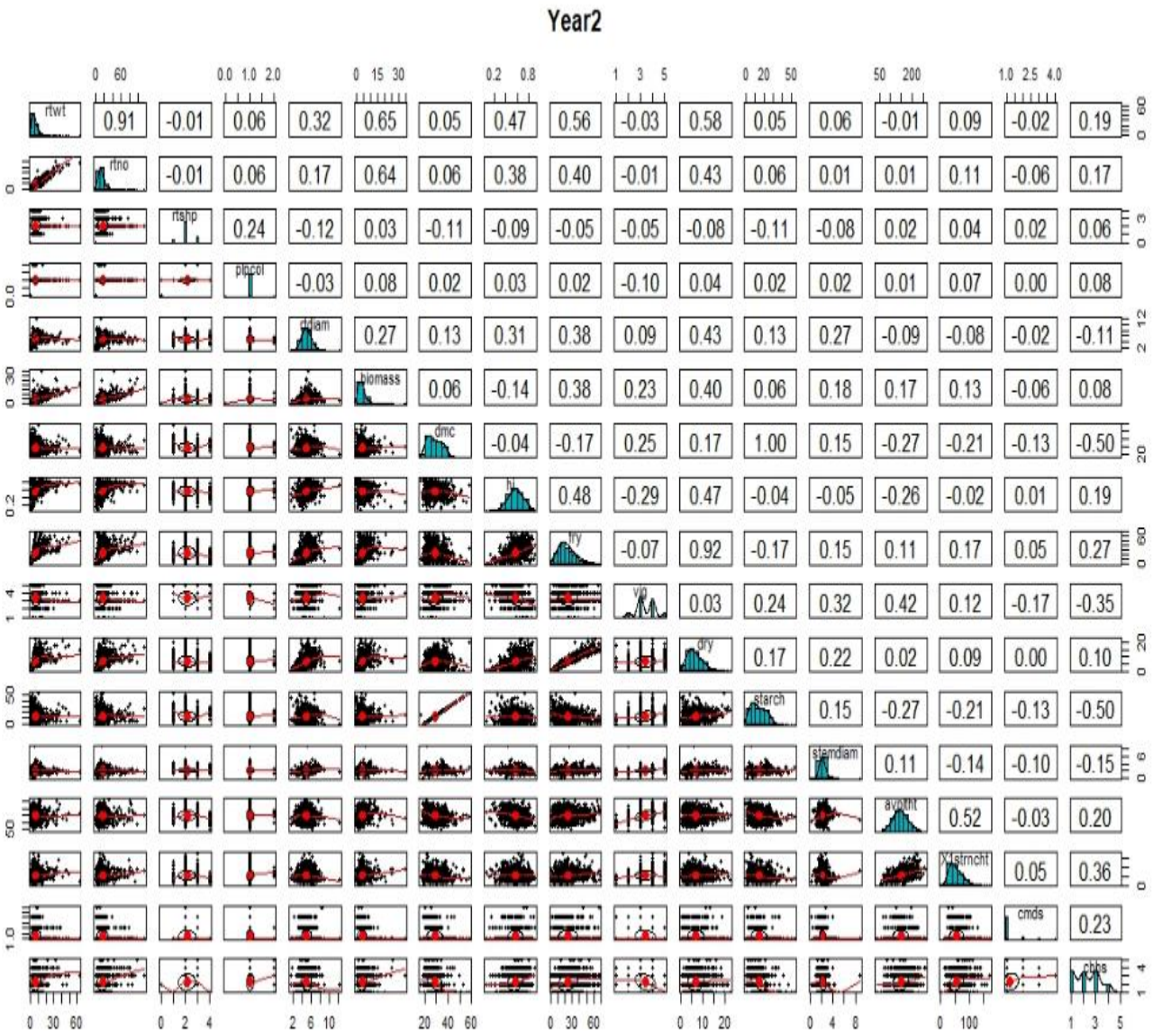


Figure 6.2: Pearson's correlation coefficient among fresh storage root yield, other yield components s for the three harvesting stages in year 2

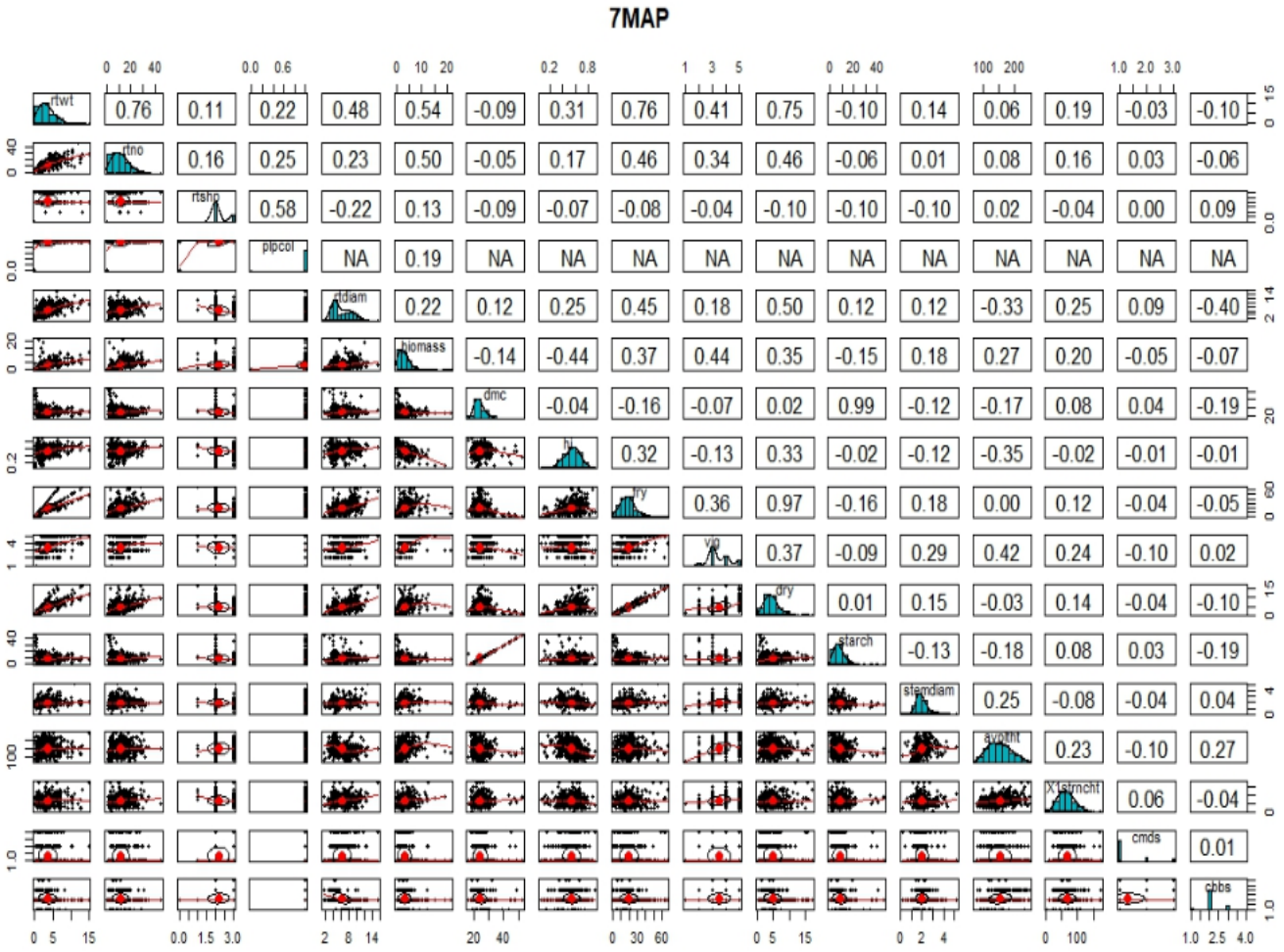


Figure 6.3: Correlation of yield, yield related and morphological traits at in F_1 population 7 MAP

6.3.2 Simple statistics of yield, yield related traits, and diseases

Simple statistics of the genotypes from F_1 population evaluated at Umudike over a 2-year period for different traits are presented in Table 6.3. Maximum values obtained for fresh storage root yield was 69 t/ha, 320 cm for plant height, 52.83% for dry-matter content, 0.91 for harvest index, 46.05% for starch content, 5 for CBB, 4 for CMD, 22.5% for dry root yield, 115 for number of roots, 63.2 kg for root weight, 15.88 cm for root diameter, 8.8 cm for stem diameter, and 5.00 for plant vigour.

Across genotypes, fresh root yield ranged between 0.5 t/ha and 69 t/ha, dry-matter content between 16.77% and 52.83%, starch content ranged between 0.09 % and 46.05%, cassava bacterial blight 1 to 5, CMD 1 to 4, dry root yield 0.22% to 22.50%, number of root ranged between 0 to 115, root weight 0 kg to 63.2 kg, root diameter 1.55 cm to 15.55 cm, stem

diameter 0.2 cm to 8.80 cm, and plant vigour 1 to 5 (Table 6.4). Mean across genotypes for the various traits were: 27.48 t/ha for fresh root yield, for plant height 159.1 cm, for dry-matter content 26.29%, harvest index 0.56, starch content 10.72%, cassava bacterial blight 2.5, CMD 1.32, dry root yield 7.25%, number of roots14.78, for root weight 6.95 kg, root diameter 5.95 cm, stem diameter 2.11 cm, and plant vigour 3.25 cm.

Table 6.3: Simple statistics of yield, yield related traits, and diseases of the F_1 population and five checks evaluated for early root bulking in Umudike, Nigeria in the 2016/17 and 2017/18 growing seasons

Traits	Min	Max	Mean
Fresh storage root yield (t/h)	0.5	69	27.48
Plant height (cm)	63	320	159.1
Dry-matter content (%)	16.77	52.83	26.29
Harvest index	0.06	0.91	0.56
Starch content (%)	0.09	46.05	10.72
Cassava bacterial blight	1	5	2.5
Cassava mosaic disease	1	4	1.32
Dry root yield (t/h)	0.22	22.5	7.25
Root number	0	115	14.78
Root weight (kg)	0	63.2	6.95
Root diameter (cm)	1.55	15.55	5.95
Stem diameter (cm)	0.2	8.8	2.11
Plant vigour	1	5	3.25

6.3.3 Principal component coefficient of the various traits with principles of the various yield-related traits

The Eigen values of the first five PCs and the correlation coefficients of the yield traits with the PCs are presented in Tables 4 and 5 for the first and second year. The first five PCs accounted for 68% and 71% in the first and second year, respectively (Tables 6.4 and 6.5). In the first year, principal component 1 accounted for 28% of the variation, and indicated that root weight, number of roots, total plant biomass, fresh root yield, dry root yield, and plant height were the main contributors. Principal component 2, accounting for 13% of the variation, had dry-matter content and starch content as the main contributors, and all were positively correlated, while

PC3, accounting for 11% of variation, had root diameter, harvest index, and plant height as main factors. PC4 contributed 9% of the variation, and indicated stem diameter as the main factor, while PC5 contributed 7% of the variation, and had root number as the main factor (Table 6.4). In the second year (Table 6.5), principal component 1 accounted for 25% of the variation, and indicated that root weight, root number, plant biomass, fresh root yield, and dry root yield were the main contributors. PC2 contributed 18% to the variation, and dry-matter content, harvest index, plant vigour, and starch content were the main contributors. PC3 accounted for 13% variation with the main factors being dry-matter content, starch content, and stem diameter, while PC4 accounted for 9% variation, with stem diameter being the main factor. PC5 accounted for 7% variation, and stem diameter was the main contributor.

Table 6.4: Principal component analysis in F_1 population evaluated for early bulking in year 1

Traits	PC1	PC2	PC3	PC4	PC5
Root weight (kg)	-0.39	0.09	-0.20	0.05	0.15
Root number	-0.33	0.11	-0.11	0.05	0.41
Root diameter (cm)	-0.12	-0.05	-0.36	-0.24	-0.23
Biomass (kg)	-0.37	0.04	0.16	-0.10	0.18
Dry-matter content (%)	0.00	0.65	0.09	-0.14	-0.07
Harvest index	0.01	0.02	-0.61	0.25	-0.06
Fresh root yield (t/h)	-0.39	-0.09	-0.20	0.01	-0.06
Vigour	-0.29	-0.16	0.29	-0.17	-0.11
Dry root yield (t/h)	-0.40	0.05	-0.19	0.01	-0.06
Starch content (%)	0.00	0.65	0.09	-0.14	-0.07
Stem diameter (cm)	-0.27	-0.17	0.22	-0.35	-0.15
Plant height (cm)	-0.30	-0.01	0.31	0.24	-0.13
Eigen values	2.20	1.48	1.38	1.20	1.09
Eigen values as proportion of total variance	0.28	0.13	0.11	0.09	0.07
Cummulative % of total variance	0.28	0.41	0.52	0.61	0.68

Table 6.5: Principle component Analysis in F_1 population evaluated for early bulking in year 2

Traits	PC1	PC2	PC3	PC4	PC5
Root weight (kg)	-0.42	0.03	-0.19	0.06	-0.02
Root number	-0.38	0.12	-0.13	0.21	0.01
Root diameter(cm)	-0.26	-0.29	0.05	-0.28	-0.02
Biomass (kg)	-0.38	0.20	0.04	0.02	-0.11
Dry-matter content (%)	0.06	0.34	-0.48	-0.09	-0.25
Harvest index	-0.09	-0.40	-0.21	0.06	0.20
Fresh root yield (t/h)	-0.33	-0.29	-0.12	-0.25	-0.02
Plant vigour	-0.24	0.35	0.22	-0.13	0.08
Dry root yield (t/h)	-0.35	-0.21	-0.23	-0.21	-0.10
Starch content (%)	0.06	0.34	-0.48	-0.10	-0.25
Stem diameter (cm)	-0.03	-0.01	0.31	-0.35	-0.45
Plant height (cm)	-0.26	0.29	0.23	-0.13	0.18
Eigen values	2.06	1.73	1.47	1.21	1.11
Eigen values as proportion of total variance	0.25	0.18	0.13	0.09	0.07
Cummulative % of total variance	0.25	0.42	0.55	0.64	0.71

6.3.4 Identification and selection for early-bulking genotypes in F_1 population and five checks

Fresh root yield ranged between 3.47 and 44.39 t/ha, 5.87 and 64.16 t/ha, and 10.14 and 66.38 t/ha, respectively at 7, 10, and 12 MAP (Table 6.6), while their mean yields were 19.69 t/ha, 31.83 t/ha, and 31.00 t/ha. There was a significant yield increase at 10 and 12 MAP. The coefficients of variation for yield at 7, 10, and 12 MAP were 32, 33, and 39%, respectively, while the percentage yield mean increase between 7 to 10 months was 12.14 t/ha, representing 23.56% (Table 6). Coefficient of variation among the genotypes for fresh storage root yield was almost the same at 7 MAP (32%) and 10 MAP (33%). The variation was a little bit higher between 7 and 12 MAP (39%) than 10 and 12 MAP (Table 6.6). It was observed that out of the 134 genotypes evaluated for root bulking in the F_1 population, 90 genotypes had a percentage FRSY less than 100% at 10 MAP over 7 MAP, while 44 of the genotypes had a yield increase higher than 100% at the same time of harvest. Eighteen genotypes had a negative FRSY increase at 10 MAP over 7 MAP. A FSRY increase at 10 MAP over 7 MAP, 90 genotypes can be regarded as early-bulking genotypes, 44 genotypes as late bulkers, while 18 of the early bulkers that had a negative percentage increase as type1 early bulkers. The results also show

that 59 genotypes had a yield increase in FSRY that was less than 100%, while 76 genotypes had a yield increase in FRSY that was more than 100% at 12 MAP over 7 MAP (Table 6.7). In other words, 59 genotypes were identified as early bulkers, while 76 were late bulkers. It was observed that 21 genotypes had a negative percentage yield result at 12 MAP over 7 MAP (Table 6.8), while 38 genotypes showed a positive yield increase at 12 MAP over 7 MAP (Table 6.8). The genotypes that had a negative percentage yield value can be classified as type1 early bulkers, because there was a reduction in yield before they reached their respective time of harvest; and it was observed that rotting of tubers had already set in when they were harvested at either 10 or 12 MAP. However, they completed their cycle of maturity before harvest. Percentage yield increase ranged between -61.56 and 300.88% at 10 MAP over 7 MAP, while percentage yield increase at 12 MAP over the yield at 7 MAP ranged between -63.77 and 370% (Figs. 6.3 and 6.4).

Table 6.6: Summary for descriptive analysis of fresh root yield of 129 genotypes and five check varieties evaluated at Umudike Nigeria for earliness in storage root bulking

Parameter	7 MAP¥	10 MAP	12 MAP
Range	3.47-44.39	5.87-64.16	10.14-66.38
CV (%)	32	33	39
Mean	19.69	31.83	31.00
Mean yield increase (t/ha)	12.14 (23.56%)		

*MAP = months after planting

Table 6.7: Yield and percentage yield increase of early-bulking F_1 cassava genotypes at 10 MAP over 7 MAP genotypes

Genotype	10 MAP	7 MAP	total yld	increase	% increase
146B	20.65	13.64	34.29	7.01	51.39
200B	25.85	16.79	42.64	9.06	53.96
073B	35.27	22.52	57.79	12.75	56.62
235B	26.85	17.14	43.99	9.71	56.65
171B	37.71	24.00	61.71	13.71	57.13
178B	29.75	18.88	48.63	10.87	57.57
001B	22.60	14.13	36.73	8.47	59.94
LR	43.92	27.25	71.17	16.67	61.17
121B	27.00	16.73	43.73	10.27	61.39
071B	27.61	17.09	44.70	10.52	61.56
099B	33.77	20.75	54.52	13.02	62.75
016B	40.00	24.25	64.25	15.75	64.95
068B	25.07	15.17	40.24	9.90	65.26
117B	34.13	20.63	54.76	13.50	65.44
220B	39.27	23.27	62.54	16.00	68.76
096B	25.00	14.75	39.75	10.25	69.49
106B	34.67	20.21	54.88	14.46	71.55
036B	45.77	26.38	72.15	19.39	73.50
197B	37.25	21.38	58.63	15.87	74.23
014B	48.00	27.25	75.25	20.75	76.15
223B	28.38	15.94	44.32	12.44	78.04
033B	33.00	18.50	51.50	14.50	78.38
261B	38.58	21.34	59.92	17.24	80.79
MR	34.58	19.04	53.62	15.54	81.62
023B	35.27	19.14	54.41	16.13	84.27
044B	45.13	24.17	69.30	20.96	86.72
193B	35.18	18.75	53.93	16.43	87.63
010B	36.94	19.52	56.46	17.42	89.24
108B	41.27	21.75	63.02	19.52	89.75
138B	37.12	19.52	56.64	17.60	90.16
059B	35.39	18.46	53.85	16.93	91.71
167B	46.67	24.29	70.96	22.38	92.14
053B	6.70	3.47	10.17	3.23	93.08
148B	29.77	15.27	45.04	14.50	94.96
185B	42.38	21.52	63.90	20.86	96.93
169B	38.52	19.50	58.02	19.02	97.54

Table 6.7 cont: Yield and percentage yield increase of early-bulking F_1genotyoes at 10 MAP over 7 MAP

Genotype	10 MAP	7 MAP	total yld	increase	% increase
062B	32.75	32.27	65.02	0.48	1.49
046B	25.77	23.93	49.70	1.84	7.69
020B	35.63	32.60	68.23	3.03	9.29
180B	13.72	12.54	26.26	1.18	9.41
229B	15.42	13.83	29.25	1.59	11.50
095B	16.04	14.25	30.29	1.79	12.56
015B	21.25	18.63	39.88	2.62	14.06
199B	22.61	19.73	42.34	2.88	14.60
105B	31.17	26.36	57.53	4.81	18.25
110B	24.42	20.38	44.80	4.04	19.82
065B	26.71	22.25	48.96	4.46	20.04
196B	24.18	19.84	44.02	4.34	21.88
182B	21.63	17.38	39.01	4.25	24.45
155B	25.58	20.42	46.00	5.16	25.27
237B	27.75	21.77	49.52	5.98	27.47
120B	34.73	26.77	61.50	7.96	29.73
262B	25.56	19.63	45.19	5.93	30.21
039B	20.38	15.58	35.96	4.80	30.81
159B	26.27	20.02	46.29	6.25	31.22
112B	32.11	24.27	56.38	7.84	32.30
057B	18.47	13.89	32.36	4.58	32.97
042B	29.98	22.52	52.50	7.46	33.13
075B	28.92	21.58	50.50	7.34	34.01
177B	44.93	33.48	78.41	11.45	34.20
151B	40.23	29.88	70.11	10.35	34.64
111B	40.53	30.04	70.57	10.49	34.92
211B	36.33	26.75	63.08	9.58	35.81
097B	42.15	30.86	73.01	11.29	36.58
076B	30.93	22.42	53.35	8.51	37.96
168B	35.50	25.25	60.75	10.25	40.59
018B	30.70	21.25	51.95	9.45	44.47
025B	31.00	21.13	52.13	9.87	46.71
082B	55.02	37.27	92.29	17.75	47.63
040B	37.77	25.25	63.02	12.52	49.58
PAR1	33.38	22.13	55.51	11.25	50.84

Table 6.7 cont. Yield and percentage yield increase of early-bulking F_1 genotypes at 10 MAP over 7 MAP

Genotype	10 MAP	7 MAP	total yld	increase	% increase
017B	16.50	16.75	33.25	-0.25	-1.49
028B	12.00	16.52	28.52	-4.52	-27.36
034B	18.27	29.89	48.16	-11.62	-38.88
055B	5.87	15.27	21.14	-9.40	-61.56
058B	12.23	12.71	24.94	-0.48	-3.78
061B	19.25	21.42	40.67	-2.17	-10.13
104B	40.54	41.04	81.58	-0.50	-1.22
119B	21.00	26.48	47.48	-5.48	-20.69
125B	19.50	21.35	40.85	-1.85	-8.67
127B	33.31	44.39	77.70	-11.08	-24.96
142B	13.58	19.04	32.62	-5.46	-28.68
207B	24.00	28.00	52.00	-4.00	-14.29
216B	25.92	35.71	61.63	-9.79	-27.42
243B	18.37	20.95	39.32	-2.58	-12.32
247B	9.15	19.11	28.26	-9.96	-52.12
254B	17.00	23.33	40.33	-6.33	-27.13
260B	8.96	22.54	31.50	-13.58	-60.25
PAR2	22.02	23.02	45.04	-1.00	-4.34

Table 6.8: Yield and percentage yield increase of early-bulking F_1 cassava genotypes at 12 MAP over 7 MAP

genotype	Yld7M (t/ha)	Yld12M (t/ha)	Total Yld (t/ha)	Yldincrease	%Yld increase 7M
196B	19.84	19.44	39.28	-0.4	-2.02
106B	20.21	19.77	39.98	-0.44	-2.18
267B	15.02	14.58	29.6	-0.44	-2.93
171B	24.00	22.16	46.16	-1.84	-7.67
025B	21.13	19.25	40.38	-1.88	-8.90
065B	22.25	20.25	42.5	-2	-8.99
105B	26.36	23.11	49.47	-3.25	-12.33
125B	21.35	18.63	39.98	-2.72	-12.74
154B	19.63	16.09	35.72	-3.54	-18.03
119B	26.48	21.32	47.8	-5.16	-19.49
246B	17.00	13.67	30.67	-3.33	-19.59
095B	14.25	11.43	25.68	-2.82	-19.79
254B	23.33	16.45	39.78	-6.88	-29.49
028B	16.52	11.45	27.97	-5.07	-30.69
262B	19.63	13.48	33.11	-6.15	-31.33
138B	19.52	12.94	32.46	-6.58	-33.71
104B	41.04	24.64	65.68	-16.4	-39.96
071B	17.09	10.14	27.23	-6.95	-40.67
163B	19.02	10.96	29.98	-8.06	-42.38
230B	19.02	10.52	29.54	-8.5	-44.69
034B	29.89	10.83	40.72	-19.06	-63.77

Table 6.8 cont

genotype	Yld7M (t/ha)	Yld12M (t/ha)	Total Yld (t/ha)	Yld increase	%Yld increase 7M
042B	22.52	33.76	56.28	11.24	49.91
073B	22.52	33.42	55.94	10.90	48.40
039B	15.58	23.09	38.67	7.51	48.20
211B	26.75	39.12	65.87	12.37	46.24
099B	20.75	30.31	51.06	9.56	46.07
197B	21.38	30.82	52.2	9.44	44.15
100B	18.13	25.71	43.84	7.58	41.81
181B	20.27	28.69	48.96	8.42	41.54
062B	32.27	45.27	77.54	13	40.29
219B	12.98	18.2	31.18	5.22	40.22
207B	28.00	39.05	67.05	11.05	39.46
243B	20.95	29.19	50.14	8.24	39.33
132B	12.54	17.09	29.63	4.55	36.28
082B	37.27	50.59	87.86	13.32	35.74
120B	26.77	36.04	62.81	9.27	34.63
127B	44.39	59.51	103.9	15.12	34.06
177B	33.48	44.17	77.65	10.69	31.93
018B	21.25	28	49.25	6.75	31.76
097B	30.86	40.54	71.4	9.68	31.37
052B	18.5	23.98	42.48	5.48	29.62
111B	30.04	38.9	68.94	8.86	29.49
216B	35.71	45.15	80.86	9.44	26.44
112B	24.27	30.49	54.76	6.22	25.63
LR	27.25	34.04	61.29	6.79	24.92
146B	13.64	16.76	30.4	3.12	22.87
016B	24.25	29.4	53.65	5.15	21.24
005B	15.04	17.98	33.02	2.94	19.55
237B	21.77	25.31	47.08	3.54	16.26
122B	21.46	24.76	46.22	3.3	15.38
263B	16.5	19	35.5	2.5	15.15
193B	18.75	20.5	39.25	1.75	9.33
020B	32.6	35.04	67.64	2.44	7.48
MR	19.04	20.35	39.39	1.31	6.88
078B	18.5	19.67	38.17	1.17	6.32
229B	13.83	14.58	28.41	0.75	5.42
014B	27.25	28.31	55.56	1.06	3.89
247B	19.11	19.77	38.88	0.66	3.45
168B	25.25	25.73	50.98	0.48	1.90

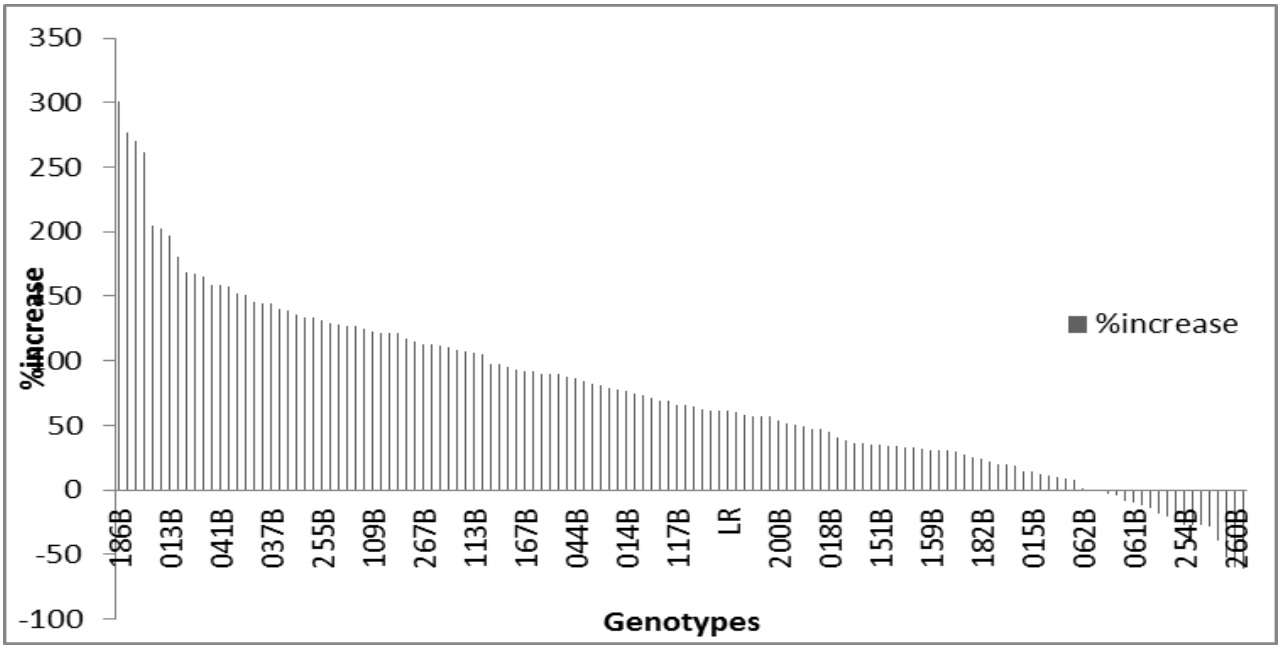


Fig. 6.4: Percentage yield increase in fresh storage root yield of 129 F_1 cassava genotypes and 5 checks at 10 MAP over 7 MAP evaluated for early storage root bulking in Umudike humid forest agroecology of Nigeria in 2016/17 and 2017/18 seasons

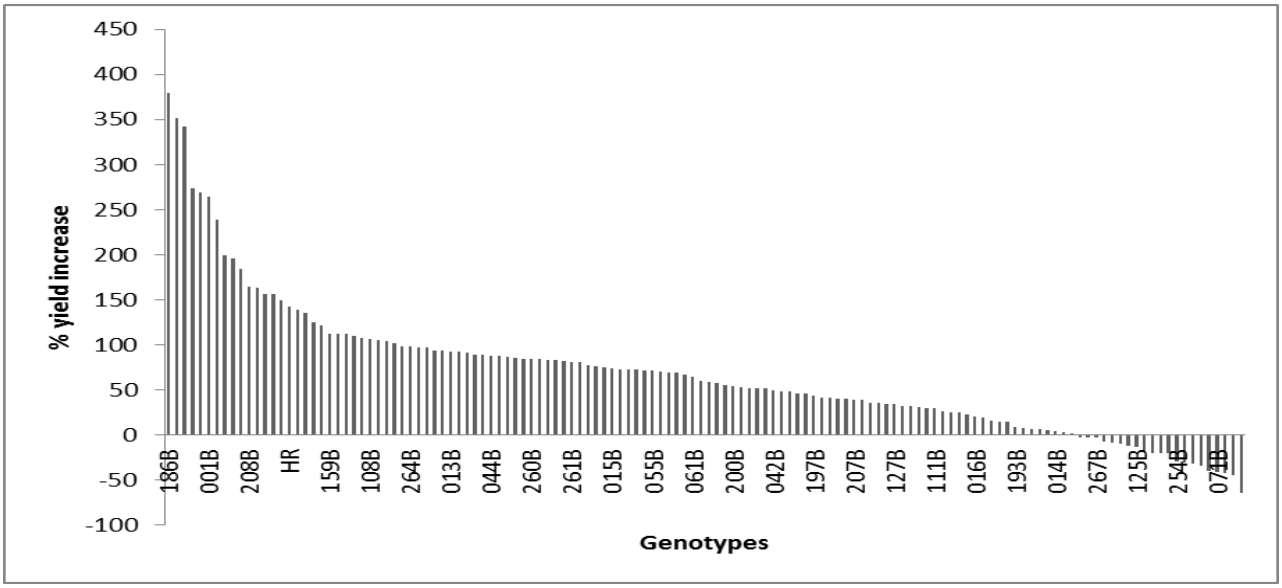


Figure 6.5: Percentage yield increase in fresh storage root yield of 129 F_1 genotypes and 5 checks at 12 MAP over 7 MAP evaluated for early storage root bulking in Umudike humid forest agroecology of Nigeria in the 2016/17 and 2017/18 seasons

6.3.5 Mean values of traits with harvest time

The values of traits showed that most of the traits had performed better at 12 MAP when compared with the performance at 7 and 10 MAP (Fig. 6.5), except for some traits such as plant vigour, whose mean value score was highest at 7 MAP (3.37), followed by the score at 10 MAP (3.18). The lowest mean score for vigour was at 7 MAP; height at first branching, with scores of 78.64 cm at 10 MAP and 75.26 at 12 MAP; and stem diameter, with mean scores of 2.22 cm at 10 MAP and 2.19 cm at 12 MAP.

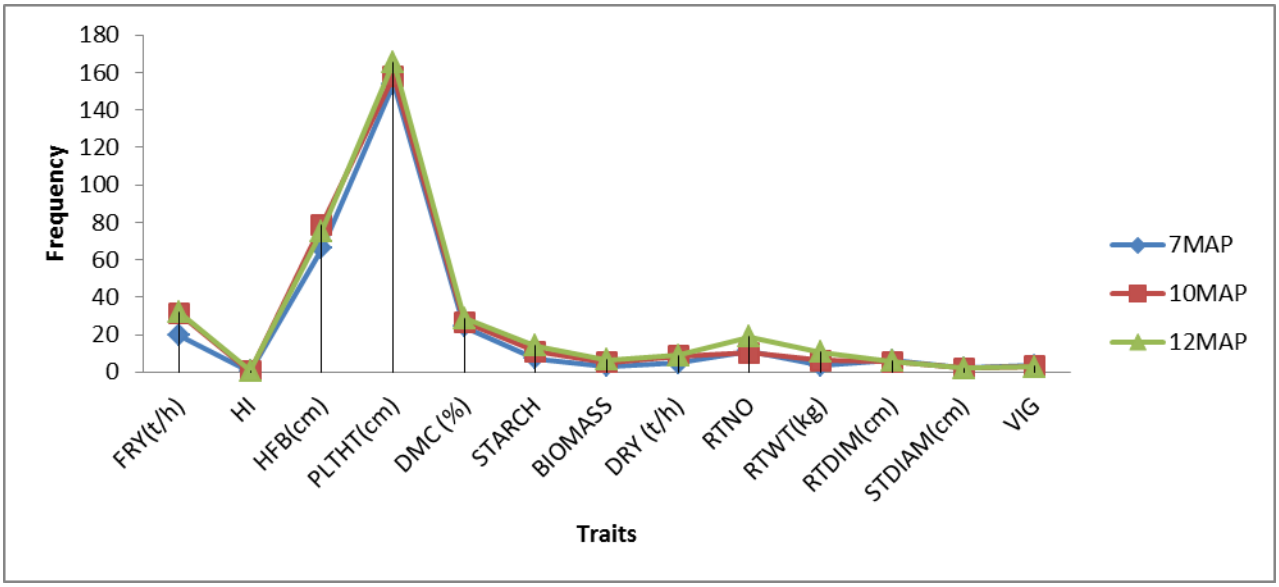


Figure 6.6: Mean trait values at different harvest time

*FRY = fresh root yield, HI = harvest index, HFB = height at first branching, DMC = dry-matter content, Biomass = total plant biomass, DRT = dry root yield, RTNO = Root number, RTWT = root weight, RTDIAM = root diameter, STDIAM = stem diameter, VIG = plant vigour

6.3.6 Mean values of traits across the years

The performance of traits over years was shown in Fig. 6.6. The result showed that mean values of traits such as fresh root yield, height at first branching, plant height, dry-matter content, starch content, dry root yield, root diameter, stem diameter, and vigour were higher in

the first year than in the second year, though the difference was significant. Only two traits (root weight and root number) had higher mean values in the second year compared to the first year.

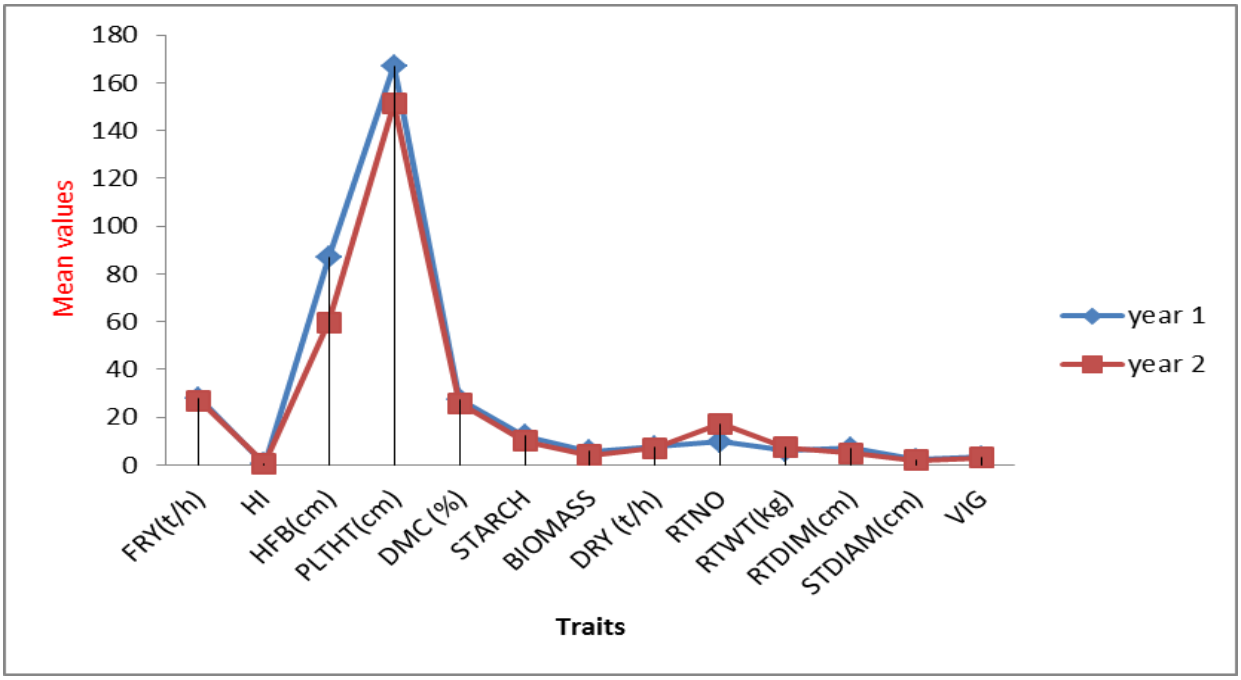


Figure 6.7: Mean trait distribution over years

6.3.7 Estimates of phenotypic, genotypic and environmental variance components

The variance components of genotype and environment were determined for each trait and for each harvest date separately. The variance components for genotypes in all three harvest times were higher for all traits than that of environment (Table 6.9). Height at first branching, plant height, and fresh root yield consistently attained high genotypic and phenotypic variances at harvest times. Similarly, for all the traits, a large proportion of the phenotypic component of variance was accounted for by the genotypic component of the variance as reflected by H_b being > 20% for all traits harvested at 7 MAP, > 30% for all traits harvested at 10 MAP, and > 60% for all traits harvested at 12 MAP. Phenotypic coefficient of variation ranged between 16.54% for dry-matter content up to 113.2% for harvest index at 7 MAP; 14.32% for dry-matter content and 66.73 for total plant biomass at 10 MAP; 17.98% for dry-matter content and 118.55% for root number at 12 MAP; whereas the genotypic coefficient of variation ranged between 11.00% and 111.62% at 7 MAP; 10.18% and 46.29% at 10 MAP; and 15.33 and

99.88% at 12 MAP (Table 6.9), respectively. Phenotypic coefficient of variation in general was higher than the corresponding genotypic coefficient of variation for all the traits evaluated at the various harvest time. However, dry-matter content had the lowest value for PCV and GCV at various months of harvest. The estimates of genetic variability within the population were measured by the broad sense heritability. H_b estimates were high in most of the traits evaluated in the three harvest times, but the highest values for all traits were recorded at 12 MAP. The highest H_b estimates at 7, 10, and 12 MAP were recorded by harvest index, plant vigour, and dry root yield, while the lowest H_b estimates were recorded by stem diameter, stem diameter and plant height at 7, 10, and 12 MAP, respectively. In general, broad sense heritability ranged between 24.16% for stem diameter and 97.22% harvest index for all traits at the three harvest times.

Table 6.9: Estimates of variance components and broad sense heritability for 13 traits in F_1 cassava genotypes evaluated for early root bulking on three separate harvest times at Umudike

Traits	GV	PV	VE	PCV	GCV	$H_2b\%$
7 MAP						
HFB	263.5	602.35	338.85	37.13	24.56	43.74
PLTHT	303.75	768.85	465.1	18.04	11.33	39.5
DMC	7.21	16.29	9.08	16.54	11	44.26
FRY	39.94	85.45	45.51	47.73	32.62	47.74
HI	0.35	0.36	0.01	113.2	111.62	97.22
STCH	12.71	29.11	16.4	65.64	43.36	43.66
BIOM	3.12	5.77	2.65	75.09	55.2	54.02
DRY	2.49	5.2	2.71	49.26	36.12	47.67
RTNO	20.16	41.68	21.52	58.06	40.37	48.37
RTWT	2.93	4.89	1.97	64.07	49.6	59.92
RTDIAM	0.65	1.4	0.75	19.27	13.14	46.52
STDIAM	0.053	0.221	0.168	23.51	11.55	24.16
VIG	0.302	0.59	0.288	21.76	15.55	51.07
10 MAP						
HFB	605.25	987.15	381.9	40.23	31.5	61.3
PLTHT	850	1301.65	451.65	22.86	18.48	65.31
DMC	7.27	14.39	7.12	14.32	10.18	50.53
FRY	137.1	225.05	87.95	46.47	36.27	60.91
HI	0.01034	0.01744	0.0071	24.08	18.54	59.29
STCH	12.895	25.52	12.63	46.09	32.76	50.53
BIOM	6.32	13.13	6.805	66.73	46.29	48.13
DRY	10.25	17.17	6.92	48.46	37.58	59.69
RTNO	11.45	26.27	14.82	47.9	31.62	43.59
RTWT	8	14.63	6.63	61.38	45.4	54
RTDIAM	1.0575	1.7275	0.67	23.33	18.28	61.22
STDIAM	0.15	0.4049	0.2549	28.83	17.54	37.046
VIG	0.486	0.7005	0.2145	26.29	21.89	69.37
12 MAP						
HFB	923.05	1317.6	394.55	47.51	39.77	70.05
PLTHT	1026.5	1589.5	563	23.67	19.025	64.50
DMC	19.595	26.875	7.30	17.96	15.33	72.91
FRY	198	254.70	56.70	49.73	43.84	77.74
HI	0.017385	0.02326	5875	25.92	22.41	74.74
STCH	34.75	47.7	12.9524	48.91	41.75	72.85
BIOM	30.405	46.975	16.57	95.19	76.58	65.00
DRY	19.56	24.735	5.175	53.34	47.92	79.11
RTNO	384.05	508.4	124.35	118.55	99.88	75.54
RTWT	100.08	141.25	41.17	107.84	90.78	70.85
RTDIAM	1.75	2.45	0.35	25.6	21.64	71.43
STDIAM	0.382	0.547	0.165	33.33	27.85	69.80
VIG	0.28685	0.4268	0.13995	22.48	18.43	67.20

*MAP = months after planting, HFB = height at first branching (cm) PLTHT = plant height (cm), FRY = fresh storage root yield (t/ha), HI = harvest index, STCH = starch content (%), BIOM = total plant biomass (kg), DRY = dry root yield (t/ha), RTNO = root number, RTWY = root weight (kg), RTDIAM = root diameter (cm), STDIAM = stem diameter (cm), VIG = plant vigour, GE = genotypic variance, PE = phenotypic variance, PVE = phenotypic coefficient of variation, GVE = genotypic coefficient of variation, H_2b = broad sense heritability

6.3.7 Evaluation of early root bulking at Umudike and Kano at 12 MAP

6.3.7.1 Combined analysis of variance across location at 12 MAP

The genotype, location, and year mean square in the combined ANOVA were all highly significant (P < 0.001) for all traits (Table 6.10). The interactions, genotype x location, genotype x year mean squares were also highly significant (P < 0.001) for all the traits. Location x year mean squares were highly significant (P < 0.001) for all traits except dry root yield (< 0.05), but were not significant for total plant biomass and root weight. Genotype x location x year mean squares were all highly significant (P < 0.001), except for root weight, which was significant at (P < 0.05). Coefficients of variation ranged between 16.1% for root diameter and 76.3% for root weight.

Table 6.10: Combined analysis of variance of mean squares for yield and other agronomic traits of F_1 cassava genotypes and five checks evaluated for early root bulking at Umudike and Minjibir Kano at 12 MAP in the 2016/17 and 2017/18 growing seasons

Source of variation	DF	FRY	HI	HFB	PLTHT	DMC	STARCH
Genotype (g)	126	520***	0.05***	2922.5***	3206.3***	55.44***	97.97***
Location (L)	1	23745***	1.98436***	223439***	224272***	4177.3***	7415.7***
year (y)	1	3649***	3.34***	109226***	233595***	475.03***	774.68***
g x L	101 (25)	378.7***	0.034***	2369.5***	2719.0***	36.94***	65.48***
g x y	116 (10)	374.2***	0.036***	1898.7***	1782.9***	37.38***	66.70***
L x y	1	5150.6***	1.26***	74724***	190540***	2747.6***	4859.7***
g x Lx y	40 (86)	265.5***	0.024***	2835.2***	2241.5***	36.76***	65.20***
cv (%)		36.90	18.50	36.0	20.6	13.00	31.70

Table 6.10: contd. Combined analysis of variance of mean squares for yield and other agronomic traits of F1 cassava genotypes and 5 checks evaluated for early root bulking at Umudike and Minjibir Kano at 12 MAP in the 2016/17 and 2017/18 growing seasons

Source of variation	DF	BIOM	DRY	RTNO	RTWT	RTDIM	STDIAM	VIG
Genotype (g)	126	59.01***	53.82***	574.2***	141.31***	5.74***	1.20***	1.47***
Location (L)	1	871.96***	1008.38***	8951.3***	8624.14***	259.98***	13.32***	105.7***
year (y)	1	1262.96***	287.15***	14580.8***	1317.20***	84.75***	8.45***	2.63***
g x L	101 (25)	57.55***	35.39***	526.5***	148.43***	2.94***	0.86***	0.77**
g x y	116 (10)	42.56***	35.48***	226.1***	49.64***	3.73***	1.06***	1.51***
L x y	1	11.52ns	67.49**	30547.9***	2075.73ns	307.75***	6.09***	47.71***
g x Lx y	40 (86)	35.78***	23.28***	278.9***	64.37**	1.91***	0.58***	0.86***
CV (%)		68.3	40	65.5	76.3	16.1	23.2	19.3

6.3.7.2: Relationships among yield and other yield-related traits in Kano and Umudike at 12 MAP

Pearson's correlation coefficient was used to determine the relationships among traits at Kano and Umudike at 12 MAP, as shown in Figures 6.7 and 6.8. Most of the agronomic traits were highly and significantly correlated with each other in both locations at 12 MAP. Work in these two locations was centred on yield. In Kano, fresh root yield strongly and positively correlated with dry root yield (r = 0.94) (Fig. 6.8). Fresh root yield also correlated positively with other yield-related traits such as root weight (r = 0.56), root number (r = 0.44), root diameter (r = 0.47), total plant biomass (r = 0.36), harvest index (r = 0.44), stem diameter (r = 0.12), plant height (r = 0.22), and height at first branching (r = 0.16). In Umudike at 12 MAP, fresh root yield similarly had a strong relationship with dry root yield (r = 0.92). Other traits that correlated positively with fresh root yield in Umudike at 12 MAP included root weight (r = 0.47), root number (r = 0.28), root diameter (r = 0.41), total plant biomass (r = 0.26), harvest index (r = 0.38), stem diameter (r = 0.09), and plant height (r = 0.08).

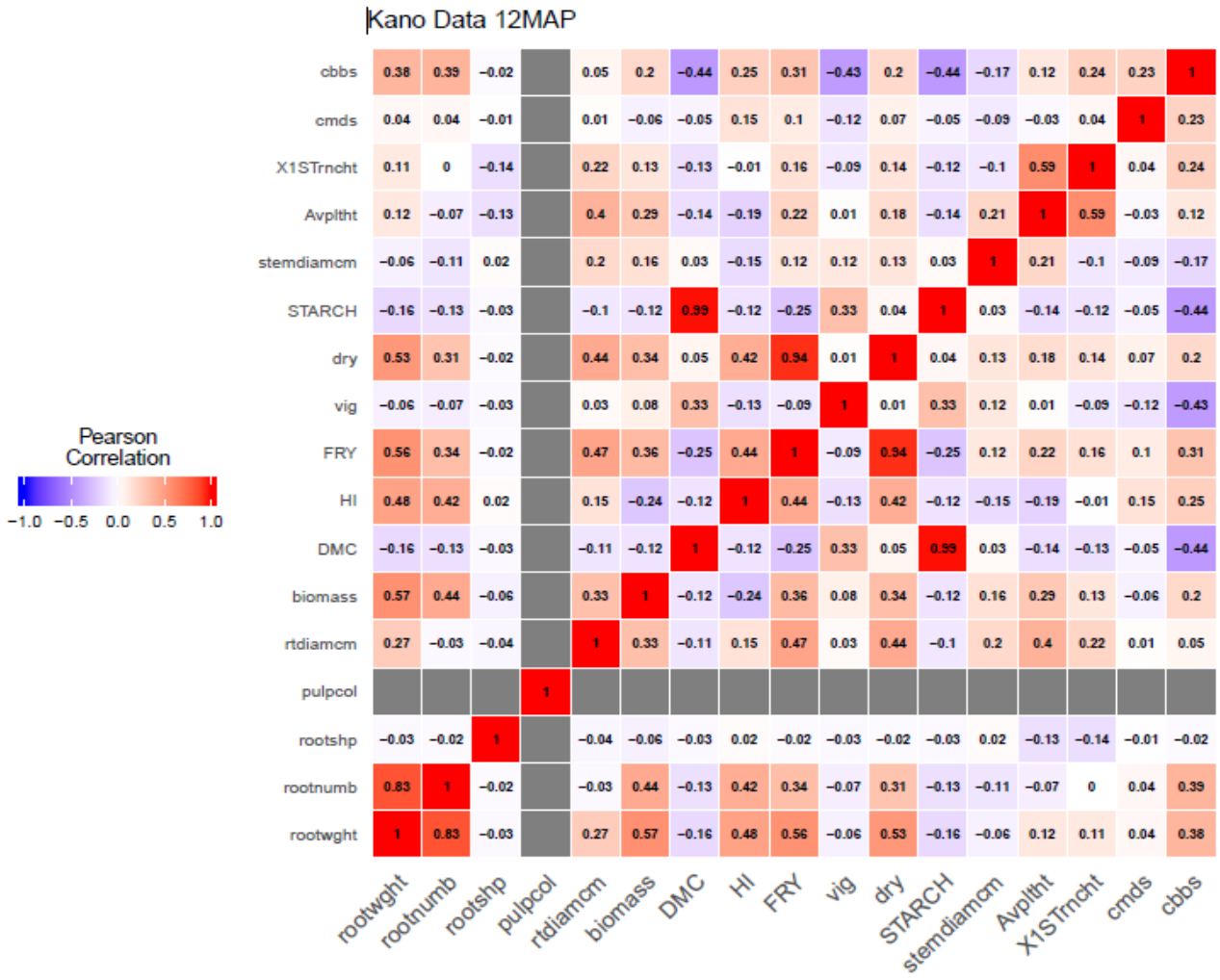


Figure 6.8: Correlation coefficient of yield and other agronomic traits of F_1 population at 12 MAP in Kano

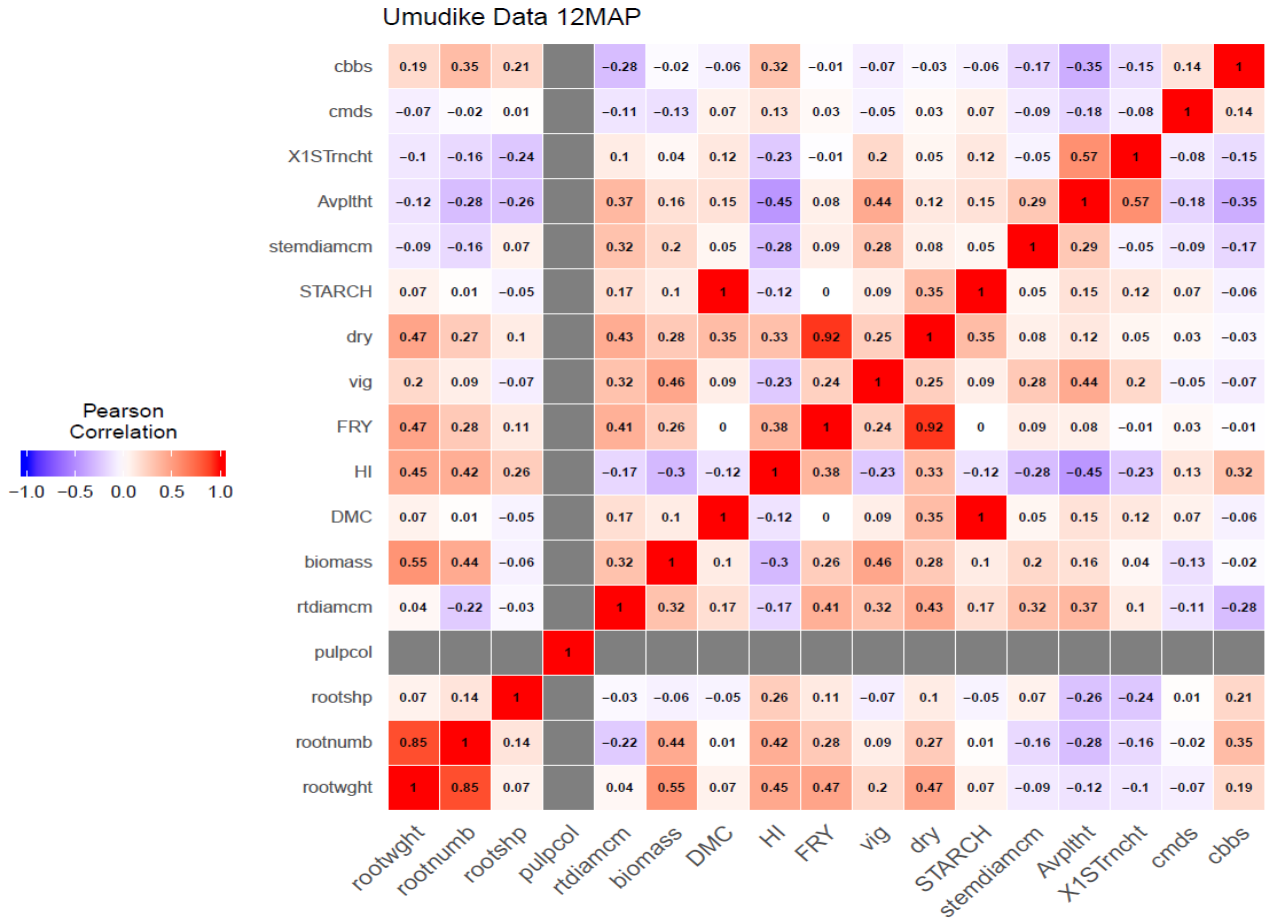


Figure 6.9: Correlation coefficient of yield and other agronomic traits of F_1 population at 12 MAP in Umudike

6.3.9 Location by genotype interaction effects

Twenty representative genotypes were selected from the 129 genotypes to determine the effect on location x genotype interaction of the various traits. Mean plant height for genotype ranged between 88.33 cm and 197.82 cm in the Kano location, while in Umudike, the mean ranged between 113.98 cm and 216.25, while the mean for the representative genotypes for the two locations was 148.247 cm (Table 6.11). It was observed that mean value for plant height in Umudike was higher than the mean value for plant height in Kano. In DMC, mean value ranged between 27.82% and 37.84% in the Kano location, while the range was 23.46% to 36.86%. The mean value across genotypes in Kano was higher than the mean DMC in the Umudike location. The mean FRY for the representative genotypes ranged between 11.29 t/ha to 36.54 t/ha in Kano, while in Umudike, the mean range was 20.89 t/ha to 53.92 t/ha. The overall mean value for fresh root yield across the genotypes for the two locations was 30.105 t/ha. Results also

showed that the mean yield performance in Umudike was higher than that of yhe Kano location (Table 6.12).

The mean harvest index in the representative genotypes ranged between 0.39 and 0.62 in Kano, while in Umudike it ranged between 0.45 and 0.84. The mean value for the harvest index in Umudike was higher than the mean value for harvest index in Kano (Table 6.12), while the mean of genotypes across the two locations was 0.57.

The mean starch content ranged etween 12.75 and 24.96% in Kano; and between 6.93 and 24.77% at Umidike. The mean value for starch content was higher in Kano than the mean value for starch content in Umudike by over 19% (Table 6.13).

Mean plant biomass for the genotypes across the two locations was 6.95 kg. The biomass in Umudike was found to be higher than that of Kano. The range value for Kano was 0.92 to 11.88 kg, while that of Umudike was 2.45 to 19.18 kg (Table 6.13).

The mean root number across all the genotypes in the two locations was 19.12 kg. The mean value of the root number for the representative genotypes was higher in Umudike when compared with the root number in Kano (Table 6.14). The mean root number in Kano ranged between 1.64 kg and 34.67 kg, while that in Umudike ranged between 0.60kg and 53.47 kg.

The mean root weight was higher in Umudike than the root weight in Kano (Table 6.14). The overall mean value across the two locations was 9.60 kg. The mean root weight of the representative genotypes ranged between 2.99 kg and 13.14 kg in Kano, while in Umudike it ranged between 2.38 kg and 34.74 kg. The mean root weight in Umudike was higher than the mean root weight in Kano.

The mean root diameter and stem diameter of the representative genotypes is shown in Table 6.15. The mean root diameter in Kano was 5.25 cm, while in Umudike it was 6.81 cm (Table 6.15). The mean across the genotypes for the two locations was 6.03 cm, while the range values were 3.81 and 6.61 cm in Kano; and 4.82 cm to 11.03 cm in Umudike (Table 6.15). The range values in stem diameter for the two locations were 1.79 to 3.06 cm in Kano; and 0.78 to 2.87 cm in Umudike. The mean value for stem diameter in Kano was higher than the mean

value for stem diameter in Umudike by 4.82%, while the overall mean value across the representative genotypes was 2.28 cm (Table 6.15).

The mean score for plant vigour was higher in Kano than in Umudike (Table 6.16). The overall mean score across all the representative genotypes was 3.31. The range values were 3.0 to 4.25 in Kano and 2.23 to 4.05 in Umudike.

Table 6.11: Effect of location x genotype interaction on plant height and dry-matter content

	PLTHT (cm)			DMC (%)		
	Location			Location		
Genotype	Kano	Umudike	Mean	Kano	Umudike	Mean
001B	132.8	155.43	144.115	35.24	32.77	34.005
002B	112.83	188.75	150.79	36.99	25.31	31.15
005B	88.33	200.69	144.51	37.84	26.17	32.005
010B	145.75	165.7	155.725	32.79	29.32	31.055
013B	197.82	146.8	172.31	35.17	27.92	31.545
014B	118.63	154.01	136.32	27.82	24.07	25.945
016B	103	156.30	103	34.49	30.73	32.61
017B	150.88	164.05	157.465	30.63	30.41	30.52
018B	136.45	164.8	150.625	36.98	28.33	32.655
020B	142.48	169.75	156.115	34.44	23.46	28.95
023B	92.04	121.49	106.765	32.36	33.52	32.94
025B	143.82	161.6	152.71	35.09	29.11	32.10
027B	179.7	209.16	194.43	37.54	36.86	37.20
028B	123	113.98	118.49	33.25	28.75	31.00
033B	135	114.49	124.745	29.95	25.38	27.665
034B	141.82	185.55	163.685	32.8	28.75	30.775
036B	117.25	216.25	166.75	33.11	25.38	29.245
037B	165.75	131.29	148.52	35.69	36.28	35.985
039B	114.25	184.4	149.325	33.62	26.64	30.13
040B	164.93	172.18	168.555	30.65	24.32	27.485
Mean	135.3265	164.23	148.2475	33.8225	28.674	31.24825

Table 6.12: Effect of location x genotype interaction on fresh storage root yield and harvest index

	FRY (t/ha)			HI		
	Location			Location		
Gentype	Kano	Umudike	Mean	Kano	Umudike	Mean
001B	22.5	50.98	36.74	0.55	0.71	0.63
002B	21.25	52.79	37.02	0.54	0.66	0.6
005B	11.29	20.81	16.05	0.47	0.49	0.48
010B	20.09	37.37	28.73	0.55	0.56	0.555
013B	32.3	35.5	33.9	0.4	0.68	0.54
014B	26.5	28.3	27.4	0.58	0.67	0.625
016B	17.94	29.4	23.67	0.52	0.53	0.525
017B	19.59	29.56	24.575	0.56	0.52	0.54
018B	23.34	24.5	23.92	0.39	0.65	0.52
020B	25.48	35.04	30.26	0.55	0.64	0.595
023B	36.54	46.1	41.32	0.59	0.68	0.635
025B	20.58	26.5	23.54	0.43	0.55	0.49
027B	30.63	39.92	35.275	0.62	0.71	0.665
028B	29.5	24.89	27.195	0.48	0.73	0.605
033B	16.29	45.47	30.88	0.45	0.84	0.645
034B	22.6	32.12	27.36	0.61	0.7	0.655
036B	30.89	48.79	39.84	0.54	0.64	0.59
037B	16.46	40.32	28.39	0.49	0.62	0.555
039B	22.75	24.17	23.46	0.51	0.45	0.48
040B	31.26	53.92	42.59	0.45	0.64	0.545
Mean	23.889	36.3225	30.10575	0.514	0.6335	0.57375

Table 6.13: Effect of location x genotype interaction on starch content and total biomass

	STARCH (%)			BIOM (kg)		
	Location			Location		
Gentype	Kano	Umudike	Mean	Kano	Umudike	Mean
001B	22.6	19.32	20.96	4.05	8.3	6.175
002B	24.96	9.4	17.18	5.02	7.35	6.185
005B	26.08	10.51	18.295	3.47	4.96	4.215
010B	19.35	14.76	17.055	5.9	16.15	11.025
013B	22.55	12.93	17.74	11.88	2.45	7.165
014B	12.75	7.75	10.25	3.53	6.35	4.94
016B	21.62	13.01	17.315	4.2	15.55	9.875
017B	16.48	12.87	14.675	4.6	9.15	6.875
018B	24.88	16.6	20.74	8.13	2.82	5.475
020B	21.55	16.19	18.87	9.82	11.65	10.735
023B	18.78	13.42	16.1	3.45	5.28	4.365
025B	22.44	6.93	14.685	7.0	6.4	6.7
027B	25.7	20.34	23.02	6.1	7.92	7.01
028B	19.97	14.47	17.22	8.67	2.85	5.76
033B	15.59	24.77	20.18	3.7	19.18	11.44
034B	19.44	14.04	16.74	2.92	4.81	3.865
036B	19.79	9.48	14.635	6.25	4.67	5.46
037B	23.2	23.96	23.58	3.1	6.16	4.63
039B	20.46	11.17	15.815	6.72	14.73	10.725
040B	16.5	8.09	12.295	8.66	4.16	6.41
Mean	20.7345	14.0005	17.3675	5.8585	8.0445	6.9515

Table 6.14: Effect of location x genotype interaction on root number and root weight

	RTNO			RTWT (kg)		
	Location			Location		
Genotype	Kano	Umudike	mean	Kano	Umudike	mean
001B	12.91	41.91	27.41	5.22	23.57	14.395
002B	19.75	15.66	17.705	6.38	14.18	10.28
005B	13.5	0.6	7.05	2.97	2.38	2.675
010B	22.75	36.84	29.795	7.2	19.38	13.29
013B	19.81	10.16	14.985	9.29	4.99	7.14
014B	12.03	30.75	21.39	5.03	16.67	10.85
016B	16.75	39.25	28	4.53	20.35	12.44
017B	23.5	23.5	23.5	5.55	9.83	7.69
018B	1.64	8.66	5.15	2.78	4.77	3.775
020B	34.67	40.75	37.71	13.14	18.95	16.045
023B	9.25	15.33	12.29	4.97	10.79	7.88
025B	19.34	16.25	17.795	4.88	6.82	5.85
027B	17.13	23.06	20.095	9.36	15.13	12.245
028B	22	6.28	14.14	9.1	6.24	7.67
033B	12.59	53.47	33.03	4.38	38.74	21.56
034B	7.8	13.76	10.78	4.23	9.98	7.105
036B	14.25	15.75	15	8.07	9.02	8.545
037B	5.66	24.01	14.835	3.02	12.66	7.84
039B	17	35.25	26.125	6.67	12.18	9.425
040B	9.1	2.27	5.685	5.22	5.54	5.38
Mean	15.5715	22.6755	19.1235	6.0995	13.1085	9.604

Table 6.15: Effect of location x genotype interaction on root diameter and stem diameter

	RTDIAM (cm)			STDIAM (cm)		
	Location			Location		
Genotype	Kano	Umudike	Mean	Kano	Umudike	Mean
001B	5.44	7.9	6.67	2.75	1.73	2.24
002B	5.14	6.05	5.595	1.8	2.36	2.08
005B	4.35	7.02	5.685	1.84	2.17	2.005
010B	4.61	6.21	5.41	2.79	2.28	2.535
013B	6.58	7.18	6.88	2.93	1.51	2.22
014B	5.27	7.1	6.185	2.76	4.07	3.415
016B	5.03	5.95	5.49	1.99	1.86	1.925
017B	4.74	6.66	5.7	2.57	2.03	2.3
018B	4.61	6.75	5.68	2.32	2.3	2.31
020B	5.49	6.49	5.99	2.45	2.18	2.315
023B	3.81	4.82	4.315	2.01	0.78	1.395
025B	4.33	5.69	5.01	1.79	2.77	2.28
027B	5.29	6.29	5.79	2.2	1.97	2.085
028B	5.39	5.49	5.44	2.69	1.56	2.125
033B	5.45	11.03	8.24	2.45	1.49	1.97
034B	6.5	7.51	7.005	2.58	1.84	2.21
036B	5.19	7.05	6.12	3.06	2.89	2.975
037B	6.24	7.72	6.98	2.48	2.14	2.31
039B	4.99	5.28	5.135	2.43	2.83	2.63
040B	6.61	7.93	7.27	1.93	2.71	2.32
Mean	5.253	6.806	6.0295	2.391	2.1735	2.28225

Table 6.16: Effect of location x genotype interaction on plant vigour

	VIG		
	Location		
Genotype	Kano	Umudike	Mean
001B	2.98	2.98	2.98
002B	4.25	3.5	3.875
005B	3.5	3.85	3.675
010B	3.5	3.55	3.525
013B	3.52	2.23	2.875
014B	3.53	3.03	3.28
016B	3.5	3.25	3.375
017B	3.5	2.98	3.24
018B	2.44	2.48	2.46
020B	3.21	3.78	3.495
023B	3.75	3.11	3.43
025B	3.28	3.2	3.24
027B	4.01	3.39	3.7
028B	3.75	2.26	3.005
033B	3	4.05	3.525
034B	3.03	3.48	3.255
036B	4	3.25	3.625
037B	3.75	2.65	3.2
039B	3	3.41	3.205
040B	3.47	2.97	3.22
Mean	3.4485	3.17	3.30925

6.3.10 GGE Biplot analysis of genotype by environment interaction in F_1 cassava genotypes

GGE biplot was constructed using the first two principal components (PC1 and PC2) derived from subjecting the environment-centred data to singular-value decomposition (Yan and Rajcan, 2002). Results of the GGE biplot also showed that the first two principal components (PC1 and PC2) justified 92.7% of the sum of squares with PC1 = 87% and PC2 = 5.7% for fresh root yield; 79% with PC1 = 64.9% and PC2 = 14.1% for starch content; 79.2% with PC1 = 65.1% and PC2

= 14.1% for dry-matter content; 88.4% with PC1 = 80.4% and PC2 = 8% (Figures 6.10, 6.11, 6.12, and 6.13).

6.3.10.1 Winning genotype and mega-environment in fresh storage root yield

The vertex genotypes in this study were 078B, 119B, 070B and 040B, 066B and 036B (Fig. 6.9). Another important feature in Fig. 6.10 is that it indicated environmental groupings, which suggested the possible existence of different mega-environments. The environment group within each sector and the genotypes at the polygon's extremity characterised the mega-environments (Yan and Rajcan, 2002). Based on the biplot analysis, of four environments, two mega-environments were suggested in Fig. 6.10. The first mega-environment contains environments UMUYR1 and KanoYR1, with genotypes 066B and 078B being the winners. The second environment contains environment UMUYR2, with genotype 040B and 036B being the winner in that environment. Thus, the genotypes 066B and 078B performed better in the environment of UMUYR1 and Kanoyr1, while 040B and 036B performed better in UMUYR2.

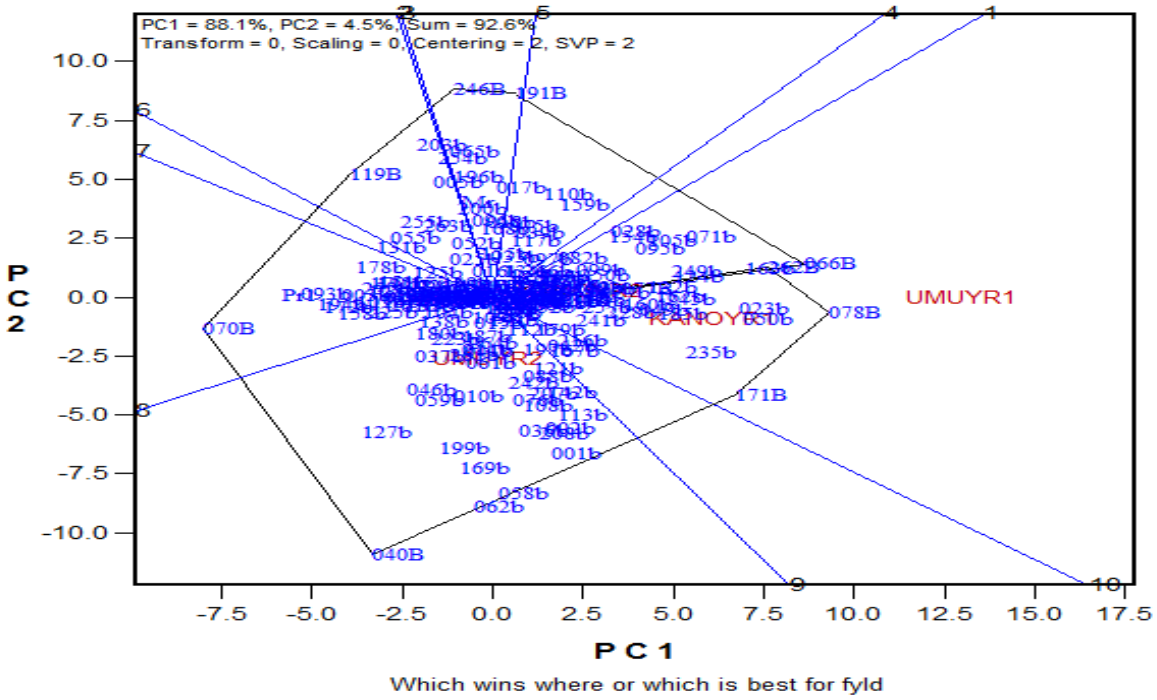


Figure 6.10: Polygon views GGE biplot based on symmetrical scaling for the which-won-where patterns of genotypes and environments for fresh storage root yield

6.3.10.2 Winning genotype and mega-environment in starch content and dry-matter content

The vertex genotypes in this study for starch and dry-matter content were the same (172B, 027B, 260B, and 109B) (Figures 6.11 and 6.12). Based on the biplot analysis, of four environments, three mega-environments were suggested. The first mega-environment contains environments UMUYR1, with genotypes 172B and 109B being the winners. The second environment contains environments KANOYR1 and KANOYR2, with genotype 027B being the winner in that environment. Thus, the genotypes 027B performed better in the environments of KANOYR1and KANOYR2, while 172B and 109B performed better in UMUY1.

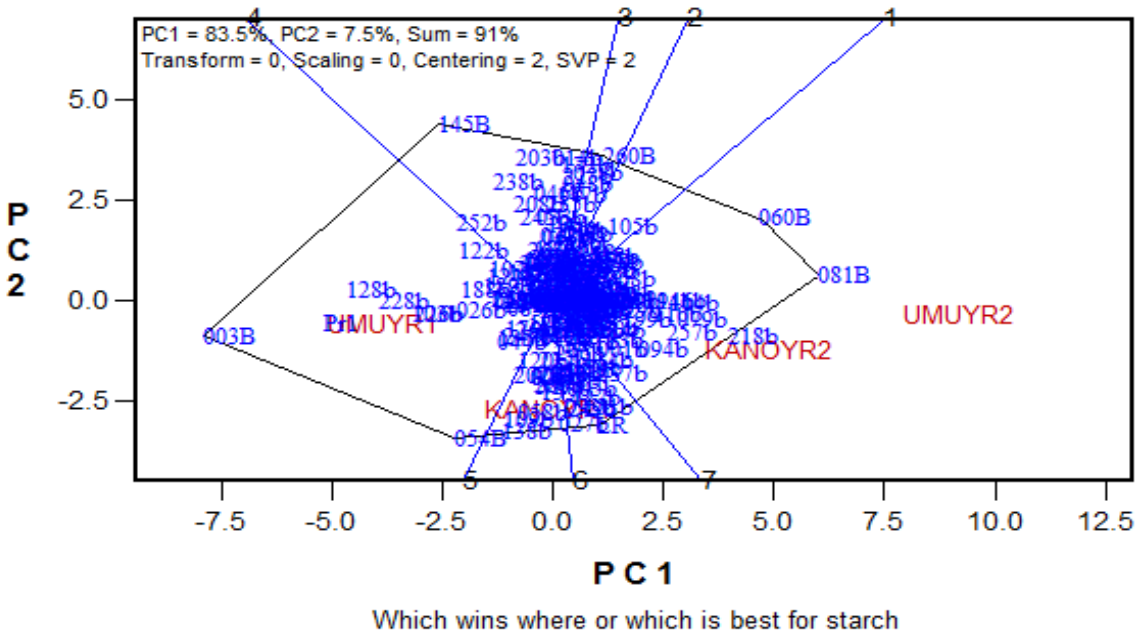


Figure 6.11: Polygon views GGE biplot based on symmetrical scaling for the which-won-where patterns of genotypes and environments for starch content

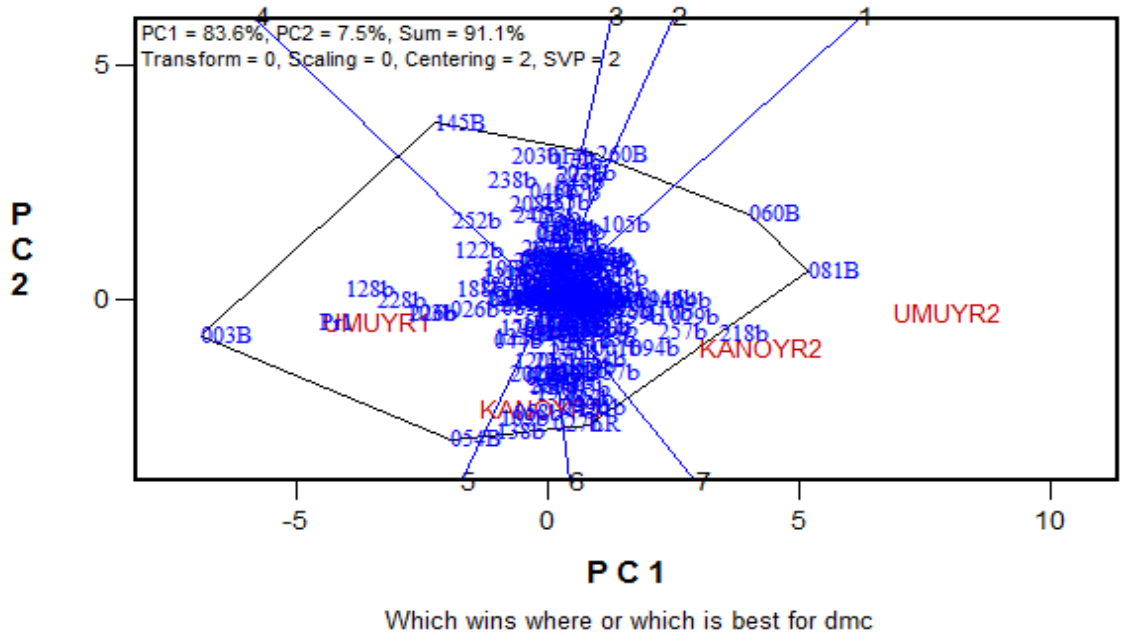


Figure 6.12: Polygon views GGE biplot based on symmetrical scaling for the which-won-where patterns of genotypes and environments for dry-matter content

6.3.10.3 Winning genotype and mega-environment in root number

Results of the analysis showed that the vertex genotypes for root number were 113B, 109B, 193B, and 207B (Fig. 6.13). The first mega-environment contains environment UMUYR1 with the genotype 113B. The second environment contains UMUYR2 and KANOYR2 with genotype 109B. The third environment was KANOYR1 with genotype 193B and 207B. The results showed that genotype 133B performed better in terms of root number in the UMUYR1 environment, and 109B was the winner in KANOYR2 and UMUYR2, while 193B and 207B won in KANOYR1 (Fig. 6.13).

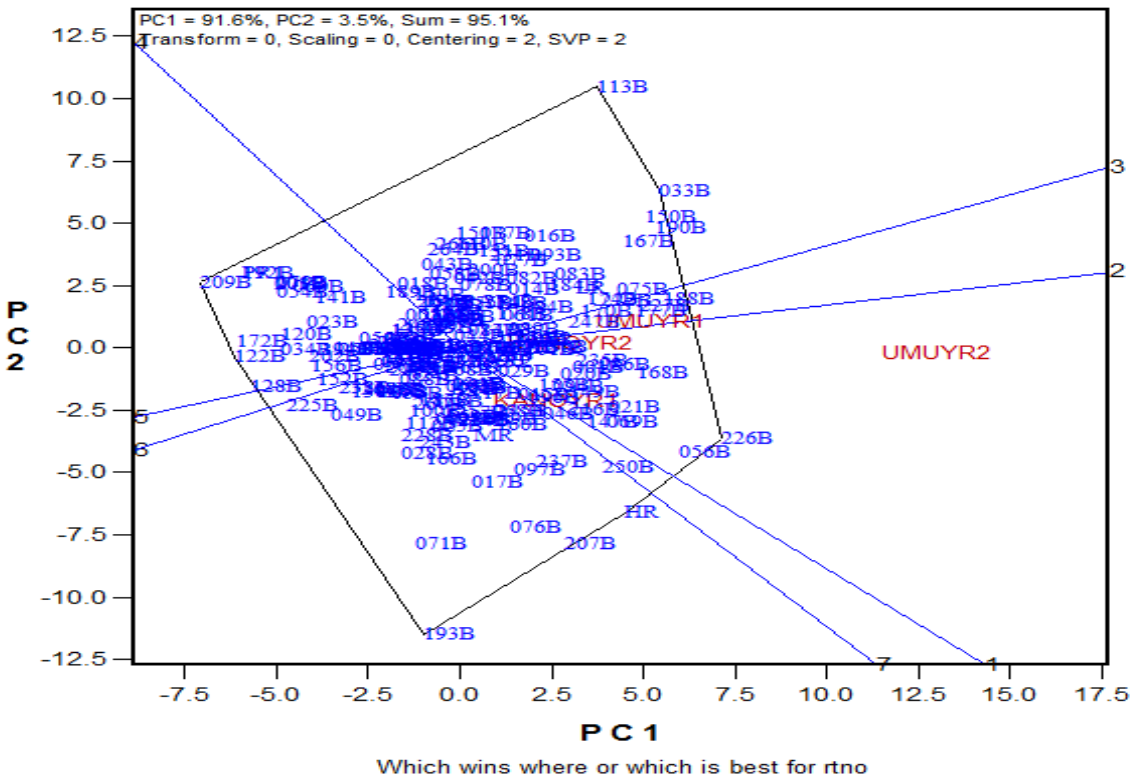


Figure 6.13: Polygon views GGE biplot based on symmetrical scaling for the which-won-where patterns of genotypes and environments for root number

6.3.10.4 Mean performance and stability of genotypes in fresh root yield

Fig. 6.13 represents the biplot of stability and mean performance of 125 genotypes for fresh root yield. The average tester coordinates (ATC X-axis) or the performance line passes through the biplot origin with an arrow indicating the positive end of the axis. The ATC Y-axis or the stability axis passes the plot origin with a double arrow head, and is perpendicular to the ATC X-axis. The average yield of the genotypes is estimated by the projections of their markers to the ATC X-axis (Aminn *et al.,* 2011). Considering the above description, genotype 078B and 066B had the highest mean yield, while genotype 070B had the poorest mean yield. Greater projection unto ATC Y-axis, regardless of the direction, means greater instability. Therefore, genotypes 040B, 078B, and 070B are regarded as unstable. The visible genotype from the graph, which combined good performance with stability, is 171B because of its closeness to the mean yield and short projection of the genotype marker line (Fig. 6.14).

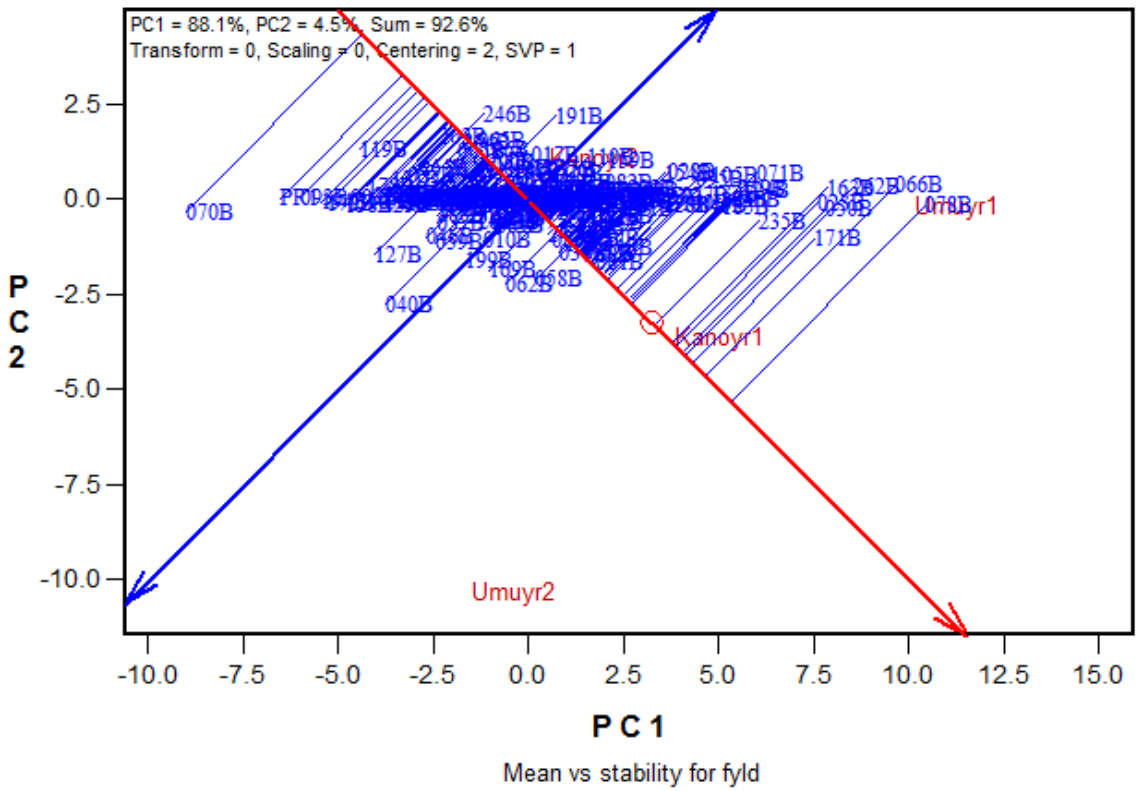


Figure 6.14: Average environment coordination (AEC) views of the GGE biplot based on environment-focused scaling for the mean performance and stability of genotypes for fresh root yield in F_1 population

6.3.10.5 Mean performance and stability of genotypes in starch and dry-matter content

Genotypes 027B, 194B, 146B, 109B, 110B, 076B, and 133B had the highest mean yield, while poor yield was recorded from genotypes 172B, 122B, and 260B (Figures 6.15 and 6.16). The performances of the genotypes 172B, 122, 027B, and 194B were the most variable (least stable), whereas genotypes 109B, 076B, and 194B were stable and at the same time achieved high starch and dry-matter content.

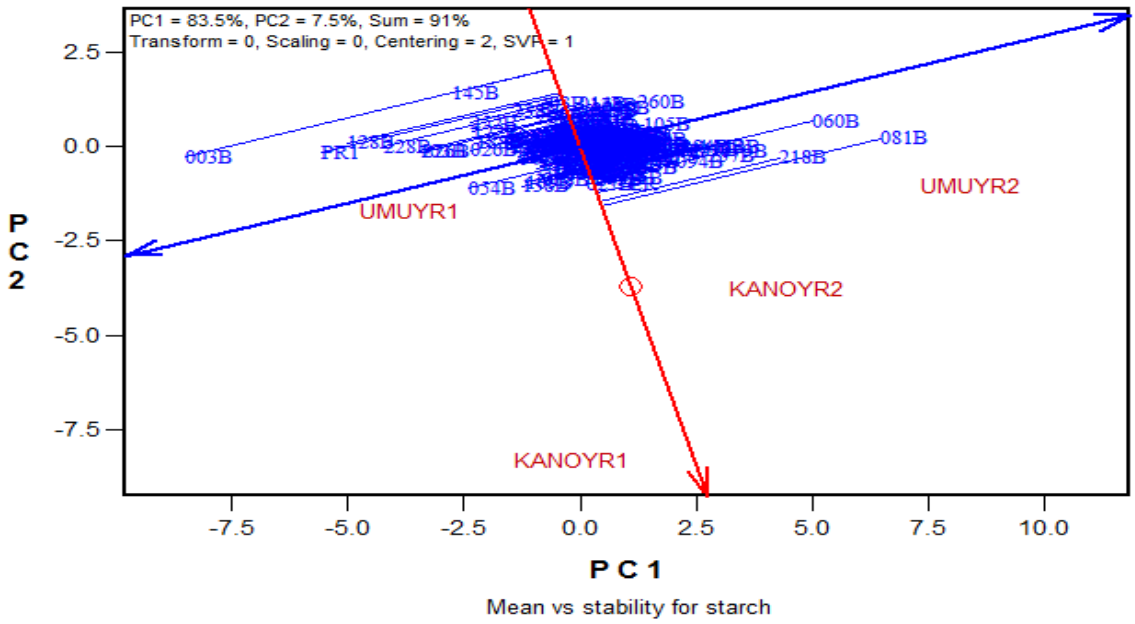


Figure 6.15: Average environment coordination (AEC) views of the GGE biplot based on environment-focused scaling for the mean performance and stability of genotypes for starch content in F_1 population

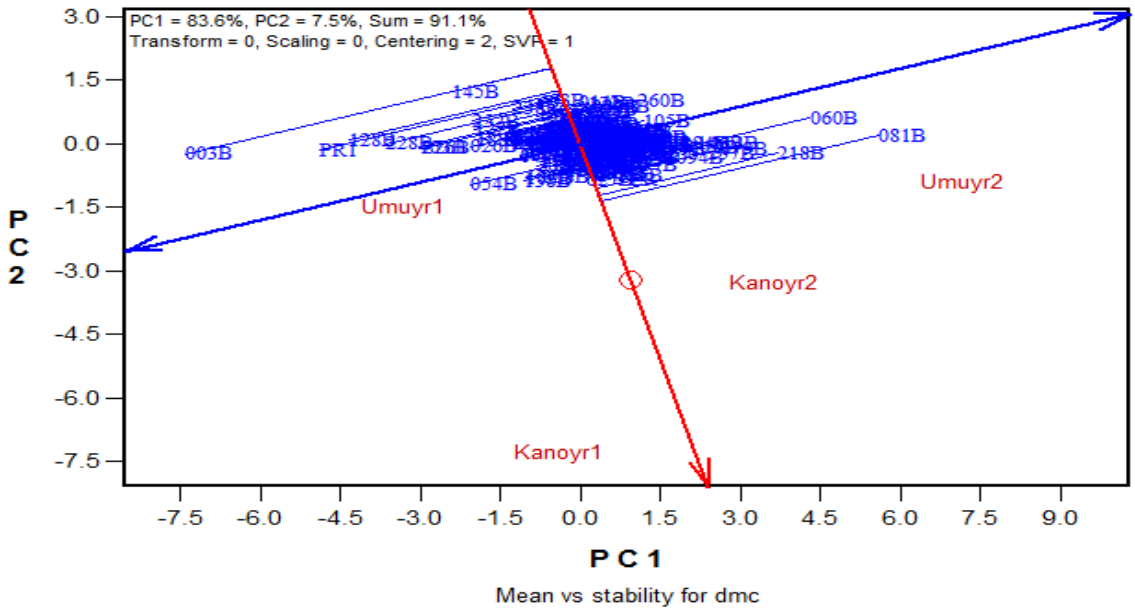


Figure 6.16: Average environment coordination (AEC) views of the GGE biplot based on environment-focused scaling for the mean performance and stability of genotypes for dry-matter content in F_1 population

6.3.10.6 Mean performance and stability of genotypes in root number

Mean performance and stability of genotypes for root number are shown in Fig. 6.17. The results show that the genotypes with a high mean root number were 109B, 168B, HR, 207B, 001B, 020B, 247B, LR, 113B, 127B, and 076B. The genotype with the poorest root number was 146B. The genotype that was highly unstable was 113B, though with a high mean root number, while the genotypes that were stable with a high mean root number were 168B, HR, 001B, 020B, 247B, 207B, and 076B.

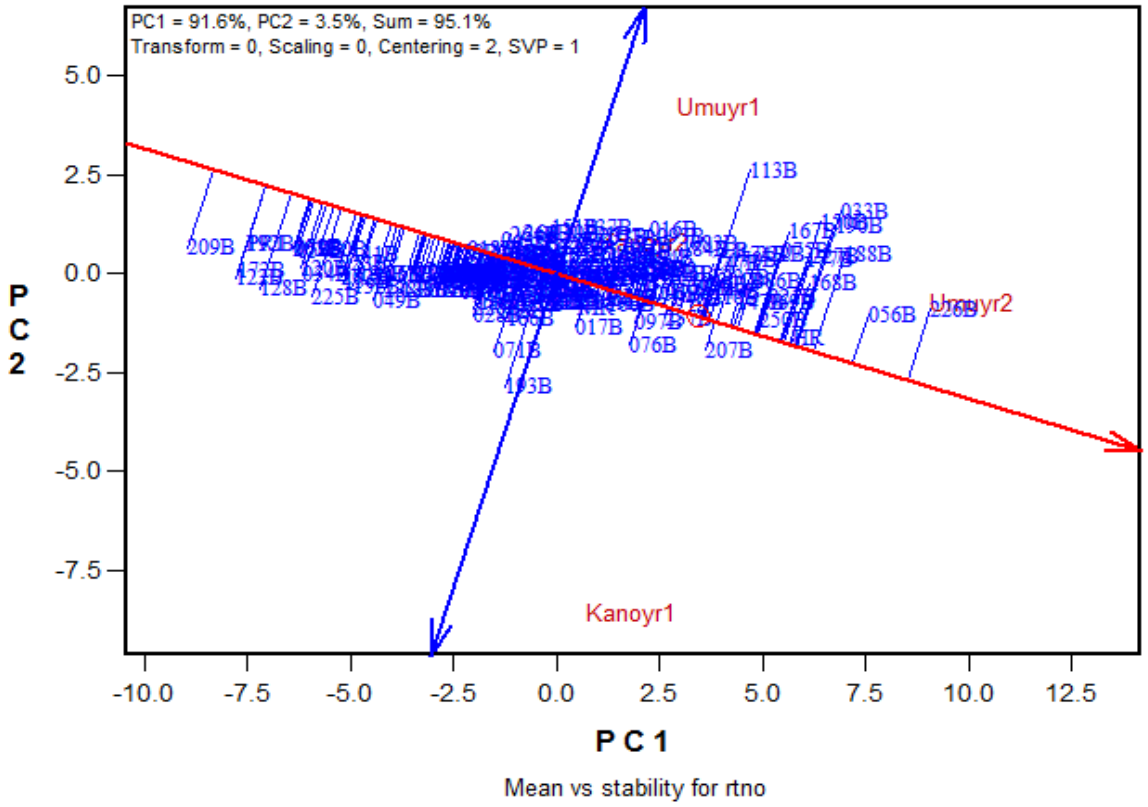


Figure 6.17: Average environment coordination (AEC) views of the GGE biplot based on environment-focused scaling for the mean performance and stability of genotypes for root number in F1 population

6.4 Discussion

The research was aimed at identifying early-bulking cassava genotypes in the F_1 population and also to find out the traits associated with early storage root bulking. According to El-Sharkawy (2004), cassava plants started root bulking at three months after planting, but roots become a

major sink only between 180 and 300 days after planting. Genotypic- mean square was significant for all traits, indicating that the genotypes evaluated were different; and by hybridising among them, genetic advance would be achieved for all traits. Significant differences between harvest times for all the traits evaluated showed differences in the growth and development of the test genotypes over time. Similar results had been reported by Ngeve (2002) to determine the effect of harvest time and test environment on cassava storage root yields and culinary qualities. They found significant differences among genotypes and locations for fresh storage root yield and root number. Significant mean squares for location for all traits indicated that locations were different to each other and consequently, this had significant influence on the performance of the genotype for the various traits. This calls for the need for a decentralised breeding scheme for cassava in Nigeria and other cassava producing countries. The significant genotype x location effects for the traits is an indication that some of the genotypes had a specific adoption of one or more of the locations (Egesi *et al.,* 2007; Aina *et al.,* 2009), while the significant differences of genotype by harvest time interaction was a clear indication of differences regarding the bulking rates and patterns. This had also been previously reported by Okechukwu and Dixon (2009), Kamau *et al.* (2011) and Okogbenin *et al.* (2013).

From results obtained for majority of the traits fresh root yield (FRY), was lowest when plants were harvested at 7 MAP, and generally higher at each subsequent harvest time. This trend reflects the dynamics in cassava growth and development (Cock *et al.,* 1979; Ekanayake *et al.,* 1998; Lahai *et al.,* 1999; Alves, 2002), and dry mass accumulation in the roots as affected by source-sink relations (Alves, 2002; Lahai and Ekanayake, 2009).

Cassava established through stem cuttings grows through phases; the vegetative phase is characterised by rapid growth of stem and foliage; the root bulking phase characterised by the rapid growth of storage roots; and the senescence phase (Cock *et al.,* 1978; Ekanayake *et al.,* 1998). At every growth and development phase in cassava, dry mass production and partitioning between plant organs occurs with respect to growth stages of the plant. Partitioning of dry mass is important in cassava, because of the trend in development from leaves to storage roots; and thus, the assimilates are partitioned between these plant parts (Cock, 1984; Alves, 2002). The distribution pattern of photo-assimilates among the different organs of cassava changes during the growth cycle, with shoots dominating in the first 3 to 5 MAP, while the storage roots become the major sink for assimilates during the rest of the growth cycle (El-Sharkawy, 2003). In this study, root bulking was gradual in the first five months, with most of the

bulking in terms of fresh root yield for the majority of the cultivars having taken place by 7 and 10 MAP.

Phenotypic correlation analysis revealed that other agronomic traits (harvest index, plant vigour, dry root yield, stem diameter, plant height, root diameter, root number, and storage root weight) highly correlated with FSRY, indicating their interdependence and importance in influencing fresh storage root yield. Similar results were reported by Okogbenin *et al.* (2006), who found that early-bulking yield in cassava was highly influenced by harvest index, foliage, root diameter, and root number. This means that these traits can be used to select for early bulking during selection. The high positive correlations between fresh storage root yield and dry root yield, and root number agree with the reports of Suja *et al.* (2009), Okechukwu and Dixon (2009), and Parkes *et al.* (2013).

Principal component analysis was used to explain the relative contribution of the various traits to the genotypes' performance. Combining the important traits (root weight, root number, plant biomass, fresh root yield, dry root yield, dry-matter content, plant height, starch content, harvest index, and stem diameter) as revealed by PCA, can be used in the selection stage by breeders to select for early bulking, to shorten the breeding cycle, and enhance yield productivity. Number of roots and plant height occurred twice in the two PCs in year 1 analysis, while dry-matter content, starch content, and stem diameter occurred twice and three times in the PCs in the second year, respectively. The other parameters were present in one of the PCs, indicating their relative importance to early bulking in cassava. It also indicated that breeders should seriously consider including root number, plant height, and stem diameter in their selection indices when evaluating their choice at the advanced selection stages. Root weight, root number, total plant biomass, fresh root yield, dry root yield, dry-matter content, harvest index, and starch content were the major contributors to PC1 and PC2. PC1 and PC2 are key traits in cassava breeding, and are normally used in selection for high storage, fresh storage yield (Kawano *et al.,* 1998; Ojulong, 2006).

The wide range observed at 7, 10, and 12 months after planting across two seasons indicates the variation among the evaluated genotypes for fresh storage root yield at each plant age of harvest. A similar result has also been reported by Bunmi *et al.* (2017), who observed a wide range of variation among the genotypes evaluated in different planting ages in two locations. The mean difference in yield was higher at 10 MAP over 7 MAP than 12 MAP over 7 MAP,

indicating that there were more genotypes identified as early bulkers than late bulkers when harvested at 10 MAP. The negative percentage increase in FSRY observed in some of the genotypes shows that they must have initiated storage root development by reaching their maximum yield within a short growing period. This confirms similar observations made by Bitai and Lian (1978) in sweet potato. They discovered that the bulking of the storage roots of early maturing cultivars declines in the early (or later) period of growth, whereas for the late maturing cultivars, the bulking rate increases at the middle or during later growth periods. In other words, they can be termed as 'type one early maturing genotypes', because of the reduction in yield when harvested at 10 months and even at 12 months. The late-bulking genotypes are better harvested between 10 to 12 MAP, as there is not much difference in their yield, while the early-bulking genotypes are better harvested at 7 MAP. In this study, the early-bulking genotypes identified in this study are better harvested at 7 MAP to avoid field weathering or rot, and decline in yield. This applies especially to the type one early bulkers because some of them had very high yield when harvested at 7 MAP, which was found to decline at 10 MAP harvest.

The extent of environmental influenceon any character is indicated by the magnitude of the differences between the genotypic and phenotypic coefficient of variation (Akinwale *et al.,* 2011). Large differences reflect high environmental influence while small differences reflect high genetic influence. The PCV estimates for all traits were higher than the GCV estimates indicating the considerable role of the environment on the expression of these traits; hence variation of these traits was not only genetically determined, but was also due to environmental effects. This has also been reported by Akinwale *et al.,* 2010; Ntawuruhunga and Dixon, 2010, Manu *et al.,* 2013. Deshmukh *et al.,* (1986) stated that PVC and GCV values more than 20% are regarded as high whereas values less than 10% are considered to be low while values between 10 and 20% are regarded to be medium. Based on this description, most of the traits had high PVC and GCV, few traits had medium values for PVC and GCV while none of the traits had low PCV and GCV. The high GCV values signify the possibility of improving these traits through selection. However, the difference between PVC and GCV for all traits was relatively low, indicative of low environmental influence. (Akinwale *et al.,* 2010). The degree of success in selection depends on the magnitude of variations determined through heritability estimates and genetic advance (Panse, 1957). Estimates of genetic variability in the F_1 population were quantified by the broad sense heritability estimates. If heritability of a trait is very high (about 80% or more), selection for such characters could be fairly easy, because there would be a close correspondence between the genotype and the phenotype due to the relative small

contribution of the environment to the phenotype. Selection for characters with low heritability (40% or less) may be difficult, due to the adverse effect of the environment on the phenotype. Bhateria *et al.* (2006) classified heritability estimates as high (> 50%), medium (30%-50%), and low (< 30%). Most of the traits attained high and medium heritability at each of the harvest times, except for stem diameter, whose heritability was low at 7 MAP, but medium and high at 10 and 12 MAP, respectively. The high genetic variation in F_1 population studied is a strong indication of genetic variability and low environmental variance, showing a great number of additive gene effects in the inheritance of these characters, and these traits can be passed to progeny (Asante and Dixon, 2002). The high heritability of the traits signifies that these traits can be selected using simple selection procedures. The low to medium estimate shows that these traits were strongly influenced by the environment, suggesting that direct phenotypic selection for these traits might be ineffective.

With respect to trait performance in different locations at 12 MAP, it was observed that most of the traits performed better in Umudike, except for dry-matter content, starch content, plant vigour, and stem diameter. The high performance of dry-matter content and starch content in the Kano location could be attributed to the low rainfall distribution in the area. This could also be the reason for low storage root yield compared to the yield in a more humid area.

Graphically presenting the 'which-win-where' pattern is important for studying the possible existence of different environments in a region (Guach and Zobel, 1997; Yan, 2001). The polygon view of a biplot is the best way to visualise the interaction patterns between genotypes and environments, and effectively interpret a biplot. GGE biplot was used to identify the best-performing genotypes in different environments, the mean performance, and stability. The identification of high-yielding adaptive cultivars in various environments will help to improve in breeding the high-yielding cassava genotypes in multiple environments.

6.5 Conclusion

This study was conducted to identify early-bulking cassava varieties in the F_1 population, and the traits significantly associated with early storage root bulking. Fresh root yield was significantly associated with morphological and productivity traits such as harvest index, root weight, number of roots, stem diameter, root diameter, biomass, and dry root yield, while principal component analysis identified important traits such as root weight, root number, plant

biomass, fresh root yield, dry root yield, dry-matter content, plant height, starch content, harvest index, and stem diameter, which can be used by breeders in the selection for early root bulking in cassava.

There was high genetic variability among genotypes for fresh root yield and all other traits evaluated, indicating that significant progress would be achieved in selecting for these traits, even with simple phenotypic selection procedures. In the multiple environments, genotype 171B was stable for fresh root yield; 109B, 076B, and 194B were stable with a high mean starch and dry-matter content; while 168B, HR, 001B, 020B, 247B, 207B, and 076B were relatively stable over the environments. The early-bulking genotypes identified in this study will be better harvested at 7 MAP, while the late bulkers are better harvested between 10 to 12 MAP. The early-bulking genotypes identified in this study should be evaluated further in more locations, especially dry ecology, to test them for yield stability before being released to farmers.

CHAPTER 7

QTL MAPPING AND BREEDING FOR EARLY BULKING IN F_1 CASSAVA GENOTYPES

ABSRACT

Worldwide, cassava feeds an estimated population of 800 million people directly or indirectly. The food security role in averting famine has necessitated the need for cassava varieties that can be harvested early in contrast to the late yielding varieties. Early-bulking (EB) varieties shorten the growth period from planting to harvesting, have a better fit in the environments with a short rainy season, and they reduce exposure to biotic and abiotic stresses, thereby increasing productivity. Late-bulking cultivars occupy land for extended periods of time, and consequently, the land cannot be effectively utilised for the sequential cultivation of other crops. Given the expanding importance of cassava as food, feed, and industrial crop, genotypes with high root yield and early bulking are considered strategic in the fight to alleviate hunger and poverty in rural drought-prone areas, and are thus highly desired in the production of cassava. Therefore, the objectives of this study were to identify early-bulking cassava genotypes, the traits significantly associated with early storage root bulking, and QTLs linked to early bulking at 7 months after planting (MAP). In total, 135 cassava genotypes were evaluated at Umudike for storage root yield during two planting seasons at three plant ages of 7, 10, and 12 MAP. Analysis of variance indicated a significant effect of genotype, harvest time, interactions between genotype and harvest time, genotype and year, harvest time and year on yield, and other yield-related traits. There was a positive correlation between fresh root yield and other yield-related traits. Principal component analysis indicated root weight, root number, total biomass, fresh root yield, plant height, and dry root yield were the main contributors. Estimates of genetic variance showed high genetic variability in the F_1 population. Cassava genotypes with more than 100% increase in fresh root yield at 12 MAP over the yield at 7 MAP, and 10 MAP over the yield at 7 MAP were regarded as late bulkers, while those with less than 100% increase were regarded as early bulkers. On this basis, 108 genotypes were identified as early bulkers at 10 MAP over 7 MAP, while 59 were identified as early bulkers at 12 MAP over 7 MAP. Composite interval mapping (CIM) identified 16 QTLs in year 1, and 12 QTLs in year 2 associated with traits for early bulking at 7 MAP. There was co-localisation of multiple QTLs in various traits such as dry root yield and fresh root yield; dry-matter content and starch; plant vigour and root diameter. The QTLs explained phenotypic variation ranging between 4.2% and 11.40% in the first year; and 3.96% and 18.88% in the second year. Two major QTLs were also

identified with PVE > 14%. Gene actions revealed additive and over-dominant gene actions. The identification of these loci will aid in breeding for early root bulking in cassava via marker-assisted selection.

Keywords: Composite interval mapping; early storage root bulking; *Manihot esculenta*; quantitative trait loci; QTL mapping

7.1 Introduction

Cassava (*Manihot esculenta Crantz*) is the third most important source of carbohydrates in the tropics after rice and maize (Huang et al., 2001) and the sixth most important crop in terms of global annual production (FAO, 2010). Widely grown in tropical Africa, Asia, and Latin America, cassava is the developing world's fourth most important crop, with production in 2006 estimated at 226 million tonnes (FAO, 2009, 2012). It is the staple food of nearly a billion people in 105 countries, where the root provides as much as a third of daily calories (FAO, 2009), and the annual consumption is greatest in Africa. It is also a major staple food for about half of the Nigerian population, and nearly 50 million tonnes of fresh cassava is produced annually in the country (FAO, 2008).

Bulking in cassava refers to the swelling or thickening of the storage roots as a result of excess assimilates after vegetative growth (Okogbenin, 2013). Early bulking has been used as concept to describe early maturing or early-ready cassava varieties that are harvestable at 7-8 months after planting (MAP). The role of food security in eliminating potential famine among the rapidly growing populations has necessitated the need to breed a crop for early-yielding in contrast to late-yielding cassava varieties. Early bulking is currently considered a key requirement for cassava to make the transition from being a traditional food crop to a cash crop or an industrial one (Okogbenin and Fregene, 2002). It is also important to develop cassava as an early-bulking variety in situations where there is mounting pressure on available agricultural land forcing farmers to increase production, and in semi-arid regions where early-bulking cultivars can be harvested after one cycle of rain. According to Nweke *et al.* (1994), cassava's long growth cycle makes it relatively difficult for the crop to be available to be harvested in a short time. Rainfall patterns in some parts of the tropics, where rain lasts for about 6 months or less provides a clear distinction between rainy and dry seasons, thereby making cassava cultivation in such areas difficult. Late bulking (LB) is a major factor leading to farmers abandoning improved cassava genotypes in sub-Saharan Africa due to demographic and market pressures, while

early bulking (EB) is one of the most important traits of interest of farmers in cassava growing areas. There is an increasing trend and concern towards developing early maturing cassava varieties as a result of the increasing demand for early cultivars by farmers (Chikoti, 2011; Tumuhimbise *et al.,* 2012; Kamau, 2011; Bassey and Gamalliel, 2013).

In dry ecology, drought imposes slow crop development, which causes the harvest of cassava to extend beyond 12 MAP. Early bulking is therefore seldomly considered as a measurable trait in marginal environments, but it implied having to identify good bulkers under stress rather than identifying early maturing varieties (Okogbenin *et al.,* 2013). Thus, evaluating early bulking for drought tolerance is used in selecting good varieties with potential good yield at 12 MAP.

In the absence of direct plant shoot traits to follow in the selection of early-bulking cassava, indirect methods such as the use of fresh storage root yield (FRY) has been used for assessing early bulking in cassava (Kawano, 1987; Bunmi *et al.,* 2017). Hershey (2012) reports that genotypes with the highest FSRY at an early harvest time tend to be the highest yielders at later stages, and based on this, high yield is co-selected with early bulking.

Early root bulking in cassava has been found to be highly influenced by harvest index, foliage, root diameter, and root number (Okogbenin *et al.,* 2006). They also stated that starch initiation time, storage root diameter, plant height, harvest index, number of storage roots, and plant vigour were all significantly correlated with dry storage root yield, and further showed that storage root diameter, dry foliage mass, and harvest index were the most important factors for storage root bulking. This suggested that both the source and sink capacities were important in determining early bulking. They concluded that one should select for high harvest index, dry foliage mass or both when breeding for early bulking. Kawano (1990) and Ojulong *et al.* (2010) showed that the harvest index is a better trait to select for early root bulking than storage root yield. In other words, there is no adequate information on the various factors that may adequately define cassava early bulking.

Many important agronomic traits such as yield, drought tolerance, and some forms of disease resistance are controlled by many genes. In other words, they are quantitative traits each with small effects (quantitative trait loci, QTLs), which makes it difficult to breed for these traits. Cassava has a long breeding cycle of 8-10 years, making it very difficult to derive at a new improved variety. Cassava's genetic improvement can be made more efficient through the use of easily assayable genetic or DNA markers. One of the most important applications of

molecular marker technology in relation to genetic improvement of cassava involves the use of marker-assisted selection (Heman, 2015). Marker-assisted selection enables a precise identification of genotypes without environmental influence and aids in the reduction of the breeding cycle. It saves a lot of time in the breeding process. It aids in the reduction of large breeding populations at the seedling stage as a result of the selection made possible by the use of markers at such an early stage. This is important in cassava breeding, because of the length of the growing cycle, and the expense involved in the evaluation process.

Mapping and isolation of QTLs are important for efficient plant breeding by marker-assisted selection (MAS), and for gaining a better understanding of the molecular mechanisms underlying the traits (Hiroki *et al.,* 2013). A lot of genetic studies have been conducted to identify QTLs in monogenic traits such as resistance to pests and diseases in cassava, but little work has been done into the quantitative traits such as early bulking. Olasanmi *et al.* (2013) used bulked segregant analysis (BSA) to identify SSR markers associated with early bulking. The study discovered 9 SSR markers, which were closely linked to early bulking in cassava. Genetic studies for early bulking had also been conducted in the F_1 cross of non-inbred parents, using RFLP markers, though it was expensive and labour intensive (Okogbenin and Fregene, 2002). In the study, they identified QTLs controlling early bulking in dry foliage weight, harvest index, and root diameter. They went further to analyse the F_2 population for early bulking, using 122 segregating SSR markers, of which they identified QTLs in dry storage root yield, fresh foliage, and harvest index (Okogbenin *et al.,* 2006).

Genetic markers such as RFLPs and SSRs have been deployed to identify QTLs linked to early bulking in F_1 and F_2 cassava genotypes, respectively (Okogbenin *et al.,* 2002; Okogbenin *et al.,* 2006), but SNPs had not been used for this purpose in cassava. SNPs presently are mostly used in genotyping, because they are highly polymorphic, highly abundant in the genomes, and provide the highest map resolution when compared with other marking systems (Jones *et al.,* 2007).

We describe phenotypic evaluation of bulking in cassava at 7, 10, and 12 MAP; traits driving early bulking in F_1 cassava genotypes, and genetic analysis of early bulking using KASPar SNPs markers in the F_1 population.

7.2 Materials and Methods

A set of 129 F_1 cassava genotypes developed from intra-specific cross between two improved cassava varieties (IITA-TMS980505 and IITA-TMS980581) and five checks (IITA-TMS30572, TME 419, IITA-TMS9102324, IITA-TMS980505, and IITA-TMS980581) were evaluated for storage root yield at 7, 10, and 12 months after planting (MAP) in two growing seasons (2016/2017 and 2017/2018) in Umudike (humid forest). IITA-TMS980505 used as a female parent is an early-maturing cassava variety with moderate flowering ability, while IITA-TMS980581, the male parent, is also an early-maturing cassava variety with profuse flowering ability. The experimental design was an alpha lattice replicated twice. Twenty stakes of each genotype were planted in each season, in a plot of 20 m^2 (5 m x 4 m) at a spacing of 1 x 1 m, giving a density of 10,000 plants/ha. The plots were weeded as required to minimise competition for nutrients, moisture, and sunlight between weeds and the cassava plants.

7.2.1 Data collection

The cassava genotypes were evaluated for CMD and CBB at appropriate growth stages. To evaluate the F_1 progenies for early bulking, three sequential harvests were done at 7, 10, and 12 MAP. During each harvest, the genotypes were evaluated for plant height, plant vigour, stem diameter, number of storage roots, storage root diameter, storage root weight, dry-matter content of storage roots (DMC), and root starch content. Some of the data collected at harvest were used to estimate total biomass, fresh storage root yield (FRY), dry storage root yield (DRY), and harvest index (HI) for each genotype. The morphological and productivity traits evaluated have been described in Chapters 4 and 6

7.2.2 Genotyping

DNA was extracted from freshly harvested leaves of each cassava genotype, using LGC extraction kits. The mapping populations were genotyped by LGC Genomics Laboratory, United Kingdom. The LGC genotyping high-throughput platforms for SNPs were used for this process as described in the LGC website (www.lgcgenomics.com). SNP markers developed for cassava in another GCP-linked project at the University of Maryland and IITA were validated, and subsequently converted to a KASPar-based platform. KASPar assay offers the simplest and most effective way to determine SNP genotypes in the laboratory. It was used because of its flexibility, cost effectiveness, and the fact that it can be carried out on undefined sets of markers. FlapJack, a multi-platform tool for interactive visualisation of high throughput genotype data was used to check the data quality, marker information across loci for each genotype, and across the

population (genotype) per marker. Good data quality would imply good DNA quality, and excellent genotyping as well as reliable results. Segregation of the markers was achieved using an SNP viewer. The markers were tested for segregation ratio, using the Chi square test (test for deviation from expected Mendelian segregation for each marker), and the best segregating and informative markers were used in mapping. A total of 1,845 SNPs were made available, and 94% (1,740) were successfully converted to the KBiosciences system. The 1,740 SNPs were then used to test parents of the mapping population as well as the mapping population for polymorphism with the allele-specific primers designed for each SNP using the Snaper Tool. A total of 505 SNP markers out of 856 polymorphic markers, derived at from mapping population B, were subjected to linkage analysis. The genetic linkage map was calculated with SNP markers using "CP option" of JoinMap Version 4.1 (Van Ooijen, 2006), which is appropriate for outcrossing species, since both parents are heterozygous, and the segregation linkage phase is unknown. JoinMap 4.1 was used to find the order of the markers in the linkage groups. Following the calculation of pairwise recombination frequencies, linkage groups were identified, using the logarithm of odds (LoD) score of independence between pairs of loci at a threshold of 10.

7.2.3 Marker-trait analysis and QTL mapping

QTL analysis with the phenotypic data of the F_1 lines at 7 MAP was carried out by single marker regression analysis, using R/QTL (Arends *et al.,* 2010) at a LoD threshold of ≥ 3.0. Composite interval mapping with the Bayesint model was used for QTL detection through R/qtl V1.37-11 (Broman, 2014). The peak, map position, confidence interval, estimated effects of the QTLs, interactions among detected QTLs, phenotypic variation explained (PVE), additive (A) and dominant components for each QTL were retrieved from the analysis. The D/A ratio of 0-0.20, 0.21-0.80, 0.81-1.20 or > 1.20 explained for additive (A), partial dominance (PD), dominance (D) and over-dominant (OD) mode of gene action were estimated as described by Stuber *et al.* (1987). Analysis of the QTLs was carried out, using R/qtl software package (Broman *et al.,* 2003).

7.2.4 Data analysis

The collected data were subjected to descriptive analysis, using the Microsoft Excel software, and analysis of variance (ANOVA), using SAS software (version 9.0). Pearson's phenotypic correlations between traits were performed to determine the traits' relationships. Principle component analysis was used to determine variation among the traits, and the traits that are

driving early bulking. Estimates of variance components were obtained by equating the observed mean squares from ANOVA with their expected mean squares (EMS). Phenotypic and genotypic coefficients of variation were computed, using the Excel package.

Genotypic variance component:

$$\sigma^2 g = MSg - {}^{MSe}/_{r}$$

Where M S g is genotypic mean square, MSe is error mean square and r is replication.

Environmental variance component:

$$\sigma^2 e = {}^{MSe}/_{r}$$

Phenotypic variance component:

$$\sigma_p^2 = \sigma_g^2 + \sigma_e$$

Genotypic and phenotypic coefficients of variation were calculated according to the method suggested by Burton and Dewane (1953) as

Genotypic coefficient of variation (GCV)

$$GCV = \frac{\sqrt{\sigma_g^2}}{\bar{x}} \times 100$$

Phenotypic coefficient of variation (PCV)

$$PCV = \frac{\sqrt{\sigma_p^2}}{\bar{x}} \times 100$$

Where $\bar{x}\ is\ the\ grand\ mean\ value\ of\ the\ trait$

Broad sense heritability (h^2)

$$h^2 = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

Table 7.1: Ecological factors and study conditions of Umudike location used for the evaluation of cassava genotypes for early root bulking in the 2016/2017 and 2017/2018 growing seasons

Ecological factors and study conditions	Umudike
Latitude	5°29'N
Longitude	7°24'N
Altitude (above sea level)	120 m
Agroecology	Humid forest
Annual rainfall (amount)	2200 mm
Temperature (mean)	26°C
Relative humidity range	50-95%.
Soil classification	Dystric Luvisol

7.3 Results

7.3.1 Mean squares for storage root yield and other agronomic traits at three harvest ages in Umudike during the 2016/2017 and 2017/2018 growing seasons

There were significant differences among the genotypes for fresh storage root yield (FRY at 7, 10, and 12 MAP (Table 7.2). It was also observed that age at harvest had a significant effect on yield and other agronomic traits at $P \leq 0.001$. There was a significant difference among the genotypes across seasons for all traits evaluated, except for fresh root yield. For the interaction between genotype and harvest age, there was a significant difference for all the traits evaluated. Both genotype x year (G x Y) and harvest age x year (HA x Y) interactions were found to have highly a significant effect ($P \leq 0.001$) on all traits, except for root weight for G x Y, and plant vigour for HA x Y. All traits were significantly ($P \leq 0.001$) influenced by the interaction G x HA x Y. Coefficient of variation ranged between 15.4% (dry-matter content) and 80% (root weight). Other traits that had a high level of variation were plant biomass (78.6%), and root number (73.1%).

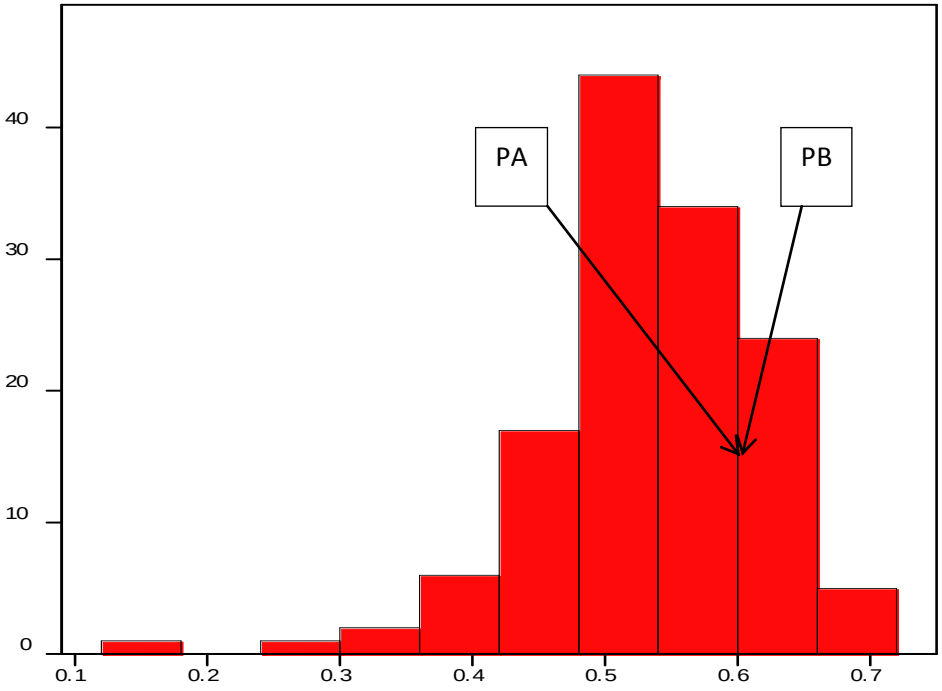
7.3.2 Frequency distribution of clones at 7 MAP

Frequency distribution of the genotypes for the traits studied is shown in Fig. 7.1. All the traits showed continuous distribution, which is common for quantitative traits. None of the traits fit a normal distribution, but were slightly skewed to the right, except for the harvest index, which was skewed to the left.

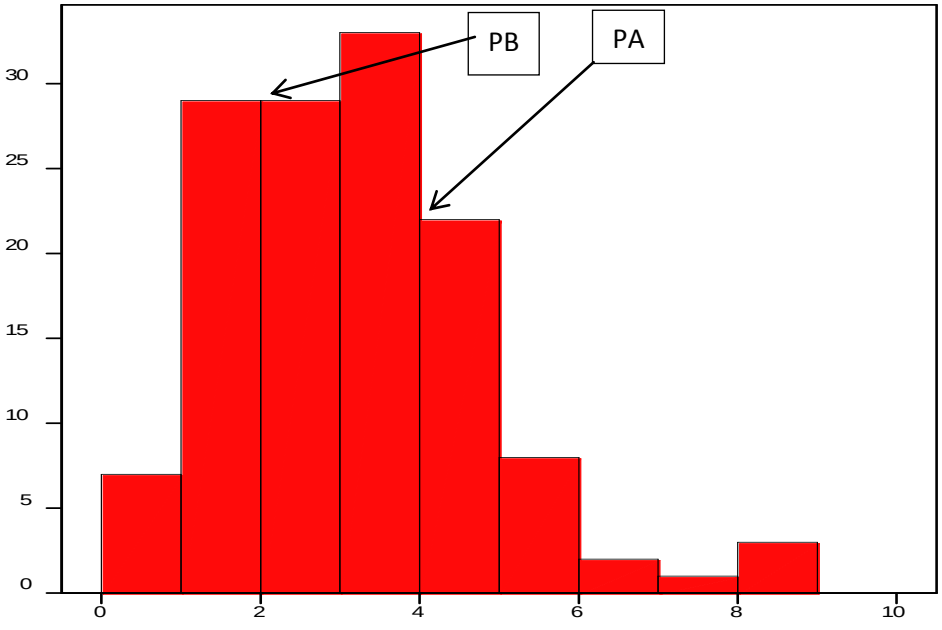
Table 7.2: Mean squares for yield and other agronomic traits of 129 F_1 cassava genotypes and checks evaluated for early root bulking at Umudike, Nigeria in the 2016/2017 and 2017/2018 growing seasons

Source of variation	Genotype (G)	Harvest age (HA)	year (Y)	G X HA	G X Y	HA X Y	G X HA X Y	CV
DF	133	2	1	247	133	2	151	
FRY (t/h)	638.5***	24684.7***	385.9ns	301.5***	335.4***	2764..4***	239***	41.6
HI	0.06***	0.61***	1.71***	0.03***	0.04***	2.58***	0.02*	21.3
HFB (cm)	3266.3***	223967***	29774***	1437.2***	2247.9***	22137.4***	1562.2***	36.8
PLTHT (cm)	4535.2***	19018***	100617***	1461.2***	2676.7***	303395***	903.8ns	19.7
DMC (%)	56.36***	2794.1***	1046***	34.93***	39.54***	133.57***	27.5***	15.4
STARCH (%)	99.86***	4918***	1855.4***	62.24***	69.98***	228.93***	48.74***	49.3
BIOM (kg)	67.41***	1566.04***	1019.34***	31.98***	32.15***	90.34***	23.09***	78.6
DRY (t/h)	48.73***	2853.5***	159.75***	25.81***	23.48***	144.17***	18.39***	42.8
RTNO	515.6***	11755.82***	23134.52***	301.58***	125.41***	21137.28***	132.3***	73.1
RTWT (kg)	142.59***	6697.38***	808.92***	85.66***	34.8ns	3049.58***	30.7ns	80
RTDIM (cm)	6.77***	55.68***	1805.5***	2.42***	4.37***	353.14***	2.03***	18.4
STDIAM (cm)	1.09***	7.8***	1.11ns	0.72***	0.93***	1.81**	0.5**	29.5
VIG	2.43***	65.25***	21.35***	0.72***	1.53***	0.57ns	0.7***	21.2

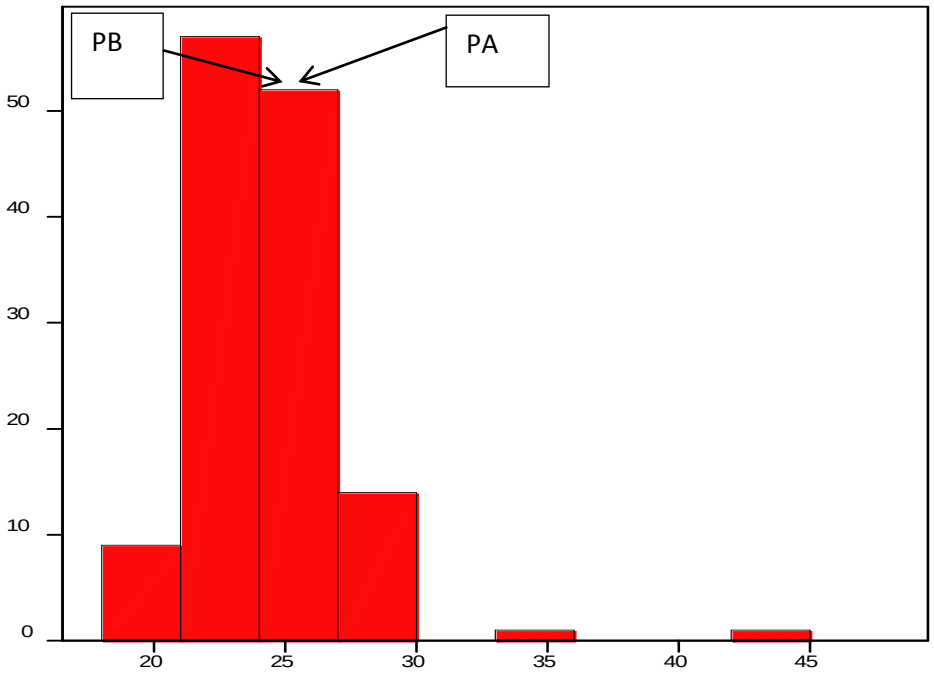
FRY = fresh root yield, HFB = height at first branching, PLTHT = plant height, DMC = dry-matter content, STARCH = starch content, BIOM = plant biomass, DRY = dry root yield, RTNO = root number, RTWT = root weight, STDIAM = stem diameter, VIG = plant vigour, ns = not significant, *, ** & *** = significant at 5, 1, & 0.1%



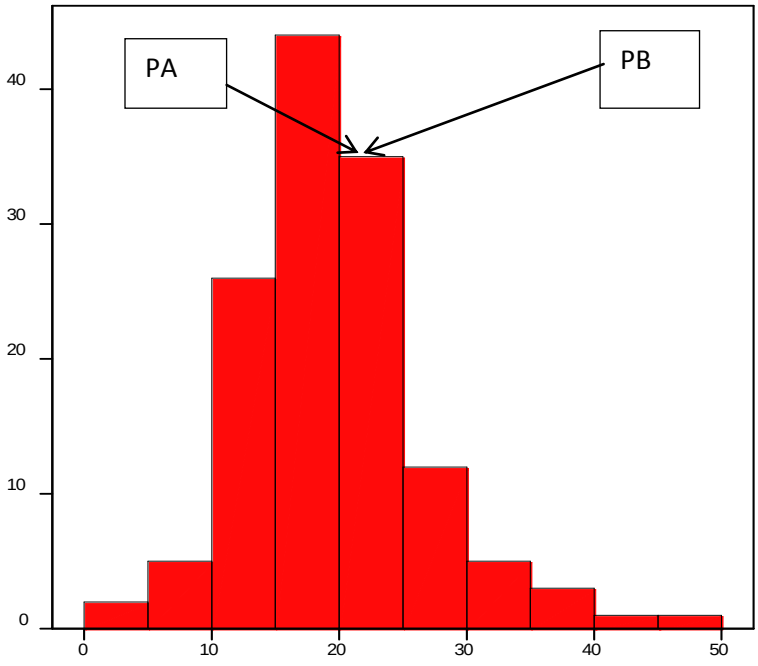
Harvest index



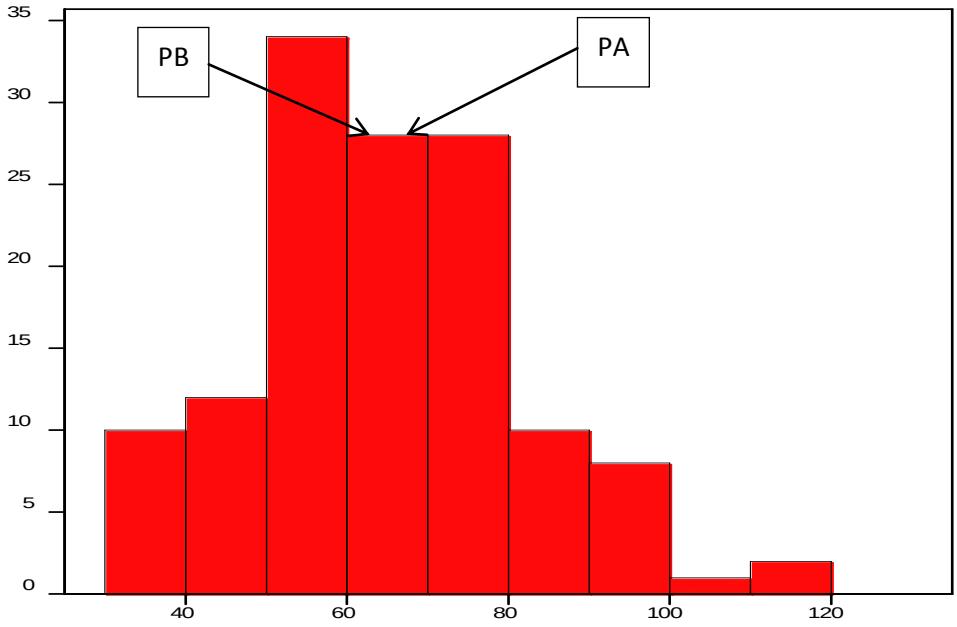
Biomass



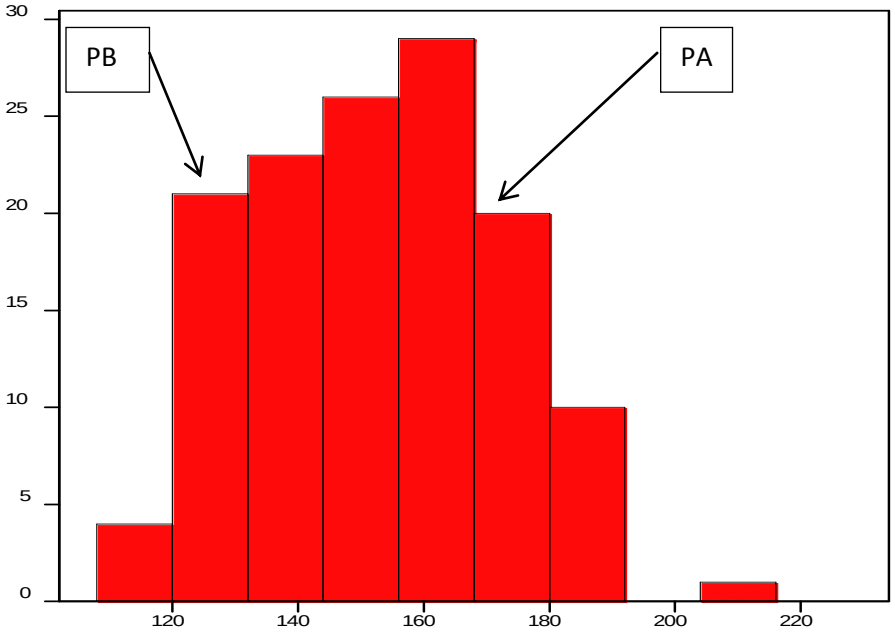
Dry matter content (%)



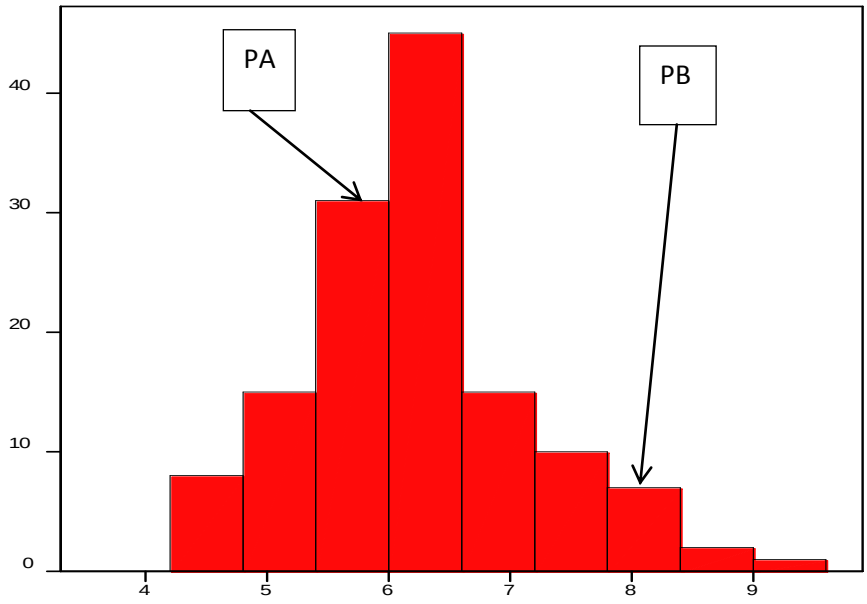
Fresh root yield (t/h)



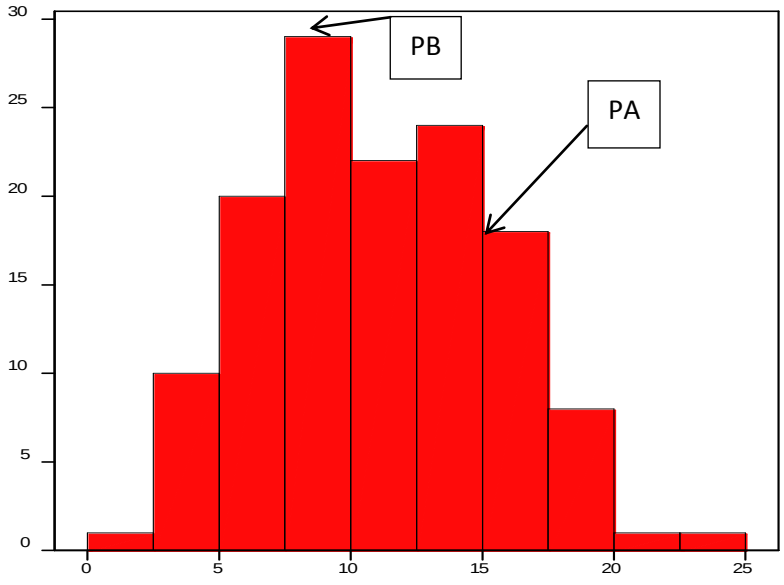
Height at first branching



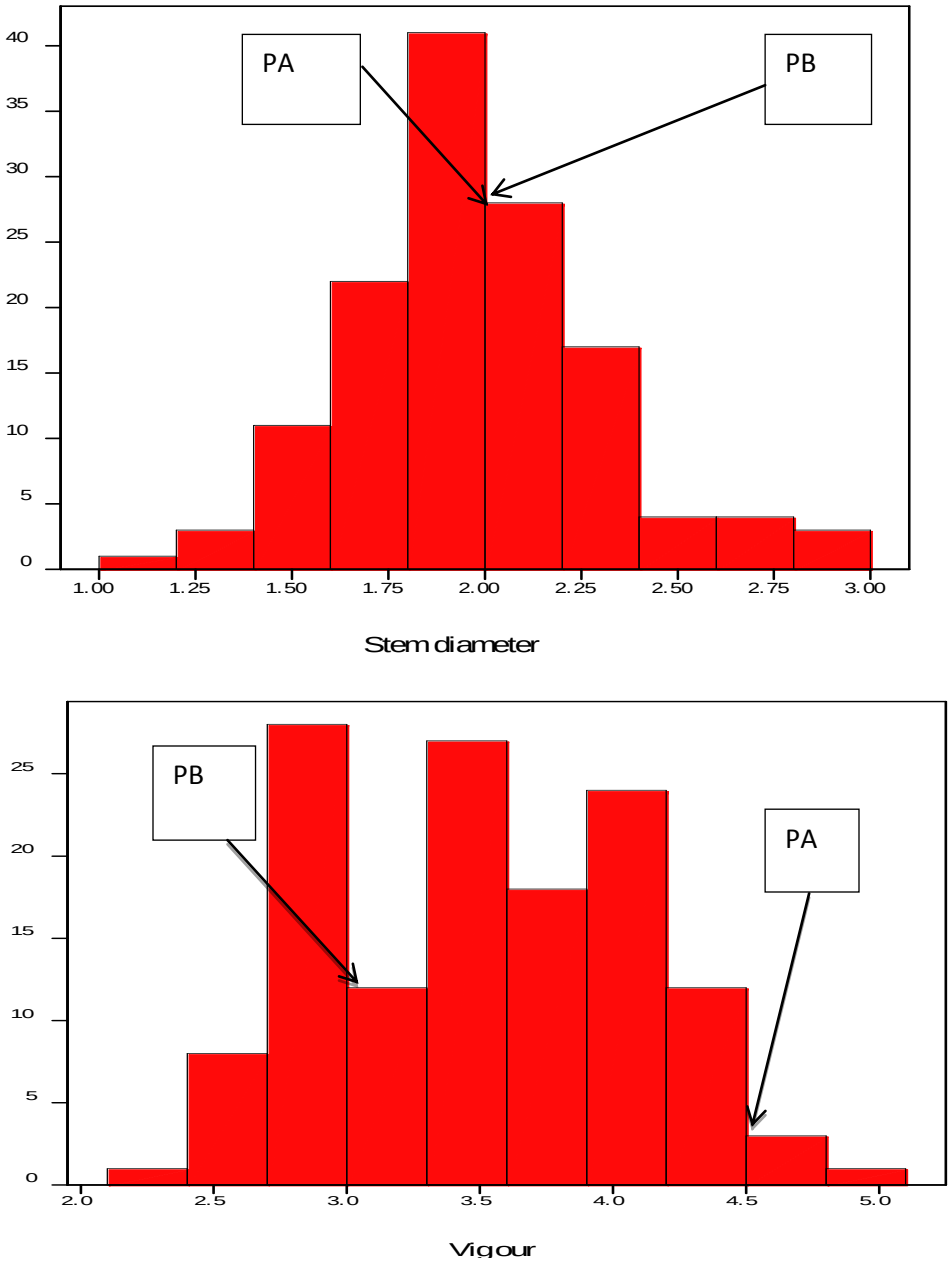
Plant height



Root diameter



Number of roots



PA= TMS98/0505; PB= TMS98/0581

Figure 7.1: Frequency distribution of morphological and productivity traits in F_1 population at 7 MAP

7.3.3 Relationships among traits in three planting ages

Pearson's correlation coefficients among the traits over two years for the three planting ages are shown in Fig. 7.2. There was a positive correlation between each pair of most of the traits (morphological and productivity). Most of the productivity traits significantly correlated with each

other. Fresh storage root yield correlated positively with harvest index, dry root yield, stem diameter, Plant height, height at first branching, root diameter, plant biomass, root number, and root weight; but negatively with dry-matter content, and starch content. The highest correlation coefficient was between fresh root yield and dry root yield (r = 0.94), followed by biomass (r = 0.41), harvest index (r = 0.35), root diameter (r = 0.34), and root number (r = 0.33). Other traits that correlated positively with fresh root yield included stem diameter (r =0.20), plant height (r = 0.22), and plant vigour (r = 0.06). Similarly, stem diameter correlated positively with root weight, root diameter, biomass, fresh storage root yield, plant vigour, dry root yield, starch content, and plant height; but negatively with CMD and CBB. It was observed that stem diameter had moderate to weak correlations with most of these traits. Plant vigour correlated positively but only moderately with plant biomass, dry-matter content, starch content, stem diameter, and plant height (r = 0.16, 0.11, 0.29, 0.11, 0.16, and 0.21, respectively), but was negatively correlated with CMD and CBB.

7.3.4 Relationship between fresh storage yield and other agronomic traits at 7 MAP

Fresh root yield was highly and positively correlated with root weight and dry root yield, with r values of 0.76 and 0.97, respectively at 7 MAP. Other traits that were positively correlated with fresh root yield at 7 MAP included root number (r = 0.43), biomass (r = 0.42), harvest index (r = 0.31), vigour (r = 0.37), stem diameter (r = 0.17), and height at first branching (r = 0.15). Fresh root yield was also found to relate negatively with starch content, dry-matter content, plant height, CMD and CBB (Fig.7.3).

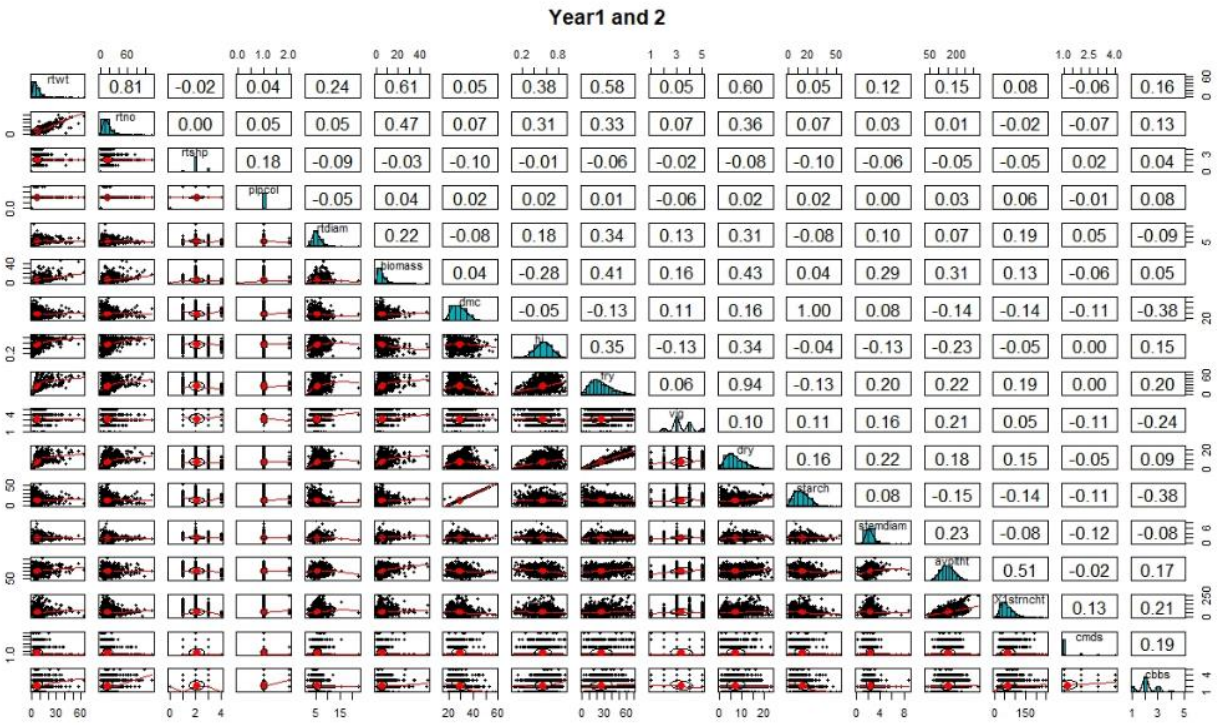


Figure 7.2: Pearson's correlation coefficient among fresh storage root yield and other yield components over years for the three planting ages

*Rtwt = root weight; rtno = root number; rtdiam = root diameter; dmc = dry-matter content; hi = harvest index; fry = fresh root yield; dry = dry root yield; stemdiam = stem diameter; avpltht = plant height; vig = plant vigour; xstbranch = height at first branching; cmds = cassava mosaic disease severity; cbbs = cassava bacterial blight severity

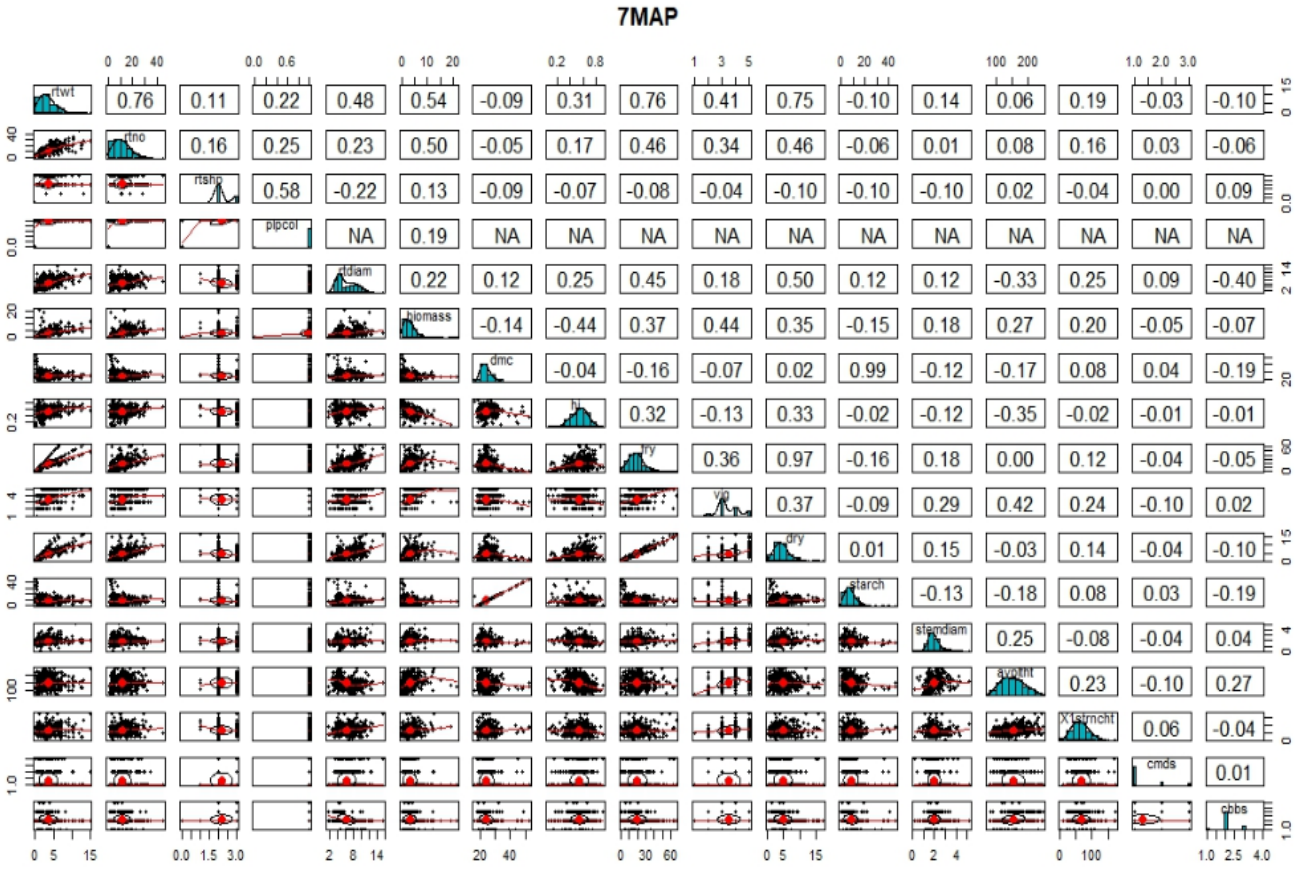


Figure 7.3: Pearson's correlation coefficient among fresh storage root yield and other yield components at 7 months after planting

*Rtwt = root weight; rtno = root number; rtdiam = root diameter; dmc = dry-matter content; hi = harvest index; fry = fresh root yield; dry = dry root yield; stemdiam = stem diameter; avpltht = plant height; vig = plant vigour; xstbranch = height at first branching; cmds = cassava mosaic disease severity; cbbs = cassava bacterial blight severity

7.3.5 Root yield, yield-related traits and response to diseases among cassava genotypes evaluated at Umudike in the 2016/2017 and 2017/2018 growing seasons

Simple statistics of the genotypes from F_1 population evaluated in Umudike over a 2-year period for the different traits are presented in Table 7.3. Results showed that there was a wide variation for the traits evaluated among the genotypes, which further explained variations among the genotypes. High mean values (> 25) across genotypes were seen in some important traits such as fresh root yield and dry-matter content. There were low mean disease scores across genotypes for CMD and CBB, signifying a low level of severity among the genotypes.

Table 7.3: Simple statistics of yield, yield-related traits, and response to diseases of cassava genotypes in an F_1 population and checks evaluated for early root-bulking in Umudike, Nigeria in the 2016/17 and 2017/18 growing seasons

Traits	Min	Max	Mean
Fresh root yield (t/ha)	0.5	66.38	27.48
Plant height (cm)	63	320	159.1
Dry-matter content (%)	16.77	52.83	26.29
Harvest index	0.06	0.91	0.56
Starch content (%)	0.09	46.05	10.72
Cassava bacterial blight	1	5	2.5
Cassava mosaic disease	1	4	1.32
Dry root yield (t/ha)	0.22	22.5	7.25
Root number	0	115	14.78
Root weight	0	63.2	6.95
Root diameter (cm)	1.55	15.55	5.95
Stem diameter (cm)	0.2	8.8	2.11
Plant vigour	1	5	3.25

7.3.6 Identification and selection for early-bulking genotypes in F_1 population and five checks

Fresh root yield ranged between 3.47 and 44.39; 5.87 and 64.16, and 10.14 and 66.38 t/ha, respectively, at 7, 10, and 12 MAP (Table 7.4), while their mean yields were 19.69, 31.83, and 31.00 t/ha. There was a significant increase in yield at 10 and 12 MAP over that of 7 MAP. Coefficient of variation (CV) among the genotypes for fresh storage root yield was almost the same at 7 (32%) and 10 MAP (33%), while at 12 MAP, the CV was 39%. The mean yield increase between 7 and 10 months was 12.14 t/ha, representing 23.56%, while the yield increase between 10 and 12 MAP was -0.83 t/ha (Table 7.4). The yield, yield increase, and percentage yield increase of the genotypes at 10 MAP over 7 MAP, and 12 MAP over 7 MAP are presented in Figures 7.4 and 7.5. It was observed that out of the 134 genotypes evaluated for root bulking in the F_1 population, 108 genotypes had a percentage yield increase of FRY less than 100% at 10 MAP over 7 MAP, while 44 genotypes had a yield increase higher than 100%. With this result, 108 genotypes were regarded as early bulkers, while 44 genotypes were late bulkers. The results also also showed that 59 genotypes had a yield increase in FRY that was less than 100%, while 76 genotypes had a yield increase in FRSY that was more than 100% at

12 MAP over 7 MAP. In other words, 59 genotypes were identified as early bulkers, while 76 genotypes were late bulkers. The percentage yield increase ranged between -61.56 and 300.88% at 10 MAP over 7 MAP (Fig. 7.4), while at 12 MAP, it ranged between -63.77 and 370% over the yield at 7 MAP (Fig. 7.5).

Table 7.4: Summary for descriptive analysis of fresh root yield of 129 genotypes and five check varieties evaluated at Umudike Nigeria for being early in storage root bulking

Parameter	7 MAP	10 MAP	12 MAP
Range	3.47-44.39	5.87-64.16	10.14-66.38
CV (%)	32	33	39
Mean	19.69	31.83	31.00
Mean yield increase (t/ha)		12.14	-0.83

*MAP = months after planting

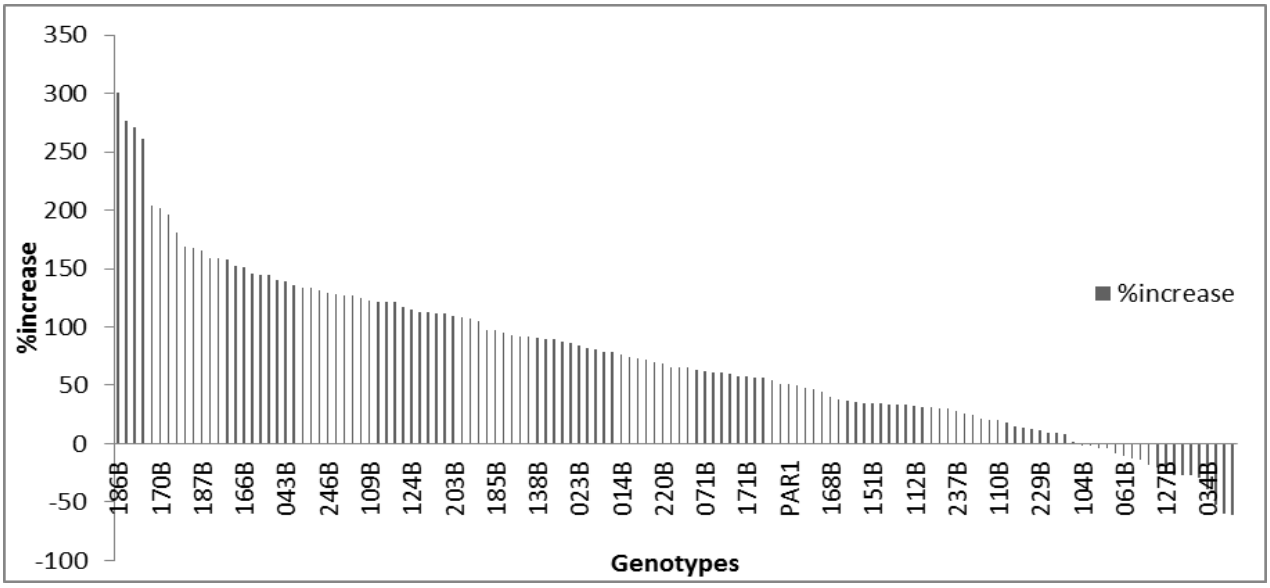


Figure 7.4: Percentage yield increase in fresh storage root yield of the F_1 cassava genotypes and checks at 10 MAP over 7 MAP, evaluated for early storage root bulking in the Umudike humid forest agroecology of Nigeria in the 2016/17 and 2017/18 growing seasons

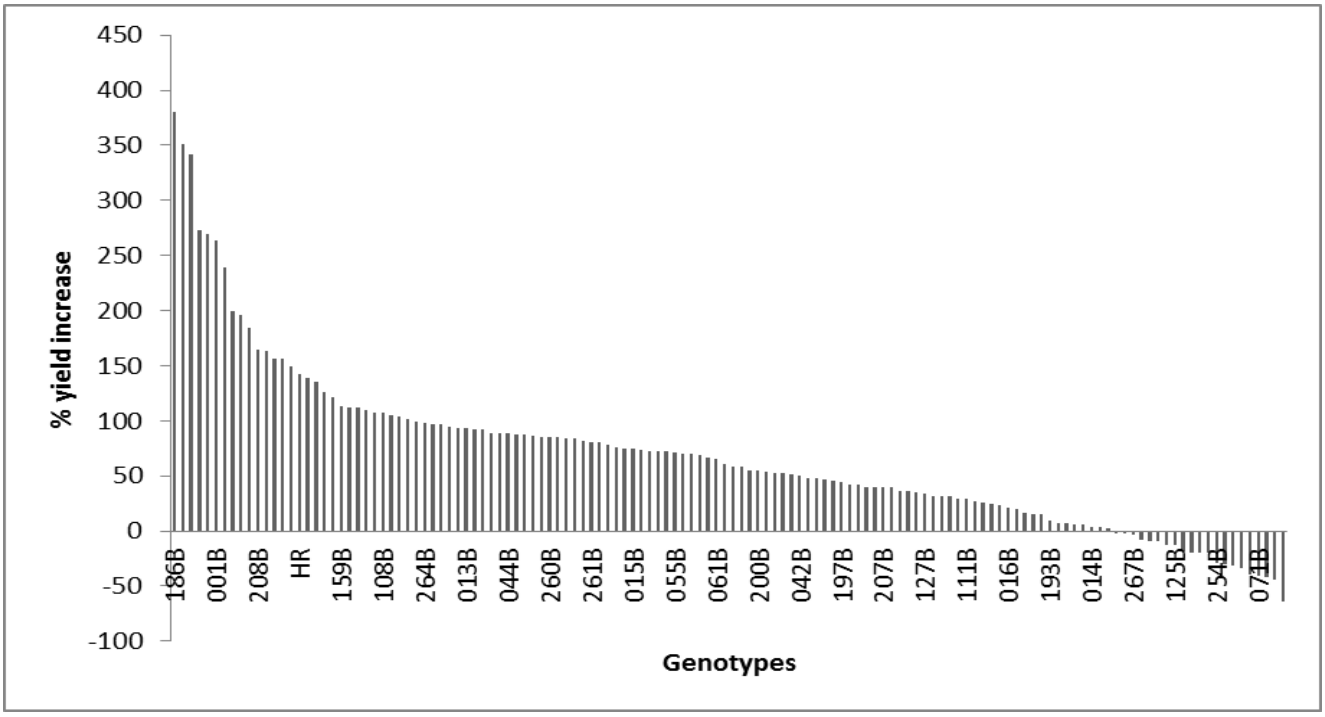


Figure 7.5: Percentage yield increase in fresh storage root yield of cassava F_1 genotypes and checks at 12 MAP over 7 MAP, evaluated for early storage root bulking in the Umudike humid forest agroecology of Nigeria in the 2016/17 and 2017/18 growing seasons

7.3.7 Genetic linkage map for traits associated with early bulking in F_1 cassava genotypes generated at Umudike

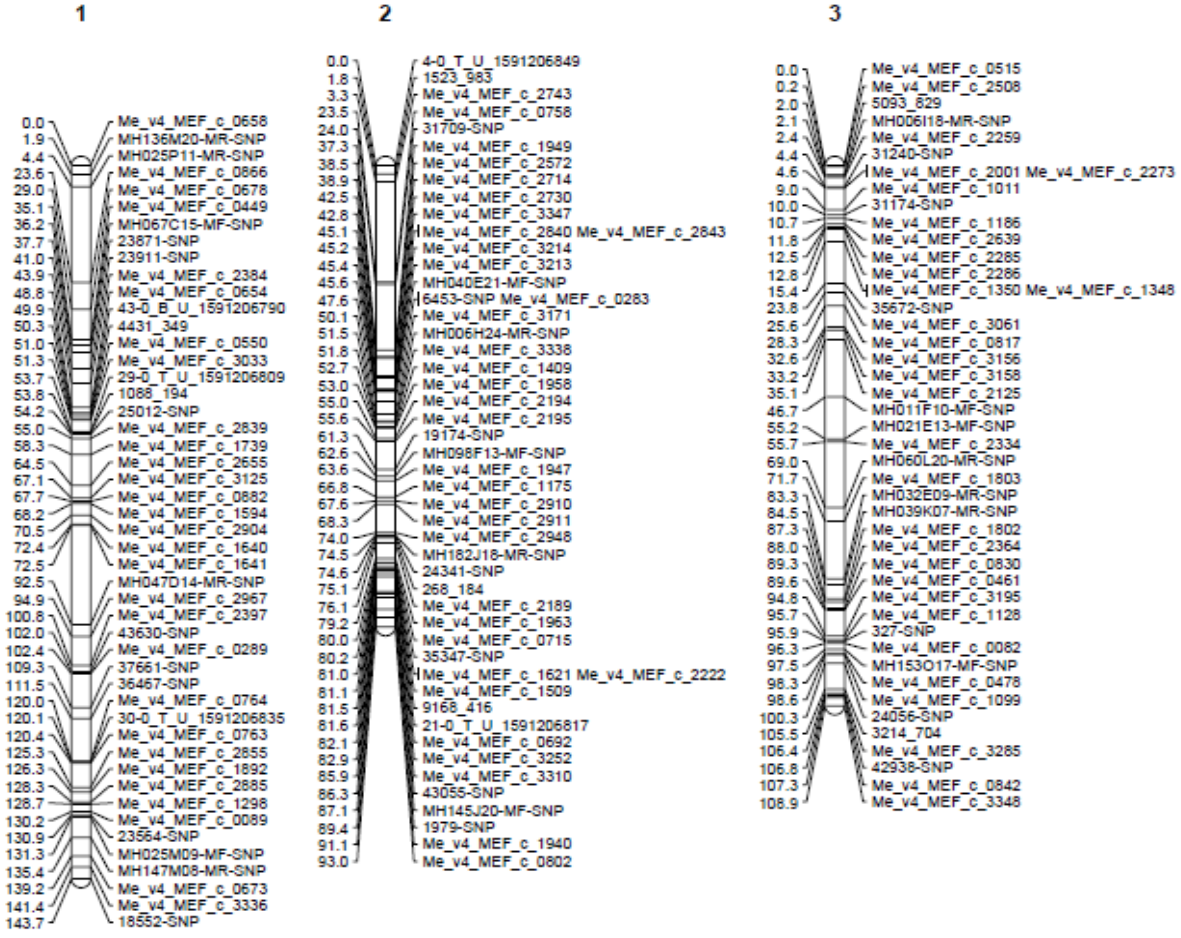
Results of the parental survey with SNPs indicated that the number of polymorphic markers in each parent was between 522 and 567 markers, while 856 polymorphic markers for the cross combination (parental pairs) were selected for the development of mapping populations (Tables 7.5 and 7.6). Genotyping the mapping population with KASPar SNP array resulted in 856 polymorphic markers (Table 7.6). After removing all ambiguous and unlinked markers, the genetic linkage map with 505 markers evenly distributed across all 21 cassava chromosomes was constructed, spanning 582.8 cM in length, with an average marker density of 3.69 cM (Fig. 7.6). Chromosome 1 was the longest (143.7 cM), while chromosome 20 was the smallest (22.5 cM). The number of markers per linkage groups ranged between 6 and 48 markers; the length of the linkage ranged between 25.6 cM and 143.7 cM, while inter-marker distance ranged between 1.92 cM and 6.69 cM. Thus, the marker distribution in the population indicated that it had good genome coverage and was suitable for QTL mapping.

Table 7.5: The number of polymorphic markers per parent

Parents	Polymorphic markers
TMS98/0505	567
TMS98/0581	522

Table 7.6: The number of polymorphic markers per cross

Cross	Population	Polymorphic markers
TMS 98/0505 x TMS 98/0581	Population B	856



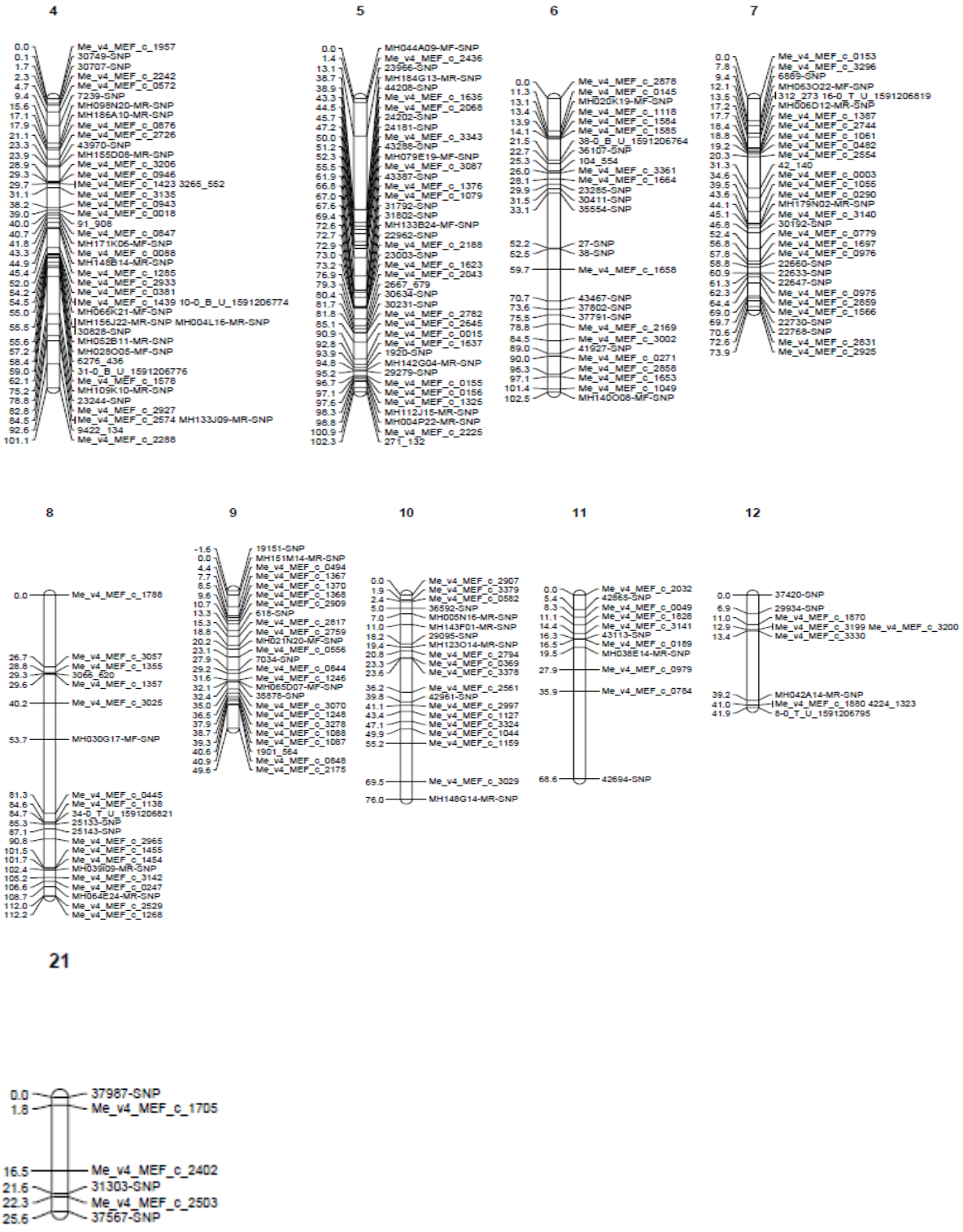


Figure 7.6: Genetic linkage map of cassava in F_1 population

7.3.8 Identification of QTL for early bulking in year 1

A total of 16 QTLs associated with early bulking in the F_1 population were identified in 9 linkage groups (LGs 1, 2, 4, 5, 6, 7, 10, 13, and 16). The significant peak values of LoD scores (LoD ≥ 3), the position of the peaks, the percentage phenotypic variance, and estimated gene actions are shown in Table 7.7. The number of QTLs identified ranged between 1 and 3. The identified QTLs were found in 8 traits. Results showed that root shape had the highest number of identified QTLs in this analysis in the first year.

7.3.8.1 Biomass

Analysis resulted in the identification of two QTLs (c5.loc73.2 and c10.loc52.0). This implies that the first QTL was located on chromosome 5 at 73.2 cM, while the second was located at chromosome 10 at 52.0 cM. The QTLs accounted for 9.3% and 11.4% of the phenotypic variation, respectively. The two loci stemming from the parent TMS980581 reduced the biomass, with the QTL on LG5 having an additive effect, while the QTL on LG 10 had an over-dominance effect. The QTL alleles influencing this trait were from parent genotype TMS98/0505. Although *c10.loc52.0* was identified for its over-dominance gene action, an additive gene action could not be excluded.

7.3.8.2 Dry root yield (DRY)

Analysis identified two QTLs (c2.loc81.5 and c4.loc49) located on chromosomes 2 and 4. All the QTLs had an increasing effect. The two QTL alleles responsible for the increased phenotypic values for this trait in the population were from TMS980505, while TMS980581 reduced the dry root yield. The phenotypic variances explained by the QTLs were 4.87 and 4.20, respectively. The two QTLs detected for dry root yield expressed over-dominance gene actions.

7.3.8.3 Fresh root yield (FRY)

Two significant QTLs were found for this trait and mapped at the same LGs (2 and 4) as with DRY. The phenotypic variations explained by the QTLs were 4.21 and 4.60%, respectively. The alleles driving the yield were from parent genotype TMS98/0505. Both loci QTLs had decreasing effects. The same gene actions (additive gene actions) were associated with this trait (Table 7.7). Results showed that while the two QTLs reflected additive gene actions, a dominance gene action should not be excluded.

7.3.8.4 Height at first branching (HFB)

Three different regions of the genome were found to be significantly associated with this trait. The QTLs were found on LGs 1, 5, and 13. The phenotypic variances explained by these QTLs ranged between 5.93 and 10.14%. Two QTLs (*c1.loc111.5* and *c5.loc93.0*) increased the height at first branching; and the alleles involved were derived from TMS980505. The other QTL, *c13.loc70.0*, decreased the height at first branching; and the alleles involved were derived from TMS98/0581. Except for QTL *c5.loc93*, which had an over-dominance gene action, the other QTLs exhibited additive gene actions (Table 7.7). Although results indicated an additive gene action for *c13.loc70.0*, the possibility of a dominance gene action for this QTL could also not be excluded.

7.3.8.5 Root number (RTNO)

Two QTLs located in LGs 2 (*c2.loc81.6*) and 6 (*c6.loc17*) were identified for root number. The phenotypic variance explained by c2.loc81.6 was 6.82%, while c6.loc17 had a PVE of 10.12%. The loci *c2.loc81.6* increased the root number, with the QTL on LG2 having an over-dominant effect, while the QTL on LG 6 reduced the root number and had an additive effect. The QTL alleles influencing this trait were from parent genotype TMS98/0505. Although *c6.loc17* tended to show additive gene effects, dominance gene effects could not be excluded in the QTL.

7.3.8.6 Root shape (RTSHP)

This trait revealed several QTLs in the population. Three regions, representing different linkage groups, were found to be significantly linked to this trait. The LGs include 20, 4, and 13. The LoD scores ranged between 3.0 and 3.45. The PVE ranged between 5.63% and 7.6%. All the QTLs had additive gene actions. Two of the QTLs (*c4.loc55.5* and *c13.loc68*) increased the root shape, while *c20.loc36* caused a reduction in root shape. Though *c20.loc36* showed an additive gene action, a dominance gene action should not be excluded.

7.3.8.7 Stem diameter (STMDIAM)

One QTL significantly associated with stem diameter was found on LG 16. The phenotypic variation accounted for by the QTL was 5.1. The QTL (*c16.loc0.32*) reduced the stem diameter; and this was coming from the parent TMS980581. Though the QTL detected an additive gene action, a dominance gene action should not be neglected.

7.3.8.8 Vigour (VIG)

This trait revealed one highly significant QTL in the mapping population. The QTL (*c7.loc0.0*) controlling vigour was located in LG 7, and accounted for 10.74% of the phenotypic variation. The QTL exhibited an additive gene action and reduced vigour. This meant that the allele that reduced vigour came from the parent TMS980581, while the allele that increased vigour was derived from the parent TMS980505. Although the QTL showed an additive gene effect, a dominance gene effect should not be excluded.

7.3.9 Co-localisation of QTLs in year 1

Results indicated that one QTL was identified for more than one trait. For example, QTL c2.loc81.5 was found to be associated with dry root yield and fresh root yield (Table 7.8).

7.3.10 Identification of QTLs for early bulking in year 2

In the second year, 12 QTLs were identified (Table 7.9) in eight linkage groups (1, 3, 7, 8, 13, 15, and 16). The identified QTLs were found in eight traits. Additional QTLs were identified in some traits where QTLs were identified in year 1, while QTLs were also identified in new traits such as root diameter, starch content, and dry-matter content in the second year (Table 7.9). The identified QTLs ranged between 1 and 3.

7.3.10.1 Dry root yield (DRY)

The second year's analysis resulted in the identification of one QTL *(c7.loc34.6),* implying that the QTL was located on chromosome 7 at 34.6 cM. The QTL accounted for a high phenotypic variation of 14.02%. The allele influencing this trait (positive effect and significant at $P \leq 0.01$) was from TMS98/0505. The QTL showed an additive gene action.

7.3.10.2 Fresh root yield (FRY)

Two highly significant QTLs were found for this trait and mapped to LGs (7 and 16). One of the QTLs was matched in the same linkage groups with DRY (LG7). The QTLs explained a high phenotypic variation of 18.88% and 12.01%, respectively. The alleles driving yield were from parent genotype TMS98/0505. Both loci QTLs had increasing effects. One of the QTLs *(c7.loc34.6*) showed an additive gene effect, while the other QTL *(c16.loc70*) showed an over-dominance gene effect.

7.3.10.3 Height at first branching (HFB)

A region of the genome was found to be significantly associated with this trait. The QTL controlling HFB was located in LG 15, and accounted for 10.52% of phenotypic variation. The

QTL had a reducing effect, coming from the parent TMS980581. The QTL exhibited an over-dominance gene action and reduced HFB. Though the QTL had an over-dominance gene action, an additive gene action should not be omitted.

7.3.10.4 Stem diameter (STDIAM)

This trait revealed several QTLs in the population. Three regions, representing different linkage groups, were found to be significantly linked to this trait. The LGs included 3, 7, and 8. The LoD scores ranged between 3.14 and 4.04. The PVE ranged between 3.98% and 8.24%. Two of the QTLs *(c3.loc170* and *c8.loc87*) increased the stem diameter, which came from the parent TME980505, while one QTL *(c7.loc64*) reduced the stem diameter coming from the parent TMS980581. All QTLs had an over-dominant gene action. Though analysis revealed that the QTL *c7.loc64* had an over-dominance gene action, an additive gene action should not be excluded.

7.3.10.5 Vigour (VIG)

One QTL, significantly associated with plant vigour, was found on LG 3. The phenotypic variation accounted for by the QTL was 12.08%. The QTL allele influencing plant vigour was from parent plant TMS98/0581. The QTL detected for plant vigour showed an over-dominance gene action.

7.3.10.6 Dry-matter content (DMC)

Analysis identified two QTLs (*c1.loc3* and *c10.loc 53*) located on LG 1 and 10. The two QTL alleles responsible for the increased phenotypic values for this trait in the population were from both parents (meaning one QTL from either parent). The allele for *c1.loc3* originated from TMS98/0505, and was mapped in the interval mk001-mk003, which explained 8.97%. The allele in respect of *c10.loc53* originated from TMS98/0581, and similarly explained 8.26% of phenotypic variance. The two QTLs detected for dry-matter content showed an over-dominant gene action. Although results indicated an over-dominance gene action for *c10.loc53*, the possibility of an additive gene action for this QTL could also not be excluded.

7.3.10.7 Root diameter (RTDIAM)

This trait revealed one QTL in the mapping population. The significant QTL found for this trait was found mapped to the same LGs (13) as was plant vigour. The QTL *(c3.loc67*) controlling root diameter accounted for 11.77% of the phenotypic variation. The QTL exhibited dominance

gene action and an increased root diameter. The favourable allele was derived from genotype TMS98/0505. Results indicated that the QTL showed an additive gene action.

7.3.10.8 Starch content

A significant QTL for this trait was found mapped in the same LG (1) as with DMC, and accounted for 10.19% of phenotypic variation. The QTL showed a dominant gene action and increased starch content coming from the parent TMS980505. An additive gene action should not be discounted, even though the analysis revealed an over-dominance gene action.

7.3.11 Co-localisation of QTLs in year 2

Results indicated that a number of the identified QTLs were applicable for more than one trait. Most of the traits identified with this phenomenon were dry root yield and fresh root yield; dry-matter content and starch content; plant vigour and root diameter. For example, *c7.loc34.6* was found for fresh root yield and dry root yield, with the same marker interval in the second year. Similarly, QTL *c1.loc3* was involved in dry-matter content and starch, while QTL *c13.loc72* was responsible for plant vigour and root diameter, respectively (Table 7.10).

Table 7.7: Composite interval analysis for early bulking year 1

Trts[a]	QTL[b]	Chro[c]	Flank Markers	Position	Lod	Add[d]	Dom[e]	Gene action	%PVE[f]	P-value[g]
BIOM(kg)	*c5.Loc73.2*	5	mk192-mk215	73.2	3.47	-0.029	16.9	A	9.3	0.0006***
	c10.Loc52.0	10	mk346-mk355	52	4.16	-0.02	-0.41	OD	11.4	0.00013***
DRY(t/ha)	*c2.Loc81.5*	2	mk050-mk092	82	3.48	0.0046	1.06e+04	OD	4.87	0.025*
	c4.Loc49	4	mk170-mk180	49	3.25	0.00043	1.61	OD	4.2	0.042*
FRY(t/ha)	*c2.Loc81.5*	2	mk089-mk091	82	4.02	-0.0411	8.34e+04	A	4.21	0.036*
	c4.Loc50	4	mk170-mk190	50	3.16	-0.0212	8.77	A	4.6	0.027*
HFB(cm)	*c1.Loc111.5*	1	mk033-mk035	111.5	6.43	5.35	-9.09	A	5.93	0.0087**
	c5.Loc93	5	mk192-mk222	93	3.67	1.83	35.1	OD	10.14	0.00038***
	c13.Loc70.0	13	mk393-mk405	70	3.02	-0.23	36.68	A	6.26	0.0068**
RTNO	*c2.Loc81.6*	2	mk090-mk092	82	3.38	0.25	86180	OD	6.82	0.0043**
	c6.Loc17	6	mk237-mk238	17	4.5	-2.02	6.28	A	10.12	0.00036***
RTSHP	*c4Loc55.5*	4	mk171-mk177	55.5	3.45	0.021	-0.039	A	6.84	0.0036**
	c13.Loc68	13	mk388-mk394	68	3.02	0.0044	-0.14	A	5.63	0.0044**
	C20.Loc36	2	mk512-mk514	36	3	-0.028	0.45	A	7.6	0.0021**
STMDIAM(cm)	*c16.Loc0.32*	16	mk451-mk456	0.32	3.77	-2.92e-05	0.00159	A	5.1	0.029*
VIG	*c7.Loc0.0*	7	mk260-mk261	0	3.36	-0.083	1.754	A	10.74	0.00052***

*a = Traits; b = Individual QTLs; c = Chromosome where the marker QTLs are located; d = Additive gene effects; f = Marker significantly associated with trait variation; g = probability of the association between a QTL and marker; BIOM = Biomass; DRY = Dry root yield; FRY = Fresh root yield; HFB = Height at first branching; RTNO = Number of roots; RTSHP = Root shape; STMDIA = Stem diameter; VIG = Plant vigour; NS is not significant, *, **, & *** is significant at 5, 1, & 0.1%.

Table 7.8: QTL identified for more than one trait

QTL	Trait	Interval
c7.loc34.6	DRY, FRY	mk272-mk274

Table 7.9: Composite interval analysis for early bulking year 2

Trts	QTL	Chrom	Flank Mark	Position	Lod	Add	Dom	GA	%PVE	P-value
DRY (t/ha)	*c7.Loc34.6*	7	mk272-mk274	34.6	4.24	1.0221	-0.99	A	14.02	5.7e-05***
FRY (t/ha)	*c7.Loc34.6*	7	mk272-mk274	34.6	6.06	6.85	-6.66	A	18.88	2.81e-07***
	c16.Loc70	16	mk468-mk469	70	3.76	0.0002	3.13	OD	12.01	4.51e-05***
HFB (cm)	*c15.Loc26*	15	mk444-mk445	26	3.11	-0.22346	-0.8068	OD	10.52	0.00090***
STDIAM (cm)	*c3.Loc107*	3	mk113-mk145	107	3.36	8.28e-05	7.05e-02	OD	4.53	0.0201*
	c7.Loc64	7	mk260-mk287	64	3.14	-3.39e-03	-1.43e-02	OD	8.24	0.000989***
	c8.Loc87	8	mk302-mk303	87	4.04	5.52e-04	1.30e-01	OD	3.96	0.0324*
VIG	*c13.Loc72*	13	mk388-mk396	72	3.6	-0.00227	-15.285	OD	12.05	0.00025***
DMC (%)	*c1.Loc3*	1	mk001-mk003	3	3.23	0.007312	0.2123	OD	8.97	0.00147**
	c10.Loc53	10	mk354-mk355	53	3.01	-0.01471	-0.05419	OD	8.26	0.00239**
RTDIAM (cm)	*c13.Loc72*	13	mk395-mk406	72	3.51	0.02	-22.096	A	11.77	0.00036***
STARCH (%)	*c1.Loc3*	1	mk001-mk003	3	3.01	2.03e-07	5.57e-06	OD	10.19	0.0011**

*DRY = dry root yield; FRY = fresh root yield; HFB = height at first branching; STDIAM = stem diameter; VIG = plant vigour; DMC = dry-matter content; RTDIAM = root diameter.

Table 7.10: QTLs identified for more than one trait in year 2

QTL	Trait
c2.loc81.5	DRY, FRY
c1.loc3	DMC, STARCH
c13.loc72	VIG, RTDIAM

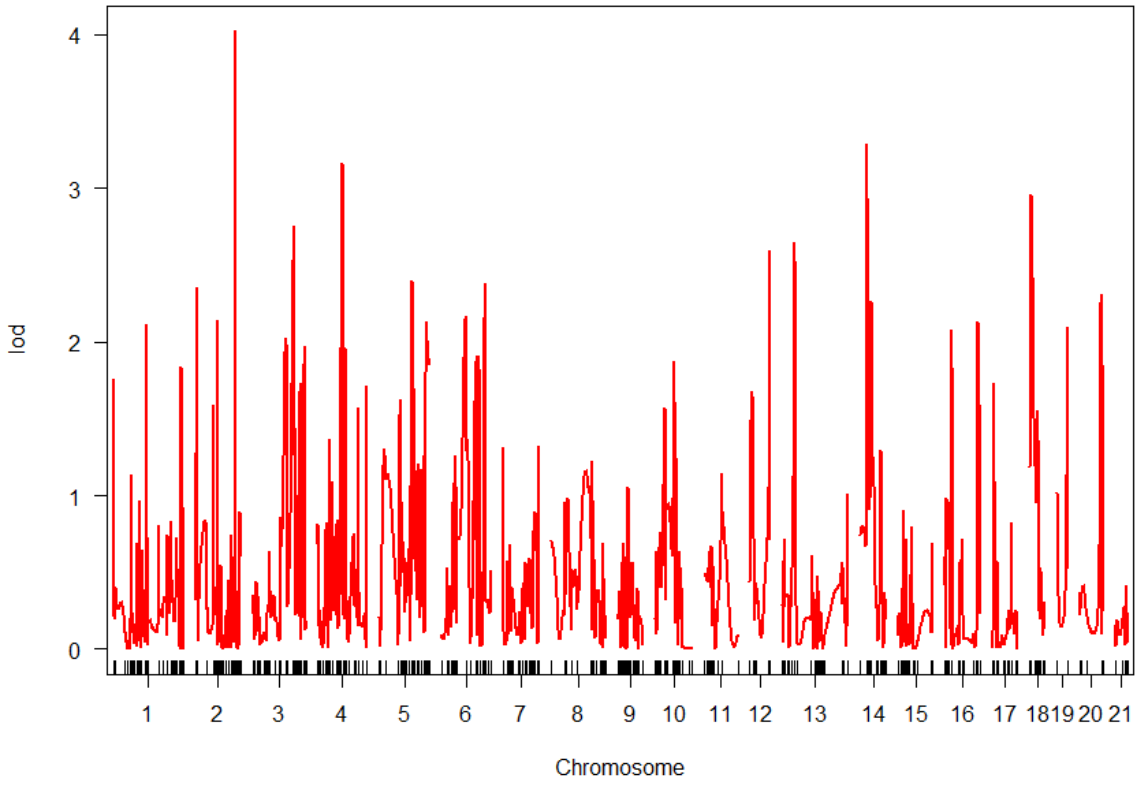


Figure 7.7: QTL peaks for linkage group (LG) 1-21 (fresh root yield year 1)

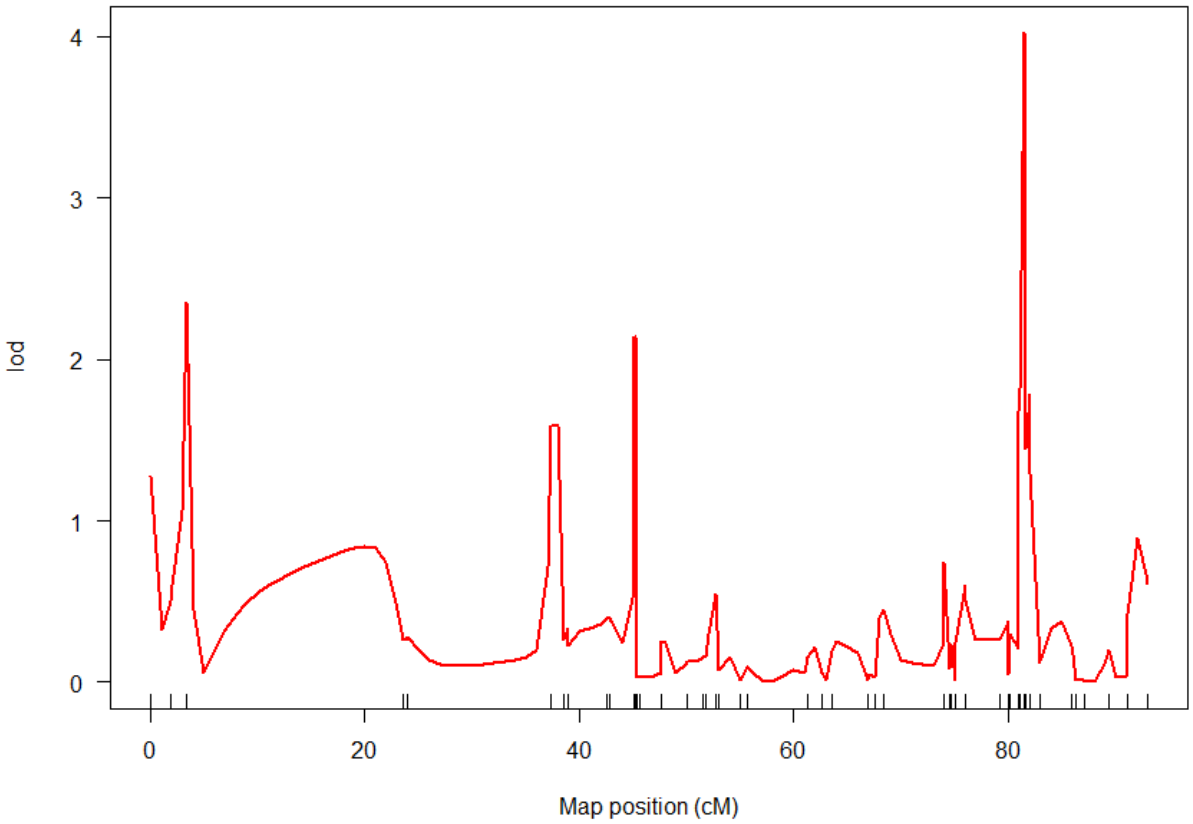


Figure 7.8: QTL peak (c2.loc81.5) and map position (fresh root yield year 1)

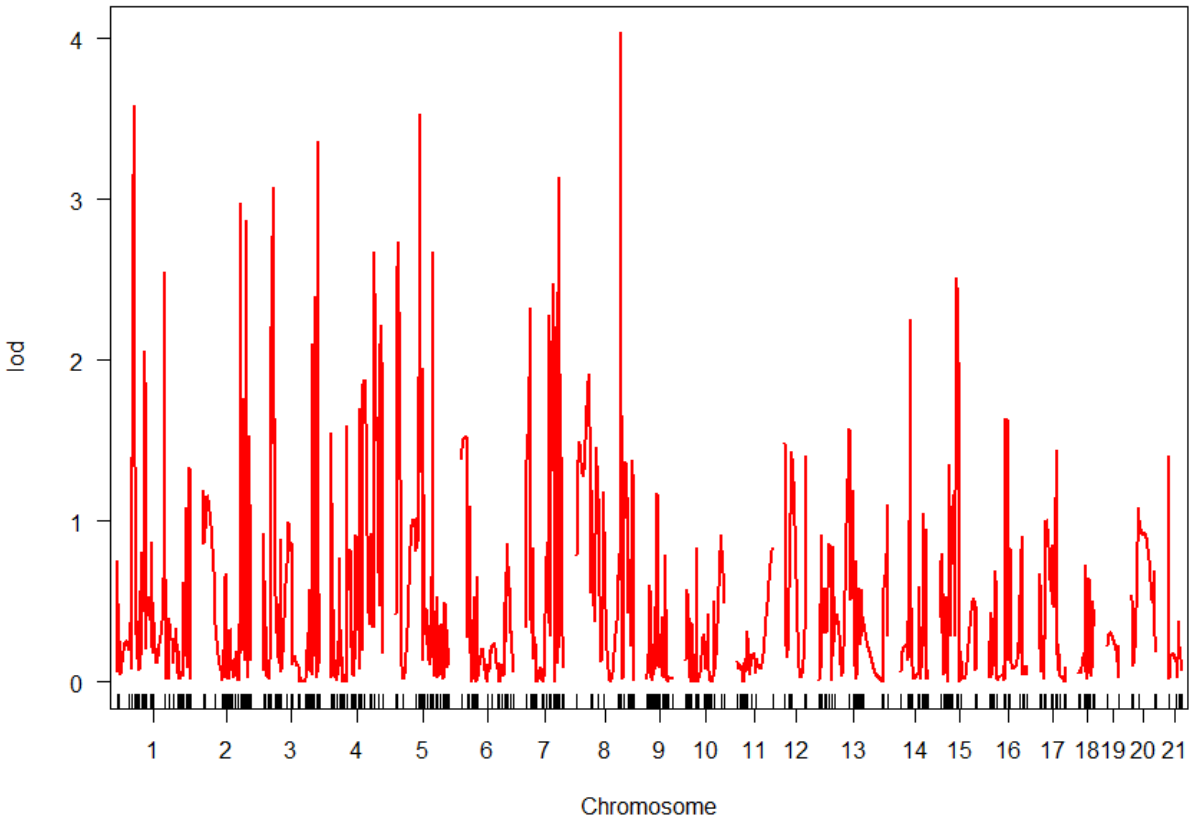


Figure 7.9: QTL peaks for linkage group (LG) 1-21 (stem diameter year 2)

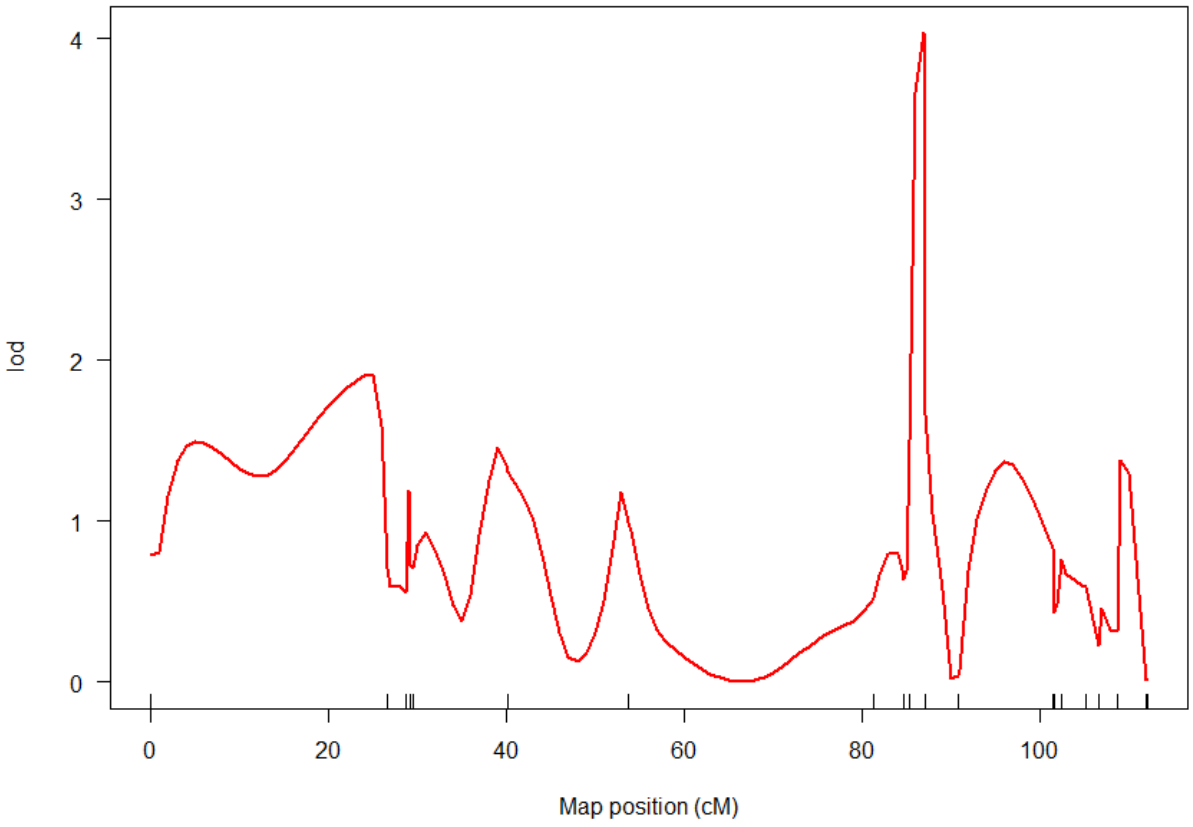


Figure 7.10: QTL peak (c8.loc87) and map position (stem diameter year 2)

7.4 Discussion

The significant mean square among the genotypes for all traits indicated the variation in the population for the traits; and hybridisation among them can further lead to a genetic advance for all the traits. The significant difference among the genotypes for fresh storage root yield indicated the differences in yield among the genotypes for early storage root bulking. The significant effect of interaction between genotype and harvest age on fresh root yield (FRY) suggested that age at harvest influences the cassava genotypes differently in terms of the trait. This had been reported previously by Okechukwu and Dixon (2009), Kamau *et al.* (2011), and Okogbenin *et al.* (2013). The highly significant correlation observed between FRY and other agronomic traits (harvest index, plant vigour, dry root yield, stem diameter, plant height, root diameter, root number, and storage root weight) in this study indicated their interdependence,

and importance in influencing FRY. Similar results had been reported by Okogbenin *et al*. (2006), Kundy *et al.* (2014), and Ntawurunga *et al.* (2001). This implied that these traits can be used to select for early bulking during selection.

The wide range observed at 7, 10, and 12 months after planting across the two seasons indicated a wide range of variation among the genotypes for fresh storage root yield at each plant age of the harvest. A similar result has already been reported by Olasanmi *et al.* (2017), when they observed a wide range of variation of genotypes evaluated at different plant ages in two locations. The Mean yield increase was higher at 10 MAP over 7 MAP than at 12 MAP over 7 MAP, indicating that there were more genotypes identified as early bulkers than late bulkers when harvested at 10 MAP. The percentage yield increase of less than 100% observed in some of the genotypes showed that they must have initiated storage root development earlier, thereby reaching their maximum yield within a short growing period. This confirmed similar observations made by Bitai and Lian (1978) in sweet potato. They discovered that bulking in early-maturing cultivars declined in the early (or later) period of growth, whereas for the late-maturing cultivars, the bulking rate increased at the middle or later growth periods. Similar results had also been confirmed by Olasanmi *et al.* (2017), when they identified early-bulking cassava varieties at 12 MAP over 7 MAP, with a yield increase of less than 100%. The late-bulking genotypes are better to be harvested between 10 to 12 MAP, while the early-bulking genotypes are better to be harvested at 7 MAP. In this study, the early-bulking genotypes identified in this study are better to be harvested at 7 MAP to avoid field weathering, rot, and a reduction in yield.

The study identified 28 QTLs from the 2-year analysis in 11 traits. Some of the QTLs linked with productivity traits (biomass, dry root yield, fresh root yield, root number, and root diameter) that are related to early bulking, corroborate similar results by Okogbenin (2006; 2002), who identified QTLs in productivity traits (fresh foliage, dry root yield, harvest index, and root diameter) being linked to early bulking in F_1 and F_2 populations. All the identified QTLs were highly significant at $P < 0.01$, signifying that they were linked to the identified traits. In other words, these markers can be used in the selection and breeding for these traits. The explained phenotypic variance varied from small to moderate and major effects. Two QTLs for fresh root yield and dry root yield for a relatively large amount (> 14%) were thus major QTLs identified in this study (Collard et al*.,* 2005). Root yielding is directly influenced by the rate and duration of bulking and has been used to access early bulking in cassava (Olasanmi *et al.,* 2017; Tershey *et al.,* 2012; Okogbenin and Fregene, 2002).

Some QTLs were found to control more than one trait. This is in agreement with the observed correlations between fresh root yield and dry root yield, dry-matter content and starch, root diameter and plant vigour found in this study. Likelihood intervals affecting more than one trait were also identified (Tables 7.8 and 7.10). This suggested that either some QTLs had pleiotropic effects or that different QTLs affecting these traits tended to be clustered together into closely linked groups (Okogbenin *et al.,* 2006). The localisation of QTL or gene pleiotropic effects have been reported in other QTL mapping studies (Okogbenin and Fregene, 2003; Okogbenin *et al.,* 2006).

This study identified useful QTL alleles from both parents, which may help define useful genetic backgrounds for improved early-bulking varieties, if combined efficiently in good haplotypes, in a good recurrent selection programme.

The QTLs identified two different gene actions (additive and over-dominant). Almost 50% of the QTLs had over-dominant gene action, signifying that this mode of gene action was critical to performance. Thus, this was in agreement with broadly held views that superiority in field performance was highly linked to its heterozygosity (Fukuda *et al.,2010* Collins *et al.,* 2008;. Cebellos *et al.,* 2004). A good number of additive gene actions were also found, implying that breeding needs to explore these genes through selfing and a recurrent selection scheme to increase allele frequencies for such loci. Cassava is highly heterozygous, and as a result of this, a different gene mode is expected. The utilisation of both the additive and dominant gene actions through identified QTLs can be exploited for rapid genetic gain and for yield improvement in cassava. A previously constructed linkage map of cassava in an F_1 population using KASPar SNPs was used for this study (Ewa *et al.,* unpublished). Based on this genetic map, 22 significant QTLs were identified under humid environment conditions, controlling early bulking. The same genetic map has also been reported to have identified QTLs controlling drought tolerance in marginal environments (Ewa *et al.,* unpublished). This implies that KASPar-derived tools are suitable for the development of a dense map in cassava.

7.5 Conclusion

This study was carried out to identify early-bulking cassava varieties in an F_1 population. Traits significantly associated with early storage root bulking were identified and a genetic analysis for early bulking conducted. Fresh root yield was significantly associated with other agronomic traits such as harvest index, root weight, number of roots, stem diameter, root diameter, biomass, and

dry root yield, while principal component analysis identified important traits such as root weight, root number, plant biomass, fresh root yield, dry root yield, dry-matter content, plant height, starch content, harvest index, and stem diameter as traits that can be used in the selection for early root bulking in cassava by breeders. The early-bulking genotypes identified in this study will be better to be harvested at 7 MAP, while the late bulkers are better to be harvested between 10 to 12 MAP. The early-bulking genotypes identified in this study should be evaluated further at more locations to test them for yield stability before being released to farmers. The study identified 16 and 12 QTLs in year one and two respectively. The identification of these loci will aid in breeding for early root bulking in cassava via marker assisted selection in cassava.

CHAPTER 8

ESTIMATE GENETIC IMPROVEMENT FOR DROUGHT TOLERANCE IN CASSAVA GENOTYPES

ABSTRACT

Cassava is a perennial tropical crop, cultivated for its starchy tuberous roots. The crop can adapt to drought-prone areas and has the ability to grow in a depleted and marginal soil. However, drought has been identified as one of the threats to cassava production in marginal environments. The heterozygous nature of the crop and parental lines used to generate new segregating progeny population make it difficult to identify parents with good breeding values. Selection is an important breeding strategy used to improve many economic traits in several varieties of crops. Selection aids in the improved frequency of desirable genes in a given population, and helps to maintain the high genetic variability in heterozygous population. A second population (population C) for drought tolerance was developed from crosses, using parents selected through the selection index (classical breeding) and optiMAS (molecular breeding tool). Most of the traits evaluated ($P < 0.001$) varied significantly among genotypes. Fresh root yield positively correlated with yield-related and morphological traits, while morphological traits positively correlated with each other. PC1 and PC2 identified fresh root yield, root weight, plant biomass, dry root yield, plant height, the number of scars, the number of leaves, plant vigour, scar level, harvest index, length of leafless stem, and length of stem with leaves as traits that were driving drought tolerance. There was a better performance in traits such as plant biomass, root number, dry-matter content, number of scars, number of leaves, leaf retention, and length of stem with leaf in the second population (population C) than the first population (population B). Twenty superior genotypes were selected from both crosses, which will be incorporated in the breeding programmes for further evaluation.

Keywords: breeding programmes; drought tolerance; optiMAS; population C; selection

8.1 Introduction

Cassava (*Manihot esculenta Crantz*) is a crop of significant economic and social relevance. Along with rice, maize, sugarcane, and banana/plantain, it is among the most important sources of carbohydrates in the diet in many tropical countries (Burns *et al.,* 2010). Cassava is a key food security crop in sub-Saharan Africa, and in several Asian and American countries such as Indonesia, Brazil, Paraguay, and Haiti. Cassava is the second most important source of starch worldwide (Stapleton, 2012); Thus, it is also a key commodity for agro-industrial processes, including the production of carburant ethanol and cooking fuel (Anyanwu *et al.,* 2015)

All parts of this remarkable crop can be exploited. Its most commonly used product is the starchy root. Its foliage has an excellent nutritional quality for animal and human consumption, and offers great potential for further exploitation in food and feed. The stems are used for commercial multiplication through cuttings, along with minor applications such as mushroom culture.

The cassava plant grows exceptionally well in low fertility and drought-prone environments (Cock, 1984). Although cassava is adaptable to marginal soils with low fertility and irregular rainfall conditions, the challenges posed by global climate change have had a negative impact on this crop's productivity (Oliveira *et al.,* 2015). Increased incidence and severity of drought has made it difficult for breeding programmes to select drought-tolerant genotypes, or to garner a full understanding of the mechanisms associated with drought stress. Drought is a quantitative trait, which makes it difficult to breed and select drought-tolerant genotypes (El-sharkawy, 2005, 2007; Budak *et al.,* 2013; Okogbenin *et al.,* 2013). The availability and use of high-yield cassava varieties that are tolerant to water stress may contribute positively towards the product offer, particularly in more sensitive environments prone to climate change (Eder Jorge de Oliveira *et al.,* 2015).

Genetic improvement of crops is the science of applying genetics, plant breeding principles, and biotechnology to improve plants. It has been effective in achieving improved yields, and disease resistant and improved nutritional quality in crops. Modern scientific approaches integrate both laboratory and field research, where genomics, bioinformatics, quantitative genetics, and biotechnology increase the efficiency of selection and breeding for better crop varieties. The International Institute of Tropical Agriculture (IITA) has played a major role in the genetic improvement of cassava over the last 40 years (IITA Annual Report, 2013). Most of these

improvements were achieved through conventional breeding, which is a very lengthy process, because of cassava's long growth cycle and low rate of multiplication. It takes five to six years from the time cassava is crossed to generate new recombinant progenies, which go through field evaluations, to the time new parents are selected for the next crossing cycle.

In order to cut short this lengthy breeding process, modern biotechnology has aided in the development and adaptation of the rapidly advancing next-generation sequencing technologies by generating high-density molecular markers that unravel the crop's genetic diversity, and locating genomic regions that control quantitative and qualitative breeding traits (IITA Annual Report, 2013). The National Root Crops Research Institute (NRCRI) Umudike, Nigeria had also embarked on the use of next-generation markers in genomic selection, a new breeding method that uses statistical modelling to predict how a plant will perform, even before it is field tested.

One of the challenges in cassava breeding relates to the parents to be used in generating new germplasm, because of the time required to evaluate segregating progeny; and the large genetic variation generated with each cross, due to the highly heterozygous nature of the crop (Ceballos *et al.,* 2015). Cassava breeders typically apply phenotypic recurrent selection, as is common for clonally propagated crops (Burton, 1992; Grüneberg *et al.,* 2009; Lebot, 2010; Quero-García *et al.,* 2010; Ceballos *et al.,* 2012). Because of the low multiplication rate of cassava from stem cuttings, it takes several years to have enough planting material available for replicated multi-location evaluations under the conventional propagation systems (Ceballos *et al.,* 2004, 2012). A typical selection cycle requires two years to produce the progeny (botanical seeds) of planned crosses, and six consecutive years of field evaluation. Initial phenotypic evaluations are based on unreplicated trials grown in one or, at most, two locations. Critical selection decisions need to be taken during this lengthy process. Breeders try to reconcile the practical need to reduce the large number of genotypes in the early stages of selection with the awareness that selection based on unreplicated trials is prone to large experimental errors.

Selection is an important breeding strategy practised to improve many economic traits in several varieties of plants. Selection during the first clonal evaluation stage has the advantage of obtaining information that allows an estimate to the general combining ability of the progenitors, shortening the length of the evaluation process, improving the probabilities of identifying superior germplasm, and helping to detect new potential traits that can be incorporated into the breeding criteria (Ceballos *et al.,* 2015). Many different variables are relevant and need to be considered in determining the kind of germplasm that the selection process must achieve.

Selecting for fresh root yield is often more effectively achieved through the indirect selection for correlated traits (Kawano, 2003; Kawano *et al,* 1998). To facilitate the process of selection, CIAT has implemented its selection index approach (Baker, 1986), combining key variables. Weights varying from one to ten are assigned to variables, depending on the importance of the variable and on how it meets the objectives of the research. The selection index is used to sort genotypes from best to worse. The best genotypes can serve as parents that will be used in developing new progeny.

Current developments in plant genotyping lead to major progress in the knowledge of genetic architecture of traits of interest (Valente *et al.,* 2013). With the increasing use of molecular markers in plant breeding programmes, it is important to develop decision-support tools to help breeders implement marker-assisted selection (MAS) projects.

OptiMAS, a decision support tool, has been developed to help breeders create a given ideal genotype, assembling favourable alleles from diverse parental origin, and to help breeders and geneticists make rapid and effective selection decisions. Alogarithms are deployed to trace parental QTL alleles identified as favourable throughout the selection generations, using information given by markers located in the vicinity of the estimated QTL position. OptiMAS also computes the probabilities of parental alleles, and using the probabilities, it proposes easy ways of identifying the best candidate for selection (Valente *et al.,* 2013). This tool appears promising to accelerate the genetic gain in plant breeding programmes.

Therefore, we report the use of a second population developed by using parents from an OptiMAS selection and the selection index method to determine traits significantly associated with drought tolerance, assess genetic variability in the second population, and select superior genotypes that can be incorporated into breeding programmes.

8.2 Materials and Methods

8.2.1 Development of second population

A second population (designated as population C), which is an advancement over the first population (population B), was developed. Two selection methods were used in selecting parents used for the second population. These included the OptiMAS and index selection methods. This second population is expected to result in superior varieties.

8.2.1.1 OptiMAS selection method

OptiMAS, a decision support tool, was used to identify genotypes that have the best haplotypes. Superior genotypes and their contributing QTLs were identified, and information produced on associations between physiological traits and yield performance in drought. Parents used in developing population C were selected for the best set of QTL combinations (haplotypes) with a view to increase the frequency of useful alleles.

8.2.1.2 Selection index method

Important traits that represent useful parameters for assessing breeding values were considered as variables for the selection index equation. Thus, the selection index method was calculated, based on the most important variables. The variables were weighted, based on the importance criteria in the breeding objectives. The selection index was constructed by using standardised deviation units (Steel and Torrie, 1960) to avoid the problems related to the magnitudes involved when measuring different variables. The selection index was calculated using the formular, $SI = (X_1*W_1) + (X_2*W_2) + (X_3*W_3) + (X_n*W_n)$, where W_1. W_2, W_3….W_n were the respective weights for each variable. X_1, X_2, X_3…..X_n = different variables, while each of the variables were standardised using the classical statistical formula (Steel and Tome, 1960): $X' = (Xi-\mu) / St.Dev$, where X‘ is the standard value; Xi is the original value; μ is the mean of the population; St.Dev. is the standard deviation for the variables analysed. The calculation was as follows:

$$SI = (FRY*10) + (DRY*10) + (DMC*10) + (HI*8) + (VIG*7) - (CMD*7)$$

Where SI = selection index; FRY is fresh root yield; DRY is dry root yield; HI is harvest index; VIG is plant vigour; CMD is cassava mosaic disease. Negative values were used for those variables where lower values represented the most desirable phenotypes. Since SI was estimated using the standardised values, a positive SI meant a performance better than the average, while a negative one meant a poor performance.

8.2.2 Plant materials and field experimental design

A total of 152 progenies were used in this study, developed from all possible combinations from selected parents, using optiMAS and the selection index methods of selection. The parents of the developed progeny (population C) were used as check. Cassava cuttings of uniform length (20-30 cm) were planted in an augmented design. It was a single row planting of five plant

stands per plot. The stakes were placed horizontally in the soil at the recommended 1 m spacing within plants per row, and 1 m separation between the rows (Ng and Ng, 2002). For homogenous germination and plant establishment, supplementary irrigation was applied, and water stress was imposed 90 days after planting (DAP) by withholding irrigation. Cassava should be established for three months under rainfall or irrigation before tolerance to drought can be measured effectively (Okogbenin *et al.,* 2013). The trial was established in November 2017 and harvested in November 2018.

8.2.3 Data collection

8.2.3.1 Phenotypic evaluation

For phenotyping activities, several data were collected on the field. There were several traits thought to be associated with drought tolerance in cassava (El-Sharkawy and Cadavid, 2002; Setter and Fregene, 2007), but it was not known to what extent these traits conferred improved root yield under drought conditions, the principal trait of interest to farmers. The current approach was to obtain precise phenotypic measurement of root yield under drought conditions as the primary measurement for drought tolerance. A list of agro-morphological descriptors used to phenotype cassava, their method, and the schedule of measurement (Fukuda *et al.,* 2010; Okogbenin *et al.,* 2013), as well as all phenotypic / morphological and productivity traits evaluated have been described in Chapters 3 and 4.

8.2.4 Data analysis

All phenotypic data were analysed with Genstat application, using analysis of variance procedures to determine variations among genotypes. Correlation analysis was used to determine traits strongly and significantly influencing drought tolerance. Principal component analysis was used to determine the traits that were the main contributors to drought tolerance and productivity traits.

8.3 Results

8.3.1 Weather conditions during the period of the experiment

The total rainfall recorded during the period of the experiment was 616 mm, with an average of 47.39 mm (Table 8.1). Monthly fluctuations in temperature and relative humidity were also recorded (Table 8.1). There was no rainfall recorded for a period of six months (November, December 2017 and January, February, April, and November 2018) from when the experiment

was established until harvest. The highest amounts of rainfall (111.90 mm and 329.00 mm) were recorded in the months of July and August (Table 8.1). Average minimum temperatures and maximum temperatures were 20.00°C and 34.10°C, respectively, with a mean temperature of 27.05°C during the experimental period, while the average minimum relative humidity and maximum relative humidity were 7.52% and 21.08%, with mean of 14.30%. The trend of rainfall and relative humidity distribution are shown in Fig. 8.1. The amount of relative humidity increased with the increase in rainfall. There was a consistent drought period (November 2017 to February 2018). A very limited amount of rainfall (1.20 mm) was observed in the month of March, but rain started falling from May. There was an increase in rainfall from May to August, while there was a decline in rainfall from September to November, when the experiment was harvested.

Table 8.1: Rainfall, temperature and relative humidity in Minjibir, Kano

		Temperature (°C)			Relative humidity (%)		
Months	Rainfall (mm)	Min	Max	Mean	Min	Max	Mean
NOV	0.00	15.50	30.40	22.95	7.40	14.60	11.00
DEC	0.00	17.30	29.80	23.55	7.40	11.70	9.55
JAN	0.00	13.20	26.20	19.70	7.40	10.90	9.15
FEB	0.00	17.50	35.60	26.55	7.10	11.80	9.45
MARCH	1.20	18.90	39.30	29.10	7.00	10.10	8.55
APRIL	0.00	24.90	40.80	32.85	7.20	10.70	8.95
MAY	29.10	25.90	37.60	31.75	7.60	25.90	16.75
JUNE	65.00	24.40	35.80	30.10	7.70	37.60	22.65
JULY	111.90	22.50	31.80	27.15	8.30	39.60	23.95
AUG	329.00	22.10	30.90	26.50	9.00	41.30	25.15
SEPT	72.30	22.50	33.10	27.80	7.60	28.00	17.80
OCT	7.50	20.90	36.40	28.65	7.10	21.50	14.30
NOV	0.00	14.40	35.60	25.00	7.00	10.30	8.65
Average	47.39	20.00	34.10	27.05	7.52	21.08	14.30
Total	616.00						

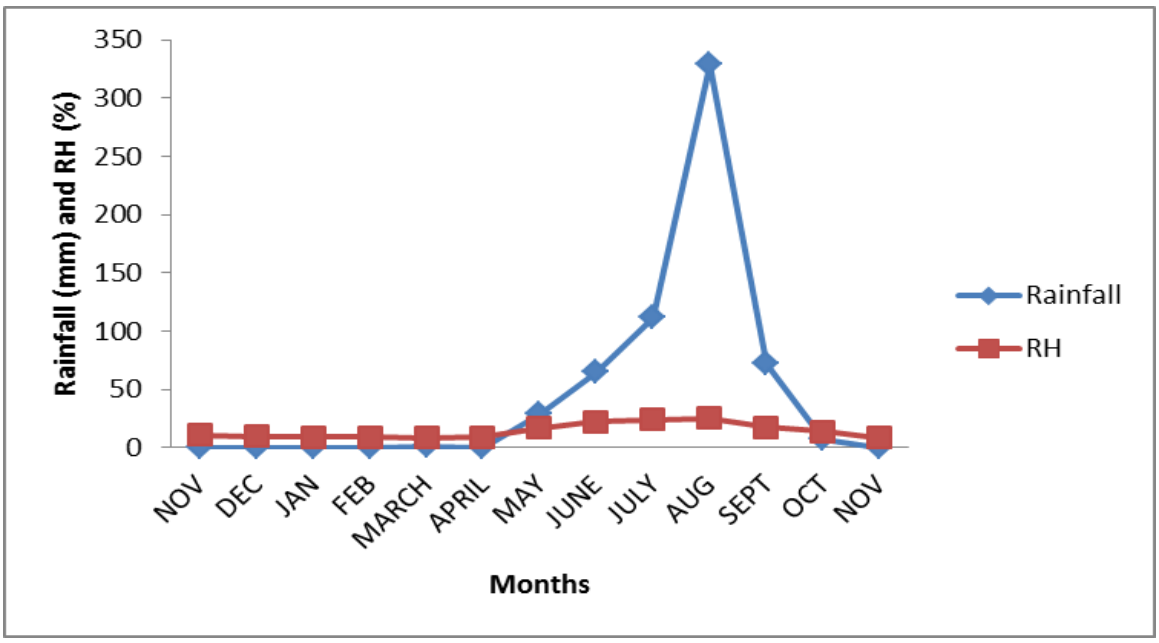


Figure 8.1: Rainfall and relative humidity distribution during the period of the experiment (November 2017 to November 2018)

8.3.2 Development of population C

8.3.2.1 OptiMAS selection (molecular breeding)

The molecular scores for each QTL and additional findings of interest are presented in Table 8.2. Ten superior genotypes, which will be used to produce the next generation, were elected using the OptiMAS selection. The selection was performed, based on the genetic values and utility criterion. Some genotypes (053B, 072B, 134B, 157B, and 166B) were considered as homozygous favourable for 3 QTL (No (+/+) = 3); homozygous unfavourable for only 1 QTL (No (-/-) = 1); no heterozygous QTL (No (+/-) = 0); and 5 QTL (No (?) = 5) were uncertain. Some of their molecular score (MS) at QTL positions were 1 in QTL1, QTL3, and QTL8; and 0 in QTL2, QTL4, QTL5, QTL6, QTL7, and QTL9. The rest of the genotypes (233B, 247B, 250B, 259B, and 264B) were homozygous favourable for 2 QTL (No (+/+) = 2); homozygous unfavourable for only 1 QTL (No (-/-) = 1); no heterozygous QTL (No (+/-) = 0); and 6 QTL (No (?) = 6) were uncertain. The MS at QTL positions for the genotype 233B were 1 in QTL1 and QTL8, while 0 in the rest of the QTLs. The MS at QTL positions of other genotypes (233B, 247B, 250B, 259B, and 264B) were 1 in QTL3 and QTL8, while 0 in QTL1, QTL2, QTL4, QTL5, QTL6, QTL7, and QTL9.

Table 8.2: Prediction of genetic values

Id	Ms	weight	UC	No(+/+)	No(-/-)	No(+/-)	No (?)	QTL1	QTL2	QTL3	QTL4	QTL5	QTL6	QTL7	QTL8	QTL9
053B	0.3333	0.3333	3	3	1	0	5	1	0	1	0	0	0	0	1	0
072B	0.3333	0.3333	3	3	1	0	5	1	0	1	0	0	0	0	1	0
134B	0.3333	0.3333	3	3	1	0	5	1	0	1	0	0	0	0	1	0
157B	0.3333	0.3333	3	3	1	0	5	1	0	1	0	0	0	0	1	0
166B	0.3333	0.3333	3	3	1	0	5	1	0	1	0	0	0	0	1	0
233B	0.2222	0.2222	2	2	1	0	6	1	0	0	0	0	0	0	1	0
247B	0.2222	0.2222	2	2	1	0	6	0	0	1	0	0	0	0	1	0
250B	0.2222	0.2222	2	2	1	0	6	0	0	1	0	0	0	0	1	0
259B	0.2222	0.2222	2	2	1	0	6	0	0	1	0	0	0	0	1	0
264B	0.2222	0.2222	2	2	1	0	6	0	0	1	0	0	0	0	1	0

*Id = genotypes; Ms = molecular score; UC = utility criterion; QTL = quantitative trait loci

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8.3.2.2 Selection index (classical breeding)

Ten genotypes were selected after harvest, using the selection index method (classical breeding) (Table 8.3). Fresh root yield for these genotypes ranged between 29.42 t/ha and 82.76 t/ha; dry-matter content ranged between 26.07% (216B) and 36.50% (196B); while dry root yield ranged between 7.98 t/ha (196B) and 27.09 t/ha (216B). Variables that were relevant and defined the objective of the study were considered in shaping the kind of genotypes that the selection process had to achieve.

Table 8.3: Genotypes selected using the selection index method (classical breeding)

Genotypes	FYLD	DMC	DYLD	SI
159B	82.76	34.99	23.77	100.89
170B	79.18	32.26	24.52	64.20
235B	56.18	30.29	18.54	61.03
128B	57.18	32.84	17.47	60.40
142B	66.06	35.50	18.46	59.61
015B	65.18	35.86	18.27	58.34
261B	46.72	32.56	14.32	53.84
196B	29.42	36.50	7.99	53.84
216B	71.18	26.07	27.09	53.19
250B	61.18	27.88	21.85	50.35

8.3.3 Population C genotypes (second population)

Different cross combinations were used to develop the second population. These included the optiMAS X selection index (OPT*SI), the selection index X selection index (SI*SI), optiMAS X optiMAS (OP*OP), and the selection index X optiMAS (SI*OP). A total of 277 seeds were developed from the crosses (Table 8.4). They were planted in a seedling nursery in the greenhouse before being transferred to the field at 6WAP. The results showed that a total number of 152 genotypes survived in the field (Table 8.4). These surviving genotypes were used to constitute the drought trial for proper field evaluation for drought tolerance.

Table 8.4: Population C genotypes

Female	Male	Cross type	No seeds	No surviving genotypes
053B	170B	OPT*SI	20	12
170B	166B	OPT*SI	10	4
235B	015B	SI*SI	10	3
170B	235B	SI*SI	15	6
159B	170B	SI*SI	20	6
235B	166B	SI*SI	20	6
261B	142B	SI*SI	8	2
170B	235B	SI*SI	7	2
015B	170B	SI*SI	6	2
235B	170B	SI*SI	70	55
264B	247B	OPT*OPT	18	14
166B	247B	OPT*OPT	19	11
247B	053B	OPT*OPT	11	7
261B	235B	SI*SI	8	2
015B	247B	SI*OPT	7	1
261B	159B	SI*SI	9	1
072B	053B	OPT*OPT	9	1
053B	264B	0PT*OPT	10	1
	Total		277	152

8.3.4 Analysis of variance

Analysis of variance indicated a highly significant ($P < 0.001$) genotype effect for all the traits measured, except for scar level, branch height, root number, root weight, length of stem with leaves, and cassava green mite (Table 8.5). This indicated the existence of genetic variability for selection and further improvement.

Table 8.5: Analysis of variance of mean squares for productivity and morphological traits in population C

Attributes	Genotype	Error	VR
SCARLEV	561.2	353.3	1.59ns
BIOM (kg)	13.60	4.95	2.75***
BRHT (kg)	303.0	709.4	0.99ns
DMC (%)	19.09	7.24	2.63***
DRY (t/h)	22.52	9.04	2.49***
FRY (t/h)	217.99	79.17	2.75***
HI	0.021	0.01	2.75***
NOLVES	11799	4361	2.71***
NOSCARS	7064	2689	2.63***
PLTHT (t/h)	787.7	380.8	2.07***
RTNO	71.78	43.67	1.64ns
RTWT (kg)	11.45	7.55	1.52ns
WTLV (cm)	279.80	228.00	1.23ns
CMD	0.52	0.28	1.84**
CBB	0.55	0.24	2.24***
CGM	0.33	0.25	1.32ns
VIG	0.90	0.28	3.20***
BRLEV	0.62	0.21	2.85***

*ns = not significant; ** = significant at P < 0.01; *** = significant at P < 0.001; SCARLEV = scar level; BIOM = total plant biomass; BRHT = branch height; DMC = dry-matter content; FRY = fresh root yield; DRY = dry root yield; HI = harvest index; NOLVES = number of leaves; NISCARS = number of scars; PLTHT = plant height; RTNO = root number; RTWT = root weight; WTLV = length of stem with leaves; CMD = cassava mosaic disease; CBB = cassava bacterial disease; CGM = cassava green mite; VIG = plant vigour; BRLEV = branching level

8.3.5 Relationship between traits in the second population (population C)

Fresh root yield was positively correlated with yield-related traits and morphological traits, but negatively correlated with CMD (Fig. 8.2). Fresh root yield had a very high positive correlation with dry root yield. It was also found to be positively correlated with other yield-related traits such as root number, root weight, plant biomass, and harvest index. Fresh root yield had a high positive correlation with morphological traits such as number of scars, and number of leaves

(Fig. 8.2). Other morphological traits that positively correlated with fresh root yield included plant height, length of leafy part of stem, length of leafless part of stem, leaf retention, level of branches, and scar level. Root number, root weight, plant biomass, and dry root yield were also found to be positively correlated with morphological traits such as plant height, length of stem with leaves, length of leafless part of stem, number of scars, number of leaves, plant vigour, leaf retention, branching level, and scar level. The strongest correlation was found between root weight and plant height, root weight and length of stem with leaf, root weight and number of scars, root weight and number of leaves, root weight and plant vigour, total plant biomass and plant height, total plant biomass and number of scars, total plant biomass and number of leaves, total plant biomass and vigour, dry root yield and number of scars, dry root yield and number of leaves, and dry root yield and vigour (Fig. 8.2). All morphological traits positively correlated with each other, except for the correlations between leaf retention and length of leafless part of stem, scar level and branch height, which were negatively correlated. CMD was found to be negatively correlated with all the morphological traits.

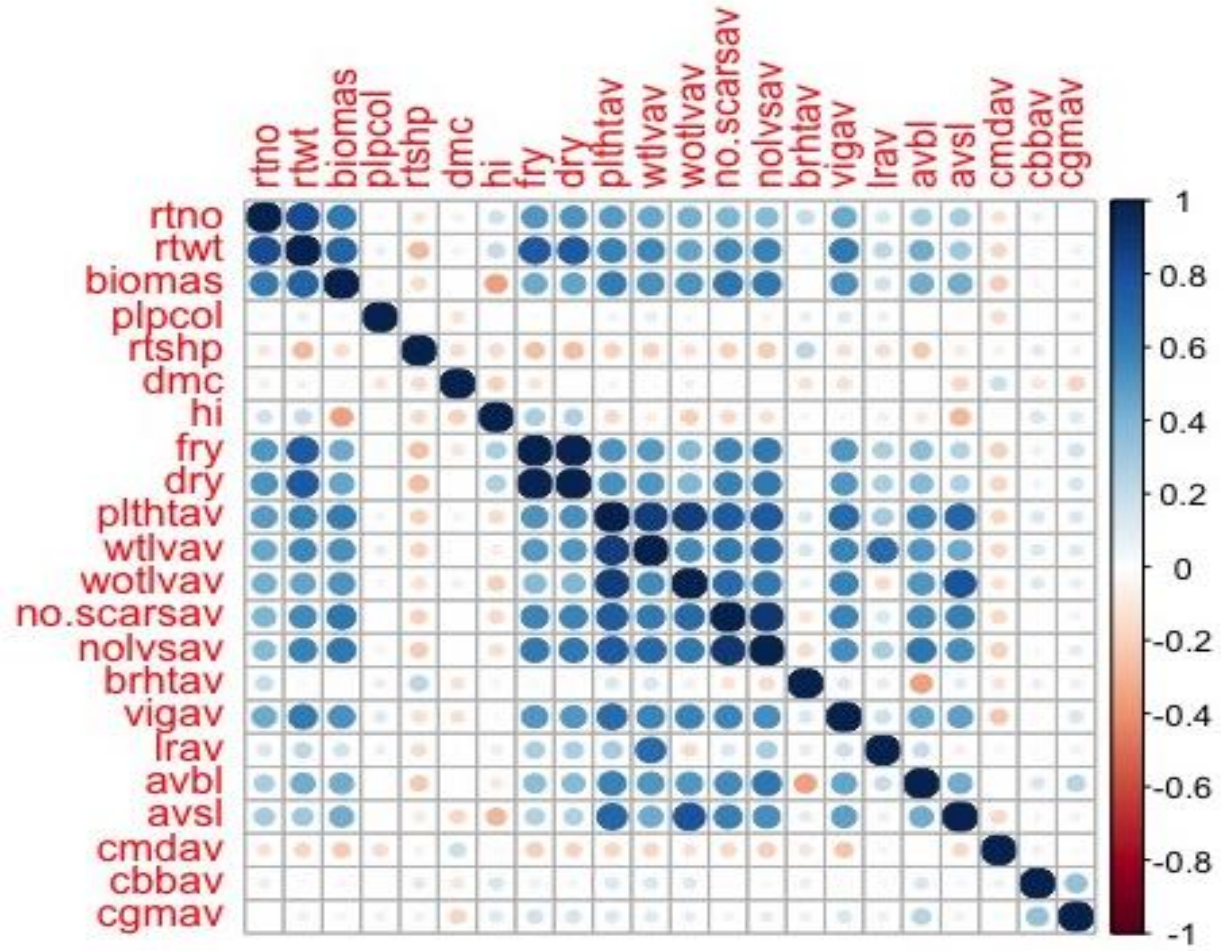

Figure 8.2: Correlation coefficient of yield and morphological traits in second population

*rtno = root number; rtwt = root weight; plpcol = pulp colour; rtshp = root shape; dmc = dry-matter content; hi = harvest index; fry = fresh root yield; dry = dry root yield; plthtav = plant height; wtlvav = part of stem with leaf; wotlvav = leafless part of stem; noscarsav = number of scars; nolvsav = number of leaves; brhtav = height at first branching; vigav = plant vigour; lrav = leaf retention; avbl = level of branching; avsl = scar level; cmdav = cassava mosaic disease; cbbav = cassava bacterial blight; cgmav = cassava green mite

8.3.6 Principal component analysis

Principle component analysis, using productivity and morphological traits, indicated that the first six PCs (PC1 to PC6) cumulatively accounted for 72% of the total variation (Table 8.6). Traits with Eigen values greater than one were considered as contributing significantly to the variation among genotypes. The first PC with an Eigen value of 2.82 accounted for 36% of the variation. The traits that contributed most to the variation were root weight, total plant biomass, fresh root

yield, dry root yield, plant height, length part of stem with leaf, length of leafless part of stem, number of scars, number of leaves, and plant vigour. The second PC (PC2) contributed 10% of total variation, and had harvest index, fresh root yield, dry root yield, and scar level as the main contributors. The third PC (PC3), with 8% of total variation, was associated with dry-matter content, and branch height. The fourth PC contributed 7% of the total variation, and was associated with root number, branch height, and branching level. The fifth and sixth PCs contributed 6% and 5% to the total variations, respectively, and were associated with length of stem with leaves, leaf retention, root number, the dry-matter content, and branching level. Some traits were found in more than one PC. Root number was found in PC4 and PC6; fresh root yield and dry root yield were found in PCs 1 and 2; dry-matter content was found in PC3 and PC6; while branching height was found in PCs 3, 4, and 6.

Table 8.6: Principal component analysis in second population (popC)

Traits	PC1	PC2	PC3	PC4	PC5	PC6
RTNO	-0.23	0.18	-0.10	-0.23	0.13	-0.31
RTWT (kg)	-0.29	0.24	0.05	-0.17	0.14	-0.10
BIOM (kg)	-0.27	-0.12	0.01	-0.18	-0.02	-0.08
DMC (%)	0.03	-0.11	0.42	-0.09	-0.19	-0.38
HI	0.02	0.53	0.00	0.07	0.24	0.04
FRY (t/h)	-0.27	0.33	0.07	-0.05	0.15	0.03
DRY (t/h)	-0.27	0.31	0.11	-0.06	0.14	-0.01
PLTHT (t/h)	-0.32	-0.14	-0.09	0.08	-0.13	-0.07
WTLF (cm)	-0.29	0.03	-0.02	0.07	-0.44	-0.04
WOTLV (cm)	-0.27	-0.28	-0.14	0.06	0.19	-0.09
NOSCARS	-0.30	-0.15	0.10	0.02	0.07	0.06
NOLVS	-0.30	-0.10	0.14	0.08	-0.03	0.08
BRAHT (cm)	-0.01	0.11	-0.51	-0.24	-0.22	-0.36
VIG	-0.27	-0.01	-0.12	-0.06	0.01	0.08
LVRET	-0.11	0.25	0.11	0.06	-0.70	0.05
BLEV	-0.23	-0.12	0.18	0.36	0.03	0.15
SCALEV (cm)	-0.23	-0.34	-0.17	-0.01	0.13	0.01
Eigen value	2.82	1.47	1.32	1.23	1.16	1.06
Proportion of variance	0.36	0.10	0.08	0.07	0.06	0.05
Cumulative proportion (%)	0.36	0.46	0.54	0.61	0.67	0.72

*RTNO = root number; RTWT = root weight; BIOM = total plant biomass; DMC = dry-matter content; PLTHT = plant height; WTLF = length of stem with leaf; WOTLV = length of leafless stem; NOSCARS = number of scars; NOLVS = number of leaves; BRHT = branch height; VIG = plant vigour; LVRET = leaf retention; BLEV = branching level; SCALEV = scar level

8.3.7 Descriptive statistics of productivity and morphological traits in population C

Results of the phenotypic statistics, including mean, skewness, minimum and maximum are shown in Table 8.7. Skewness values showed that most of the traits were positively skewed, except for plant vigour and cassava green mite, which were negatively skewed. Height at first branching, total plant biomass, scar level, CMD, cassava green mite, and leaf retention were strongly skewed towards the high values, while other traits were moderately skewed. Low standard deviation was recorded in all the morphological and productivity traits measured in the second population. In other words, the value of the standard deviation was lower than the value of the mean of the traits. The data range for each trait measured revealed a wide variation in the second population. The mean values of the parents and progenies in the population revealed that the parents performed better in most of the traits, except for harvest index, branching level, and CMD (Table 8.8). Table 8.9 shows the trait performance of populations B and C. Results indicated that the first population performed better in traits such as plant vigour, root weight, fresh root yield, harvest index, dry root yield, height at first branching, scar level, plant height, height of leafless stem, and branching level, while the second population performed better in the other evaluated traits.

Table 8.7: Simple statistics of productivity and morphological traits in second population with checks

Traits	Min	Max	Mean	SD	SKEW
RTNO	2	44	15.7	8.18	0.31
RTWT (kg)	0.10	15.20	4.66	3.27	0.83
BIOM (kg)	0.10	20.00	4.60	3.48	1.58
DMC (%)	23.12	51.48	33.15	4.22	0.89
HI	0.07	1.00	0.50	0.14	0.11
FRY (t/h)	0.67	68.00	20.25	13.80	0.93
DRY (t/h)	0.26	23.30	6.67	4.48	0.88
PLTHT (t/h)	32.00	167.10	97.27	26.96	0.02
WTLV (cm)	8.20	94.10	41.43	15.64	0.35
WOTLV (cm)	12.00	98.30	56.08	16.18	0.14
NOSCARS	17.00	392.30	141.97	80.70	0.92
NOLVS	4.40	562.30	189.58	106.38	0.85
BRANCHT (cm)	13.10	140.00	34.57	18.36	2.67
VIG	1.00	5.00	3.24	0.82	-0.21
BLEV	0.50	4.00	1.88	0.68	0.33
SCALEV (cm)	10.00	189.75	66.79	21.96	1.28
CMD	1.00	3.00	1.46	0.73	1.21
CBB	1.00	5.00	2.33	0.71	0.73
CGM	1.00	4.00	2.62	0.59	-1.03
LVRET	19.84	93.75	45.15	10.39	1.10

Table 8.8: Mean values of parents and progenies in population C

	Mean		
Traits	progenies	parents	%diff
RTNO	14.75	18.18	10.42
RTWT (kg)	4.18	5.91	17.15
BIOM (kg)	4.27	5.55	13.03
DMC (%)	32.98	33.66	1.020
HI	0.51	0.5	-0.99
FRY (t/h)	19.78	21.45	4.05
DRY (t/h)	6.45	7.25	5.84
PLTHT (cm)	93.83	107.56	6.82
WTLV (cm)	39.67	46.64	8.08
WOTLV (cm)	54.34	61.3	6.02
NOSCARS	141.43	145.28	1.34
NOLVS	188.2	195.24	1.84
BRANCHT (cm)	33.19	36.01	4.08
VIG	3.15	3.4	3.82
BLEV	1.88	1.76	-3.3
SCALEV	64.82	72.83	5.82
CMD	1.49	1.39	-3.47
CBB	2.39	3.67	21.12
CGM	2.62	2.62	0
LVRET	44.96	45.71	0.83

Table 8.9: Mean values of traits in population B and population C with checks

	PopB	PopC	
Traits	Mean	MEAN	% diff.
VIG	3.27	3.24	-0.46
RTWT (kg)	5.18	4.66	-5.28
BIOM (kg)	4.43	4.6	1.88
RTNO	12.36	15.7	11.90
FRY (t/h)	23.67	20.25	-7.79
HI	0.53	0.5	-2.91
DMC (%)	31.93	33.15	1.87
DYLD (t/h)	7.67	6.67	-6.97
SCARNO	106.41	141.97	14.32
NLVS	47.92	189.58	59.65
HFB (cm)	47.27	34.57	-15.52
SCARLEV	122.56	66.79	-29.45
LVRET	20.79	45.15	36.94
PLTHT (cm)	154.01	97.27	-22.58
HLS (cm)	141.23	56.08	-43.16
WTLV (cm)	9.85	41.43	61.58
BLEV	3.14	1.88	-25.10

8.3.8 Selection of best performing genotypes in population C

The best performing genotypes in the population C were selected, using the selection index method (Table 8.10). Different variables were measured in different units. To avoid variables measured in higher magnitude as a result of higher weight, all the variables were standardised. Based on these calculations and the ranking, 20 superior performing genotypes were selected from this population. The genotypes and traits considered for selection are shown in Table 10. The range of value for DMC for the selected genotypes was between 28.36% and 35.02%; FRY was between 25.67 and 68.00 t/ha; DRY between 8.51 and 23.30 t/ha. Most of the genotypes stemmed from a cross between the selection index's female parent and male parent, followed by optiMAS female parent and optiMAS male parent. The reason for this may be that more seeds were generated from SI*SI compared to OP*OP.

Table 8.10: Best performing genotypes in population C

s/no	clone	female	male	crosstype	DMC	HI	FRY	DRY	VIG	CMD	SI
1	327C1	159B	170B	SI*SI	34.27	0.63	68.00	23.30	3.00	1.00	82.08
2	386C1	235B	170B	SI*SI	28.96	0.59	64.00	18.54	4.00	1.00	63.31
3	392C1	235B	170B	SI*SI	33.16	0.48	47.33	15.69	5.00	1.00	56.81
4	383C1	235B	170B	SI*SI	32.16	0.47	50.67	16.29	4.50	1.00	53.64
5	361C1	235B	170B	SI*SI	32.75	0.69	45.00	14.74	3.50	1.00	52.32
6	411C1	264B	247B	OP*OP	28.36	0.53	50.00	14.18	5.00	1.00	47.70
7	349C1	235B	170B	SI*SI	32.52	0.57	52.00	16.91	2.67	1.00	47.12
8	409C1	264B	247B	OP*OP	34.17	0.49	40.00	13.67	4.00	1.00	42.06
9	359C1	235B	170B	SI*SI	32.43	0.71	36.00	11.67	3.75	1.00	41.70
10	315C1	170B	166B	SI*OP	34.68	0.64	36.00	12.48	3.25	1.00	40.76
11	371C1	235B	170B	SI*SI	33.77	0.41	43.00	14.52	4.00	1.00	40.30
12	357C1	235B	170B	SI*SI	30.84	0.70	39.00	12.03	3.50	1.00	38.39
13	338C1	261B	142B	SI*SI	31.16	0.51	50.00	15.58	4.00	2.00	38.37
14	368C1	235B	170B	SI*SI	35.02	0.57	30.67	10.74	3.83	1.00	34.55
15	380C1	235B	170B	SI*SI	34.47	0.62	42.50	14.65	3.75	3.00	33.77
16	421C1	166B	247B	OP*OP	30.29	0.45	40.50	12.27	4.50	1.00	32.49
17	320C1	170B	235B	SI*SI	31.05	0.67	42.00	13.04	2.33	1.00	31.86
18	345C1	235B	170B	SI*SI	33.14	0.72	25.67	8.51	3.83	1.00	30.91
19	341C1	015B	170B	SI*SI	33.38	0.46	35.00	11.68	4.75	2.00	27.75
20	419C1	166B	247B	OP*OP	33.67	0.59	36.67	12.35	4.50	3.00	27.16

*SI*SI = selection index*selection index; OP*OP = optiMAS*optiMas; SI*OP = selection index*optiMas; DMC = dry-matter content (%); HI = harvest index; FRY = fresh root yield (t/ha); DRY = dry root yield (t/ha); VIG = plant vigour; CMD = cassava mosaic disease

8.4 Discussion

Drought tolerance is an important research subject pertaining to cassava, as climate change has raised concerns about global drought problems, and places significant demands on crop breeding programmes (Aina *et al.,* 2007; De Oliveria *et al.,* 2017). The weather conditions described in Table 8.1 and Fig. 8.1 in Minjibir, Kano make it appropriate for field-based drought experiments. In addition to the weather conditions present in the experimental site, the 6-month total drought period, intermixed between the long and short rainfall seasons, further supports use of the site to conduct drought experiments.

In seasonal dry and semi-arid environments with less than 700 mm of annual rainfall, improved cassava can achieve dry root yields of three t/ha (El-Sharkawy, 2006). Therefore, cassava is often considered an assurance and major food security crop for resource-poor smallholder farmers living off marginal lands (Kamukondiwa, 1996). Cassava grows best in areas with a temperature range of between 24°C and 30°C (IITA, 1990), but it can tolerate temperature ranges of between 16°C and 38°C (Cock, 1984; Alves, 2002), which is in line with the temperature range of the present study, where the mean minimum temperature over a year was 20°C, while the mean maximum temperature was 34.1°C.

Most of the traits evaluated in this study significantly ($P < 0.001$) varied within genotypes. This suggested that the evaluated cassava genotypes had adequate genetic variability. El-sharkawy (2007) had subjected series of cassava genotypes to water stress, and found that some genotypes were tolerant to drought, while others were susceptible. Variability among genotypes also indicated that selection of desirable characters among these genotypes will lead to significant progress in cassava breeding schemes.

Studies on correlation enable breeders to know the mutual interrelationships between various characters, on which selection can be used for genetic improvement. In this study, the correlation coefficient (r) was used to study the relationship between cassava traits (Kawano, 1990). Fresh root yield was positively correlated with other yield traits, showing interdependency (Ojulong, 2008). Dry-matter content was not positively correlated with fresh root yield, indicating that dry-matter content is not an important indicator of storage root yield in cassava (Ntawuruhunga *et al.,* 2010). Fresh root yield was found to have a strong positive correlation with root weight, dry root yield, number of scars, and number of leaves. This means that any increase in these traits will lead to an increase in yield. Ntawuruhunga *et al.* (2001) observed similar results. They reported that average root weight and average number of roots per plant had a strong and positive effect on cassava root yield. Shedding of leaves in cassava invariably increases its number of leaf scars. Cassava adjusts to water stress by reducing its leaf canopy (Connor *et al.,* 1981; El-sharkawy and Cock, 1987) to reduce evapotranspiration. Hence, leaf shedding is an effective adaptation mechanism to moisture stress (Okogbenin *et al.,* 2013). Cassava reduces water loss through closing its stomata (Setter and Fregene, 2007), and decreasing leaves through leaf shedding (Alves and Setter, 2000; Burns *et al.,* 2010). Cassava is quick to resume its growth when conditions are again more favourable. Such rapid recovery in leaf growth has been reported by many researchers (Connor *et al.,* 1981; Baker *et al.,* 1989; El-Sharkawy, 1993), when new leaf growth increases light interception and canopy photosynthesis,

thus compensating for previous losses in biomass, particularly root yield. This could be an explanation for the positive correlation between fresh root yield, number of scars, and number of leaves. Leaf retention was poorly correlated with yield, and root weight. This may be the result of the genotypes' tendency to retain at least a small number of leaves near the shoot apex at the end of the experiment, thus providing enough photosynthetic productivity. The positive correlation between leaf retention and yield agrees with the findings by Lenis *et al.* (2006) who reported that increased longevity or improved leaf retention is a possible means to increase cassava productivity. These traits can be used as an indirect selection for yield in a dry ecology. Other yield-related traits such as root weight, plant biomass, and dry root yield were also found to be positively correlated with some morphological traits, signifying that an increase in these morphological traits leads to an increase in the yield-related traits. Thus, when considering the selection for root weight, plant biomass, and dry root yield in a dry ecology, these morphological traits should be considered. CMD negatively correlated with fresh root yield, yield-related traits, and most of the morphological traits. This indicated that an increase in CMD reduced yield, yield components, and most of the morphological traits. CMD has been regarded as a threat in cassava production, as it causes a reduction in yield. Generally, diseases are known to reduce storage root yield in cassava (Hahn *et al.,* 1979). Earlier studies on CMD (Egesi *et al.,* 2006) have associated it with a significant yield reduction due to the reduction in leaf area, and hence light interception.

Principal component analysis was used to explain the contributions of various traits to the genotype's performance. Breeders can combine all the identified traits, as revealed by the PCA analysis, in the selection for drought-tolerant cassava genotypes.

The better performance of the parents in some of the traits showed the superiority of the selected genotypes that were used to develop the progeny, while better performance of progeny than their parents in some traits showed the additive gene effect on the progeny from their parents. The mean value of some traits were higher in the population C when compared to population B, which signifies that cassava is highly heterozygous, and there was segregation among the progeny. This also signified the additive gene effect, whereby genes were transferred from the parent of these genotypes to their progeny. The results showed that the parents of these genotypes from the first population were able to transfer genes of desirable traits to their offspring. These genotypes can be used further in gene introgression to breed for a more drought-tolerant cassava genotype. The selected better-performing genotypes from population C will be incorporated into the NRCRI cassava breeding scheme.

One of the most important questions to be answered in plant breeding relates to the parents to be used in the generation of new germplasm. Cassava is highly heterozygous in nature, and therefore, it takes a lot of time to evaluate a segregating population with large variation generated from each cross. The population C used in this study was developed from selected parents, using a classical breeding method (selection index) and a molecular breeding method (optiMAS selection). Breeding a new variety of cassava usually takes 10 years, due to its long growth cycle (12 to 18 months). In using the selection index, relevant variables were considered with weightings assigned to each of the variables. The higher the weighting, the more important the variable. The selection index in this study was used to select the 10 best genotypes among 260 genotypes evaluated. The selection index was used to sort the genotypes from best to worst. It had been used in several breeding processes such as in the seedling evaluation stage, the clonal evaluation stage, the preliminary yield trial stage, the advanced yield trial phase, and the uniform yield trial phase to select the best-performing genotypes among the many genotypes that were evaluated. It had been used to select cassava genotypes with high yield, high dry-matter content, resistant to pest, and tolerant to drought (Kawano, 2003; Jennings and Iglesias, 2002; Egesi *et al.,* 2006; Mcsween *et al.,* 2006; Ewa *et al.,* unpublished data). Information derived from the selection index supplies the general performance of each genotype and can also be useful for identifying parents that tend to produce superior performing progenies. The selection of genotypes using optiMas was based on molecular scores, weighted (MS), or the utility criterion. Genotypes were selected regarding their molecular scores for the QTLs. Molecular score 1 of the selected genotypes at various QTL levels showed that the individuals were homozygous for the favourable alleles, and they were the ideal genotypes. Uncertainty could be the result of missing data. A value of 0.75 was selected for the probability threshold to be considered as homozygous (un) favourable or heterozygous at the QTL position. The favourable and unfavourable alleles located in some QTL position signified that the high level of segregation among the genotypes in the crosses made, which is common in cassava. With the increasing use of markers in breeding programmes, it is important to develop decision support tools to help breeders in implementing their marker-assisted selection (MAS) (Valente *et al.,* 2013). OptiMAS has been developed for the possibility to consider multi-allelic context, which opens new prospects to further accelerate genetic gain by assembling favourable alleles issued from diverse parents (Valente *et al.,* 2013). The marker-assisted selection helped to increase the precision of the selection, leading to more rapid genetic gain, fewer cycles of phenotypic evaluation, and thus enabling the reduction of the time needed for varietal development.

8.5 Conclusion

In conclusion, most of the traits significantly varied within genotypes suggesting that the evaluated cassava genotypes had adequate genetic variability. PC1 and PC2 identified fresh root yield, root weight, plant biomass, dry root yield, plant height, number of scars, number of leaves, plant vigour, harvest index, scar level, length of leafless stem and length of stem with leaves as traits driving drought tolerance. There was a better performance in traits such as plant biomass, root number, dry matter content, number of scars, number of leaves and leaf retention in the second population (POP C) than in the first population (POP B) signifying additive gene effect, whereby genes were transferred from the parent of these genotypes to their progeny. These genotypes can be used further in gene introgression to breed for a more drought-tolerant cassava genotype. Twenty promising genotypes were selected using selection index. The selected genotypes stemmed from crosses between parents selected from classical breeding, and parents from a marker-based selection. The MARs-bred superior genotypes from this experiment will be subjected to NRCRI uniform yield trials (UYT). The superior genotypes (from the mapping population C) selected from the NRCRI UYT, will then be advanced for the release of the genotypes as varieties in Nigeria. The novel germplasm will be available for sharing with other national programmes.

CHAPTER 9

GENERAL CONCLUSIONS AND RECOMMENDATIONS

This study addressed all the objectives of the study. It summarises the findings generated from from the study as well as makes recommendations for possible future research work.

Objective 1: Identify traits driving the physiological basis of drought tolerance in F_1 cassava genotypes.

Hypothesis: There are no traits significantly associated with drought tolerance in the F_1 cassava genotypes.

Conclusion: In this study, there were positive correlations between physiological, morphological, and other yield-related traits with fresh root yield. This meant that all the traits were important for an economic yield of cassava in a drought environment. Principal component analysis identified traits such as scar level, number of scars, height at first branching, root weight, fresh root yield, root number, and dry-matter content as traits driving drought tolerance in F_1 cassava genotypes. Therefore, the hypothesis in this study is rejected.

Recommendation: It is recommended to combine the important traits as revealed by the principal component analysis, as this can be used in the early selection stage by breeders to select drought-tolerant cassava genotypes, and enhance the yield and productivity in marginal environments.

Objective 2: To identify traits linked with early bulking in the F_1 population.

Hypothesis: There are no traits associated with early bulking in cassava.

Conclusion: The study identified important traits such as root weight, root number, plant biomass, fresh root yield, root number, plant biomass, dry root yield, dry-matter content, plant height, harvest index, and stem diameter as traits linked with early root bulking in cassava. Therefore, the hypothesis in this study is rejected.

Recommendation: The early-bulking genotypes identified in this study should be evaluated further in more locations to test them for yield stability before being released to farmers.

Objective 3: To identify, estimate effects, and validate quantitative traits loci (QTLs) for drought tolerance and early bulking in the F_1 cassava genotypes.

Hypothesis: The QTLs linked to drought and early-bulking traits cannot be mapped.

Conclusions: The study identified and mapped 27 and 30 QTLs in first and second year, respectively for morphological/physiological and productivity traits related to drought tolerance. Results also showed that 28 QTLs were mapped for early root bulking in both years. Therefore, the hypothesis in this study is rejected.

Recommendation: The future of molecular breeding in Africa will be driven by highly informative QTLs, which will result in the accumulation of desirable alleles through successive recombination activities for the development of superior genotypes with good genetic gain for improved productivity for drought and early root bulking in cassava.

More QTL studies should be initiated to support identification of more QTL for more environments and capture yet unidentified QTLs and other interactive effects with the aid of decision software tools. This will support a recurrent selection combining several sets of QTLs (haplotypes) per trait in a marker-assisted recurrent selection as opposed to the few in MAS.

Objective 4: Estimate genetic improvement for drought tolerance in the F_1 population.

Hypothesis: Genetic improvement for drought tolerance cannot be estimated in an advanced population.

Conclusion: Twenty promising genotypes were selected from the second population (population C). These selected genotypes stemmed from crosses between parents selected from classical breeding, and parents from a marker-based selection, all evolving from the first population (population B). Therefore, the hypothesis from this study is rejected.

Recommendation: The selected superior genotypes will be subjected to an NRCRI uniform yield trial (UYT). Selected genotypes from the NRCRI UYT can be advanced for varietal release in Nigeria. The novel genotypes will be made available for sharing with other national and international breeding platforms.

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Appendix

Field preparation/planting/ data collection in pictures

Full irrigation of the marked out field before planting in Minjibir, Kano

Sign post for proper identification of the experimental trial in Minjibir, Kano

Sorting of genotype into plots in Minjibir, Kano

Placement of metal label for proper identification in Minjibir, Kano

Close view of planting procedures in Minjibir, Kano

Full irrigation of the field after planting in Minjibir, Kano

Data collection in Minjibir, Kano

Data collection with my supervisors in Minjibir, Kano

Yield (421C1) at 12 months after planting (MAP)

Yield (320 C1) at 12 months after planting