

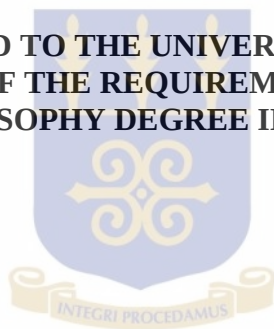
**GENETIC ANALYSIS OF POSTHARVEST PHYSIOLOGICAL DETERIORATION IN
CASSAVA (*Manihot esculenta* Crantz) STORAGE ROOTS**

BY

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**THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN
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SCHOOL OF AGRICULTURE
COLLEGE OF AGRICULTURE AND CONSUMER SCIENCES
UNIVERSITY OF GHANA
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DECEMBER, 2013

DECLARATION

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

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ABSTRACT

Postharvest physiological deterioration (PPD) is a serious abiotic stress in cassava that renders the roots unmarketable, thereby reducing the economic value of the crop. This study was undertaken to determine farmers' perception of PPD and identify cassava genotypes with delayed PPD that can be used for better shelf life improvement of cassava. A Participatory Rural Appraisal was conducted among 490 respondents that comprised farmers, processors, marketers and consumers in 12 communities in Ghana using Focus Group Discussion and semi-structured interview. Subsequently, 150 cassava accessions were assembled and assessed for genetic variability for PPD and other economic traits. Genetic diversity was assessed using morphological and molecular markers. A total of 19 qualitative and 195 SNP markers were used for the diversity studies. The Mantel test was used to assess the relationship between morphological traits and SNP markers. The 150 cassava accessions were evaluated in an Alpha lattice design to identify genotypes with delayed PPD using Booth's method. Forty genotypes exhibiting comparatively low deterioration, high yield and dry matter were selected and evaluated in three locations for two years to assess genotype by environment interaction effect (GxE) on the expression of PPD. Genetic parameters including genotypic and phenotypic coefficient of variation and broad sense heritability were estimated using variance components to determine the feasibility of improving PPD through selection. Five accessions were selected and used for physicochemical and functional analysis of cassava day zero and seven after harvest to assess physiological and chemical changes during storage. The PRA revealed postharvest losses as one of the major production constraints. Genetic diversity estimated showed a moderate to high diversity among the 150 genotypes. Ninety six per cent of the SNP markers were

polymorphic with a mean PIC value of 0.297 suggesting that these markers could be used for diversity assessment in cassava. Using the Jaccard's and Nei's similarity matrices, a higher mean similarity coefficient was obtained for the morphological traits (0.54) than was observed for the SNP markers (0.42). Dendrograms generated from both morphological and molecular analyses clustered the genotypes into five and three clusters respectively although there were disagreements between them. The correlation coefficient based on the Mantel test though significant was low ($r=0.38$, $p=0.001$). Significant variability also existed among accessions for PPD suggesting there were genotypic differences among accessions which could be used for PPD improvement. Genotypes K25, SW/00/064 and DMA00/031 with PPD scores of 9.1%, 9.6% and 9.9% respectively were identified, however, no accession with complete tolerance or resistance to PPD was identified. Genotype by environment analysis indicated the significance of genotype, location and year effects on all the traits studied. Genotype was more important for dry matter content than the other main effects while location and year effects were more important for fresh root yield and postharvest physiological deterioration respectively. The GGE biplot identified genotypes for specific locations for all the traits. Genetic variability components estimated revealed higher PCV than their corresponding GCV for all the traits studied. Broad sense heritability estimate was low for PPD ($>20\%$) indicating that this trait is not under genetic control.

DEDICATION

This piece of work is dedicated to my husband Frank, my children Nana Kweku and NanaYaa, my parents Mr. and Mrs. Thompson and my siblings for their prayers and support throughout the course of my studies.



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LIST OF ABBREVIATIONS

AMMI	Additive Main Effect and Multiplicative Interaction
AOX	Alternative Oxidase
CIAT	International Center For Tropical Agriculture
COSCA	Collaborative Study of Cassava in Africa
CRI	Crops Research Institute
DMC	Dry Matter Content
DNA	DeoxyriboNucleic Acid
EST	Expressed Sequence Tag
FAO	Food and Agriculture Organization of United Nations
FOASTAT	Food and Agriculture Organization of United Nations, Statistics Department
FGD	Focus Group Discussion
GCP	Generation Challenge Program
GCV	Genotypic Coefficient of Variation
GEI	Genotype by Environment Interaction
GNA	Ghana News Agency
H ²	Broad sense Heritability
HCN	Hydrogen Cyanide
HR	Hypersensitive Response
IITA	International Institute Of Tropical Agriculture
KASPar	Competitive Allele Specific PCR
MAS	Marker Assisted Selection

MOFA	Ministry of Food and Agriculture
NRI	Natural Resources Institute
PCV	Phenotypic Coefficient of Variation
PGRRI	Plant Genetic Resources Research Institute
PIC	Polymorphic Information Content
PPD	Postharvest Physiological Deterioration
PCR	Polymerase Chain Reaction
PRA	Participatory Rural Appraisal
QTL	Quantitative Trait Loci
RH	Relative Humidity
ROS	Reactive Oxygen Species
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeats
TMS	Tropical Manihot Species
UPGMA	Unweighted Group Method using Arithmetic Average
UV	Ultraviolet

CHAPTER ONE

1.0 GENERAL INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is currently the sixth most important food crop (FAO, 2008) in the world. It is the third most important source of carbohydrates in Africa (Owolade *et al.*, 2006), the most important food crop in Nigeria and the second most important crop in Ghana (Adjei-Nsiah, 2010). It serves as food for over 800 million people worldwide (Nassar *et al.*, 2002), providing about 500 calories daily for more than 70 million people (Chavez *et al.*, 2005). It is estimated that 250 million people in sub-Saharan Africa (SSA) derive half of their daily calories from cassava (FAO, 2008). Cassava has a high efficiency in carbohydrate production, as well as being tolerant to drought and thrives well on even poor soils making it an attractive crop especially to small scale farmers. It gives carbohydrate production per hectare about 40% higher than rice and 25% more than maize (Agwu and Anyaeche, 2007). The roots are used for animal feed, industrial starch production and income generation for many small-scale farmers (Kawano, 2003). The roots and leaves are available all year round (Ntawuruhunga *et al.*, 2006), thus cassava is an important food security crop, especially in drought-prone areas (Chavez *et al.*, 2005).

Ghana is the third largest producer of cassava in Africa (FAO, 2006). It is the most highly produced root crop and the cheapest starch staple of the Ghanaian consumer diet with per capita consumption averaging 159.2 kilograms per year (MoFA, 2009). Cassava occupies a key position in Ghana's agricultural economy contributing 22% of the agricultural gross domestic product (MoFA, 2011). It is an important crop in terms of area occupied and total production. It is produced by over 70% of Ghanaian farmers and consumed by more than 80% of the

population (Parkes *et al.*, 2012), indicating its importance in food security. The status of cassava has changed from a traditional food crop to an important industrial crop (Nweke *et al.*, 2002; Dixon and Ssemakula, 2008). Cassava has been identified as an important crop in Ghana that could generate desired economic growth and alleviate poverty (Dapaah, 1991; 1996; Nweke, 2004). There is also a continuous growing importance of cassava in Ghana for industrial use (Dapaah, 1991; Manu-Aduening *et al.*, 2006).

Local demand for cassava in Ghana increased 6.3% per year between 2005 and 2009 (FAOSTAT, 2010). This demand is expected to continue to grow strongly due to its wide ranging applications. In addition to being a key staple of the Ghanaian diet, it is also used by the breweries and other industries. This, therefore, necessitates that, demands for cassava for both human consumption and industries are met in order to promote economic growth in Ghana. However, several factors affect the ability of cassava to achieve the increasing demand. Notably of these factors is the short shelf life of cassava termed postharvest physiological deterioration (PPD).

PPD is an oxidative reaction that starts immediately after harvesting when the root is detached from the mother plant (Beeching *et al.*, 1994; Reilly *et al.*, 2001). It resembles typical changes associated with the plant's response to wounding. PPD starts from the central vascular bundles of the root, spreads to the adjacent storage parenchyma and subsequently stored starch undergoes structural changes (Plumbley and Rickard, 1991). The roots deteriorate 24-48 hours after harvest and subsequently the roots change colour. Visible signs of PPD are vascular streaking with blue or black discoloration rendering the roots unpalatable and unmarketable (Beeching *et al.*, 1994). PPD is physiological in nature and not caused by microbes. Despite the efforts of breeders in

improving the crop through the vibrant cassava breeding program in Ghana, cassava is still challenged by the problem of PPD.

Losses due to PPD have a huge impact on the potential of the crop for processing and export. As a result, cassava is primarily used in staple foods such as ‘fufu’ (boiled and pounded into a thick paste), ‘gari’ (roasted granules), and ‘agbelima’ (fermented paste). In 2007, nearly 30% of the produce was lost due to PPD (FAO, 2008). It is estimated that, world postharvest losses in cassava is 15 - 30% (FAO, 2002; Sayre *et al.*, 2011) and losses due to PPD range from 5 - 30% of harvested roots (Wenham, 1995; Zidenga *et al.*, 2012) depending on the variety, climatic conditions and distance between the farmer and the consumer. Postharvest loss for root crops was estimated to be 30 - 60% (GNA, 2012) in Ghana, and losses of 25.6 - 35% were reported for cassava (MoFA, 2011). PPD also results in market price reduction on three to four day old roots and high pricing on fresh roots. Eventually this encourages consumers to choose alternative supplies of carbohydrates, increasing dependency on other imported food. In order to increase cassava production to meet rising demand, research towards delaying PPD is important as it would minimize the risk of root losses.

As part of the collaborative study of cassava in Africa (COSCA) by the International Institute of Tropical Agriculture (IITA) in collaboration with the Natural Resources Institute (NRI) in 1992, some work on PPD was carried out. Since then, there has not been any such study on PPD in Ghana. As a result, there is no current information on PPD. It was important carrying out PRA to determine farmers’ indigenous knowledge, current status and perception of stakeholders on PPD. Variability among cassava varieties for PPD has been reported (Ekanayake and Lyasse, 2003; Aristizabal and Sánchez, 2007). Importance of germplasm collection and screening for PPD

tolerance as a major step in breeding for delayed PPD in cassava has been emphasized by Morante *et al.* (2010). Most farmer cultivars are susceptible to PPD. Collecting and screening germplasm, could lead to identification of superior cultivars with delayed PPD that could be used in hybridization schemes in Ghana. Improvement in the storability of the crop will help ensure constant root quality and better storage life. Prior to improving the shelf life of farmer cultivars, it is important to identify and select genotypes with delayed PPD that could be used as parents in breeding programs towards the development of delayed PPD genotypes.

Cassava roots in storage undergo physiological changes that affect the root quality. Changes in cassava root starch in storage have been reported (Sanchez *et al.*, 2013). Since PPD is also physiological, it would be appropriate to determine the physiochemical and functional changes in cassava roots during PPD. This would help in understanding the relationship between root quality and physiological deterioration.

Although cassava thrives well in wide-ranging environments, it exhibits different growth behaviors in different environments due to variations in climatic conditions (Egesi *et al.*, 2007). Genotype by environment analysis on some traits has been reported in cassava (Aina *et al.*, 2009; Akinwale *et al.*, 2010), however, there are no reports on GxE analysis on physiological deterioration. Evaluating cassava genotypes for delayed PPD under different climatic conditions would help in identifying the contributions of the main effects to total variation as well as identifying specific and wide adapting genotypes.

Reducing postharvest root quality losses associated with PPD would increase the market value of the roots and improve the livelihood of stakeholders involved in cassava cultivation in Ghana. The aim of this study was to exploit the genetic diversity among cassava cultivars for PPD,

identify and select parental material with constant root quality and storage life that could be used in cassava shelf life improvement in the future.

1.1 Research Objectives

The research objectives were to:

- a. Elicit stakeholders' cassava production constraints, their perception and knowledge on Postharvest Physiological Deterioration
- b. Assess the diversity and rate of Postharvest Physiological Deterioration among cultivars
- c. Determine the effects of Postharvest Physiological Deterioration on the physicochemical properties of cassava storage roots
- d. Determine the effects of genotype by environment interaction on PPD

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The cassava crop

2.1.1 Taxonomy

Cassava belongs to the plant family Euphorbiaceae, with several commercial species (Hershey, 2005). It is characterised by lactiferous vessels composed of secretory cells for latex production. Some of these are the rubber tree (*Hevea brasiliensis*), castor oil plants (*Ricinus comunis*), root crops (*Manihot spp*) and ornamentals (*Euphorbia spp*) (Ekanayake *et al.*, 1997; Fregene *et al.*, 2006). Cassava is a dicotyledonous crop and belongs to genus *Manihot*; sub-species *Manihot esculenta* Crantz and species (*ssp.*) *esculenta* (Allem *et al.*, 2001). It is the only species from the genus that is widely cultivated for food uses (Rogers and Appan, 1973; Onwueme, 1978; Mkumbira, 2002; Nassar, 2006). The genus *Manihot* has about 98 species grouped into 19 taxonomic sections (Rogers and Fleming, 1973).

Cassava is a diploid with 36 chromosomes (Roca, 1984; Nassar, 2002), although it is believed to be a segmental allotetraploid because chromosomes at metaphase 1 and anaphase 1 show a high number of duplicated nucleolar chromosomes (Kawano, 1980). There are no genetic and cytological barriers in the species of the *Manihot* genus (Nassar, 2002), thus there can be interspecific crosses between species within the genus (Nassar, 1994; 2007). Cassava cultivars have been classified according to morphological traits and cyanogenic glucoside content; however, this classification is not completely reliable since environmental factors influence the expression of the traits.

2.1.2 Morphological characteristics

Cassava is a perennial woody shrub, 1 to 3 m in height and cultivated for its starchy tuberous roots. Stems are woody and either non-branching or branched usually with large width. Fully developed vegetative leaves have five to nine lobes, however, leaves in association with the inflorescence are invariably reduced in number of lobes (Rogers, 1965). Pigmentation of the stem varies from grey to yellow, orange or brown providing one of the most stable characteristics for differentiation of cultivars.

Cassava is monoecious and predominantly an out-crosser (Fregene *et al.*, 1997). Outcrossing is mediated by protogyny resulting in a high level of heterozygosity (Hershey and Jennings, 1992). Flowering in cassava is associated with branching, hence, an early branching genotype may start flowering as early as three months after planting while non-branching types do not flower (Hahn *et al.*, 1973). Cassava varieties are classified as nonflowering, poor flowering, moderate flowering, and profuse flowering with fruit setting as poor fruit or high fruit based on the flowering habit (Indira *et al.*, 1977). Pistillate flowers have five petals and an ovary with three loci, each of which produces one seed (Rogers, 1965). Staminate flowers have ten stamens arranged in two rings of five and do not initiate opening until the last female flower of the inflorescence has bloomed (Rogers, 1965). One male flower produces about 1600 pollen grains of which only 50% are viable (Graner, 1942).

Pollination is mostly by insects, specifically bees (Rogers, 1965) however, wind is also an important pollinating agent (Bueno, 1987). Although cassava is regarded as an allogamous species, considerable selfing may occur, especially in profusely flowering genotypes (Kawano *et al.*, 1978). The fruit is a dehiscent capsule with three locules, each containing a single seed.

2.2 Importance of cassava

2.2.1 General importance

Cassava (*Manihot esculenta* Crantz) is the only food crop among the 98 species of the genus *Manihot*. It is an important root crop and source of calories in tropical and subtropical regions of the world. It is estimated that 250 million people in sub-Saharan Africa (SSA) derive half of their daily calories from cassava (FAO, 2008). It is the most important root crop (Mkumbira, 2002; Nweke, 2004) efficient in carbohydrate production (Onwuene, 1978; Cock, 1985) with nearly the highest starch content among the root crops (Moorthy, 1994). Cassava provides a continuous food supply throughout the year through its long cropping cycle (Nweke, 1994), occupying an important position as a food and income security crop. Global production of cassava was 252 million tonnes in 2011 of which over 140 million tonnes were from Africa (FAOSTAT, 2011). The world's leading cassava producer, Nigeria, generated over 42 million tonnes in 2011 (FAOSTAT, 2011), while Ghana produced 14.2 million tonnes and was ranked second in production in West Africa.

2.2.2 Importance of cassava in Ghana

Ghana is among the African countries where cassava was first introduced. Cassava is Ghana's most highly produced root crop. It is the cheapest starch staple of the Ghanaian consumer diet occupying a key position in Ghana's agricultural economy. It contributes 22% of the agricultural gross domestic product (MoFA, 2011) and an important crop in terms of area occupied and total production. Cassava is one of the major food crops cultivated in most rural communities (Moses *et al.*, 2008). It is produced by over 70% of Ghanaian farmers and consumed by more than 80% of the population (Parkes, 2009), indicating its importance in food security.

Cassava is used primarily for human consumption and as input in the feed industry in Ghana. In 2007, food and feed uses accounted for 66% of all domestic cassava production, with the remainder being wasted (FAOSTAT, 2007). In 2010, Ghana produced more than 13 million metric tonnes of cassava, making it the most highly produced crop in the country and the second most widely grown by area (MoFA, 2011). Cassava yields in Ghana are 20 to 30% higher than regional and global averages, with potential for higher yields with improved agronomic practices (MoFA, 2011). There has been a steady but marginal increase in cassava production in Ghana over the past 5 years (MoFA, 2011). Area under cultivation of the crop has also increased by 45.8% from 592,000 ha in 1997 to 840,000 ha in 2008 (MoFA, 2012). Cassava grows particularly well in almost all the agro-climatic conditions, contributing to Ghana's stature as one of the world's largest cassava producers.

2.2.3 Cassava improvement in Ghana

Cassava was first introduced in Africa by the Portuguese in the late sixteenth century (Jones, 1959) and got to Ghana in the eighteenth century. It became the principal food for both the Portuguese and slaves and was widely cultivated and used by the people by the second half of the eighteenth century (Adams, 1957). The spread of the crop to the hinterland was slow and its importance was not realized until the serious drought in 1982/1983 when all other crops failed (Korang - Amoako *et al.*, 1987). Villages that did not experience the famine of 1983 in Ghana were those that cultivated cassava as the most important and dominant staple crop (Nweke *et al.*, 1999). Cassava therefore plays a famine preventive role in Ghana and is one of the major crops cultivated throughout the country.

Since the introduction of this crop, its improvement has been carried out by the universities, Crops Research Institute (CRI) of the Council for Scientific and Industrial Research (CSIR) and other research institutions. In spite of the long history of cassava in Ghana, its improvement has relied on varieties bred in other countries. It started in 1930 when there was an outbreak of the Cassava Mosaic Virus Disease (CMD). This led to the introduction of cultivars, particularly from East Africa. These were tested, screened and elite clones (K357, K102 and K680) were released to farmers in 1965 (Doku, 1969). Subsequently, in collaboration with the International Institute of Tropical Agriculture (IITA), a number of cultivars (mostly the Tropical *Manihot* species (TMS) genotypes) have been introduced. These were tested, screened and genotypes with high and stable yields, resistant to pests and diseases across different ecological zones in Ghana were identified. Since 1992, several of these varieties have been released to farmers, however, adoption is often low because they do not meet farmers preferred attributes (Manu-Aduening *et al.*, 2005). It therefore became important for Ghana to develop its own cassava breeding program.

Over the years, emergence of new strains of the CMD virus and high virus build up in the vegetative materials used for propagation (Nweke *et al.*, 2002; Kizito *et al.*, 2005; Peroni *et al.*, 2007) led to the then CMD resistant varieties becoming susceptible to the disease. There was the need for the development of cultivars resistant to pests and diseases with acceptable storage root quality. This led to the training of scientists in cassava crosses and in 2006, the first intraspecific crosses in Ghana started (Parkes, personal communication, 2012). To improve the landraces without altering the preferred root qualities, it was crucial that, the landraces were crossed with the IITA germplasm TME 11 which is resistant to CMD. This resulted in the introgression of the CMD resistant gene(s) into the Ghanaian landraces, which has subsequently led to a very vibrant

cassava breeding program in Ghana (Manu-Aduening *et al.*, 2013). The main focus of cassava breeding in Ghana has been on increasing yield, pests and disease resistance. Not much attention has been given to PPD. Breeding for delayed PPD in cassava would therefore maintain the market value of the crop, reduce postharvest losses as well as improve the livelihood of poor farmers.

2.3 Genetic diversity in cassava

Genetic diversity is a product of interplay of biotic factors, physical environment, artificial and plant characters (Frankel *et al.*, 1995). The *Manihot* gene pool ranges from a great variety of wild species to numerous domesticated species with very specific characteristics. Genetic diversity in cassava arises from natural hybrids between wild *Manihot* spp. and cassava cultivars as well as controlled interspecific hybrids between *M. esculenta* and several wild *Manihot* spp. (Nassar, 2002). It may also arise from mutation, migration, or polyploidy (Nassar, 1991; Colombo *et al.*, 2000). High genetic diversity of *Manihot* spp has been found in Brazil and this is due to selection for adaptation to different soils and topography (Nassar, 2003). The genetic diversity of West African's cassava collections is structured according to their adaptation to biotic and abiotic stresses, morphological characteristics, agronomic practices and postharvest use (Fregene *et al.*, 2000). Although the genetic diversity in *Manihot* spp. is high, diversity within a given geographical region may be low. This is associated with the exchange of planting materials between farmers and selection for desired traits (Asante and Offei, 2003).

Genetic diversity can be assessed using morphological and molecular markers (Mohammadi and Prasanna, 2003). Morphological traits were among the earliest markers used in germplasm

management, but they have a number of limitations, including late expression and vulnerability to environmental influences (Smith and Smith, 1992). Numerous studies have used morphological descriptors to determine the genetic diversity among cassava genotypes (Rimoldi *et al.*, 2010; Asare *et al.*, 2011). Morphological descriptors permit the easy identification and differentiation of accessions. Generally, these descriptors have high heritability, suggesting that they are expressed in different environments (Rimoldi *et al.*, 2010). However, cultivar characterization when based on morphological descriptors alone, can be subjected to errors from variations in environmental conditions, especially if the cultivars under study are of similar origin, or if some agronomic characteristics are not specific (Carvalho and Schaal, 2001; Collard *et al.*, 2005). Characterization of accessions may therefore be more reliable if molecular markers are associated with morphological traits.

Different types of molecular markers have been used to assess the genetic diversity in cassava. Molecular tools used in diversity studies in cassava include Random Amplified Polymorphic DNA (RAPDs), Amplified Fragment Length Polymorphism (AFLP), Single Nucleotide Polymorphisms (SNPs), Simple Sequence Repeats (SSRs) and Expressed Sequence Tags (ESTs) (Hurtado *et al.*, 2008; Kawuki *et al.*, 2009).

With the recent advances in high-throughput genotyping technologies, single nucleotide polymorphic markers (SNPs) are increasingly becoming markers of choice for plant genetic studies and breeding. SNPs are the most common type of genetic variation among species, representing just a difference in a single nucleotide. For example, a SNP may replace the nucleotide cytosine (C) with the nucleotide thymine (T) in a certain stretch of DNA. They are either small insertions or deletions with several key advantages. SNP markers are more abundant

in the genome, more efficient and cost-effective than SSR genotyping. This new sequencing and marker genotyping technology accelerates the pace of genetic diversity research and gains in selection through molecular breeding. There is no report on the use of SNPs markers in cassava diversity studies. As a result, SNP markers would be used to assess the level of diversity among cassava accessions.

2.4 Cassava production constraints in Africa

Despite cassava being a hardy crop and its importance in Africa, several constraints including biotic and abiotic factors affect its production. The fact that cassava has a long growth cycle and able to grow in diverse ecological zones contributes largely to its exposure to these constraints (Dixon *et al.*, 1994; Mahungu *et al.*, 1994). Addressing these constraints would increase the productivity of the crop for food, income and industrial uses.

Several pests and diseases affect cassava production in Africa. This reduces yields and makes planting material unavailable leading to loss of food and income for the farming communities (Dixon, 2003). The most important economic pests are Cassava Green Mite (CGM), Cassava Mealy Bug (CMB) (Bellotti *et al.*, 1999; Bellotti, 2002; Taylor *et al.*, 2004; Poubom *et al.*, 2005) and the African variegated grasshopper (Modder, 1994). These pests feed on the leaves thereby reducing the crop's photosynthetic ability. The most important diseases are Cassava Mosaic Disease (CMD), Cassava Brown Streak Disease (CBSD), Cassava Bacterial Blight (CBB) and Cassava Anthracnose Disease (CAD) (Poubom *et al.*, 2005). Most of these biotic constraints with the exception of CBSD, have been addressed through the use of biological agents or

breeding for resistant varieties (Bellotti, 2002; Calvert and Thresh, 2002; Hillocks and Wydra, 2002).

One major abiotic constraint of cassava production is postharvest physiological deterioration (PPD). Cassava roots have the shortest shelf life compared to other root crops (Ghosh *et al.*, 1998). To prevent roots from deteriorating, they are processed or consumed immediately after harvest. However, this is only possible on a small scale. PPD therefore becomes a serious problem when commercial quantities have to be processed immediately after harvest. This has necessitated the search for cassava genotypes with delayed PPD that can be stored for at least one week with minimum or no deterioration. This would improve the economic value of the crop and thus enhance the livelihood of resource poor farmers in Ghana.

2.5 Postharvest physiological deterioration (PPD) in cassava

Cassava roots, unlike any other root crop, have a remarkably short shelf life due to physiological deterioration. The extent of deterioration depends upon the degree of mechanical damage of the roots as well as the genotype and environmental conditions. Postharvest deterioration occurs in two phases, primary and secondary deterioration. The primary deterioration starts from the central vascular bundles of the root, spreads to the adjacent storage parenchyma and subsequently stored starch undergoes structural changes (Plumbley and Rickard, 1991). This is known as postharvest physiological deterioration of which the visible signs are vascular streaking with a blue or black discoloration rendering the roots unpalatable and unmarketable (Beeching *et al.*, 1994). This initial deterioration is physiological and biochemical and does not involve microorganisms (Noon and Booth, 1977). The secondary deterioration is due to infection

with microorganisms leading to fermentation and softening of the root tissue (Plumbley and Rickard, 1991; Wenham, 1995). PPD is much more important economically than secondary microbial deterioration because, the visible colouration of the root is used as an indication of its cooking quality making the crop difficult to sell.

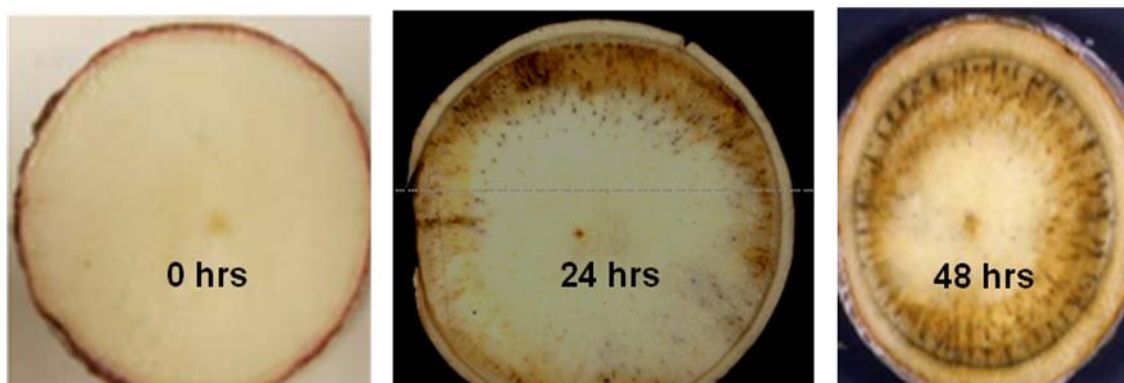


Fig. 2. 1 Cassava roots showing different levels of deterioration after harvest

Biochemical and histological changes observed in cassava during PPD parallels the wound responses in other plant systems (Bennett *et al.*, 1994).

2.5.1 Wounding and PPD

Cassava, like most other plants rely on various defense mechanisms for protection against injury from microorganisms, insects or mechanical wounding. Wounding destroys plant tissue and also provides an entry site for pathogen invasion which is a threat to the plant's survival. To effectively cope with wounding, plants have developed defensive mechanisms against wounding and pathogen attack through a cascade of responses (Bowles, 1990) leading to the formation of a protective layer at the damaged site. A typical example is the hypersensitive response (HR) by plants on pathogen attack characterized by the rapid death of the first infected host cells to restrict pathogen spread (Bowles, 1990; Chong *et al.*, 1999). Wounded tissues induce their

responses in three interrelated forms (Bennett and Wallsgrove, 1994). These are signaling, production of defensive compounds and enzymes, healing or wound repair and subsequently the inhibition of signals. These signals are produced as a direct consequence of the wounding (such as membrane peroxidation products or cell-wall fragments) or are induced by the wounding (such as jasmonic acid, abscisic acid, salicylic acid, systemin or hydrogen peroxide). Other signals that act systemically prepare the plant for the extension of wounding or pathogen invasion (these include systemin, electrical and hydraulic signals). The second phase is the production of defensive enzymes and molecules that help in the protection of the plant against pathogens or the effects of wounding. These enzymes include glucanases and chitinases that attack components of microbial cell walls, and secondary metabolites such as phenolics, that act as antimicrobials (e.g. phytoalexins) or anti-oxidants (Han *et al.*, 2001).

Wound repair occurs via the synthesis of suberin and lignin from phenolic components, callose synthesis, the insolubilisation of hydroxyproline-rich glycoproteins by hydrogen peroxide, and the formation of a wound meristem (Han *et al.*, 2001). This repair leads to the sealing of the wound and subsequently, inhibition of the production of the signals triggering the wound response, and a return of the plant to normal development. Branch pathways from general phenylpropanoid metabolism lead into all these aspects of the wound response (Dixon *et al.*, 1995). Although these aspects of the wound response are found in the cassava root, the wound repair and the resultant down- modulation of the signals are inadequate leading to a continuous cascade of wound responses that spread throughout the cassava root and observed as PPD (Beeching *et al.*, 1998). It is an abiotic stress response, typical of wound responses in other plant systems (Bennett and Wallsgrove, 1994; Dixon *et al.*, 1995). Although these responses result in wound healing and survival in other plants, this is not the case in cassava.

The storage roots of cassava are not propagules like other root crops, but rather serve primarily as repositories of carbohydrate for the plant. Tubers of yam, sweetpotato and potato accumulate storage proteins which can be broken down to support spouting. These storage proteins possess biological activities that protect the tubers against pest and abiotic stresses (Shewry, 2003). Unfortunately, cassava roots lack these storage proteins which are necessary for responding to such abiotic stress like wounding. In contrast to the detached root, wound repair does occur if the root remains attached to the plant (Mwenje, 1998), suggesting that, wound repair mechanism of the detached root was lost during evolution, since the root plays no biological function once it is detached from the mother plant.

2.5.2 Wounding in root and tuber crops

All root and tuber crops (cassava, yam, sweetpotato and taro) undergo mechanical wounding during harvest, storage and transport. This wounding leads to metabolic pathways which differ in the different crops. In sweetpotato and yam, wounding is characterized by the rapid lignification in the layers of cells at the injury site and subsequently healing of tissue (Uritani, 1999), an attribute which is either absent or inadequate in cassava. Different polyphenolic compounds have been identified in root crops in storage after wounding and infection (Uritani, 1999). Catechins were found in both taro and cassava while chlorogenic acid and isochlorogenic acid were only found in sweetpotato. Coumarins that cause UV fluorescence in cut cassava, are absent in the fresh plant and accumulate only upon damage (Wenham, 1995; Uritani, 1999). These were found in wounded cassava and sweetpotato but not in taro. The predominant form of coumarin in cassava root preceding postharvest physiological deterioration was scopoletin (Uritani, 1999). For this reason, it has been assumed that scopoletin is involved in PPD in cassava (Buschmann *et*

al., 2000; Blagbrough *et al.*, 2010; Sánchez *et al.*, 2013). Phytoalexins were produced in taro and sweetpotato as a result of microbial infection, in contrast to cassava where they were produced in the discolored region undergoing deterioration. Several key enzymes of the phenylpropanoid pathway including phenylalanine ammonia lyase (PAL), cinnamate 4-hydroxylase were induced in sweetpotato (Tanaka *et al.*, 1974) and cassava, but the activity in cassava peaked much later (Tanaka *et al.*, 1983). Levels of peroxidase, which is involved in biosynthesis of lignin as well as detoxification of hydrogen peroxide, were also found to be higher in sweetpotato than cassava. The high amount of polyphenols and lignin produced in sweetpotato tissue and taro on wounding as opposed to cassava tissue possibly explains why wound healing in cassava does not occur.

2.5.3 Biochemical and Molecular mechanisms of PPD

Biochemical changes during PPD include increases in respiration (Hirose, 1986; Sánchez *et al.*, 2013), mobilisation of starch to sugars and changes in lipid composition (Lalaguna and Agudo, 1989). There is also the increase of enzymes (Tanaka *et al.*, 1983; Buschmann *et al.*, 2000) and phytohormones (Hirose *et al.*, 1984), occurrence of a wound-induced oxidative burst (Reilly *et al.*, 2001) and the accumulation of secondary metabolites (Sakai *et al.*, 1988; Wheatley and Schwabe, 1985) which precedes the discolouration (Wheatley and Schwabe, 1985). Associated with the discoloration, coloured occlusions and tyloses are formed from the xylem parenchyma that blocks adjacent xylem vessels (Rickard and Gahan, 1983). The activity of various enzymes including dehydrogenases, peroxidases, catalases, phenylalanine ammonia lyase (PAL) and phenol oxidase increase (Hirose, 1986; Wenham, 1995).

Ethylene, a phytohormone is considered to play a role in coordinating wound and senescence responses in plants (Ecker and Davis, 1987). Secondary metabolites which accumulate during PPD include diterpene, flavan-3-ols, catechin, catechin gallate and gallic catechin and the hydroxycoumarins; esculin, esculetin, scopolin and scopoletin (Rickard, 1985; Wheatley and Schwabe, 1985). These are derived from the phenylpropanoid pathway and are localized in the apoplast of the parenchyma. Scopoletin is mainly responsible for the fluorescence in the storage parenchyma observed after cutting cassava. Its content peaks within 24 hours of injury and prior to the development of the visual symptoms of physiological deterioration (Wheatley and Schwabe, 1985). Furthermore, accumulation of scopoletin is more pronounced in cassava cultivars that are more susceptible to PPD, decreasing after 6 days due to metabolism of scopoletin to an insoluble blue-black product by peroxidation (Buschmann *et al.*, 2000; Reilly *et al.*, 2004; and Blagbrough *et al.*, 2010). Several of these metabolites show anti-microbial and or anti-oxidant activity (Sakai *et al.*, 1988; Buschmann *et al.*, 2000).

Strong evidence has been obtained for the importance of reactive oxygen species (ROS), enzymes and compounds that modulate ROS in the PPD response (Beeching *et al.*, 1998; Reilly *et al.*, 2004). ROS (e.g. hydrogen peroxide) is synthesized actively by the plant as a response to stress such as mechanical damage or as a component of defense against pathogen attack. The production of ROS is also an unavoidable consequence of aerobic respiration. Cassava roots produce ROS when mechanically damaged during harvesting resulting in an “oxidative burst” (Reilly *et al.*, 2004). This oxidative burst occurs 15 minutes after the mechanical injury and it is a reaction between anionic peroxidases, scopoletin and hydrogen peroxide, producing an insoluble blue-black precipitate explaining much of the discolouration.

The development of PPD is evidently a complex phenomenon involving multiple components. Cycloheximide inhibition of protein synthesis is an indication that, PPD is an active process involving changes in gene expression and protein synthesis. Reilly *et al.* (2004; 2007) identified some expressed sequence tags which show altered regulation of proteins and enzymes during PPD. These expressed genes are involved in cellular processes including signal transduction, reactive oxygen species turnover, cell wall repair, programmed cell death, ion, water or metabolite transport, signal transduction or perception, stress response, metabolism and biosynthesis, and activation of protein synthesis.

2.6 Evaluation of PPD in cassava roots

The method of evaluating PPD in cassava roots is a key step leading to a better understanding of events taking place during PPD and subsequently, the identification of varieties with delayed PPD. Several methods have been used to evaluate the susceptibility of cassava roots to PPD. These are:

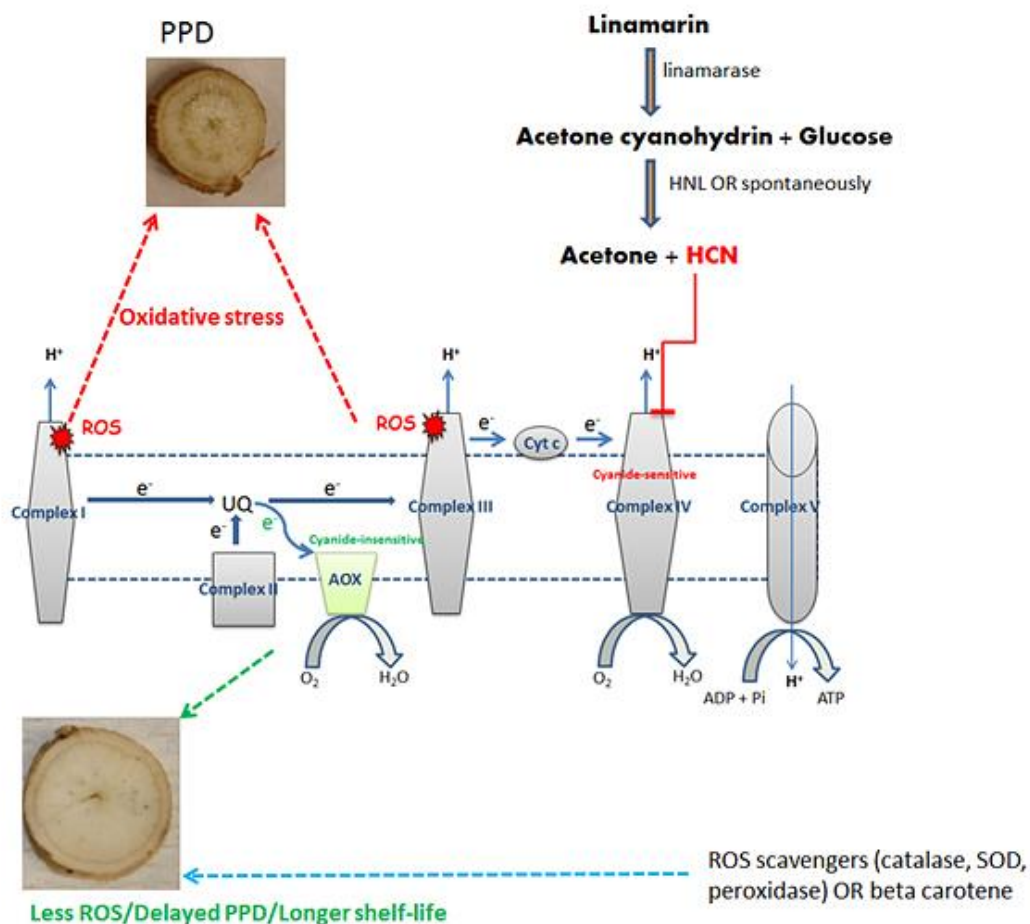
- i. subjective visual scoring using entire roots (Booth, 1977; Pino, 1979),
- ii. subjective analysis of biochemical and physiological changes in transverse sections of roots under ultraviolet (UV) light (Uritani *et al.*, 1983),
- iii. use of the severity of discoloration during PPD (Uritani *et al.*, 1983), and
- iv. scoring of transverse sections of the root by visual inspection of the root pith during PPD (Wheatley, 1982)

All these strategies are based on subjective evaluations and there is the need to implement an objective quantitative and systematic phenotypic evaluation of PPD (Han *et al.*, 2001; Estevao,

2007). Buschmann *et al.* (2000) and Oirschot *et al.* (2000) have suggested the measuring of UV fluorescent compound (hydroxycoumarins) as biochemical marker to assess PPD susceptibility. Significant differences of hydroxycoumarin have been found among cassava varieties using High Pressure Liquid Chromatography (HPLC), however, these differences do not correlate with PPD susceptibility (Buschmann *et al.*, 2000; Oirschot *et al.*, 2000; Salcedo *et al.*, 2010). Another alternative to evaluate PPD is the measurement of the sugar/starch ratio (Oirschot *et al.*, 2000) where there is a strong correlation between them. However, it would be difficult for this method to be widely accepted since sugar/starch ratio in cassava is strongly affected by environmental and geographical conditions. The two common methods currently used for PPD evaluation are the Booth's and Wheatley's methods. This study tends to validate these methods to identify which could be the most appropriate method for identifying cassava genotypes with delayed PPD.

2.7 Biosynthetic pathway of PPD in cassava

Cassava produces cyanogenic glycosides, which break down to release cyanide. Both PPD and cyanogenesis are triggered by mechanical injury of cassava storage roots (McMahon *et al.*, 1995; Reilly *et al.*, 2004). Mechanical damage that occurs during harvesting initiates cyanogenesis by bringing linamarin and linamarase in contact and subsequently the release of cyanide.



Source: Zidenga *et al.*, 2012

Fig. 2.2 Mechanism and control of PPD in cassava

The cyanide (HCN) released inhibits mitochondrial respiration by inhibiting complex IV in the mitochondrial electron transfer chain. Inhibition of complex IV causes a burst of reactive oxygen species (ROS) production at complexes I and III (Zidenga *et al.*, 2012). It is this oxidative burst that causes PPD. While ROS play a role in signal transduction cascades that activate defence-related gene expression, it also causes cellular damage directly by rapidly oxidizing cellular components (Zidenga *et al.*, 2012). Overexpressing the plant alternative oxidase (AOX), which is insensitive to cyanide, prevents over reduction of complexes I and III, thus lowering ROS

production and delaying PPD. Reduction of ROS to control PPD can also be achieved by overexpression of ROS scavengers (Zidenga *et al.*, 2012).

2.8 Strategies to overcome PPD

Currently, three main approaches are being used to overcome PPD in cassava. These are the use of improved storage methods, conventional breeding and genetic engineering (Westby, 2002).

2.8.1 Storage techniques

Several traditional marketing and storage systems have been adapted to prevent root perishability on a small scale. PPD becomes a serious problem considering large scale production, since there is no technique available to store and preserve cassava roots commercially (Aristizabal and Sánchez, 2007). The systems adapted include establishment of processing centers very close to areas of production to ensure daily supply of raw material, processing into storable forms (by peeling, grating, drying, fermentation) and the traditional trading of small quantities of roots (Westby, 2002).

In rural settings, farmers have also devised some traditional means of avoiding root losses due to PPD by leaving the roots unharvested until they are ready to be consumed, processed or marketed. The disadvantages of this method are as follows: increase in the chances of pathogen infection (Westby, 2002), unavailable land for other agricultural production and the development of woody and fibrous roots which affects taste, and also increases cooking time as well as reduction in extractable starch (Wenham, 1995; Ravi *et al.*, 1996). Another traditional practice to overcome PPD is pruning the cassava plant 2 to 3 weeks before harvest. This is carried out by

removing the leaves and stems from the plant 40 - 50 cm above the soil. Studies show that, pruning delays the onset of PPD as compared to unpruned plants (Tanaka *et al.*, 1984; Plumbley and Richard, 1991; Oirschot *et al.*, 2000). There are other traditional practices involving storage of harvested cassava roots under field conditions such as in pits, clamp silos, trenches or boxes (Ravi *et al.*, 1996; Westby, 2002). Storage of roots (300 - 500 kg) in clamp silos has also been found to be effective for four weeks (Rickard and Coursey, 1981; Westby, 2002). Roots are piled up on a layer of straw and covered with straw and soil with openings for ventilation. The moisture content of the sawdust requires careful control as too much moisture promotes fungal growth whereas too little also hastens deterioration. These methods are based on the process of curing, a common method for enhancing the storage life of other root crops (Booth, 1975) at high temperatures (25°C to 40°C) and relative humidity (80% to 85%). The use of these traditional methods though can result in delaying PPD in cassava roots, they are not adopted on a commercial scale as they are considered labour intensive, difficult to manage and are not always completely effective (Ravi *et al.*, 1996; Oirschot *et al.*, 2000).

Modern techniques currently employed on a commercial scale to extend the shelf life of cassava roots include oxygen exclusion by storing in polyethylene bags, waxing and deep freezing (Reilly *et al.*, 2004; Lebot, 2009). Storage in polyethylene bags delays PPD up to 4 weeks due to the high relative humidity (RH) inside the polythene bag which reduces transpiration and respiration (Ravi *et al.*, 1996; Owiti, 2009). Another common method is dipping whole roots in paraffin wax for 45 seconds at 90°C - 95°C after treatment with fungicide. This method has also been reported to prolong the shelf-life up to a month (Aristizabal and Sánchez, 2007; Lebot, 2009). Freezing (0°C to 4°C) of the roots can also delay PPD for 2 weeks without any internal deterioration (Ravi *et al.*, 1996; Oirschot *et al.*, 2000). Alternatively, roots cut into pieces can be

stored frozen (-20°C) under deep-freeze conditions in polyethylene bags. After 4 days of freezing, roots remain palatable after thawing, although they develop some sponginess (Rickard and Coursey, 1981). These methods are used on a commercial scale in many Latin American countries (Ravi *et al.*, 1996) but in Africa, where cassava is a relatively low-cost staple food, farmers and processors cannot support the cost of such sophisticated methods for better storage.

Most of these storage methods are not being used by farmers in Ghana. It is important to carry out Participatory Rural Appraisal to find out why farmers and traders do not use these methods and possibly make enquiries on the storage methods they use.

2.8.2 Conventional breeding of cassava

Breeders have made considerable efforts in improving cassava yield and resistance to biotic and abiotic stresses through conventional breeding (Ceballos *et al.*, 2004; Onyeka *et al.*, 2004). Considerable effort has been devoted in improving cassava resistance to the African cassava mosaic virus (ACMV) through conventional and molecular breeding. However, lack of genes for resistance in existing germplasm, high heterozygosity, poor flowering leading to difficulty in coordinating flowering times between two varieties, as well as geographically specific flowering of a particular cultivar and the outcrossing nature of cassava limit the application of breeding, especially for a quantitative trait such as PPD (Jennings and Iglesias, 2002).

Conventional breeding potentially could produce cultivars with tolerance to PPD by using recurrent selection methods. Use of conventional breeding to delay PPD in cassava faces other challenges such as lack of genetic variability for PPD tolerance (Ceballos *et al.*, 2004). Unfortunately, some desirable traits are often recessive and genetically linked to undesirable

ones making trait separation difficult. There is a strong correlation between PPD and high dry matter (Jennings and Iglesias, 2002; Estevão, 2007), making breeding for PPD tolerance via conventional means a challenge. Nevertheless, there is an indication that, it is possible to break the linkage between high dry matter content and PPD (Jennings & Iglesias, 2002). Screening of numerous cassava varieties and improved lines for nutritional and agronomic traits has provided a solid platform for cassava breeding (Chávez *et al.*, 2005). Identification of varieties with delayed PPD will offer the possibility for mapping PPD related genes as well as Quantitative Trait Loci (QTLs) linked to the trait, and subsequently, the development of molecular markers for use in molecular breeding. This will hasten conventional breeding of cassava and reduce the time in developing an improved variety.

Four different sources for PPD tolerance have been identified (Morante *et al.*, 2010). The first source was identified from the wild relative of cassava, *M. walkerae* native of the United States. This led to the development of an interspecific hybrid named CW 429-1 (Blair *et al.*, 2007; Estevão, 2007; Morante *et al.*, 2010). This hybrid displays no visible signs of deterioration after 15 days of storage. Heritability estimate of PPD was 0.64 suggesting that, inheritance of the trait is controlled by additive and quantitative factors rather than qualitative factors. A second source was induced by mutagenic levels of gamma rays which putatively silenced one of the genes involved in PPD. A third source was a group of high-carotene clones. It is postulated that the antioxidant properties of carotenoids protect the roots from PPD. Finally tolerance was also observed in a waxy-starch (amylose-free) mutant. It has been postulated that, tolerance to PPD co-segregated with the starch mutation and is not a pleiotropic effect to the amylose-free condition of the starch. PPD is highly influenced by the environment, making scoring for minor differences difficult (Rodríguez, 2001). Though advances have been made, conventional

breeding has been unsuccessful in generating cassava cultivars with desirable root quality traits and improved shelf life. Although breeding and genetic experiments can provide useful information to assist solve the problem of PPD, potential biotechnological input such as the development of molecular markers could also facilitate progress. In addition, traditional breeding is usually difficult, time consuming and quite laborious and in light of these challenges to breeding for resistance to PPD, transgenic approaches look promising, but so far have failed to deliver useful products, in spite of considerable investments.

2.8.3 Genetic engineering of cassava

Biotechnological tools can be used to accelerate breeding programs. PPD is a polygenic trait which is heritable but with strong environmental interaction (Cortes *et al.*, 2002). One strategy that could facilitate breeding programs for PPD tolerance is the use of Marker Assisted Selection (MAS). Reilly *et al.* (2001) have identified, characterized and evaluated some specific genes. The correlation between PPD and these genes will allow indirect selection in breeding programs. However, in spite of these early finding, no further progress has been made. Attempts have been made to identify QTL associated with PPD. Ten putative QTLs have been identified, however, the correlations were not strong (Cortes *et al.*, 2002). It is therefore important to develop populations using parental lines with distinctly contrasting PPD responses which will be used to identify stronger QTLs.

Genetic engineering (GE) is a tool used to complement conventional breeding in crop improvement. Transferring genes from one species to another species provides a means of introducing traits of interest thereby increasing the gene pool beyond what could not be achieved by conventional breeding approaches. Thus, GE can also be used as a powerful tool to modulate

PPD in cassava roots. Key candidate genes, enzymes, compounds, and metabolic pathways that modulate PPD response have been identified. These can be manipulated via genetic modification experiments to ascertain their response to PPD. Genes for the biosynthesis of carotenoids and other antioxidants are also good candidates that can be cloned and used in transgenic cassava plants in order to determine their exact contribution to PPD tolerance. Siritunga and Sayre (2004) produced transgenic low cyanogen plants by inhibition of the synthesis of cyanogenic glycosides in the leaves. These plants have been used to investigate the mechanism of PPD in cassava which has provided the evidence for a causal link between cyanogenesis and the onset of the oxidative burst that triggers PPD. By overexpressing the cyanide-insensitive mitochondrial alternative oxidase (AOX) in cassava roots, transgenic plants which are able to delay the onset of PPD by 10 - 21 days under greenhouse and field trial conditions have been developed (Zidenga *et al.*, 2012).

2.9 Economic importance of PPD in cassava

The rapid perishability of cassava roots necessitates prompt consumption or processing of the roots immediately after harvest. In rural settings where cassava is consumed or processed immediately after harvest, PPD is not a major constraint. However, in urban centers where the roots have to be transported, time and distance (Wenham, 1995; Reilly *et al.*, 2004) from farms to markets or processing centers make PPD a more serious problem. In addition, poor roads in most of the cassava growing areas also aggravate the problem. The bulkiness of the roots results in increase in transport costs making cassava quite expensive in urban centers.

It is estimated that, world postharvest losses in cassava is 15 - 30% (FAO, 2000; Sayre *et al.*, 2011) and losses due to PPD range from 5 - 30% of harvested roots (Wenham, 1995, Zidenga *et*

al., 2012). It accounts for 10 - 12% loss in India, 5.3% in Indonesia (CIAT, 1987) and 25.6 - 35% in Ghana (MoFA, 2011). Roots with visible signs of deterioration fetch low market returns as farmers and marketers sell the roots at reduced prices. Deteriorated roots end up as animal feed with price reduction greater than 50% and economic losses due to price reduction is estimated to be 90% (Westby, 2002). In Colombia where 1.3 million tonnes are consumed fresh, approximately 10% are lost due to PPD with a reduction in price from US\$ 80 per tonne (fresh market) to US\$ 40 (animal feed). Similar losses have been reported in Africa where PPD accounts for 7 - 90% discount on 3-day old cassava in Tanzania (Ndunguru *et al.*, 1998) and 27% in Côte d'Ivoire (FAO, 1985). In Ghana, cassava is mainly used for *fufu*, but deteriorated roots are processed into *kokonte* (dried and milled cassava flour). Although this process is time consuming and strenuous, the price of *kokonte* is low compared to equal quantity of fresh cassava.

Deteriorated roots are also difficult to pound and have less desirable elasticity compared to *fufu* prepared from fresh roots. They also have poor eating and processing quality (Rickard *et al.*, 1992), takes longer to cook (Wheatley and Gomez, 1985), has an unpleasant bitter flavor and an unattractive colour. *Gari* processed from these roots has lower and less desirable swelling properties than those produced from fresh roots. PPD leads to induced respiration resulting in starch hydrolysis (Hirose *et al.*, 1984; Uritani *et al.*, 1984). It also results in reduction in starch quality and quantity (Uritani *et al.*, 1984) and causes economic and quality losses with negative impact on food and cash security. PPD therefore impacts negatively on farmers and the national economy in Ghana where cassava is a major food security crop.

CHAPTER THREE

3.0 STATUS OF CASSAVA POSTHARVEST PHYSIOLOGICAL DETERIORATION (PPD) IN SOME SELECTED COMMUNITIES IN GHANA

3.1 Introduction

Cassava is an important source of carbohydrate in Ghana with per capita production and consumption of 12,260,330 MT and 152.9 kg/annum (MoFA, 2009) respectively. Most cassava produced in Ghana is consumed unprocessed (boiled and pounded cassava) as *fufu* but there are many small-scale and few medium to large-scale enterprises processing the roots into diverse foods and products for industrial use (Manu - Aduening *et al.*, 2006). PPD is a serious problem confronting farmers, processors and consumers. To avoid PPD, storage roots have to be consumed or processed immediately after harvesting. However, this is only practical on smaller scales but not on commercial basis. Farmers use their own traditional methods to delay deterioration, for example, roots are either stored in pits or left in the soil until they are needed, processed or marketed. The disadvantage of this method is that, the land is unavailable for further production activities, there is also reduction in dry matter and palatability declines as roots become more fibrous (Rickard and Coursey, 1981). They are also labour intensive and not always effective. All these methods do not offer a practical solution to the problem of PPD.

Cassava breeders have developed and released improved varieties with desirable traits such as disease resistance and high dry matter. In some African countries, these improved varieties have had significant impact (Nweke *et al.*, 1999; Mendola, 2006; Kijima *et al.*, 2008) with 15% increase in farmers income (FAOSTAT, 2005). However, in Ghana, adoption rate of improved

varieties is very low (Manu-Aduening, 2005; Owusu and Donkor, 2012) as improved varieties do not possess preferred root quality traits. Due to this problem, participatory research in breeding approaches are very essential (DeVries and Toenniessen, 2001) and recommended as an effective approach to increasing farmer adoption of new varieties (Joshi *et al.*, 2001; Ceccarelli and Grando, 2009). It also recognizes the importance of farmers' indigenous knowledge and skills to understand the target area and identifies production constraints, which result in the breeding of demand driven crop varieties that meet the needs of farmers.

In many African countries, Participatory Rural Appraisal (PRA) tools have been used to determine farmers' preferences in many crop varieties. For example Assefa *et al.* (2005) for bean varieties in Ethiopia, Mose *et al.* (2002) for maize varieties in the moist transitional and high tropic zones in Kenya, Agwu and Anyaeche (2007) and Manu-Aduening *et al.* (2007) for cassava in Nigeria and Ghana respectively. Participatory research was undertaken to ascertain stakeholders' perception on PPD, identify farmers' preferences and cassava varieties with delayed PPD. This would help in minimizing the risk associated with root losses as well as ensure that, improved varieties meet farmers' needs.

3.1.1 Objectives

This study investigated constraints in cassava production, stakeholders' perception and knowledge of PPD, as well as identified PPD tolerant cultivars. To achieve this, the following specific objectives were to:

- a. Identify and rank key production, processing and marketing constraints among stakeholders (farmers, processors and marketers) in the cassava supply chain.

- b. Assess farmers' preferred traits in improved cassava varieties with delayed PPD
- c. Identify and validate existing storage methods used by farmers to delay PPD
- d. Examine stakeholders (farmers, marketers, processors and consumers) perception of PPD

3.2 Materials and methods

3.2.1 Study area

The agro-ecological zones and selected communities for the study are presented in Table 3.1. The study was conducted in the Rain Forest, Semi-deciduous Rain Forest, Forest Savanna Transition and Coastal Savanna agro-ecological zones in Ghana. The selected communities were Nkawie and Seidi in the Atwima Nwabiagya district of the Ashanti region, Ayigbe and Amangoase in the Wenchi district and Akrofrom, Asuayi, Aworowa and Krobo in the Techiman district, all of the Brong Ahafo region. Other communities were Ohawu and Vume in the Akatsi South district of the Volta region and Abodobi and Yeyaso in the Fanteakwa district of the Eastern region. These communities were selected because cassava farming and processing are the major occupation of the inhabitants. Research and extension linkages had already been established with the farmers.

These agro-ecological zones are characterized by bimodal rainfall pattern ranging between 800 mm to 2500 mm per annum across the zone with an average of about 950 mm per annum (Table 3.2). The major season begins from March to July for the rain forest, semi-deciduous and coastal savanna and April to July for the forest savanna transition agro-ecological zone. The minor season starts from September to October for the forest savanna transition and coastal savanna

while the rain forest and the semi-deciduous zones begin in September and ends in November. Major arable crops cultivated include maize, yam, cassava, plantain, cowpea, groundnut, soybean and rice.

Table 3. 1 Agro-ecological zones, districts and communities for PRA study

Ecozone	District	Community
Rain Forest	Fanteakwa	Yeyaso
		Abodobi
Semi-deciduous Rain Forest	Atwima Nwabiagya	Nkawie
		Seidi
Forest Savanna Transition	Wenchi	Ayigbe
		Amangoase
		Aworowa
	Techiman	Akrofrom
		Asuayi
		Krobo
Coastal Savanna	Akatsi South	Ohawu
		Vume

Table 3. 2 Rainfall data for the Agro-ecological zones

Agro-ecological zone	Rainfall (mm/annum)		
	Minimum	Maximum	Average
Rain Forest zone	1300	2500	1900
Semi-deciduous Rain Forest	1200	1600	1500
Forest Savanna Transition	1300	2200	1750
Coastal Savannah	800	1100	950

Source: Meteorological Services Department, Ghana (2010)

3.2.2 Sampling Procedure

Multi stage sampling procedure was employed in the study. One region was selected from each of the four agro-ecological zones. One district was randomly selected from a list of major

cassava growing districts in the region. From each of these districts, two communities were sampled. The target groups were farmers, processors, marketers and consumers. These stakeholders play a very important role in the cassava value chain thus their perceptions about PPD and their preferences for improved varieties with delayed PPD are important. Only farmers were involved in the focus group discussion while all of the other stakeholders were involved in the semi-structured interviews. Farmers in each community both men and women were gathered in one group for FGD, after which 15 farmers per community were randomly selected for the semi-structured interviews. Overall, 490 respondents were involved in the study consisting of 137 farmers, 103 processors, 100 marketers and 150 consumers.

3.2.3 Data collection techniques

PRA was conducted using Focus Group Discussion (FGD) and semi-structured interviews. The questionnaires for the interviews and checklist for FGD were designed by a team comprising a cassava Breeder and an Agricultural Economist. The questionnaires were first pre-tested to validate the importance of the variables and the possible responses in addressing the objectives. They were then revised to incorporate emerging issues from the pre-testing before it was finally administered to the stakeholders.

FGD was used to elicit information on constraints in cassava production and marketing, perceptions of PPD, existing methods used to delay PPD and preferences for improved varieties with delayed PPD. With the semi-structured interviews, different questionnaires were used for the different categories of stakeholders (Appendix 3.1). All the stakeholders were interviewed

individually to solicit their views on the different topics. This enabled respondents to express their opinion without undue influence from other respondents.

3.3 Data analysis

Data collected were coded and analyzed using Statistical Package for Social Sciences (SPSS). Descriptive statistics were used to summarize the qualitative data. Stakeholder perceptions of each PPD attribute were assessed by the use of a 5–point Likert scale (Likert, 1932) using perception indices (Responses: 2 = strongly agree: 1 = agree: 0 = indifferent: -1 = disagree: -2 = strongly disagree). The mean perception for each PPD attribute was measured as:

$$\bar{x} = \frac{\sum n_i x_i}{N}$$

Where n_i = number of individuals who chose the i^{th} response

X_i = the i^{th} response

N = the total number of respondents

To assess cassava production, processing and marketing constraints among stakeholders (farmers, processors and marketers), the Kendall's coefficient of concordance (W) described by Mattson (1986) was used to rank the constraints. The lower the mean rank, the more important the trait. W was computed as:

$$W = \frac{12 \sum \bar{R}_i^2 - 3N(N+1)^2}{N(N^2 - 1)}$$

Where: W = Kendall's value

N = total sample size

R = mean of the rank

The Kendall's coefficient of concordance (W) is a measure of the extent of agreement or disagreement among the rankers. The value of W is positive and ranges from zero to one. It is one when there is perfect agreement among rankers and zero when there is maximum disagreement among rankers. This approach was further applied to assess and identify farmer preferred varietal characteristics in a new cassava cultivar. However, the rankings of the varietal characteristics were done by only farmers.

3.4 Results

3.4.1 Characteristics of sampled stakeholders

The study consisted of 490 respondents of which 62% were females and 38% were males. One hundred and thirty seven (28%) were farmers, 103 (21%) processors, 100 (20%) marketers and 150 (31%) consumers (Fig. 3. 1). The respondents were aged between 18 and 70 years. Farmers consisted of 58% males and 42% females, while processors comprised of 24% and 76% males and females respectively. However, almost all the marketers (99%) were females while 62% of the sampled consumers were females.

On the whole, 17% of the farmers were from Nkawie, 18% from Seidi, 20% from Ayigbe, 12% from Amangoase, 16% from Yeyaso and the remaining proportions distributed evenly among Abodobi and Ohawu (Table 3.3).

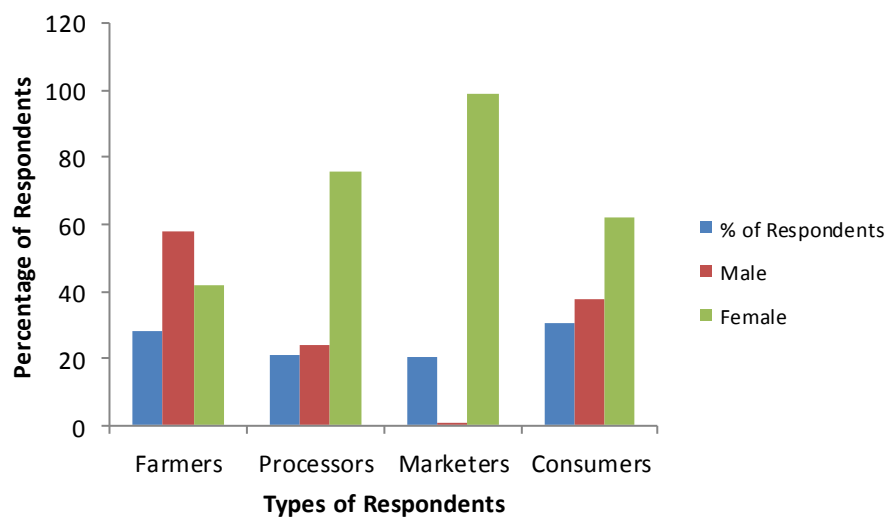


Fig. 3. 1 Gender of respondents interviewed

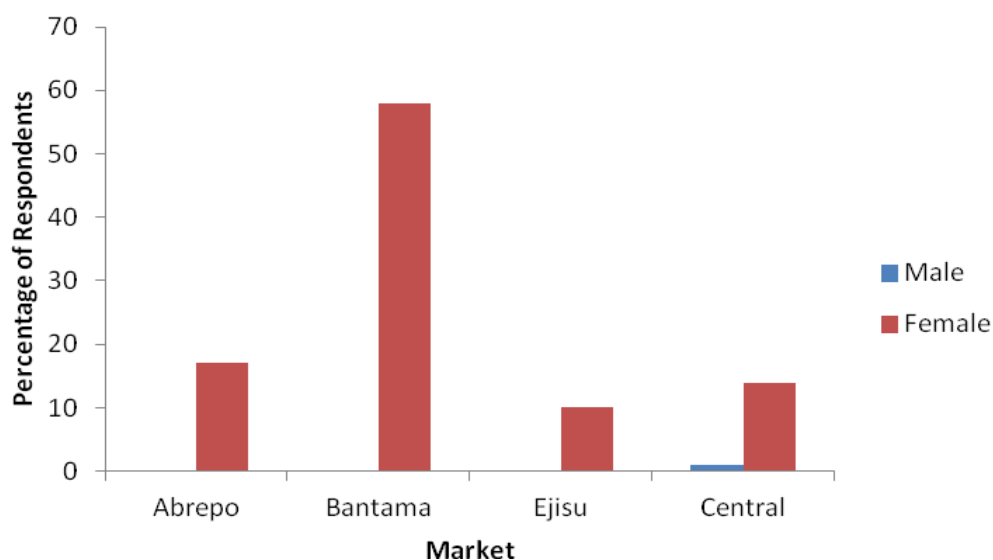
Table 3. 3 Distribution of cassava farmers by community and gender

Community	Males	Females	Total	Percentage (%)
Nkawie	14	9	23	17
Seidi	20	5	25	18
Ayigbe	10	18	28	20
Amangoase	7	10	17	12
Yeyaso	14	8	22	16
Abodobi	8	3	11	8
Ohawu	7	4	11	8
Total	80 (58 %)	57 (42 %)	137	100

In all, 42% of processors were from Aworowa, 23% from Vume, 17% from Akrofrom, 16% from Asuayi and the remaining proportion were from Krobo (Table 3.4). The marketers interviewed were from Abrepo, Bantama, Ejisu and Central markets all in Kumasi and Ejisu metropolis as presented in Fig. 3.2.

Table 3. 4 Distribution of cassava processors by community and gender

Community	Males	Females	Total	Percentage (%)
Akrofrom	2	16	18	17
Asuayi	1	15	16	16
Aworowa	11	32	43	42
Krobo	0	2	2	2
Vume	11	13	24	23
Total	25 (24 %)	78 (76 %)	103	100

**Fig. 3. 2 Distribution of marketers and their gender**

3.4.2. Cassava production constraints

3.4.2.1 Major constraints in cassava farming

Seven major production constraints were identified and ranked by farmers as shown in Table 3.5. Most of the constraints identified by farmers were related to finance. Overall, inadequate capital was ranked as the most important constraint and this was consistent across all the four agro-

ecologies. This was followed by inadequate processing centers as this was also consistent across the agro-ecologies. Due to the pivotal role played by processing in managing postharvest losses in cassava, establishment of more processing centers is vital as indicated by the rankings of the farmers.

As fresh cassava roots cannot be stored for long due to the high moisture content and had to be processed immediately after harvest in order to increase the shelf life, deterioration of roots after harvest were indicated as the next most important production constraint by the farmers.

Other constraints were high cost of labour (due to the long cropping cycle and harvesting in the dry season), pests and diseases and high cost of agrochemicals. At the agro-ecological level however, pest and diseases were priority to the farmers in the Coastal Savanna as they ranked it as the fourth most important constraint. The extent of agreement among the farmers of the rankings as indicated by the Kendall's W suggests that overall, more than 40% of the farmers are in agreement of the rankings. However, the highest agreement was recorded by farmers in the Semi-deciduous (44.7%) and the least by farmers in the Coastal Savanna (38.4%) agro-ecological zones.

3.4.2.2 Major constraints in cassava processing

Five constraints were identified and ranked by the processors as shown in Table 3.6. The processors were selected from the Coastal Savanna and the Transition agro-ecological zones. Cassava processing is very dominant in these zones hence analyses of processing constraints from these farmers is very essential to the cassava industry in Ghana. The results suggested that,

inadequate capital was ranked as the most important constraint. Deterioration of roots after harvest was ranked second after inadequate capital. High cost of labour, poor packaging of products and poor market were also identified as production constraints (in order of importance). The level of agreement among the farmers for these rankings was quite encouraging (48.4%). Processing is carried out manually using simple equipment. This makes it labour intensive and productivity is generally very low.

Table 3. 5 Ranking of cassava production constraints

Production constraints	Coastal		Forest		Semi deciduous		Transition		Overall	
	Mean Rank	Ranking	Mean Rank	Ranking	Mean Rank	Ranking	Mean Rank	Ranking	Mean Rank	Ranking
Inadequate capital	1.91	1	1.94	1	1.94	1	1.85	1	1.91	1
Inadequate processing centers/buyers	2.27	2	2.12	2	1.94	1	2.34	2	2.14	2
High cost of transportation	4.82	5	4.47	3	4.52	4	4.73	4	4.31	3
Deterioration of roots after harvest	4.27	3	4.53	4	4.48	3	3.93	3	4.58	4
High cost of labour (in the dry season)	5.09	6	4.65	5	4.69	5	4.82	5	4.74	5
Pests and diseases	4.55	4	4.79	6	5.04	6	4.84	6	4.89	6
High cost of agrochemicals	5.09	6	5.5	7	5.4	7	5.49	7	5.43	7
<i>W (p < 0.000)</i>	0.384		0.4137		0.447		0.411		0.416	

Table 3. 6 Ranking of cassava processing constraints

Processing Constraints	Coastal Savanna		Transition		Overall	
	Mean Rank	Ranking	Mean Rank	Ranking	Mean Rank	Ranking
Inadequate capital	2.0	1	1.6	1	1.95	1
Deterioration of roots after harvest	2.55	2	2.37	2	2.5	2
High cost of labour	3.36	3	3.11	3	3.49	3
Poor product packaging	4.55	4	3.54	4	4.54	4
Poor market	6.0	5	4.37	5	4.97	5
<i>Kendal's W, p < 0.000</i>	0.47		0.482		0.484	

3.4.2.3 Major constraints in cassava marketing

Five constraints were identified and ranked by the marketers as shown in Table 3.7. They ranked inadequate capital as the most important constraint. The other constraints were deterioration of roots after harvest, poor market, high cost of transportation and lack of improved storage technology for roots (in order of importance). The extent of agreement among the marketers based on the Kendall's W suggests that about 34% of the marketers were in agreement of the rankings.

Table 3. 7 Ranking of cassava marketing constraints

Production constraint	Mean Rank	Rank
Inadequate capital	2.01	1
Deterioration of roots after harvest	2.55	2
Poor market	3.33	3
High cost of transportation	3.83	4
Lack of improved storage technology	4.61	5

(W = 0.335)

3.4.3 Status of cassava postharvest physiological deterioration (PPD)

3.4.3.1 Local names for postharvest physiological deterioration in cassava roots and their meanings in the sampled communities

Local names for PPD and their meanings are presented in Table 3.8. Postharvest physiological deterioration has been referred to with different names in different local languages by the respondents. Most of the respondents (74%) could not explain the meaning of those names used to describe deteriorated cassava in their local language. About 26% of the respondents inherited these local names hence have no idea of its origin. However, some of the respondents attempted

to explain these local names. The responses obtained included; “the roots are at the initial stage of deterioration”, the roots “have been stored overnight” and the colour of the roots has changed.

Table 3. 8 Local name and meaning of PPD in the sampled communities

Community	Local name for PPD	Meaning
Abodobi	Esedepo	colour of roots have changed
Mangoase		
Ayigbe	Asodaye	roots have dreamt
Nkawie		
Seidi		
Yeyaso	Esedepo	
	Ewote	colour of roots has changed
Ohawu	Edze ashiatsu	roots are at the initial stage of deterioration

3.4.3.2 Bulk harvesting and methods for cassava root storage after harvest

Methods used by farmers to store cassava roots after harvest is presented in Fig. 3.3. Cassava farmers do bulk harvesting only when there is the availability of buyers, otherwise harvesting is mostly done in bits for contingency reasons of both sale and home consumption. Sixty eight percent of the farmers store the roots after harvest while the remaining does not store the roots but sell or consume immediately after harvest. However, 33.6% of farmers who store cassava roots after harvest, put them in sacks, 27% in polythene, 25.6% in water, 8% in pits and 5.8% leave them under shade.

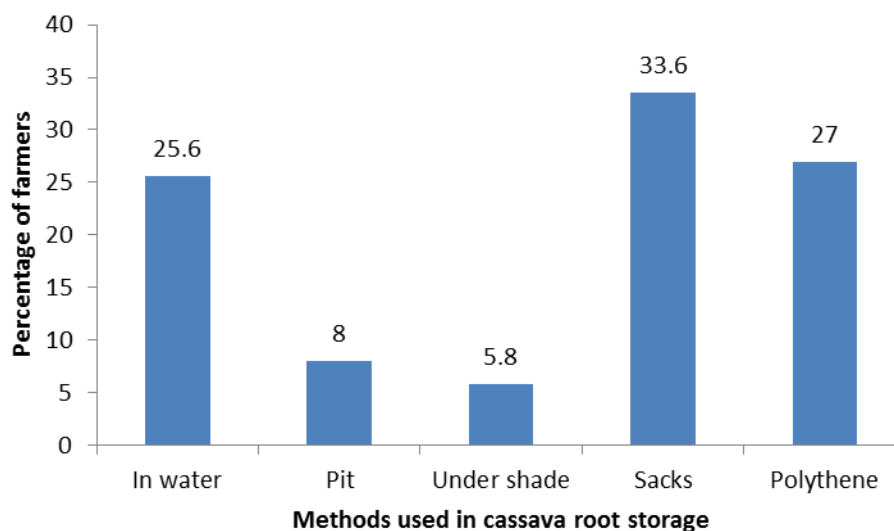


Fig. 3. 3 Farmers means of storing cassava roots after harvesting

3.4.3.3 Validation of farmers storage methods for PPD

Farmers' storage methods with the exception of storage in pits were validated (Fig 3.4). Cassava storage roots were stored under the different methods and evaluated for PPD after seven days. PPD score among the genotypes ranged from 0% - 27.5% with all genotypes stored in water exhibiting no signs of deterioration. Although genotypes stored in water showed no signs of deterioration, all the roots from all the genotypes rot by the seventh day. This implies that, storing cassava roots in water is not a reliable method that can be used beyond three days. Reaction to PPD ranged from 0% - 22.4% in polythene, while it ranged from 6.7% - 25.7% in jute and 13.3% - 24.8% in those left on shelves. Hence storage in polythene was found to be the best farmer method for delaying PPD in cassava storage roots.

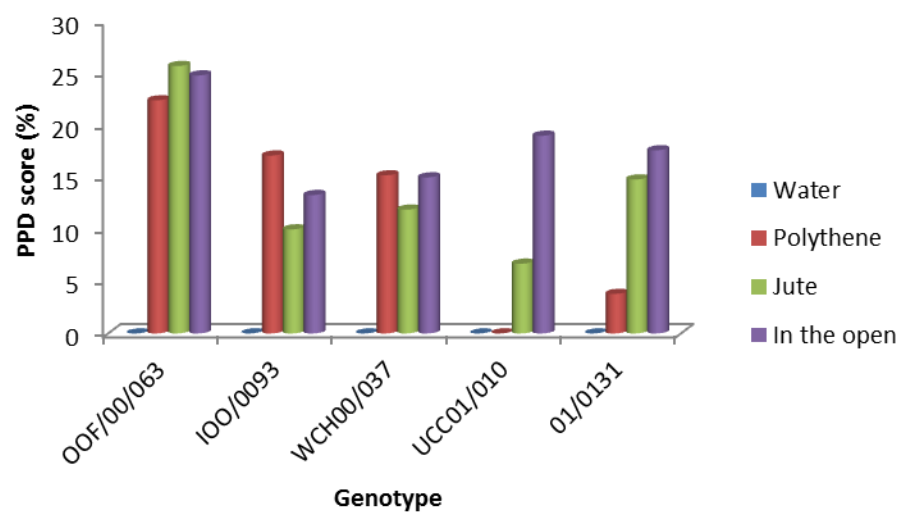


Fig. 3. 4 PPD scores for cassava genotypes

3.4.3.4 Cassava farmers' perception of the causes of PPD

The perception of cassava farmers on causes of PPD is presented in Fig. 3.5. The farmers mentioned four major causes of PPD. Thirty four percent of the respondents stated that PPD is caused by air, while 29.27%, 32.3% and 4.89% perceived that, PPD is caused by mechanical injuries, sunlight and heat respectively.

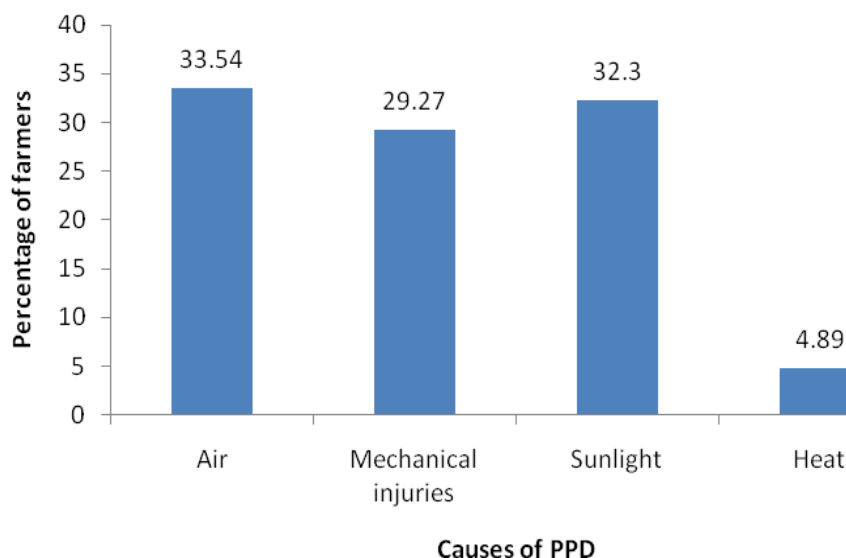


Fig. 3. 5 Causes of PPD as perceived by farmers

3.4.3.5 Stakeholders indigenous knowledge of postharvest physiological deterioration

Relevant information concerning stakeholders' indigenous knowledge on PPD is presented in Table 3.9. A significant number of them (95.3%) were aware of PPD and the corresponding changes in the roots (i.e. colour change to brown, blue-black or black and subsequently the roots becoming dry, hard and dark). They also stated that PPD starts 2-3 days after harvest, although the number of days differs between the stakeholders. The causes of PPD as perceived by stakeholders ranged from pathogens to sunlight, mechanical injuries during harvesting, atmospheric air etc. They also mentioned different methods for storing cassava roots after harvest in order to prevent deterioration. Processors and marketers normally protect the roots by covering with either polythene sheets or tarpaulin while consumers store in water, polythene bags etc. All of the respondents said emphatically that, there were no PPD tolerant cultivars, however, farmers were of the view that some cultivars deteriorate faster than others.

Table 3. 9 Farmers, processors, marketers and consumers knowledge of postharvest physiological deterioration in cassava

	Stakeholders			
	Farmers	Processors	Marketers	Consumers
Symptoms of PPD	Roots change colour to brown or blue black and becomes dry, hard and dark	Roots change colour to black or brown and becomes hard and dry	Roots change colour to blue black or brown	Roots change colour to brown and subsequently rot
Number of days for cassava roots to deteriorate after harvest	3 days	2-3 days	2-3 days	3 days
Causes of PPD	air, mechanical injuries, sunlight and heat	air, dehydration	mechanical injuries	pathogens
Methods for cassava root storage	in water or polythene bags, sacks, pits, under shade	spread on floor, sprinkle water on it, cover with polythene or tarpaulin	store in sacks or cover with polythene sheets	store in water or peel and store in freezers
Availability of PPD tolerant varieties	No PPD tolerant cassava varieties available			

3.4.3.6 Stakeholders' perception of PPD in cassava storage roots

Stakeholders' perception of PPD was assessed using a 5-point Likert scale as shown in Table 3.10. The general perception among stakeholders' was necessary to address the dynamics among them regarding those perception statements. The result signifies that stakeholders perceived some of the issues with deteriorated roots as; being difficult to pound, affects root quality, reduces market value, PPD starts 2 to 3 days after harvest and it is caused by mechanical injuries during harvesting. These perception attributes had mean score values between 0.07 and 1.17, implying that the stakeholders were in agreement with the attributes represented by these indices. All the other indices had negative mean score values (-0.28 to -0.75) indicating their disagreement with those perception attributes. However, the actual mean score values of some of

the attributes show that the stakeholders' were indifferent with the attributes. These statements were; deteriorated roots taste better, PPD is caused by microorganisms and PPD can be prevented but disagreed on the statements; PPD roots are easy to process and PPD roots can be used to prepare many dishes.

Table 3. 10 General perception on PPD

	Perception attribute	Strongly agree (2)	Agree (1)	Indifferent (0)	Disagree (-1)	Strongly disagree (-2)	Mean score
1	Deteriorated roots taste better	126	75	35	114	92	0.07
2	Deteriorated roots are difficult to pound	105	255	16	51	17	0.86
3	Deteriorated roots affect root quality	177	205	25	27	8	1.17
4	Roots deteriorate 2-3 days after harvest	163	213	36	20	7	1.15
5	Mechanical injuries cause PPD	171	176	15	61	5	1.04
6	Microorganisms cause PPD	36	106	97	89	106	-0.28
7	PPD reduce market value of roots	102	268	13	38	20	0.89
8	Deteriorated roots are easy to process	11	67	48	188	112	-0.75
9	Deteriorated roots can be used to prepare many dishes	21	62	73	174	112	-0.67
10	PPD can be prevented	54	52	112	95	118	-0.4

3.4.4. Ranking of Farmer preferred traits

The results of rankings for farmers' preferred traits by agro-ecological zones are presented in Table 3.11. Generally, the rankings by the farmers in the different agro-ecological zones reflect that for the entire sample. On the whole, high yield was identified as the most preferred varietal trait and this was similar across the agro-ecological zones. This was followed by early maturity,

high dry matter, delayed PPD, poundability, large roots, resistance to pests and diseases and peel colour (in order of importance). Generally, more than half of the farmers were in agreement of the rankings (67.1%) however, the highest agreement was recorded by farmers in the Semi-deciduous forest (69.4%) with farmers in the Coastal Savanna recording the least (64.1%). This suggests that there is not much variation in the rankings hence research efforts could focus on the overall rankings as shown in Table 3.11. From the rankings it was observed that farmers do not want to compromise on yield. They were also concerned with the long maturity of the crop with its associated high cost of labour and would, therefore, like to get varieties that matures a bit earlier than what they are currently growing. Since about 50% of the total production of cassava is consumed as *fufu*, the roots should also have good cooking qualities particularly for pounding. The peel colour was also of interest to the farmers because they perceived pink peel delays PPD more than the cream peel.

Table 3. 11 Farmer preferred cassava traits ranking

Desirable trait	Coastal		Forest		Semi deciduous		Transition		Overall	
	Mean Rank	Ranking	Mean Rank	Ranking	Mean Rank	Ranking	Mean Rank	Ranking	Mean Rank	Ranking
High yield	1.64	1	1.53	1	1.52	1	1.5	1	1.52	1
Early Maturity	3	2	2.71	2	2.77	2	2.64	2	2.7	2
High dry matter	4.36	5	4.47	5	4.65	5	4.43	5	3.59	3
Delayed PPD	3.82	4	4	4	3.4	3	3.91	4	3.76	4
Poundability	3.27	3	3.41	3	3.6	4	3.64	3	4.5	5
Large roots	5.98	6	5.98	7	6.2	7	5.76	6	5.97	6
Resistance to pests and diseases	6	7	5.91	6	5.98	6	6.02	7	6.72	7
Peel colour	7.27	8	7.26	8	7.29	8	7.18	8	7.25	8
Kendal's W, $p < 0.000$	0.641		0.666		0.694		0.663		0.671	

3.5 Discussion

The results indicated that cassava production, marketing and processing are female dominated. This could be due to the low level of education of the women and cassava production being the primary occupation in the communities where the study was carried out. Findings of this study is similar to other studies where female were observed to dominate cassava production (Adebayor and Salahu, 2007; Oyegbami *et al.*, 2010).

Some of the constraints were cross-cutting hence was among the major constraints faced by all the stakeholders. These cross-cutting major constraints ranked by the stakeholders were inadequate capital to expand their businesses, poor market in terms of their inability to negotiate the price resulting in low pricing and physiological deterioration 2-3 days after harvest. These findings were similar to findings of the survey conducted by COSCA (NRI, 1992) in which two thirds of Ghanaian farmers identified postharvest losses as a major risk factor in cassava production. This makes postharvest physiological deterioration a serious issue for farmers, processors and marketers. Other constraints identified were inadequate processing centers and high cost of transportation from farm gate to processing center/market. This could be due to poor accessibility to farmers' field, roads in bad condition and inappropriate means of transport to carry the bulky and highly perishable roots. Similar findings were obtained by Nweke *et al.* (1999), Manu-Aduening *et al.* (2006) and Fakoya *et al.* (2010).

The findings of this study indicated that, farmers have in depth knowledge of postharvest physiological deterioration in cassava than the other stakeholders. This could be explained by their experience acquired over the years and indigenous knowledge of cassava cultivation. Never the less, the other stakeholders were also aware of postharvest physiological deterioration in

cassava in terms of the discolourization and number of days before deterioration sets in. However, their level of awareness differs from each other. This could be explained by their different educational levels and indigenous knowledge of cassava production. The discolourization and the number of days it takes before postharvest physiological deterioration sets in as perceived by the stakeholders were similar to earlier studies (Plumbley and Rickard, 1991; Wenham, 1995). It was also revealed by the stakeholders that, there was no PPD tolerant cassava cultivars, however, farmers emphasized that, some cassava cultivars deteriorate faster than others. Stakeholders also believed that PPD cannot be prevented or controlled. This could be attributed to their perception of PPD being a natural process and the difficulty associated with preventing such natural occurrences.

With the perception indices, farmers, processors and marketers either agreed or disagreed to most of the attributes while consumers were indifferent on most of the attributes. This could be due to the fact that, consumers tend to buy cassava roots only when they are needed and use immediately without storing, making the issue of cassava root physiological deterioration irrelevant to them. Again, consumers buy freshly harvested roots with no signs of deterioration and would never go in for deteriorated roots, perhaps the only time they would go for deteriorated roots is when the prices has been heavily reduced. It is the middlemen who store cassava roots for processing and selling that really suffer because they buy the cassava roots in bulk.

Several researchers have designed and evaluated cassava storage methods that could extend postharvest physiological deterioration in cassava roots (Rickard and Coursey, 1981; Wheatley, 1989; Balagopalan, 2000). However, the stakeholders have not adopted any of these cassava

storage methods but have improvised their own means of storing the cassava roots. The results suggested that, storage of cassava roots in water was not an appropriate method while storage in polythene and jute sacks could delay PPD for a few days. Hence, these methods could be modified to minimize both PPD and root rot during storage. The key reasons why stakeholders use their own storage methods could be attributed to the fact that, their methods are less expensive and user friendly. Although advanced methods such as refrigeration at 3°C and coating with paraffin waxing are available, they are not viable methods in most developing countries, however, few of the consumers interviewed store cassava roots in freezers.

Although farmers', processors and marketers perceived that postharvest physiological deterioration could not be solved, their preference for an improved cassava variety with delayed PPD was obvious as delayed PPD was ranked 4th after high yield, early maturity and high dry matter. The high market value of cassava varieties with such preferred traits could probably explain the selection of those traits. This finding is similar to that of Manu - Aduening *et al.* (2005). Adequate considerations should therefore be given to these varietal attributes in the development of improved cassava varieties with delayed PPD. This would ensure that, stakeholders get the best from the cassava roots and subsequently increasing their incomes.

3.6 Conclusion

Major cassava production, processing and marketing constraints were identified. The constraints identified were inadequate capital, low pricing, physiological deterioration, inadequate processing centers, pests and diseases and high cost of labour and transportation. Farmers' preferences in cassava varieties were cultivars with good and stable yield, early maturing, high

dry matter, delayed PPD, large roots, poundable, resistance to pests and diseases and pink peel colour. Combination of these traits in a cultivar would facilitate adoption by farmers and other cassava stakeholders. Polythene and jute sacks were found to be the best storage systems. Stakeholders were aware of postharvest physiological deterioration and their indigenous knowledge could be used in solving the problem. The findings of the study suggested that, PPD is a major problem for stakeholders in the cassava supply chain that needs to be addressed. It was evident that, there are no PPD tolerant cassava cultivars available to farmers, however, some cultivars deteriorate faster than others. This suggested that, genetic diversity existed in farmers materials. Determining the extent of diversity among cassava germplasm from farmers collections, research institutes and genetic resource centers could result in the identification of new sources of tolerance for postharvest physiological deterioration as well as other important economic traits. This would improve the storability of cassava roots as well as the status of cassava stakeholders in Ghana.

CHAPTER FOUR

4.0 GENETIC DIVERSITY OF CASSAVA GERMPLASM SELECTED FOR PPD STUDY IN GHANA

4.1 Introduction

Considerable genetic variation is found in a single clone of the cassava germplasm due to its heterozygous nature. Numerous local varieties are cultivated by traditional farmers (Siqueira *et al.*, 2010; Alves *et al.*, 2011). These local varieties have different characteristics suggesting the existence of diversity within this crop. This diversity could probably be due to cross pollination, fruit dehiscence, and perhaps the occasional use of seeds in propagation (McKey *et al.*, 2010; Montero-Rojas *et al.*, 2011). In addition, the incorporation of volunteer seedlings, especially in vegetatively propagated crops by traditional farmers is an important means for increasing genetic variability, a possibility for avoiding genetic erosion (Pujol *et al.*, 2005; Raghu *et al.*, 2007). Genetic diversity gives species the ability to adapt to changing environments including new pests, new diseases and new climatic conditions. To monitor this diversity, it is important that it is measured accurately.

Numerous studies have used morphological descriptors to determine the genetic diversity among cassava genotypes (Rimoldi *et al.*, 2010; Asare *et al.*, 2011). Morphological descriptors permit the easy identification and differentiation of accessions. Generally, these descriptors have high heritability, suggesting that they are expressed in different environments (Rimoldi *et al.*, 2010). However, cultivar characterization when based on morphological descriptors alone, can be subjected to errors from variations in environmental conditions, especially if the cultivars under

study are of similar origin, or if some agronomic characteristics are not specific (Carvalho and Schaal, 2001; Collard *et al.*, 2005). Recent advances in molecular biology techniques have provided useful tools for genetic studies in several plant species. The use of molecular markers may permit the detection of genetic differences among closely related genotypes that are not affected by the environment (Collard *et al.*, 2005). Characterization of accessions may, therefore, be more reliable if molecular markers are associated with morphological traits.

Molecular markers are widely used in plant genetic research and breeding. Some of these molecular markers that have been used for genetic diversity studies in cassava include random amplified polymorphic DNA (Ferreira *et al.*, 2008; Rimoldi *et al.*, 2010), amplified fragment length polymorphism (Benesi *et al.*, 2010), simple sequence repeats (Alves *et al.*, 2011; Oliveira *et al.*, 2012; Costa *et al.*, 2013) and single nucleotide polymorphism (Kizito *et al.*, 2005; Tangphatsornruang *et al.*, 2008; Ferguson *et al.*, 2011). With the recent advances in high-throughput genotyping technologies, single nucleotide polymorphic markers (SNPs) are increasingly becoming markers of choice for plant genetic studies and breeding. SNPs are the most common type of genetic variation among species, representing just a difference in a single nucleotide. For example, a SNP may result in the replacement of the nucleotide cytosine (C) with the nucleotide thymine (T) in a certain stretch of DNA. They are either small insertions or deletions with several key advantages. SNP markers are more abundant in the genome, more efficient and cost-effective than SSR genotyping. This new sequencing and marker genotyping technology accelerates the pace of genetic diversity research and gains in selection through molecular breeding. A number of EST collections have been used to describe and detect SNPs in maize (*Zea mays* L.) (Ching *et al.*, 2002) and Soybean (*Glycine max* L. Merr.) (Zhu *et al.*, 2003). Lopez *et al.* (2005) has also detected SNPs from ESTs in cassava.

Efficient conservation and utilization of germplasm by breeders depend on the understanding of the extent, nature and structure of the available genetic diversity. There is, therefore, the need to assess and understand the genetic diversity in the cassava accessions been used in the study.

4.1.1 Specific objectives

The objectives of this study were to:

1. Assess the level of genetic diversity among cassava accessions
2. Determine the relationships between the morphological and molecular marker systems
3. Determine whether the cassava accessions would cluster based on their reaction to PPD

4.2 Materials and methods

4.2.1 Source of germplasm

A total of 150 cassava accessions were collected in June, 2010 (Table 4.1). The collection comprised 30 yellow rooted clones from International Institute of Tropical Agriculture (IITA), 13 from International Centre for Tropical Agriculture (CIAT), 76 from Plant Genetic Resources Research Institute (PGRRI), two released varieties from Crops Research Institute (CRI) and 29 accessions from farmers' field in major cassava growing regions where PRA was conducted. The accessions were collected from farmers from the Ashanti, Brong Ahafo and Volta regions in Ghana (Fig. 4.1).

4.2.2 Field establishment

The trial was established at the experimental station of the CSIR-Crops Research Institute, Kumasi, Ghana at 6°41'N, 1°28'W, in the 2010/2011 cropping season. Stem cuttings of about 20 - 25 cm were planted in two rows with 5 plants each per genotype at a distance of 1 m x 1 m between and within rows in a 10x15 alpha lattice design with three replicates. During the experiment, fertilizer was not applied and weeds were constantly controlled.



Fig. 4. 1 Locations in Ghana where cassava accessions were collected in June, 2010.

4.2.3 Morphological characterization

Evaluation was carried out on all the accessions according to the set of morphological descriptors (Table 4.2) for cassava (Fukuda *et al.*, 2010). Data for morphological characterization were taken

at three, six and nine months after planting. Morphological characterization of accessions was based on 25 qualitative traits.

Table 4. 1 List of 150 cassava accessions selected for the study

Accession	Source	Accession	Source	Accession	Source
2B-13	CIAT	01/1371-2	YR	KW 00/095	PGRRI
12B-36	CIAT	94/0006-1	YR	UCC 01/250	PGRRI
12B-38	CIAT	95/0379-2	YR	UCC01/249	PGRRI
2B-26	CIAT	01/1331-2	YR	ADW 00/004	PGRRI
9A-3	CIAT	01/1235	YR	UCC 01/504	PGRRI
12B-54	CIAT	94/0006-2	YR	SW 00/010	PGRRI
2B-116	CIAT	01/1331-1	YR	KW 00/187	PGRRI
5A-15	CIAT	01/1442-2	YR	KW 00/181	PGRRI
2B-51	CIAT	01/0131	YR	ADE 00/107	PGRRI
5B-97	CIAT	01/1649-1	YR	SW 00/050	PGRRI
2B-68	CIAT	01/1610-2	YR	DMA 00/069	PGRRI
2B-63	CIAT	01/1224	YR	DMA 01/004	PGRRI
K25	CRI	94/0006	YR	UCC01/195	PGRRI
TME 11	CRI	90/01554	YR	KW 00/045	PGRRI
DEBOR(NK)	FF	01/1610-2	YR	SW 00/192	PGRRI
AWOROWA	FF	01/1413-1	YR	SW 00/049	PGRRI
NO NAME (3)	FF	01/1797	YR	UCC 01/078	PGRRI
AGRIC	FF	01/1413-2	YR	UCC 01/270	PGRRI
AGBELIFIA	FF	01/1646	YR	AMW 00/006	PGRRI
BANKYHEMAA	FF	01/1412	YR	ADW 00/053	PGRRI
DEBOR	FF	01/1649-2	YR	DMA 00/008	PGRRI
DABODABOO	FF	01/1369	YR	ADE 030/039	PGRRI
AKOSUATUMTUM	FF	01/1442-1	YR	UCC 00/088	PGRRI
OWUDURO(1)	FF	KW 00/101	PGRRI	UCC 00/032	PGRRI
OWUDURO	FF	OFF 00/087	PGRRI	KSI 01/036	PGRRI
KROBO	FF	SW 00/068	PGRRI	BD 96/165	PGRRI
NO NAME (2)	FF	DMA 00/031	PGRRI	UCC 01/092	PGRRI
NKRUWA (AW)	FF	UCC 01/415	PGRRI	OFF 00/019	PGRRI
ESIABAYA	FF	OFF 00/023	PGRRI	AFS 00/043	PGRRI
WCH 009	FF	AFS 00/081	PGRRI	SW 00/152	PGRRI
DEBOR (1)	FF	DMA 00/034	PGRRI	SW 00/006	PGRRI
UCC	FF	BD 96/134	PGRRI	DMA 00/042	PGRRI
ABASAFITAA	FF	KSI 00/126	PGRRI	SW 00/204	PGRRI

Table 4.1 (cont'd) List of 150 cassava accessions selected for the study

Accession	Source	Accession	Source	Accession	Source
TEKBANKYE	FF	OFF 00/029	PGRRI	ADW 00/051	PGRRI
AFISIAFI	FF	WCH 00/011	PGRRI	SW 00/187	PGRRI
HUSHIVI	FF	SW 00/216	PGRRI	BD 96/009	PGRRI
01/0103	YR	UCC 01/209	PGRRI	UCC 01/111	PGRRI
01/1649-3	YR	OFF 01/146	PGRRI	AMW 00/99	PGRRI
01/1371-1	YR	BD 96/093	PGRRI	DMA 00/070	PGRRI
01/1368	YR	UCC 00/003	PGRRI	ADE 00/169	PGRRI
01/1277	YR	UCC01/218	PGRRI	KSI00/179	PGRRI
I00/0093	YR	BD 96/040	PGRRI	OFF 00/058	PGRRI

FF: Farmers' field; YR: Yellow roots; PGRRI: Plants Genetic Resources Research Institute;
CRI: Crops Research Institute

Table 4. 2 Qualitative traits used to characterize the cassava accessions

No.	Trait	No.	Trait
1	Colour of apical leaves (CAL)	14	Growth habit of stem (GHS)
2	Pubescence on apical leaves (PAL)	15	Colour of branches of adult plant (CBP)
3	Leaf retention (LR)	16	Length of stipule (LS)
4	Shape of central leaflet (SCL)	17	Shape of plant (PS)
5	Petiole colour (PC)	18	Extent of root peduncle (ERP)
6	Leaf colour (LC)	19	Root constrictions (RCON)
7	Lobe margins (LM)	20	Root shape (RS)
8	Colour of leaf vein (CLV)	21	External colour of storage root (ECR)
9	Orientation of petiole (OP)	22	Colour of root pulp (CRP)
10	Prominence of foliar scars (PFS)	23	Colour of root cortex (CRC)
11	Colour of stem cortex (CSC)	24	Ease of peeling (EP)
12	Colour of stem epidermis (CSEP)	25	Texture of root epidermis (TRE)
13	Colour of stem exterior (CSEX)		

Source: Morphological descriptors for cassava according to Fukuda *et al.* (2010).

4.2.4 Molecular characterization

4.2.4.1 DNA extraction

Genomic DNA was extracted using standard procedures according to Egnin *et al.* (1998) with slight modifications. Freshly harvested apical leaves of about 200 mg of each accession were ground in liquid nitrogen into a fine powder. Eight hundred microliters of extraction buffer (50 mM Tris HCL (pH 8.0), 300 mM NaCl, 20 mM EDTA, 20% PVP, 1.5% Sarkocine and 0.1 g/L Na₂S₂O₂) was used to lyse nuclear membranes. Proteins and polysaccharides were precipitated by adding 400 µl of 5 M potassium acetate (instead of 800 µl of phenol chloroform isomyl alcohol) as used by Egnin *et al.* (1998) and the samples centrifuged at 13,000 rpm for 10 minutes. RNA was removed by adding 4 µl RNase A (10 mg/ml), and incubated at 37°C for thirty minutes. DNA was precipitated using 700 µl of ice-cold isopropanol and centrifuged at 13,000 rpm for ten minutes. Eighty percent ethanol was used to wash DNA and centrifuged at 13,000 rpm for five minutes. Ethanol was discarded and DNA pellets were air-dried at room temperature. DNA pellets were resuspended in 200 µl 1X TE (Tris-ethylenediaminetetracetic acid) buffer after which quality of DNA was determined on 0.8% (w/v) ethidium bromide stained agarose gel. The purity and quantification of DNA was determined by measuring the absorbance at 260 nm (A₂₆₀) and 280 nm (A₂₈₀) with a spectrophotometer (Biochrom Libra S12).

4.2.4.2 SNP genotyping

KASPar technology was employed for SNP genotyping at the KBiosciences laboratories (United Kingdom). One hundred and ninety five SNP markers developed from the GCP project were

used (Appendix 4.1) for the genotyping. The genotyping assay for KASPar consisted of two reagent components (KASP Primer mix and KASP Master mix) plus the DNA sample. The KASP Primer mix was made up of two allele-specific forward primers and one common reverse primer. The KASP Master mix contained the FAM and HEX specific FRET cassette system, Taq polymerase, dNTP's, 5-carboxy-X-rhodamine, succinimidyl ester (ROX) and $MgCl_2$ in an optimized buffer solution. The KASP Primer mix was combined with the KASP Master mix and added to the DNA samples (5 - 50 ng) to be genotyped (Table 4.3) in a 96-well plate and sealed using the KubeTM heat-based sealer. The reaction was carried out in a standard thermal cycler with conditions comprising two temperatures, rather than the traditional three steps (Table 4.4). After the completion of the PCR run, the scan results were read and allele call was generated from the KlusterCaller software. SNP markers that were monomorphic or with more than 20% missing data were considered non informative, and were removed from further analysis. A total of 100 SNP markers were retained for genetic diversity analysis.

Table 4. 3 Constituent reagent volumes for making KASP genotyping mix

Component	Volume (ul)
DNA	5
Mastermix	5
Primermix	0.14
Total reaction volume	10

Table 4. 4 The KASP thermal cycling program

Temperature/Time	Number of cycles
94°C for 20 seconds	Hot-start activation
94°C for 20 seconds 61-55°C for 60 seconds (dropping 0.6°C per cycle)	10 cycles
94°C for 20 seconds 55°C for 60 seconds	26 cycles

4.3 Data analysis

The 25 qualitative traits were recorded in the 150 cassava accessions. Descriptive statistics was performed using XLSTAT (2013) and MINITAB 15 programs. The descriptive statistics generated were used to construct frequency graphs for each of the qualitative traits. Factor analysis was performed on the 25 traits to determine which trait contributed highest to the variability. The subset of the traits generated by the factor analysis was subjected to principal component analysis (PCA). PCA was employed to determine the percentage contribution of each trait to total genetic variation using GENSTAT version 12 and MINITAB 15 program. Agglomerative hierarchical clustering was performed on the Euclidean distance matrix utilizing average linkage. The Jaccard's similarity coefficients for pairwise comparisons were calculated and the similarity matrix generated was used to construct a dendrogram using the Unweighted Pair Group Method Average (UPGMA); (Sneath and Sokal, 1973). The genetic analysis package PowerMarker version 3.0 (Liu and Muse, 2005) was used to generate the following statistics: number of alleles per locus, major allele frequency, observed heterozygosity (H_O), expected heterozygosity (H_E) and polymorphic information content (PIC) (Bostein and White, 1980). PIC values were calculated with the equation: $PIC = 1 - \sum P_i^2 - \sum 2P_i^2 P_j^2$

Where: ΣP_i^2 = sum of each squared *i*th haplotype frequency.

PowerMarker was used to calculate the genetic distances among the genotypes using the Euclidean method. A neighbour-joining (NJ) algorithm (Nei, 1973) was used to construct a dendrogram from the distance matrix using the MEGA 5.2 software (Tamura *et al.*, 2007) embedded in PowerMarker. A Mantel matrix test (Mantel, 1967) was carried out to compare the extent of agreement between dendrograms derived from morphological and molecular data using the distance matrices.

4.4 Results

4.4.1 Genetic diversity analysis at the morphological level

4.4.1.1 Descriptive analysis of the qualitative traits

Frequency distribution of the qualitative traits is presented in Fig. 4.2. Genetic variability was observed among the 150 cassava accessions for most of the variables except lobe margin and stem growth habit. One hundred and forty seven (97.3%) accessions showed a smooth lobe margin while the winding type was observed in only three genotypes (2.7%). One hundred and forty seven (97.3%) accessions also showed an upright growth habit, while zigzag growth type was present in only three genotypes (2.7%). All the possible phenotypic classes described by Fukuda *et al.* (2010) were observed for most traits, except for leaf shape, petiole colour, developed leaf colour, colour of leaf vein and colour of root pulp. Root flesh colour is a trait with great commercial importance for both consumers and processors. The yellow rooted genotypes have higher concentrations of beta carotene, however, only 10% of the yellow types were observed among the genotypes. The main colours for root flesh among the accessions were white (14.3%) and cream (75.7%).

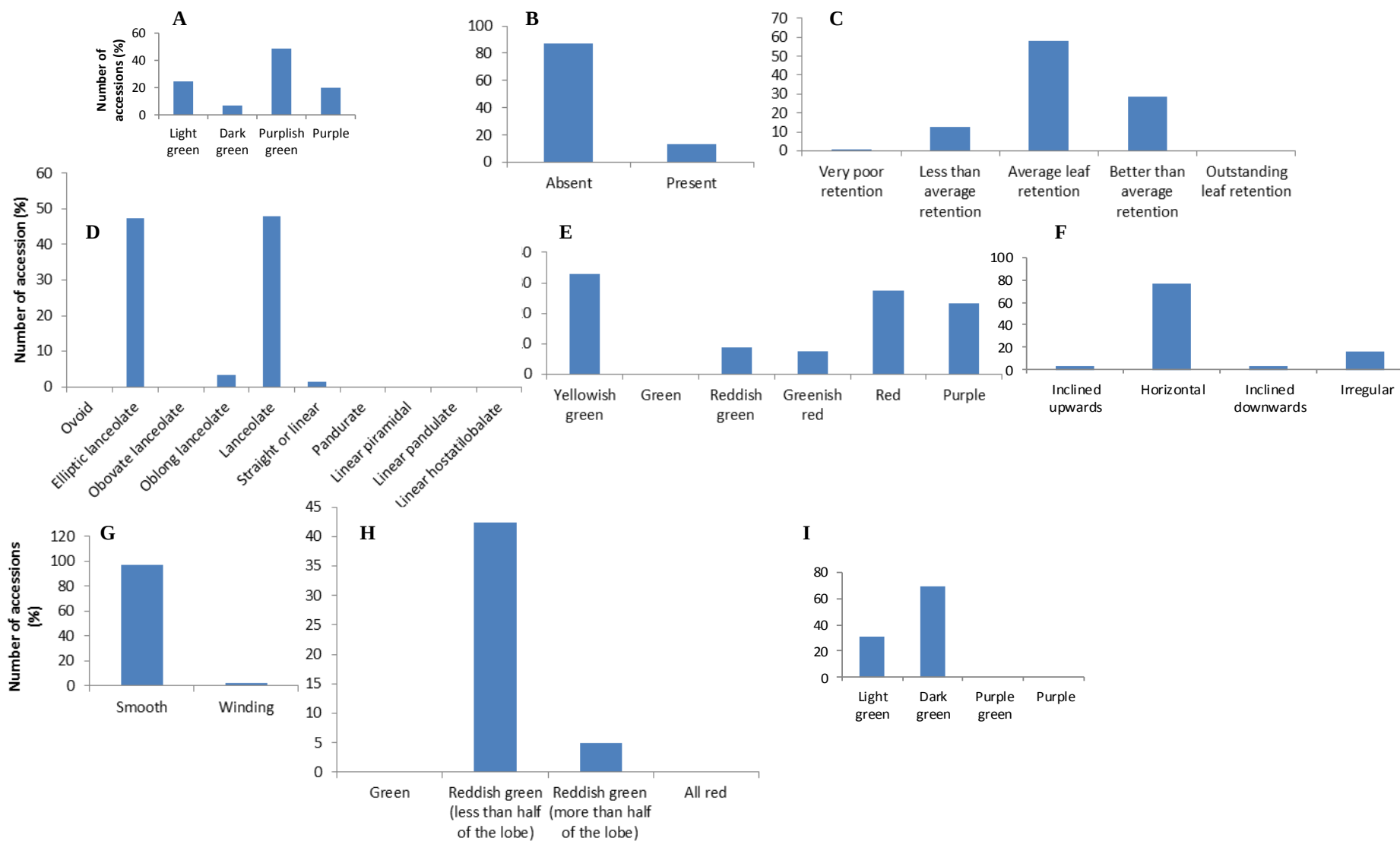


Fig. 4. 2 Morphological descriptors (Fukuda *et al.*, 2010) evaluated in cassava accessions

A: apical leaf color; B: pubescence on apical leaves; C: leaf retention; D: shape of central leaflet; E: petiole colour; F: leaf colour; G: lobe margins; I: colour of leaf vein H: orientation of petiole

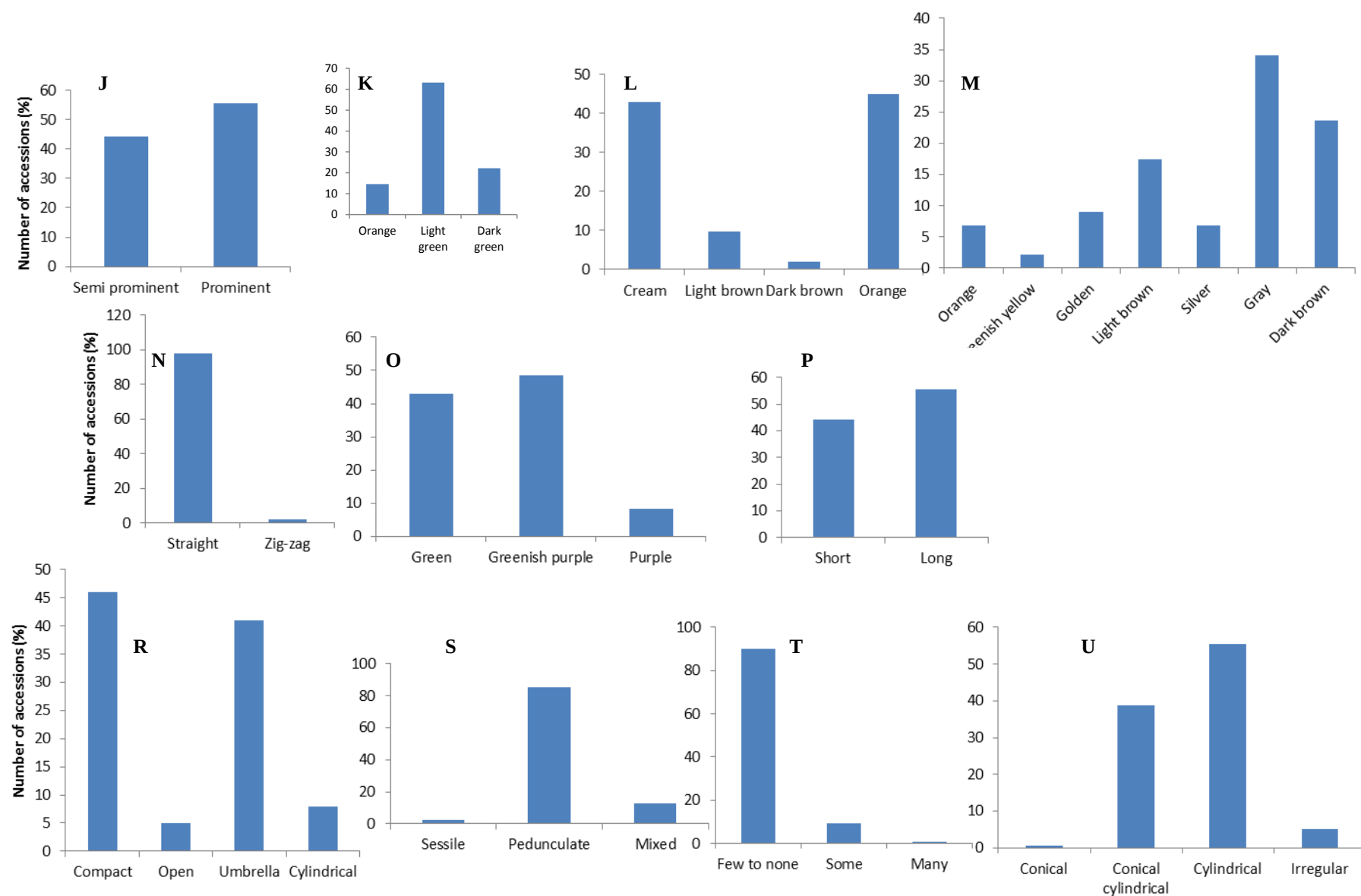


Fig. 4.2 (cont'd) Morphological descriptors (Fukuda *et al.*, 2010) evaluated in cassava genotypes. J: prominence of foliar scars; K: colour of stem cortex; L: colour of stem epidermis; M: colour of stem exterior; N: growth habit of stem; O: colour of end branches of adult plant; P: length of stipules; R: shape of plant; S: extent of root peduncle; T: root constrictions; U: root shape

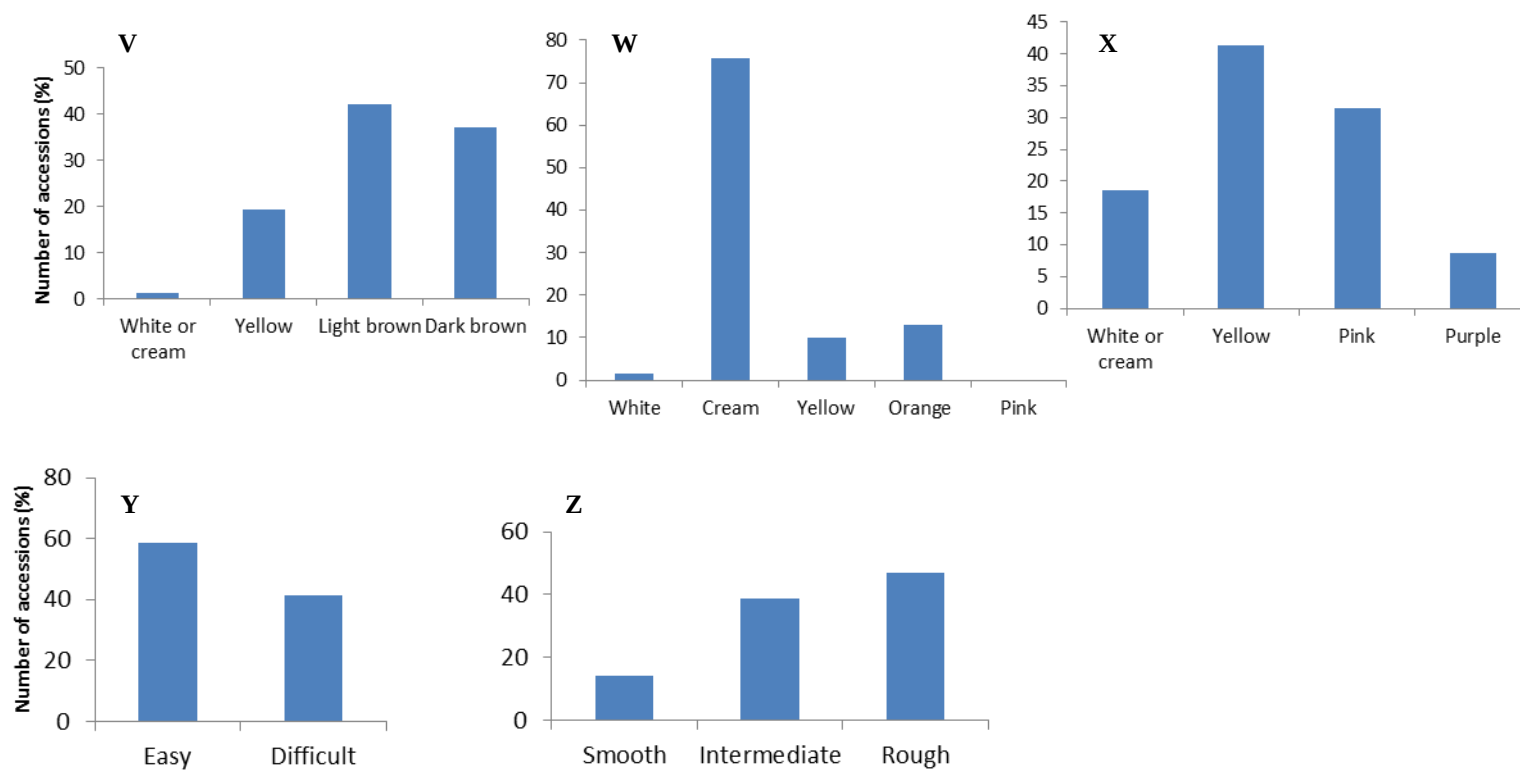


Fig. 4.2 (cont'd) Morphological descriptors (Fukuda *et al.*, 2010) evaluated in cassava genotypes. V: external colour of storage root; W: colour of root pulp; X: colour of root cortex; Y: ease of peeling; Z: texture of root epidermis

4.4.1.2 Factor analysis

The factor analysis was carried out for the 25 qualitative traits to identify the combination of traits contributing to maximum variability (Table 4.5). The first factor had a contributing factor loading of 14.3%, which was contributed by the traits colour of root pulp (CRP), leaf retention (LR), plant shape (PS), color of petiole (PC) and colour of leaf vein (CLV). The second factor had a maximum factor loading of 8.5% contributed by the traits colour of root cortex (CRC), color of apical leaf (CAL), colour of stem exterior (CSEX) and colour of branched adult plant (CBP). The third factor had a contributing factor loading of 7.3%. The variability was contributed by the traits orientation of petiole (OP), length of stipule (LS), colour of stem epidermis (CSEP) and leaf colour (LC). The fourth factor had a contributing factor loading of 6.5% contributed by pubescence of apical leaf (PAL), colour of stem cortex (CRC) and texture of root epidermis (TRE). Among these 25 variables, root shape had maximum variation (49.9%) and leaf vein colour (CLV) had the least variation (20%), whereas extent of root peduncle (ERP) and root shape (RS) had contributing variation of 47.5% and 49.9%, respectively, in total variation of 43.6%.

Table 4. 5 Factor analysis of the 25 qualitative traits

Variable	Factor1	Factor2	Factor3	Factor4	Communality	% Variance
CAL	-0.353	0.644	-0.149	-0.125	0.673	32.7
PAL	-0.190	-0.159	0.263	0.440	0.555	44.5
LR	-0.478	0.364	-0.076	-0.241	0.545	45.5
SCL	-0.373	-0.215	0.122	0.009	0.552	44.8
PC	0.782	0.225	0.121	0.079	0.793	20.7
LC	-0.518	0.035	-0.570	-0.003	0.744	25.6
LBM	-0.254	0.038	0.235	-0.284	0.661	33.9
CLV	0.706	0.364	0.212	-0.004	0.800	20.0
OP	-0.233	-0.042	0.599	-0.057	0.534	46.6
PFS	0.404	0.077	-0.165	0.147	0.559	44.1
CSC	-0.135	-0.285	-0.292	0.436	0.601	39.9
CSEP	0.203	-0.025	-0.592	0.003	0.615	38.5
CSEX	-0.107	-0.430	-0.359	0.402	0.692	30.8
GHS	-0.146	0.146	-0.111	0.163	0.612	38.8
CBP	0.456	0.620	-0.053	-0.162	0.748	25.2
LS	0.214	0.241	-0.559	-0.082	0.585	41.5
PS	0.430	-0.321	-0.119	-0.077	0.703	29.7
ERP	-0.135	-0.310	0.079	0.167	0.525	47.5
RCON	-0.121	0.244	-0.078	-0.139	0.642	35.8
RS	0.071	-0.173	-0.337	-0.120	0.501	49.9
ECR	0.420	-0.368	-0.072	-0.583	0.701	29.9
CRP	-0.534	0.131	0.019	-0.199	0.527	47.3
CRC	0.362	-0.402	0.102	-0.375	0.558	44.2
EP	0.000	0.193	-0.241	-0.068	0.618	38.2
TRE	0.331	-0.495	-0.061	-0.522	0.661	33.9
Eigenvalue	4.3	2.5	2.2	1.9		
% Variance	17.2	10.0	8.8	7.6		
% Cumulative variance	17.2	27.2	36.0	43.6		

4.4.1.3 Principal component analysis

The principal components, eigenvalues and percentage variation of the principal component analysis and correlation matrix is presented in Tables 4.6 and 4.7. Variables with more than 30% variation were selected from the factor analysis to perform the principal component analysis to

assess the relative importance of the 19 selected variables. These variables were root shape (RS), extent of root peduncle (ERP), colour of root pulp (CRP), orientation of petiole (OP), leaf retention (LR), shape of central leaf (SCL), pubescence on apical leaf (PAL), colour of root cortex (CRC), prominence of foliar scar (PFS), length of stipule (LS), colour of stem cortex (CSC), growth habit of stem (GHS), colour of stem epidermis (CSEP), ease of peeling (EP), root constrictions (RCON), texture of root epidermis (TRE), lobe margin (LBM), colour of apical leaf (CAL) and colour of stem exterior (CSEX). These variables were not correlated with each other. This indicates that, the variables did not present any redundancy in the measurements. Seven principal components were identified and accounted for 62.3% of the total variation among the accessions. The first PC axis with an eigenvalue of 2.53 accounted for 13.3 % of the total variation whereas the second, third and the forth PC axes with eigenvalues of 1.99, 1.77 and 1.61 accounted for 10.5%, 9.4% and 8.5% of the total variation respectively. The fifth, sixth and seventh PC axes with eigenvalues of 1.46, 1.32 and 1.21 accounted for 7.7%, 7.0% and 5.9% of the total variation respectively.

The first principal component with reference to its high factor loadings was positively associated with traits such as leaf retention and colour of apical leaf. The second PC was associated with leaf characteristics (colour of apical leaf, pubescence on apical leaf and leaf retention); the third with growth habit of plant, orientation of petiole as well as root constriction while the forth was associated with traits related to storage root characteristics (colour of root pulp and root constriction) and leaf lobe margins. The fifth PC with high factor loadings was associated with leaf characteristics (pubescence on apical leaf and shape of central leaf) and growth habit of the plant while the sixth PC was also associated with growth habit of plant.

Table 4. 6 Principal component analysis showing the contribution of qualitative traits to total variation among the cassava accessions

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7
CAL	0.293	0.394	0.181	-0.094	-0.053	0.084	-0.237
PAL	0.144	-0.316	0.131	-0.178	0.278	0.057	0.064
LR	0.347	0.256	0.061	0.071	-0.168	-0.150	0.161
SCL	0.182	-0.204	0.053	-0.006	-0.349	0.337	-0.213
LBM	0.279	-0.065	-0.054	0.353	-0.031	-0.104	-0.224
OP	0.280	-0.206	-0.290	0.035	0.097	-0.172	0.185
PFS	-0.250	0.148	-0.096	-0.129	0.131	-0.430	-0.156
CSC	-0.107	-0.284	0.430	-0.205	-0.099	-0.127	0.015
CSEP	-0.255	0.180	0.312	0.220	-0.138	0.193	-0.117
CSEX	-0.185	-0.317	0.388	-0.067	-0.176	-0.144	0.347
GHS	0.081	0.047	0.316	0.042	0.275	0.377	0.246
LS	-0.237	0.334	0.140	-0.157	-0.200	-0.039	-0.216
ERP	0.042	-0.288	0.140	-0.016	-0.106	-0.003	-0.435
RCON	0.112	0.077	0.243	0.405	0.225	0.029	-0.071
RS	-0.148	-0.052	0.225	0.337	0.116	-0.153	-0.182
CRP	0.360	-0.008	0.218	0.277	-0.078	-0.148	0.161
CRC	-0.250	-0.171	-0.251	0.284	-0.127	0.225	0.016
EP	-0.060	0.282	-0.019	-0.020	-0.295	0.192	0.475
TRE	-0.231	-0.141	-0.208	0.409	-0.240	0.108	0.061
Eigenvalue	2.535	1.996	1.777	1.612	1.458	1.324	1.121
%Variation	12.1	9.5	8.5	7.7	6.9	6.3	5.4
% Cumulative variance	12.1	21.6	30.1	37.8	44.7	51.0	56.4

Table 4. 7 Correlation matrix of the 19 qualitative traits used to characterize the 150 cassava accessions

Variable	CAL	PAL	LR	SCL	LBM	OP	PFS	CSC	CSEP	CSEX	GHS	LS	ERP	RCON	RS	CRP	CRC	EP
CAL	-																	
PAL	0.052	-																
LR	0.348	0.023	-															
SCL	0.042	0.110		-														
LBM	0.099	0.045	0.165	0.160	-													
OP	0.044	0.113	0.120	0.021	0.262	-												
PFS	0.167	0.137	0.056	0.175	0.139	-0.059	-											
CSC	0.014	0.115	0.144	0.087	0.113	-0.056	0.062	-										
CSEP	0.031	0.140	0.032	0.026	0.090	-0.316	0.039	0.076	-									
CSEX	0.311	0.094	0.155	0.033	0.072	-0.117	-0.071	0.310	0.124	-								
GHS	0.099	0.144	0.066	0.004	0.015	-0.050	-0.139	0.089	0.127	0.003	-							
LS	0.172	0.195	0.015	0.187	0.143	-0.320	0.210	0.060	0.231	0.047	-0.019	-						
ERP	0.140	0.132	0.135	0.126	0.118	-0.008	-0.038	0.123	0.022	0.120	-0.030	-0.100	-					
RCON	0.127	0.047	0.025	0.026	0.148	0.015	-0.084	-0.068	0.136	0.005	0.148	-0.094	0.032	-				
RS	0.062	0.059	0.070	0.073	0.033	-0.101	0.019	0.120	0.150	0.094	0.072	0.011	-0.011	0.147	-			
CRP	0.177	0.085	0.379	0.085	0.288	0.087	-0.215	0.040	0.142	0.018	0.115	-0.145	0.087	0.264	0.026	-		
CRC	0.327	0.117	0.207	0.003	0.039	0.001	-0.012	-0.017	0.056	0.008	-0.039	0.065	-0.036	-0.023	0.069	-0.138	-	
EP	0.076	0.260	0.048	0.037	0.103	-0.090	0.007	-0.157	0.134	0.022	0.022	0.135	-0.038	-0.034	0.158	-0.010	0.038	-
TRE	-0.26	-0.09	-0.13	0.034	0.033	-0.047	-0.032	-0.061	0.168	0.025	-0.142	-0.024	-0.044	-0.077	0.151	-0.061	0.319	0.016

4.4.1.4 Hierarchical clustering analysis

The dendrogram generated from the similarity matrix is presented in Figure 4.3. The genetic similarity ranged from zero to one with a mean similarity of 0.54, suggesting a moderate to high diversity among the accessions. One hundred and thirty four cassava accessions were grouped into five distinct clusters at 0.64 similarity. The colour of the stem exterior grouped the accessions into the different clusters. Accessions in clusters I and II had light brown stem colour while those in clusters III, and IV had gray and golden stems respectively. Cluster size varied from one to eighty. The largest clusters, cluster I had 60% of the accessions followed by clusters III, II and IV with 25%, 12% and 3% of the accessions respectively. Cluster V (01/1331-1) had one accession. This cluster being an outlier formed its own group probably because of its peculiar winding lobe margin, some constrictions on the roots with orange stem and root pulp.

Within cluster I, most of the accessions had straight growth habit of the stem, long stipules and difficult cassava peels while accessions in cluster II had purplish green apical leaf. With cluster III, accessions had dark greenish stem cortex with prominent foliar scars while cluster IV had accessions with few constrictions on the roots, purplish root cortex and rough textured root epidermis. The highest similarity was recorded between WCH009 (WN) and AMW00/006 (0.992) and the lowest was between Dabodabo and OFF01/146 (0.223). This is clearly seen in the dendrogram as accessions WCH009 (WN) and AMW00/006 were found in one cluster (cluster I) while accessions Dabodabo and OFF01/146 were found in clusters I and IV respectively. All the cassava accessions with the exception of those collected from CSIR-Crops Research Institute did not cluster based on their geographical locations. TME 11 and K25 from CSIR-Crops Research Institute grouped in cluster I while CIAT materials grouped in clusters I

and III. The yellow rooted clones also grouped across clusters I, II and III. Collections from farmers' fields and PGRRI also grouped across all the five clusters. Cassava accession did not also cluster based on their reaction to PPD.

4.4.2 Genetic diversity analysis at the molecular level

A total of 195 SNP markers were used to assess the genetic diversity among the cassava accessions. Of these markers, 8 were monomorphic (201-SNP, 826-SNP, 2423-SNP, 2526-SNP, Me_v4_MEF_c_2938, Me_v4_MEF_c_2317, Me_v4_MEF_c_1357 and Me_v4_MEF_c_1828) and 187 were polymorphic. SNP markers that were monomorphic or with more than 20% missing data were considered non informative, and were removed from further analysis, as a result, only 100 SNP markers were retained for genetic diversity analysis.

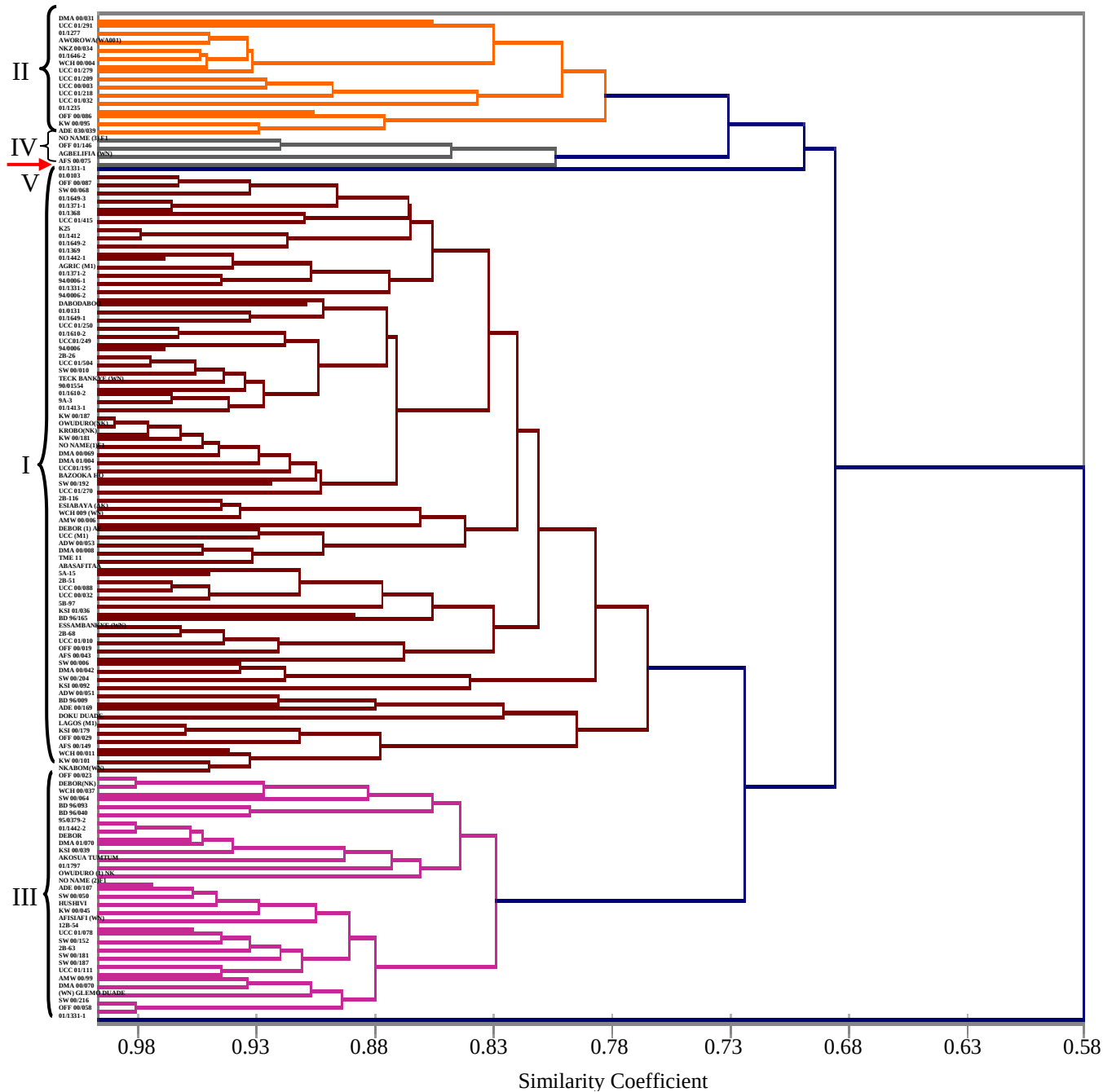


Fig. 4. 3 Dendrogram of cassava accessions based on the nineteen selected qualitative traits

Table 4.8 Dendrogram groupings of the cassava accessions based on qualitative traits

Cluster groups					
	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
01/0103	BAZOOKA HO	DMA 00/031	OFF 00/023	No Name (3) F1	01/1331-1
OFF 00/087	SW 00/192	UCC 01/291	DEBOR(NK)	OFF 01/146	
SW 00/068	UCC 01/270	01/1277	WCH 00/037	Agbelifia (WN)	
01/1649-3	2B-116	Aworowa(001)	SW 00/064	AFS 00/075	
01/1371-1	ESIABAYA (AK)	NKZ 00/034	BD 96/093		
01/1368	WCH 009 (WN)	01/1646-2	BD 96/040		
UCC 01/415	AMW 00/006	WCH 00/004	95/0379-2		
K25	DEBOR (1) AF	UCC 01/279	01/1442-2		
01/1412	UCC (M1)	UCC 01/209	DEBOR		
01/1649-2	ADW 00/053	UCC 00/003	DMA 01/070		
01/1369	DMA 00/008	UCC 01/218	KSI 00/039		
01/1442-1	TME 11	UCC 01/032	AKOSUA TUMTUM		
AGRIC (M1)	ABASAFITAA	01/1235	01/1797		
01/1371-2	5A-15	OFF 00/086	Owuduro (1) NK		
94/0006-1	2B-51	KW 00/095	NO NAME (2)F1		
01/1331-2	UCC 00/088	ADE 030/039	ADE 00/107		
94/0006-2	UCC 00/032		SW 00/050		
DABODABOO	5B-97		HUSHIVI		
01/0131	KSI 01/036		KW 00/045		
01/1649-1	BD 96/165		AFISIAFI (WN)		
UCC 01/250	Essambankye (WN)		12B-54		
01/1610-2	2B-68		UCC 01/078		
UCC01/249	UCC 01/010		SW 00/152		
94/0006	OFF 00/019		2B-63		
2B-26	AFS 00/043		SW 00/181		
UCC 01/504	SW 00/006		SW 00/187		
SW 00/010	DMA 00/042		UCC 01/111		
TECK BANKYE (WN)	SW 00/204		AMW 00/99		
90/01554	KSI 00/092		DMA 00/070		
01/1610-2	ADW 00/051		(WN) GLEMO		
9A-3	BD 96/009		DUADE		
01/1413-1	ADE 00/169		SW 00/216		
KW 00/187	DOKU DUADE		OFF 00/058		
OWUDURO(NK)	LAGOS (M1)				
KROBO(NK)	KSI 00/179				
KW 00/181	OFF 00/029				
NO NAME(1)F1	AFS 00/149				

4.4.2.1 Genetic diversity analysis using SNP markers

Summary statistics for number of alleles, observed and expected heterozygosity and polymorphic information content is presented in Table 4.8. A total of 200 alleles with an average of 2 alleles per locus were observed. The expected heterozygosity was lowest in 6922-SNP (0.062) and highest in Me_v4_MEF_c_2758 and Me_v4_MEF_c_2726 (0.5) with a mean of 0.34. The observed heterozygosity per individual SNP locus ranged from zero in Me_ve_MEF_c_2905 to 0.867 at locus Me_ve_MEF_c_2409 with a mean of 0.37. With the exception of marker Me_ve_MEF_c_2905, all other markers were heterozygous. The mean observed heterozygosity was higher than the expected heterozygosity. This tends to substantiate the heterozygote nature of most of the accessions and the fact that cassava is largely cross-pollinated. However, only 38% of the markers had observed heterozygosity values that were greater than their corresponding expected heterozygosity values. The major allele frequency of all the markers with the exception of 6922-SNP (0.968) was generally below 0.95, indicating that they were all polymorphic. PIC values ranged from 0.06 (6922-SNP) to 0.375 in eight markers with an average of 0.297 ± 0.0769 . The higher the PIC value, the more informative is the marker, hence, Me_v4_MEF_c_2909, Me_v4_MEF_c_1018, Me_v4_MEF_c_2807, Me_v4_MEF_c_2448, Me_v4_MEF_c_2286, Me_v4_MEF_c_2758, Me_v4_MEF_c_2726, 6889-SNP and 2300-SNP were found to be highly informative.

4.4.2.2 Clustering analysis

The dendrogram generated is presented in Fig. 4.4. The dendrogram revealed three main clusters with five distinct sub-clusters with a mean similarity of 0.42 suggesting a high diversity among

the accessions. Sub-clusters A, B and C were found in cluster II, while sub-clusters D and E were found in cluster III. Sub-cluster size varied from four to 77 accessions. The largest sub-clusters, C and D had 33% each of the accessions, while sub-clusters A and E had 6% each of the accessions and sub-cluster B had 29% of the accessions. The lowest similarity level (0.23) was observed between accessions 95/0379-2 and TME 11 and the highest similarity (0.90) was found between UCC00/003 and 01/1235; Debor and DMA 01/070; AFS00/043 and SW00/204.

Table 4.9 Summary statistics of genetic variation using 100 SNP markers among 150 cassava accessions

SNP marker	MAF	Na	H _e	H _o	PIC
1920-SNP	0.555	2	0.494	0.483	0.372
2216-SNP	0.879	2	0.213	0.394	0.190
2257-SNP	0.728	2	0.396	0.316	0.318
2300-SNP	0.508	2	0.500	0.552	0.375
2496-SNP	0.894	2	0.190	0.350	0.172
327-SNP	0.896	2	0.186	0.308	0.169
6331-SNP	0.839	2	0.270	0.335	0.234
6453-SNP	0.719	2	0.404	0.364	0.322
6464-SNP	0.916	2	0.154	0.136	0.142
6630-SNP	0.905	2	0.172	0.274	0.157
6780-SNP	0.627	2	0.468	0.356	0.358
6889-SNP	0.509	2	0.500	0.439	0.375
6912-SNP	0.900	2	0.180	0.300	0.164
6922-SNP	0.968	2	0.062	0.178	0.060
7138-SNP	0.798	2	0.323	0.341	0.271
7239-SNP	0.730	2	0.394	0.331	0.317
7259-SNP	0.893	2	0.190	0.364	0.172
7434-SNP	0.869	2	0.228	0.263	0.202
958-SNP	0.871	2	0.225	0.394	0.200
Me_v4_MEF_c_1018	0.513	2	0.500	0.392	0.375
Me_v4_MEF_c_1175	0.827	2	0.287	0.266	0.246
Me_v4_MEF_c_1183	0.525	2	0.499	0.587	0.374
Me_v4_MEF_c_1220	0.829	2	0.283	0.179	0.243
Me_v4_MEF_c_1246	0.705	2	0.416	0.409	0.330

Table 4.9 (cont'd) Summary statistics of genetic variation using 100 SNP markers among 150 cassava accessions

SNP marker	MAF	N _a	H _e	H _o	PIC
Me_v4_MEF_c_1977	0.655	2	0.452	0.362	0.350
Me_v4_MEF_c_2034	0.555	2	0.494	0.487	0.372
Me_v4_MEF_c_2043	0.864	2	0.236	0.223	0.208
Me_v4_MEF_c_2051	0.746	2	0.379	0.608	0.307
Me_v4_MEF_c_2120	0.852	2	0.252	0.152	0.220
Me_v4_MEF_c_2124	0.630	2	0.466	0.259	0.358
Me_v4_MEF_c_2195	0.811	2	0.306	0.245	0.259
Me_v4_MEF_c_2226	0.831	2	0.281	0.323	0.242
Me_v4_MEF_c_2236	0.746	2	0.379	0.358	0.307
Me_v4_MEF_c_2286	0.512	2	0.500	0.621	0.375
Me_v4_MEF_c_2337	0.644	2	0.458	0.441	0.353
Me_v4_MEF_c_2363	0.599	2	0.480	0.571	0.365
Me_v4_MEF_c_2366	0.545	2	0.496	0.569	0.373
Me_v4_MEF_c_2384	0.618	2	0.472	0.386	0.361
Me_v4_MEF_c_2402	0.631	2	0.465	0.398	0.357
Me_v4_MEF_c_2409	0.567	2	0.491	0.867	0.371
Me_v4_MEF_c_2419	0.562	2	0.492	0.239	0.371
Me_v4_MEF_c_2437	0.786	2	0.336	0.321	0.280
Me_v4_MEF_c_2447	0.765	2	0.361	0.252	0.295
Me_v4_MEF_c_2448	0.512	2	0.500	0.434	0.375
Me_v4_MEF_c_2456	0.766	2	0.359	0.414	0.294
Me_v4_MEF_c_2486	0.793	2	0.328	0.367	0.274
Me_v4_MEF_c_2510	0.867	2	0.231	0.233	0.204
Me_v4_MEF_c_2524	0.800	2	0.320	0.336	0.269
Me_v4_MEF_c_2552	0.640	2	0.461	0.404	0.355
Me_v4_MEF_c_2562	0.554	2	0.494	0.397	0.372
Me_v4_MEF_c_2653	0.648	2	0.456	0.575	0.352
Me_v4_MEF_c_2726	0.500	2	0.500	0.290	0.375
Me_v4_MEF_c_2748	0.902	2	0.177	0.151	0.162
Me_v4_MEF_c_2758	0.504	2	0.500	0.491	0.375
Me_v4_MEF_c_2801	0.508	2	0.500	0.411	0.375
Me_v4_MEF_c_2851	0.632	2	0.465	0.421	0.357
Me_v4_MEF_c_2873	0.661	2	0.448	0.278	0.348
Me_v4_MEF_c_2888	0.877	2	0.216	0.395	0.192
Me_v4_MEF_c_2905	0.915	2	0.156	0.000	0.144
Me_v4_MEF_c_2909	0.517	2	0.499	0.210	0.375
Me_v4_MEF_c_2990	0.638	2	0.462	0.742	0.355
Me_v4_MEF_c_3057	0.934	2	0.124	0.097	0.116
Me_v4_MEF_c_3070	0.578	2	0.488	0.359	0.369

Table 4.9 (cont'd) Summary statistics of genetic variation using 100 SNP markers among 150 cassava accessions

SNP marker	MAF	N _a	H _e	H _o	PIC
Me_v4_MEF_c_3081	0.629	2	0.467	0.403	0.358
Me_v4_MEF_c_1278	0.726	2	0.398	0.336	0.319
Me_v4_MEF_c_1320	0.799	2	0.321	0.320	0.269
Me_v4_MEF_c_1387	0.789	2	0.333	0.316	0.278
Me_v4_MEF_c_1447	0.547	2	0.496	0.388	0.373
Me_v4_MEF_c_1527	0.581	2	0.487	0.301	0.368
Me_v4_MEF_c_1566	0.586	2	0.485	0.375	0.368
Me_v4_MEF_c_1637	0.881	2	0.210	0.150	0.188
Me_v4_MEF_c_1645	0.750	2	0.375	0.347	0.305
Me_v4_MEF_c_1679	0.720	2	0.404	0.447	0.322
Me_v4_MEF_c_1892	0.909	2	0.165	0.187	0.151
Me_v4_MEF_c_1919	0.810	2	0.308	0.354	0.261
Me_v4_MEF_c_1940	0.626	2	0.468	0.513	0.359
Me_v4_MEF_c_1945	0.675	2	0.439	0.154	0.342
Me_v4_MEF_c_1947	0.860	2	0.241	0.232	0.212
Me_v4_MEF_c_1958	0.840	2	0.268	0.270	0.232
Me_v4_MEF_c_3094	0.734	2	0.391	0.385	0.314
Me_v4_MEF_c_3120	0.659	2	0.451	0.587	0.349
Me_v4_MEF_c_3131	0.768	2	0.356	0.318	0.293
Me_v4_MEF_c_3142	0.772	2	0.353	0.250	0.290
Me_v4_MEF_c_3155	0.695	2	0.424	0.406	0.334
Me_v4_MEF_c_3195	0.822	2	0.293	0.303	0.250
Me_v4_MEF_c_3197	0.652	2	0.454	0.271	0.351
Me_v4_MEF_c_3242	0.568	2	0.491	0.355	0.370
Me_v4_MEF_c_3310	0.573	2	0.489	0.366	0.370
Me_v4_MEF_c_3336	0.768	2	0.357	0.419	0.293
Me_v4_MEF_c_3338	0.722	2	0.402	0.278	0.321
Me_v4_MEF_c_3343	0.550	2	0.495	0.481	0.372
Me_v4_MEF_c_3356	0.625	2	0.469	0.233	0.359
Me_v4_MEF_c_3361	0.817	2	0.298	0.354	0.254
Me_v4_MEF_c_3376	0.817	2	0.299	0.333	0.254
Minimum	0.500		0.062	0.000	0.060
Maximum	0.968		0.500	0.867	0.375
Mean	0.713	2	0.341	0.372	0.297
STD^a	0.132		0.116	0.135	0.077
95% CI	0.021		0.019	0.022	0.012

MAF: major allele frequency; N_a: number of alleles; H_e: expected heterozygosity; H_o: observed heterozygosity; PIC: Polymorphic information content; a: Standard deviation with 95% confidence interval

All the cassava accessions did not cluster based on their geographical locations neither did they cluster based on their reaction to PPD. Accessions from CRI and the yellow rooted clones grouped across clusters II and III while collections from farmers' fields, CIAT and PGRRI also grouped across all the three clusters.

4.4.2.3 Comparison between morphological and molecular data

Accessions grouped into five distinct clusters based on the morphological data while they grouped into three main clusters with five distinct sub-clusters based on the molecular analysis. Both systems showed different accessions for lowest and highest similarities. With the morphological analysis, the lowest similarity was between Dabodabo and OFF01/146 and the highest similarity was recorded between WCH009 (WN) and AMW00/006. The lowest similarity was observed between 95/0379-2 and TME 11 and the highest similarity were found between UCC00/003 and 01/1235 for the molecular analysis. The genetic similarity ranged from 0.22 to 0.99 in the morphological analysis and 0.23 to 0.90 in the molecular analysis.

Despite these discrepancies, there were similar groupings although there were disagreements between the two dendrograms. Related accessions were grouped in the same cluster while unrelated accessions were grouped in separate clusters. BD96/093 and BD96/040 accessions which were morphologically similar (cluster III) were also observed to be genetically similar as they were grouped in the same cluster (cluster II). Similarly, accessions SW00/204 and AMW00/099 that appeared to be similar in molecular analysis (cluster II) had different characteristics and were considered as morphologically different hence, clustered into different clusters (clusters I and III). This shows the influence of environment on the phenotype. To

compare the extent of agreement between the two dendrograms, the distance matrices generated were compared using the Mantel matrix test. A significant positive correlation was found between the two data sets ($r = 0.38$; $p < 0.001$), but the correlation was rather weak. This could perhaps explain why the morphological and molecular analysis showed different accessions for both lowest and highest similarities.

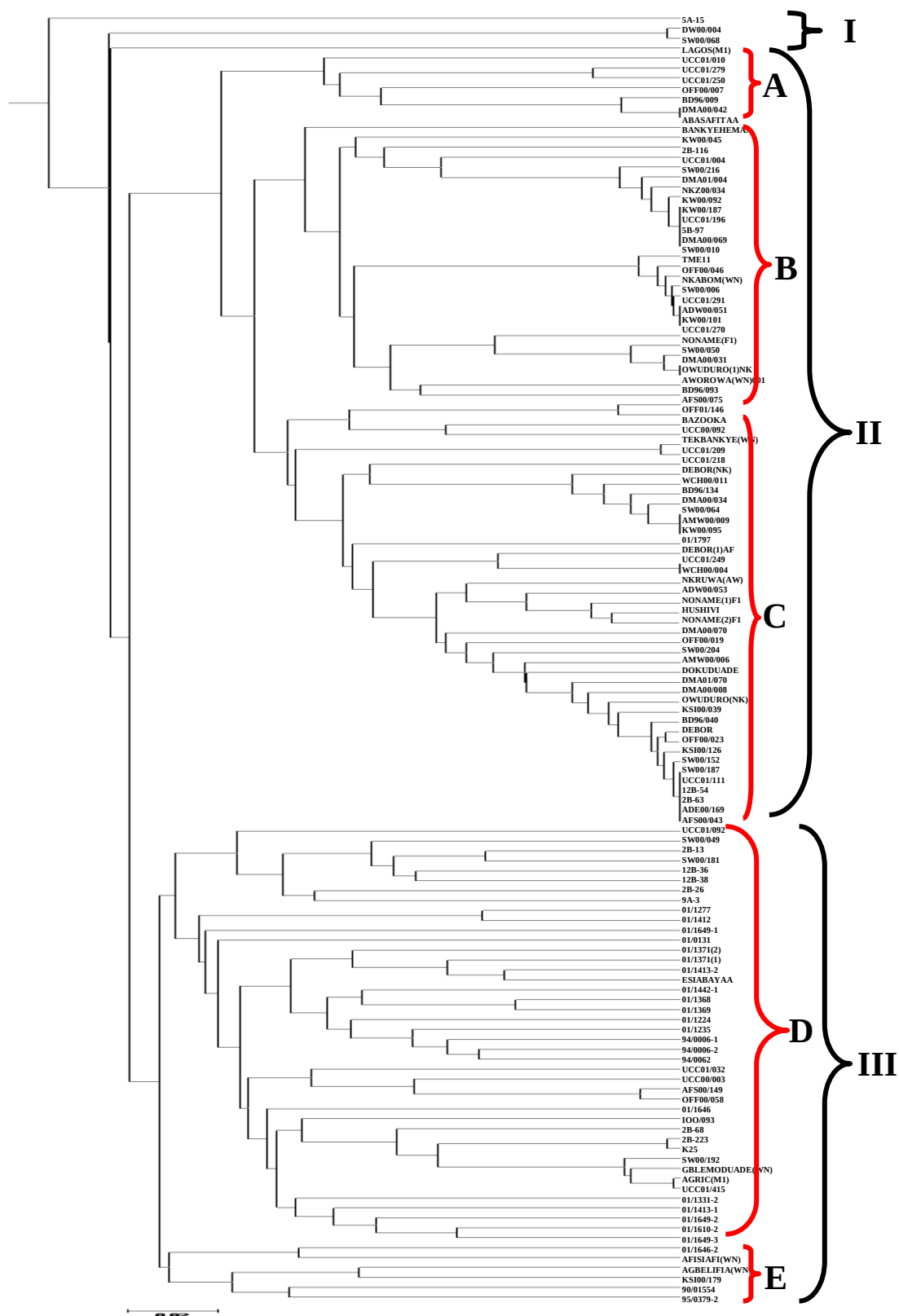


Fig. 4. 4 Dendrogram of cassava accessions based on SNP markers

Table 4.10 Dendrogram cluster groupings of the cassava accessions based on SNP markers

Cluster groups				
Cluster I	Cluster II		Cluster III	
	Sub-cluster A	Sub-cluster C	Sub-cluster D	Sub-cluster E
5A-15	UCC01/010	OFF01/146	UCC01/092	01/1646-2
DW00/004	UCC01/279	BAZOOKA	SW00/049	AFISIAFI(WN)
SW00/068	UCC01/250	UCC00/092	2B-13	Agbelifia(WN)
LAGOS(M1)	OFF00/007	Tekbankye(WN)	SW00/181	KSI00/179
	BD96/009	UCC01/209	12B-36	90/01554
	DMA00/042	UCC01/218	12B-38	95/0379-2
		DEBOR(NK)	2B-26	
	Sub-cluster B	WCH00/011	9A-3	
	ABASAFITAA	BD96/134	01/1277	
	Bankyehemaa	DMA00/034	01/1412	
	KW00/045	SW00/064	01/1649-1	
	2B-116	AMW00/099	01/0131	
	UCC01/004	KW00/095	01/1371(2)	
	SW00/216	01/1797	01/1371(1)	
	DMA01/004	DEBOR(1)AF	01/1413-2	
	NKZ00/034	UCC01/249	ESIABAYAA	
	KW00/092	WCH00/004	01/1442-1	
	KW00/187	NKRUWA(AW)	01/1368	
	UCC01/196	ADW00/053	01/1369	
	5B-97	NONAME(1)F1	01/1224	
	DMA00/069	HUSHIVI	01/1235	
	SW00/010	NONAME(2)F1	94/0006-1	
	TME11	DMA00/070	94/0006-2	
	OFF00/046	OFF00/019	94/0062	
	NKABOM(WN)	SW00/204	UCC01/032	
	SW00/006	AMW00/006	UCC00/003	
	UCC01/291	DOKUDUADE	AFS00/149	
	ADW00/051	DMA01/070	OFF00/058	
	KW00/101	DMA00/008	01/1646	
	UCC01/270	OWUDURO(NK)	IOO/093	
	NONAME(F1)	KSI00/039	2B-68	
	SW00/050	BD96/040	2B-223	
	DMA00/031	DEBOR	K25	
	OWUDURO(1)NK	OFF00/023	SW00/192	
	Aworowa(WN)001	KSI00/126	Gblemoduade(WN)	
	BD96/093	SW00/152	AGRIC(M1)	
	AFS00/075	SW00/187	UCC01/415	

4.5 Discussion

Genetic variation among genotypes is important for sustainable use of genetic resources to meet the demand for future food security as well as conservation strategies (Karuri *et al.*, 2010). Genetic variation studies are based on either morphological or molecular traits. Morphological traits are useful for preliminary evaluation as they offer a quick method of assessing the extent of variability within and between accessions (Karuri *et al.*, 2010; Lekha *et al.*, 2011). In the present study, descriptive analysis of 150 cassava accessions with 25 qualitative traits revealed the existence of morphological variation among the accessions. The findings of this study were similar to other findings. For instance, Carvalho and Schaal (2001) reported a high degree of variability among 94 cassava accessions of Brazilian origin. Raghu *et al.* (2007) in similar studies also identified a high level of variability among 58 cassava accessions based on 29 morphological traits. Similarly, Lyimo *et al.* (2012) reported a significant variability among 39 cassava accessions of Tanzanian origin with 14 morphological traits.

Out of the 25 qualitative traits used for the assessment of genetic variation, 19 were found to contribute to maximum variation by factor analysis. In a similar study Raghu *et al.* (2011) identified 24 morphological traits out of 28 traits, while Asare *et al.* (2011) identified 14 traits out of 19 based on factor analysis. These two previous studies reported six out of the 19 qualitative traits identified from this study. The discrepancy could probably be attributed to the differences in the cassava genotypes, morphological traits as well as environmental conditions under which these studies were carried out. In the present study the most discriminating traits were leaf retention, colour of apical leaf, orientation of petiole, colour of root pulp, leaf lobe margins, pubescence on apical leaf, growth habit of plant, root constriction and leaf shape. These

qualitative traits with maximum variability can be used for cassava diversity studies in Ghana. Leaf shape has been identified to be highly heritable and expresses majority of the crop's diversity (Tairo *et al.*, 2008; Karuri *et al.*, 2010). In the present study, clustering based on similarity index of the qualitative traits grouped the 150 cassava accessions into five clusters. In a similar study, Carvalho and Schaal (2001) identified 22 distinct clusters using 94 cassava accessions. Similarly, Raghu *et al.* (2007) also identified six distinct groups using 58 accessions.

Since morphological traits are influenced by the environment, molecular markers which are not influenced by the environment are preferable in genetic diversity studies (Kaemmer *et al.*, 1992; Gepts, 1993). The study by Kawuki *et al.* (2009) is currently the only published report where SNPs have been used for diversity studies in cassava. They identified, characterized some SNP markers and assessed their utilization in cassava diversity assessment. This current study is the first reported case in Ghana where SNP markers have been used in cassava diversity studies.

Using the 195 SNP markers, 96% were found to be polymorphic. The informativeness of a genetic marker is measured by the polymorphic information content. The average PIC value observed for this study was 0.297. In a previous study on cassava genetic diversity using SNPs, Kawuki *et al.* (2009) reported PIC values of 0.228 in 74 cassava accessions with 26 SNPs. The moderately high PIC value obtained for this study could be attributed to the number and type of SNP markers used. PIC values for SNP markers are generally low as observed in genetic diversity studies in other crops. For instance, Yang *et al.* (2011) reported PIC value of 0.34 in maize genotypes using 884 SNP markers. This high PIC value observed by Yang *et al.* (2011) could be attributed to large number of SNP markers used for the study.

The observed heterozygosity (0.372) was higher than the expected heterozygosity (0.341). This suggests that, the cassava accessions used for the study were heterogeneous, hence could be used for the improvement of desirable traits. Findings of this study is similar to studies of Asare *et al.* (2011), however, they used SSR markers other than SNP markers used in this present study. The average expected heterozygosity was also below 0.5 for this study and that for Kawuki *et al.* (2009). He *et al.* (2012) reported expected heterozygosity of 0.29 in rice using 353 SNPs. SNPs are biallelic markers which have a 50-50 distribution of the two alleles (Kruglyak, 1997; Kawuki *et al.*, 2009), whereas the maximum can approach one for multi-allelic markers such as SSRs. Hence the maximum expected heterozygosity observable with biallelic markers is 0.5. This explains the lower polymorphism observed in SNP markers (Vignal *et al.*, 2002). The SNP markers used were able to group the cassava accessions into three main clusters and five sub-clusters.

Although the morphological and SNP marker systems were able to separate the accessions into different clusters, both systems did not group the accessions based on geographical location and their reaction to PPD. This could be attributed to the fact that both marker systems were not correlated to PPD. Similar groupings were observed among some of the accessions although there were some disagreements between the two dendrograms. The use of molecular markers may allow a more accurate detection of differences between genotypes than morphological characterization because molecular markers are not influenced by the environment unlike morphological traits. A significant but weak positive correlation between the morphological and SNP analyses indicated that both approaches are complementary and could be used for the efficient and effective characterization of cassava accessions. In similar studies, weak correlation

between molecular and morphological characters for cassava was observed (Carvalho *et al.*, 1998; Elias *et al.*, 2004).

4.6 Conclusion

The morphological and molecular analyses were able to separate the accessions into different clusters. However, both marker systems did not cluster the accessions based on geographical location neither did they cluster them based on their reaction to PPD. Related accessions were grouped in the same cluster while unrelated accessions were grouped in separate clusters. However, there were some discrepancies between the clusters as some accessions that appeared to be similar based on molecular analysis had different morphological characteristics and were therefore grouped into different clusters based on morphological traits. The morphological and SNP markers were found to be complementary hence both approaches should be used for genetic diversity studies in cassava. The moderate to high genetic diversity among the cassava accessions observed in this study indicated the availability of desirable traits within the germplasm that could be exploited for breeding purposes.

CHAPTER 5

5.0 EVALUATION OF CASSAVA GENOTYPES FOR DELAYED POSTHARVEST PHYSIOLOGICAL DETERIORATION (PPD)

Cassava is considered as a global food security crop, likely to play a more significant role in the near future (Rosenthal *et al.*, 2012). World cassava production has increased in recent years and is expected to increase further due to higher demand as human food, animal feed and its value as raw material for industrial proposes (Sriroth *et al.*, 2010). Over the years, improvement of the crop has been mainly on traits such as yield, pests and disease resistance. Recently, efforts are being directed towards delaying PPD in cassava.

There are no PPD tolerant varieties available for farmers in Ghana. Several studies have reported the variability among cassava varieties for PPD (Buschmann *et al.*, 2000; Ekanayake and Lyasse, 2003; Aristizabal and Sánchez, 2007). Based on this information, storage roots could be evaluated for PPD over longer periods to select desirable genotypes with delayed PPD. Morante *et al.* (2010) has also emphasized the importance of germplasm collection and screening for PPD tolerance as a major step in breeding for delayed PPD in cassava. Landraces have desirable traits that can be tapped for improvement purposes. Collection and screening of cassava germplasm for PPD tolerance could lead to the identification of genotypes with longer shelf life. This can subsequently be used in the cassava improvement program in Ghana. Several methods are available for root processing for PPD evaluation, however, to identify PPD tolerant cassava genotypes, it is important to use the appropriate root processing method. Since this study is the first to carry out an elaborate work on PPD in Ghana, it is, therefore, necessary to validate the

two most common methods used in PPD evaluation to identify the appropriate method that could be used for subsequent PPD activities in Ghana.

Cassava roots in storage undergo physiological changes that may ultimately affect the root quality. Changes in root crop starches in storage have been reported for potato (Kaur *et al.*, 2007; Singh *et al.*, 2008), sweetpotato (Zhang *et al.*, 2002), yams (Akissoe *et al.*, 2004; Aishat *et al.*, 2007) and cassava (Sanchez *et al.*, 2013). Since PPD in itself is physiological, it would be appropriate to determine some of the physiochemical and functional changes in cassava processed from deteriorated cassava roots. These analyses must also be carried out on cassava genotypes classified as PPD tolerant to ensure that, these genotypes have constant root quality and better storage life after the period of storage. This will help in understanding the complex interactions and relationships between root quality and physiological deterioration.

5.1 Objectives

The objectives of this study were to:

- a. Compare methods for root processing for PPD evaluation
- b. Identify cassava genotypes with delayed PPD
- c. Determine the physicochemical and functional changes in cassava roots during storage

5.2 Materials and methods

5.2.1 Evaluation of cassava genotypes for their reaction to PPD

5.2.1.1 Study site

The study was conducted at the experimental fields of the Crops Research Institute at Fumesua near Kumasi. Fumesua is in the Semi-deciduous forest and characterized by rainfall ranging from 1200-1600 mm with temperature ranging from 22°C-31°C. Soils are dominated by the ferric acrisol type with sandy loamy and loamy clay texture.

5.2.1.2 Plant material and experimental design

One hundred and fifty cassava genotypes (Table 4.1) were used for the study. Healthy stem cuttings of 20 cm were planted in four row plots of eight plants per row at 1 m by 0.75 m spacing in the 2010/2011 planting season. Plots were separated by 1 m alleys. Materials were evaluated using a 10 x 15 alpha lattice design with three replicates with each block consisting of 15 genotypes. The cassava genotypes were grown under normal cultural practices and no fertilizers and irrigation were provided during the period of the experiment. Weeds were manually controlled with hoes and cutlasses.

5.2.1.3 Data collection and root harvest

Roots were harvested 10 months after planting (MAP). Harvesting was done manually with special care to minimize wounding since it accelerates PPD. Data were taken from the two inner rows leaving the outer rows on each side of the plot as borders. Data were collected for the traits

storage root number per plant (SRN); fresh storage root yield (FRY); biomass yield (BY) and disease severity and incidence. Harvest index (HI) was calculated as a ratio of fresh root yield to the total fresh biomass. Dry matter content (DMC) was calculated as the ratio of dry weight to fresh weight and expressed as a percentage. Dry root yield (DRY) was derived as a product of DMC and FRY (Kawano *et al.*, 1987). Reaction to Cassava Mosaic Disease (CMD) and Cassava Green Mite (CGM) were assessed 3, 4 and 5 MAP using a scale of 1-5 (IITA, 1990) to rate genotypes for resistance to the diseases and pests.

5.2.1.4 Root quality analysis

5.2.1.4.1 Root processing for PPD evaluation

Comparing the different root processing methods for PPD evaluation, five cassava genotypes were selected and used based on the availability of storage roots. The methods for processing roots for PPD evaluation consisted of two treatments which were the Booth's (Booth, 1977) and Wheatley's methods (Wheatley, 1980). Five roots from each genotype were selected using a randomized complete block design with 3 replicates. With the Booth's method, storage roots were stored as intact roots whilst with the Wheatley's method, the proximal and distal ends of each root were removed to accelerate PPD. The distal end was covered with cling film to prevent moisture loss, allowing deterioration to develop from the proximal end only. Storage roots were stored on shelves in a barn under ambient conditions where air circulated freely through the shelves. The roots were stored for seven days and scored for PPD using standard methodology for PPD quantification (Wheatley and Gomez, 1985; Sanchez *et al.*, 2006; Morante *et al.*, 2010). For the identification of cassava genotypes with delayed PPD, ten marketable sized roots were

selected for each genotype from each replication and used for PPD evaluation using the Booth's method (Booth, 1977) as described above.

5.2.1.4.2 Scoring for PPD

Scoring for PPD was a destructive process where seven 2 cm thick transversal slices were cut along the root, starting from the proximal end. A scoring scheme ranging from 1-10 was assigned to each slice, corresponding to the percentage of the cut surface showing discoloration (1 = 10%, 2 = 20%, etc.). The mean PPD score for each root was calculated by averaging the scores for the seven transversal sections. Roots showing symptoms of microbial rot was not evaluated for PPD.

5.2.1.4.3 Root dry matter content (DMC) determination

For DMC estimation, three cassava storage roots were washed and grated into chips using a mechanical cassava grater. One hundred grams of the chips were sampled in two replicates. They were oven dried at 72°C for 48 hours, weighed and DMC was estimated.

5.2.2 Physicochemical changes of cassava flour during PPD

Five genotypes were selected and used for the biochemical analysis based on the availability of storage roots. Three treatments were used for this experiment. These were immediately after harvest (0 DAH), seven days after harvest (7 DAH) and 14 days after harvest (14 DAH). Most of the storage roots for the different genotypes got rotten by day 14. This affected the quantity of

flour extracted, as a result only flour extracted from roots stored at 0 DAH and 7 DAH were retained for the analysis.

5.2.2.1 Processing of storage roots into flour

Two storage roots from each genotype were peeled, washed with water and grated into a mash using a mechanical cassava grater on 0 DAH and 7 DAH. The mash was packed into porous, woven polypropylene sacks and dehydrated by pressing with a manual screw-press. The cakes obtained were pulverized, sieved through nylon mesh and sun-dried for two days by spreading thinly on aluminum trays with periodic stirring. The granules obtained were milled and sieved through a 120 μm mesh. The resulting flour was packaged in plastic air tight bags and stored at room temperature.

5.2.2.2 Physicochemical properties determination

Flour yield was determined by weighing the amount of flour obtained from 1 kg of fresh cassava roots expressed as a percentage of the total fresh root. Proximate analysis was carried out according to the method of AOAC (2000). All samples were analyzed in triplicate.

1. Moisture Content Determination

A sample of 2 g of cassava flour was accurately weighed into a previously dried and weighed glass crucible. It was then dried in a thermostatically controlled forced convection oven (Gallenkamp, England) at 105°C overnight to a constant weight. The glass crucibles were

removed and transferred into a desiccator to cool after which they were weighed. Moisture content was determined by difference and expressed as a percentage.

2. Ash Content Determination

A sample of 2 g of cassava flour was weighed into a pre-ignited and previously weighed porcelain crucible placed in a muffle furnace (Gallenkamp, England) and ignited for 2 hours at 600°C. After ashing, the crucibles were cooled to 105°C in a forced convection oven before cooling them further to room temperature in a desiccator. The crucibles and their contents were weighed and the weight reported as percentage ash content.

3. Crude Protein Determination

About 30 - 40 mg of cassava flour was put in a digestion flask and 1g catalyst powder mixture was added. Two millilitres of concentrated sulphuric acid and 2 ml 30% hydrogen peroxide was added. This was digested for 30 minutes, cooled and distilled water was added to dissolve the solids. It was allowed to cool at room temperature and the digest was transferred into a distillation apparatus, making sure that, none remained in the flask by rinsing it five to six times in 1 - 2 ml portions of distilled water. The 125 ml Erlenmeyer flask with 6 ml boric acid solution and 3 drops indicator solution was placed under a condenser. Eight millilitres of sodium hydroxide-sodium thiosulfate solution was added. It was allowed to steam distill until about 50 ml distillate was collected. Titration was carried out until the first appearance of violet colour. Percentage nitrogen and protein was calculated using the formula:

$$\% \text{ Nitrogen} = \frac{(\text{ml HCl in sample} - \text{ml blank}) \times \text{normality HCl} \times 14.007 \times 100}{\text{Weight of sample}}$$

$$\% \text{ Protein} = \% \text{ Nitrogen} \times 14.007$$

4. Crude fat Content Determination

A sample of 2 g of cassava flour was transferred into a paper thimble plugged at the opening with glass wool and placed into a thimble holder. Two hundred milliliters of petroleum ether was measured into a weighed round-bottom flask and assembled together with the thimble holder and its contents. A Quickfit condenser was connected to a Soxhlet Extractor (Tecator, Herndon) and refluxed for 16 hours on low heat on a heating mantle. The flask was then removed and the solvent evaporated on a steam bath. The flask containing the fat was heated at 105°C in an oven for 30 minutes, cooled in a desiccator and the weight of the fat collected determined and expressed as percentage crude fat.

5. Crude Fibre Determination

Two grams of cassava flour was weighed into a round bottom flask and 100 ml of 0.25 M sulphuric acid solution was added. The mixture was boiled under reflux for 30 min and the hot solution was quickly filtered under suction. The residue was thoroughly washed with hot water until it was free of acid. The residue was transferred into a labelled flask and 100 ml of hot 0.3 M sodium hydroxide solution was added and the mixture was boiled again under reflux for 30 min and filtered quickly under suction. The insoluble residue was then washed with hot water until it was base free. It was dried to a constant weight in an oven at 100°C for 2 hours, cooled in desiccators and weighed (C1). The weighed sample was then incinerated and reweighed (C2). Percentage crude fibre content was then calculated.

6. Carbohydrate Content Determination

The nitrogen free method was used. The carbohydrate was calculated as weight by difference between 100 and the summation of other proximate parameters as Nitrogen free Extract (NFE) percentage carbohydrate.

5.2.2.3 Functional properties

1. Solubility and Swelling Power Determination

Swelling capacity was determined by the method of Barimah (1999). One gram of cassava flour was weighed into a 50 ml centrifuge tube. It was stirred and heated at 85°C in a thermostatically regulated water bath for 20 minutes with constant stirring. The tubes were removed after 20 minutes and cooled at room temperature. It was centrifuged at 2200 rpm for 15 minutes. The supernatant was decanted into a pre-weighed tube and the sedimented paste was weighed. The water in the supernatant was evaporated and the tubes were weighed after evaporation. The solubility and swelling power were calculated using the formulas:

$$\% \text{ Solubility} = \frac{\text{Weight of soluble starch} \times 100}{\text{Weight of sample}}$$

$$\text{Swelling Power} = \frac{\text{Weight of Sedimented Paste} \times 100}{(\text{Weight of sample}) \times (100 - \text{Solubility})}$$

2. Water Binding Capacity Determination

The water binding capacity of the flour samples were determined by the method of Yamazaki (1953) as modified by Medcalf and Gilles (1965). Two grams of the sample was dissolved in 40 ml distilled water in a 50 ml centrifuge tube. The solution was agitated for 1 hour on a Griffin flask shaker (Griffin and George Ltd, Great Britain) after which it was centrifuged at 2200 rpm for 10 minutes. The supernatant was decanted and the wet flour drained for 10 minutes and weighed. Water binding capacity was calculated using the formula:

$$\% \text{Water Binding Capacity} = \frac{\text{Bound Water} \times 100}{\text{Weight of sample}}$$

3. pH Determination

Ten grams of cassava flour was weighed into a beaker. Fifty millilitres of distilled water was added and stirred intermittently for 10 minutes. pH was determined using a pH meter (HANNA Instrument).

5.3 Data analysis

Data were subjected to analysis of variance (ANOVA) using the GLM procedure of Statistical Analysis System (SAS) version 9.3. A two way ANOVA was performed for the analysis of the physicochemical properties of cassava flour using GENSTAT version 12. Separation of means was carried out using Fisher's Least Significance Difference (LSD) at 5% probability levels. Pearson's correlation was carried out to determine the linear relationship between PPD and other

important traits. The linear additive model and analysis for variance for alpha lattice design is stated below:

$$Y_{ij} = \mu + \alpha_i + \beta_j + e_{ij}$$

Y_{ij} = observed value at the i th treatment and j th block

μ = overall mean

α_i = effect of treatment i

β_j = effect of block j

e_{ij} = residual

5.4 Results

5.4.1 Validation of cassava root processing methods for PPD evaluation

5.4.1.1 Temperature and relative humidity conditions during PPD evaluation

The average minimum and maximum temperatures were 22.3°C and 33.4°C respectively during the period of the experiment. Average relative humidity was 97.4% at 6:00 AM, 63.7% at 12:00 PM and 66.2% at 6:00 PM.

5.4.1.2 PPD reaction among cassava genotypes

The results for PPD reaction and analysis of variance for the different processing methods are presented in Figure 5.1 and Table 5.1 respectively. PPD scores among the cassava genotype ranged from 13.3% - 36.6% with all genotypes showing signs of deterioration after seven days of storage. Reaction to PPD ranged from 13.3% - 24.8% for the Booth's method while it ranged

from 19% - 36.6% for the Wheatley's method. Minimum and maximum deterioration was found in genotypes IOO/0093 and OFF/00/063 respectively in the Booth's method while minimum and maximum deterioration were found in genotypes UCC01/010 and 01/0131 respectively in the Wheatley's method. The results indicated that PPD scores were minimal with Booth's processing method than the Wheatley's method. This could be attributed to the fact that Wheatley's method was developed to accelerate PPD hence, Booth's method would be used for subsequent evaluation for PPD since farmers and processors store whole roots.

The analysis of variance indicated that genotypes, methodology and genotype by methodology interaction were highly significant at ($p < .001$). The relative magnitude of the main effects and the interaction was measured as a proportion of the total sum of squares. This showed that genotype accounted for 21% of the total sum of squares; methodology accounted for 38.6% and interaction had the greatest impact of 40%. This suggests that PPD reaction is largely depended on the genotypes as well the type of processing method used prior to the evaluation. This implies that the appropriate processing method should be selected and used for PPD evaluation in order to achieve the best results.

Table 5. 1 Analysis of variance for PPD evaluation processing methods

Source of variation	DF	SS	% Explained	MS	F	P
Genotype	4	316.58	21.00	79.14	581.15	<.001
Method	1	581.68	38.59	581.68	4271.25	<.001
Genotype. Method	4	605.39	40.16	151.35	1111.34	<.001
Residual	18	2.45	0.16	0.14		
Total	29	1507.41				

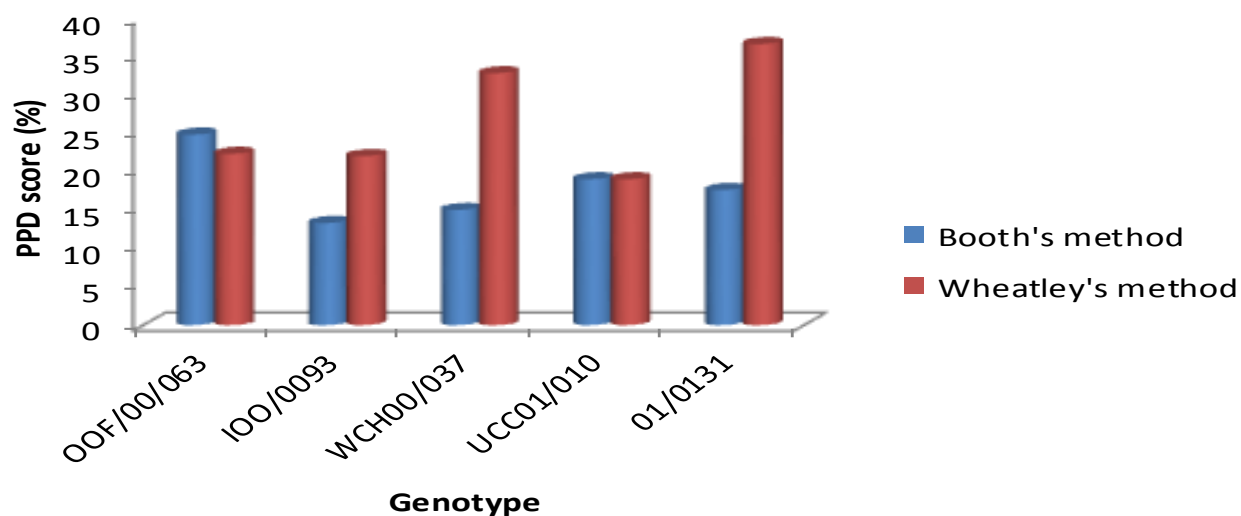


Fig. 5. 1 PPD scores for cassava genotypes

5.4.2 Characterization of cassava genotypes based on their reaction to PPD

5.4.2.1 Mean performance for Postharvest Physiological Deterioration

Mean performance and analysis of variance for PPD are presented in Tables 5.2 and 5.3 respectively. Cassava roots totally rotten due to microbial infection were not scored for PPD, since this tends to underestimate the assessment. The cassava genotypes evaluated showed a wide range of variation within and among genotypes after seven days of storage.

Mean PPD ranged from 9.1% to 60.1% with a grand mean and standard deviation of $22.87\% \pm 7.61$ respectively. The lowest and highest reaction to PPD was recorded in genotypes K25 and Bankyehemaa respectively. Based on the cassava storage roots reaction to PPD, the genotypes were grouped into three classes namely low PPD, moderate PPD and high PPD (Fig. 5.2). Genotypes within the low PPD class had $<10\%$ deterioration score whilst those in the

moderate and high PPD classes had scores that ranged from 10% - 30% and 30% - 60% respectively. Cassava genotypes K25, SW/00/064 and DMA00/031 had 9.1%, 9.6% and 9.9% deterioration rates respectively and therefore belonged to the low PPD group.

Analysis of variance for PPD showed significant effects ($P < 0.05$) for genotypes. For the proportion of the sum of squares, genotype accounted for 54.8% of the total sum of squares. This suggests that, variation observed for physiological deterioration was mainly due to the genotypic differences among the cassava genotypes.

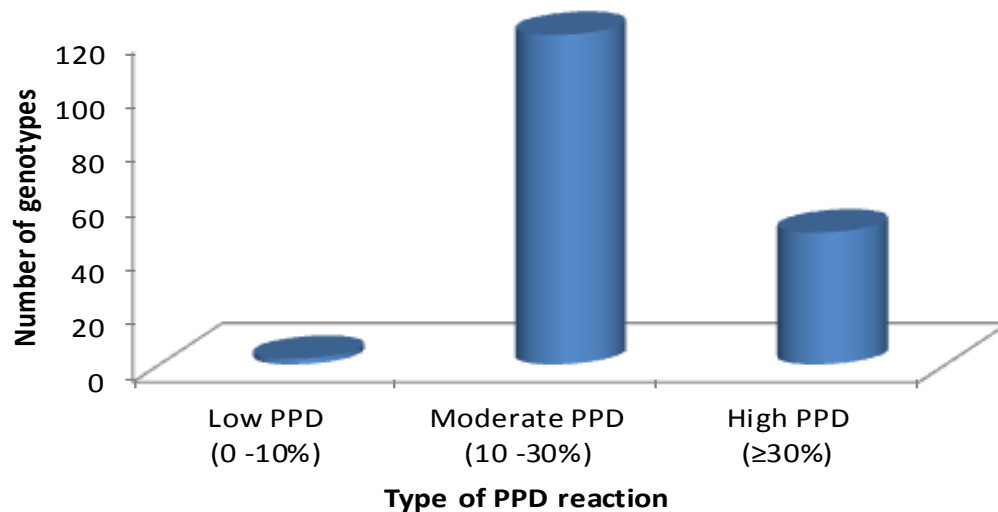


Fig. 5. 2 Characterization of cassava genotypes based on PPD reaction

Table 5. 2 Mean postharvest physiological deterioration (%) of 150 cassava genotypes evaluated

Genotype	PPD (%)	Genotype	PPD (%)	Genotype	PPD (%)
01/0103	21.00	2B-51	21.80	BAATIA(AK)	30.20
01/0131	32.50	2B-68	15.50	BANKYHEMAA(WN)	60.10
01/1224	18.30	5A-15	43.60	BAZOOKA(HO)	35.00
01/1235	27.10	5B-97	28.20	BD 96/009	20.10
01/1277	22.10	90/01554	13.90	BD 96/040	29.50
01/1331-1	23.30	94/0006	15.50	BD 96/093	23.70
01/1331-2	12.30	94/0006-1	20.20	BD 96/134	28.40
01/1368	13.70	94/0006-2	16.70	BD 96/165	24.40
01/1369	16.40	95/0379-2	20.90	DABODABO	31.00
01/1371-1	32.70	9A-3	17.50	DEBOR	24.80
01/1371-2	25.00	A TUMTUM (M1)	31.00	DEBOR(1)AF	19.50
01/1412	32.60	ABASAFITAA (HO)	19.00	DEBOR(NK)	20.90
01/1413-1	23.70	ADE 00/107	30.90	DMA 00/008	23.10
01/1413-2	35.70	ADE 00/169	32.80	DMA 00/031	9.90
01/1442-1	14.80	ADE 030/039	24.90	DMA 00/034	21.10
01/1442-2	20.20	ADW 00/004	29.10	DMA 00/042	25.80
01/1646-1	21.90	ADW 00/051	25.40	DMA 00/048	16.30
01/1646-2	19.30	ADW 00/053	19.00	DMA 00/069	20.80
01/1649-1	24.60	AFISIAFI (WN)	25.60	DMA 00/070	20.50
01/1649-2	15.00	AFS 00/043	12.90	DMA 01/004	22.30
01/1649-3	21.50	AFS 00/075	20.70	DMA 01/070	15.00
01/1797	16.70	AFS 00/081	25.50	DOKU DUADE	22.20
12B-36	25.70	AFS 00/149	28.70	ESIABAYA(AK)	21.00
12B-38	21.10	AGBELIFIA (WN)	18.10	ESSAMBANKYE(WN)	24.80
2B-13	42.40	AGRIC (M1)	17.30	GBLEMODUADE(WN)	37.00
2B-166	20.00	AMW 00/006	20.90	HUSHIVI	21.80
2B-223	18.10	AMW 00/099	33.80	I00/0093	29.40
2B-26	13.80	AWOROWA(WN001)	31.30	K25	9.10
KROBO(NK)	12.60	OFF 00/019	14.60	KW 00/101	31.80
KSI 00/039	24.00	OFF 00/023	20.80	KW 00/181	38.80
KSI 00/092	30.70	OFF 00/029	25.00	KW 00/187	26.30
KSI 00/126	17.80	OFF 00/058	43.60	LAGOS(M1)	25.60
KSI 00/179	27.60	OFF 00/063	16.00	NKABOM(WN)	15.80
KSI 01/036	27.80	OFF 00/086	21.50	NKRUWA(AW)	27.20
KW 00/045	31.80	OFF 00/087	22.80	NKZ 00/034	23.10
KW 00/095	19.60	OFF 01/146	23.10	NO NAME(1)F1	18.60

Table 5.2 (cont'd) Mean postharvest physiological deterioration (%) of 150 cassava genotypes evaluated

Genotype	PPD (%)	Genotype	PPD (%)
OWUDURO(NK)	20.90	UCC 00/032	36.30
SW 00/006	40.90	UCC 00/088	19.00
SW 00/010	28.90	UCC 01/010	25.30
SW 00/049	16.80	UCC 01/032	25.50
SW 00/050	30.60	UCC 01/078	13.00
WCH 009(WN)	17.50	UCC 01/092	13.60
OWUDURO(1)NK	20.90	UCC 01/111	16.60
NO NAME(2)F1	19.80	UCC 01/195	28.20
NO NAME(3)F1	35.90	UCC 01/209	21.70
SW 00/064	9.60	UCC 01/218	16.20
SW 00/068	22.50	UCC 01/249	17.30
SW 00/152	25.60	UCC 01/250	25.30
SW 00/181	13.50	UCC 01/270	39.20
SW 00/187	26.50	UCC 01/279	22.80
SW 00/192	24.30	UCC 01/291	21.70
SW 00/204	21.70	UCC 01/415	14.00
SW 00/216	21.90	UCC 01/504	24.80
TECKBANKYE(WN)	21.30	UCC(M1)	34.90
TME 11	27.80	WCH 00/004	16.00
UCC 00/003	23.40	WCH 00/011	22.90
Grand Mean	22.87	WCH 00/037	19.60
SE	1.06		
LSD	17.0		
CV	22.6		

Table 5. 3 Analysis of variance for postharvest physiological deterioration

Source of variation	Df	Mean squares	Contribution to SS (%)
Genotype	148	0.025*	54.8
Blk (Rep)	29	0.023*	
Residual	219	0.016	
Total	396		

Df: degrees of freedom *significant at 5%

5.4.3 Mean performance for root dry matter content

Mean performance and analysis of variance for root dry matter content are presented in Tables 5.4 and 5.5 respectively. Mean root dry matter content ranged from 22% - 38.9% with a grand mean and standard deviation of 31.9%±4.04 respectively. Lowest mean was recorded in genotype 01/1368 while the highest mean was recorded in genotype 5B-97. Analysis of variance for root dry matter showed significant effects ($P < 0.05$) for genotypes and contributed 26.2% of the total sum of squares.

Table 5. 4 Mean root dry matter content (%) of 150 cassava genotypes evaluated

Genotype	DMC (%)	Genotype	DMC (%)	Genotype	DMC (%)
01/0103	33.30	01/1797	29.00	ADE 030/039	28.30
01/0131	31.70	12B-36	34.10	ADW 00/004	35.50
01/1224	22.90	12B-38	31.10	ADW 00/051	36.00
01/1235	24.70	2B-13	38.70	ADW 00/053	36.00
01/1277	26.40	2B-166	30.10	AFISIAFI (WN)	27.10
01/1331-1	27.30	2B-223	35.80	AFS 00/043	31.10
01/1331-2	24.80	2B-26	30.00	AFS 00/075	33.30
01/1368	22.00	2B-51	26.20	AFS 00/081	37.30
01/1369	27.30	2B-68	36.10	AFS 00/149	35.20
01/1371-1	27.30	5A-15	38.80	AGBELIFIA (WN)	25.40
01/1371-2	27.40	5B-97	38.90	AGRIC (M1)	26.50
01/1412	26.50	90/01554	29.60	AMW 00/006	35.80
01/1413-1	34.30	94/0006	33.60	AMW 00/099	37.30
01/1413-2	24.20	94/0006-1	26.20	AWOROWA(WN001)	35.00
01/1442-1	24.00	94/0006-2	26.30	BAATIA(AK)	34.10
01/1442-2	26.30	95/0379-2	26.50	BANKYHEMAA(WN)	31.20
01/1646-1	30.60	9A-3	24.60	BAZOOKA(HO)	36.80
01/1646-2	26.90	A TUMTUM (M1)	31.10	BD 96/009	35.90
01/1649-1	28.00	ABASAFITAA (HO)	31.70	BD 96/040	35.30
01/1649-2	24.30	ADE 00/107	27.60	BD 96/093	33.20
01/1649-3	26.90	ADE 00/169	36.20	BD 96/134	35.20
BD 96/165	36.90	OFF 00/086	33.10	DMA 00/031	33.40
DABODABO	38.40	OFF 00/087	34.40	DMA 00/034	36.00
DEBOR	34.60	OFF 01/146	33.70	DMA 00/042	33.00

Table 5.4 (cont'd) Mean root dry matter content (%) of 150 cassava genotypes evaluated

Genotype	DMC (%)	Genotype	DMC (%)	Genotype	DMC (%)
DEBOR(1)AF	33.50	OWUDURO(1)NK	31.10	DMA 00/048	34.80
DEBOR(NK)	27.70	OWUDURO(NK)	30.90	DMA 00/069	35.40
DMA 00/008	31.30	SW 00/006	33.60	DMA 00/070	38.30
DMA 01/004	34.90	NO NAME(2)F1	36.10	NO NAME(1)F1	35.20
DMA 01/070	34.70	NO NAME(3)F1	35.50	UCC 01/010	29.50
DOKU DUADE	35.20	OFF 00/019	33.40	UCC 01/032	29.70
ESIABAYA(AK)	28.40	OFF 00/023	34.50	UCC 01/078	33.50
ESSAMBANKYE(WN)	35.10	OFF 00/029	37.50	UCC 01/092	36.00
GBLEMODUADE(WN)	29.10	OFF 00/058	29.80	UCC 01/111	37.50
HUSHIVI	28.30	SW 00/010	33.50	UCC 01/195	37.30
I00/0093	22.90	SW 00/049	34.90	UCC 01/209	33.30
K25	22.30	SW 00/050	35.40	UCC 01/218	31.50
KROBO(NK)	29.60	SW 00/064	30.20	UCC 01/249	32.50
KSI 00/039	34.60	SW 00/068	34.90	UCC 01/250	29.10
KSI 00/092	37.20	SW 00/152	31.80	UCC 01/270	36.30
KSI 00/126	32.40	SW 00/181	35.40	UCC 01/279	30.20
KSI 00/179	31.90	SW 00/187	29.90	UCC 01/291	31.00
KSI 01/036	29.50	SW 00/192	33.80	UCC 01/415	28.90
KW 00/045	26.50	SW 00/204	35.60	UCC 01/504	32.70
KW 00/095	35.70	SW 00/216	36.30	UCC(M1)	34.70
KW 00/101	30.50	TECKBANKYE(WN)	31.80	WCH 00/004	26.80
KW 00/181	29.30	TME 11	30.90	WCH 00/011	35.30
KW 00/187	29.10	UCC 00/003	33.50	WCH 00/037	31.20
LAGOS(M1)	30.80	UCC 00/032	37.40	WCH 009(WN)	35.00
NKABOM(WN)	36.60	UCC 00/088	33.10	OFF 00/063	29.80
NKRUWA(AW)	32.10				
Grand Mean	27.00				
SE	4.56				
LSD	7.4				
CV	4.80				

Table 5. 5 Analysis of variance for root dry matter content

Source of variation	Df	Mean squares	Contribution to SS (%)
Genotype	148	0.008*	26.2
Blk(Rep)	29	0.007	
Residual	221	0.004	
Total	399		

Df: degrees of freedom *significant at 5%

5.4.4 Mean performance for fresh root yield

Mean performance and analysis of variance for fresh root yield are presented in Tables 5.6 and 5.7 respectively. Mean fresh root yield ranged from 14.5t/ha - 38.7t/ha with a grand mean and standard deviation of $28.6\% \pm 4.04$ respectively. Lowest yield was recorded in genotype Owuduro while the highest yield was recorded in genotype UCC01/250. Analysis of variance for fresh root yield showed significant effects ($P < 0.05$) for genotypes and contributed 32.4% of the total sum of squares.

Table 5. 6 Mean fresh root yield (t/ha) of 150 cassava genotypes evaluated

Genotype	FRY (t/ha)	Genotype	FRY (t/ha)	Genotype	FRY (t/ha)
01/0103	32.30	2B-26	23.60	AWOROWA(WN001)	26.80
01/0131	30.30	2B-51	21.90	BAATIA(AK)	29.50
01/1224	30.70	2B-68	24.50	BANKYEHMAA(WN)	27.50
01/1235	29.60	5A-15	26.30	BAZOOKA(HO)	29.60
01/1277	22.50	5B-97	23.10	BD 96/009	23.70
01/1331-1	21.50	90/01554	28.80	BD 96/040	25.30
01/1331-2	25.80	94/0006	32.10	BD 96/093	20.10
01/1368	29.10	94/0006-1	32.80	BD 96/134	22.70
01/1369	26.50	94/0006-2	28.70	BD 96/165	29.00
01/1371-1	23.20	95/0379-2	29.50	DABODABO	21.30
01/1371-2	32.40	9A-3	23.70	DEBOR	25.80
01/1412	25.30	A TUMTUM (M1)	32.20	DEBOR(1)AF	27.30
01/1413-1	24.80	ABASAFITAA (HO)	32.00	DEBOR(NK)	24.30
01/1413-2	26.80	ADE 00/107	25.50	DMA 00/008	21.50
01/1442-1	26.30	ADE 00/169	28.50	DMA 00/031	22.30
01/1442-2	23.80	ADE 030/039	23.70	DMA 00/034	26.30
01/1646-1	32.70	ADW 00/004	22.20	DMA 00/042	27.70
01/1646-2	25.50	ADW 00/051	27.30	DMA 00/048	28.70
01/1649-1	31.70	ADW 00/053	26.80	DMA 00/069	27.20
01/1649-2	27.30	AFISIAFI (WN)	26.30	DMA 00/070	29.50
01/1649-3	23.30	AFS 00/043	26.30	DMA 01/004	24.50
01/1797	26.80	AFS 00/075	24.50	DMA 01/070	25.80
12B-36	27.50	AFS 00/081	31.50	DOKU DUADE	36.80
12B-38	24.30	AFS 00/149	32.30	ESIABAYA(AK)	32.20
12B-54	25.80	AGBELIFIA (WN)	31.50	ESSAMBANKYE(WN)	37.90
2B-13	23.40	AGRIC (M1)	25.50	GBLEMODUADE(WN)	37.30
2B-166	25.50	AMW 00/006	36.50	HUSHIVI	25.50
2B-223	22.30	AMW 00/099	32.60	I00/0093	24.70

Table 5.6 (cont'd) Mean fresh root yield (t/ha) of 150 cassava genotypes evaluated

Genotype	FRY (t/ha)	Genotype	FRY (t/ha)
K25	28.70	SW 00/064	29.10
KROBO(NK)	27.70	SW 00/068	26.70
KSI 00/039	29.80	SW 00/152	21.00
KSI 00/092	34.70	SW 00/181	30.50
KSI 00/126	22.20	SW 00/187	22.50
KSI 00/179	37.60	SW 00/192	31.20
KSI 01/036	29.30	SW 00/204	33.70
KW 00/045	24.30	SW 00/216	34.40
KW 00/095	16.70	TECKBANKYE(WN)	26.80
KW 00/101	37.70	TME 11	29.40
KW 00/181	24.70	UCC 00/003	32.40
KW 00/187	31.50	UCC 00/003	38.20
LAGOS(M1)	31.30	UCC 00/032	31.40
NKABOM(WN)	35.50	UCC 00/088	22.80
NKRUWA(AW)	29.70	UCC 01/010	31.60
NKZ 00/034	33.70	UCC 01/032	33.50
NO NAME(1)F1	21.50	UCC 01/078	22.50
NO NAME(2)F1	24.70	UCC 01/092	34.80
NO NAME(3)F1	29.60	UCC 01/111	25.80
OFF 00/019	35.50	UCC 01/195	33.10
OFF 00/023	27.50	UCC 01/209	34.20
OFF 00/029	23.30	UCC 01/218	33.30
OFF 00/058	35.60	UCC 01/249	37.80
OFF 00/063	37.10	UCC 01/250	38.70
OFF 00/086	34.20	UCC 01/270	34.50
OFF 00/087	24.50	UCC 01/279	32.70
OFF 01/146	23.30	UCC 01/291	35.80
OWUDURO(1)NK	14.50	UCC 01/415	32.20
OWUDURO(NK)	32.10	UCC 01/504	27.70
SW 00/006	33.50	UCC(M1)	33.80
SW 00/010	38.40	WCH 00/004	25.50
SW 00/049	32.50	WCH 00/011	30.70
SW 00/050	33.70	WCH 00/037	32.50
Grand mean	26.70		
SE	15.47		
LSD	10.50		
CV	20.80		

Table 5. 7 Analysis of variance for fresh root yield

Source of variation	Df	Mean squares	Contribution to SS (%)
Genotype	148	0.038*	32.4
Blk(Rep)	29	0.156	
Residual	221	0.028	
Total	399		

Df: degrees of freedom *significant at 5%

5.4.5 Correlation between PPD and related traits

Correlation matrix for postharvest physiological deterioration, root dry matter content and fresh root yield is presented in Table 5.8. The results of Pearson's correlation indicated that PPD was negatively correlated with fresh root yield ($R^2 = -0.028$, $P = 0.441$), positively but weakly associated with dry matter content ($R^2 = 0.214$, $P < 0.01$). This suggests that when DMC is high then PPD would also be high, however, it is possible to identify some genotypes with low PPD and high DMC. It was observed from the results that, some genotypes with high DMC also had high deterioration rates and vice versa, confirming the positive correlation between DMC and PPD. This included Bankyehemaa with DMC of 31.2% and PPD deterioration rate of 60.1%; and Bazooka with DMC and PPD deterioration rate of 36.8% and 35% respectively. Similarly, genotypes with low DMC were also observed to have low reaction to PPD. On the other hand, there were a few genotypes that had high DMC but moderate reaction to PPD. These included DMA00/031 with DMC of 33.4% and PPD of 9.9%; SW/00/064 with DMC of 30.2% and PPD of 9.6% and AFS00/043 with DMC of 31.1% and PPD of 12.9%.

Table 5. 8 Correlation matrix for postharvest physiological deterioration, dry matter content and fresh root yield

	FRY	DMC	PPD
FRY			
DMC	-0.163 (0.222)		
PPD	-0.028 (0.441)	0.214 (0.000)	

Pearson correlation and p-value in parenthesis

5.4.6 Physicochemical changes in cassava storage roots during Physiological deterioration

5.4.6.1 Proximate analysis of cassava flour during Physiological deterioration

The results and analysis of variance for the proximate analysis are presented in Tables 5.9 and 5.10 respectively. All the parameters considered with the exception of crude protein content were highly significant for genotype, days of storage and the genotype by storage interaction effects. This implied that there were genetic differences among the cassava genotypes and the days of storage also had significant effect on the ash, carbohydrate, fat, fibre and moisture content considered.

The fat content in the cassava flour ranged from 0.93% in 01/1224 to 3.83% in DMA01/070 on day one and reduced in all the genotypes ranging from 0.5% (SW00/064) to 1.72% (DMA01/070) on day seven. Ash content is a measure of the mineral element content in the crop that is translocated into the roots. It ranged from 3.3% in genotype UCC01/010 to 4.96% in 01/1224 on day one, and ranged from 2.86% to 5.5% on day seven. There was an inconsistent trend for the ash content on day seven as it reduced in most of the genotypes except Debor (1) AF and 01/1224. The ash content was, however, observed to be higher (with the exception of

UCC01/010 on day seven) for all the different storage periods than the maximum 3% specified by Codex Alimentarius Commission for edible cassava flour.

Moisture content of the flour ranged from 8.08% in Debor (1) AF to 9.27 % in 01/1224 on day one and reduced in all the genotypes ranging from 6.76% in genotype 01/1224 to 7.31% in genotype DMA01/070 on day seven. The moisture content for the two storage periods was all lower than the 12% specified by Codex standards for edible cassava flour. The carbohydrate content ranged from 83.1% in genotype DMA01/070 to 85.84% in UCC01/010 on day one after harvest, and ranged from 80.41% (01/1224) to 85.16% (SW00/064) on day seven. There was an inconsistent trend of the carbohydrate content after seven days of storage, as the amount reduced in the other genotypes and peaked in genotypes Debor (1) AF and DMA01/070.

Crude protein content was non-significant for all the sources of variation. This suggested that there were no genetic differences among the cassava genotypes and the days of storage also had no effect on crude protein content. It ranged from 1.55% (UCC01/010) to 1.75% (Debor (1) AF on day one to 1.1% (DMA01/070) and 1.79% (UCC01/010) on day seven. There was an inconsistent trend of the crude protein content after seven days of storage, as the amount peaked in the other genotypes but reduced in genotypes DMA01/070 and SW00/064. Crude fibre content ranged from 0.72% to 1.3 on day one after harvest and from 0.97% to 2.11% in genotypes DMA01/070 and 01/1224 respectively on day seven. Crude fibre content peaked in most of the genotypes but reduced in genotypes UCC01/010 and 01/1224 after seven days of storage. The crude fibre in this study was, however, lower in both storage periods (with the exception of genotype 01/1224) than the 2% upper limit specified for edible cassava flour by Codex.

5.4.6.2 Functional analysis of cassava flour during Physiological deterioration

The results for the functional analysis are presented in Tables 5.11 and 5.12. Solubility of cassava flour is important in baking, as flour with high solubility yield soggy dough with low cohesiveness. Genotypes and storage period were highly significant at ($p < 0.001$). This indicated that there was a differential response of the genotypes to the period of storage. Solubility of the flour ranged from 16.28% (Debor (1) AF)) to 27.2% (01/1224) on day one and reduced in all the genotypes ranging from 13.44% (DMA01/070) to 20.47% (01/1224) on day seven.

Water binding capacity is an important parameter in the consistency of products in the bakery industry. The analysis revealed that, genotypes and genotype by period of storage interaction were significant at ($p < 0.001$) and ($p < 0.05$) respectively. Water binding capacity of the flour ranged from 231.3% (DMA01/070) to 289.5% (01/1224) on day one and reduced in all the genotypes but peaked in genotypes 01/1224 and DMA01/070 on day seven.

Swelling capacity determines the extent to which cassava flour increase in volume when soaked in water in relation to its initial volume. The higher the swelling capacity, the greater its suitability for use in foods. The analysis revealed that, the swelling capacity was highly significant for genotype and period of storage ($p < 0.001$) and genotype by storage interaction ($p < 0.01$). This suggested that, the genotypes responded to the period of storage. Swelling power was in a range of 25.07 (SW00/064) to 27.67 (UCC01/010) on day one and reduced in all the genotypes on day seven.

The quality of cassava flour as it influences the taste is determined by pH. pH less than four indicates an appreciable level of fermentation. The analysis of variance revealed pH to be

significantly affected by the genotypic differences among the cassava genotypes used for the study and not by the period of storage. The flour samples had pH levels greater than five, indicating that, the cassava flour prepared from the different storage periods were of good quality. The values of pH were in the range of 6.07 to 7.2 on day one and 6.18 to 7.03 on day seven in genotypes 01/1224 and Debor (1) AF respectively.

Table 5. 9 Proximate analysis of cassava flour at different storage periods

Genotype	% MOISTURE		% ASH		% FIBRE		% FAT		% PROTEIN		% CARBOHYDRATE	
	0DAS	7DAS	0DAS	7DAS	0DAS	7DAS	0DAS	7DAS	0DAS	7DAS	0DAS	7DAS
DMA01/070	8.49 (0.12)	7.31 (0.44)	3.39 (0.05)	3.01 (0.05)	0.72 (0.09)	0.97 (0.09)	3.83 (0.11)	1.72 (0.92)	1.65 (0.39)	1.11 (1.01)	83.1 (0.36)	84.7 (1.01)
Debor(1)AF	8.08 (0.12)	6.81 (0.19)	3.79 (0.05)	3.86 (0.01)	1.28 (0.03)	1.03 (0.42)	2.43 (1.30)	1.25 (0.09)	1.75 (0.05)	1.78 (0.42)	83.93 (1.49)	83.99 (0.42)
01/1224	9.27 (0.07)	6.76 (0.09)	4.96 (0.07)	5.55 (0.06)	1.3 (0.11)	2.11 (0.08)	1.82 (0.03)	1.14 (0.05)	1.64 (0.05)	1.72 (0.34)	83.52 (1.56)	80.41 (0.34)
SW00/064	8.47 (0.07)	7.26 (0.11)	3.54 (0.12)	3.05 (0.04)	1.02 (0.06)	1.14 (0.13)	0.93 (0.21)	0.5 (0.08)	1.69 (0.09)	1.68 (0.39)	85.56 (0.37)	85.16 (0.39)
UCC01/010	9.03 (0.07)	6.89 (0.20)	3.3 (0.06)	2.86 (0.08)	1.28 (0.11)	1.04 (0.06)	1.18 (0.02)	1.04 (0.07)	1.55 (0.28)	1.79 (0.05)	85.84 (0.08)	83.33 (0.05)
LSD (genotype)	0.21		0.09		0.15		0.52		0.38		0.77	
LSD (treatment)	0.14		0.06		0.09		0.33		0.24		0.49	

Mean of three replicates with standard deviation in parentheses

Table 5. 10 Analysis of variance for the proximate analysis of cassava flour

Source of Variation	DF	Mean square					
		%ASH	%CARBOHYDRATE	%FAT	%FIBRE	%PROTEIN	%MOISTURE
Genotype	4	4.380***	9.500***	3.301***	0.592***	0.128NS	0.302***
Days of storage	1	0.213***	5.723***	3.927***	0.1464**	0.010NS	20.733***
Gen.xDays of storage	4	0.220***	5.573***	1.693***	0.284***	0.132NS	0.584***
Residual	18	0.006	0.399	0.184	0.01441	0.097	0.031

***significant at 0.1% NS: not significant

Table 5. 11 Functional analysis and pH of cassava flour at different storage periods

Genotype	WBC		SOLUBILITY		SWELLING POWER		pH	
	0DAS	7DAS	0DAS	7DAS	0DAS	7DAS	0DAS	7DAS
01/1224	289.5 (2.56)	315.6 (15.45)	27.2 (3.26)	20.47 (0.74)	25.37 (0.67)	10.86 (0.34)	6.07 (0.00)	6.185 (0.007)
Debor(1)AF	248.6 (2.07)	244.5 (13.3)	16.28 (1.86)	15.43 (1.91)	25.35 (1.39)	16.03 (1.56)	7.235 (0.007)	7.025 (0.007)
DMA01/070	231.3 (22.72)	241.8 (36.44)	18.83 (5.72)	13.44 (1.43)	27.49 (1.49)	16.46 (0.29)	6.92 (0.01)	6.975 (0.007)
SW00/064	283.5 (4.5)	245.6 (12.98)	18.68 (5.21)	13.67 (0.34)	25.07 (0.81)	12.89 (1.01)	6.24 (0.01)	6.195 (0.02)
UCC01/010	275.9 (5.07)	241.5 (8.49)	16.69 (2.54)	13.5 (0.49)	27.67 (0.11)	16.32 (1.03)	6.245 (0.03)	6.34 (0.14)
LSD (genotype)	19.78		3.49		1.24		0.08	
LSD (treatment)	12.51		2.21		0.78		0.05	

WBC: water binding capacity; Mean of three replicates with standard deviation in parentheses

Table 5. 12 Analysis of variance for the functional analysis of cassava flour

Source of Variation	DF	Mean square			
		SOLUBILITY	SWELLING POWER	WBC	pH
Genotype	4	78.34***	18.494***	3819.4***	0.849***
Days of storage	1	134.434***	1022.797***	472NS	0.00002NS
Gen.xDays of storage	4	7.756NS	5.389**	1165.6*	0.018**
Residual	18	8.295	1.042	266	0.002398

WBC: water binding capacity; *significant at 5% **significant at 1% ***significant at 0.1% NS: not significant

5.5 Discussion

Evaluation of the cassava genotypes revealed a high frequency of rotten roots as a result of microbial deterioration. This could be due to the long storage period that promoted the attack of microbes including fungi.

A number of methods have been proposed for root processing prior to PPD evaluation. This present study revealed that the processing method was significant. This implied that identification of cassava genotypes for delayed PPD required the use of appropriate PPD processing method. The Booth's method was less labor intensive, convenient and had minimal PPD scores than the Wheatley's processing method. Storage of whole cassava roots without necessarily cutting the extremes could probably explain the minimal deterioration observed with the Booth's method. This finding emphasizes that cutting the extremes of the roots and covering the distal end with cling film in the Wheatley's method, contributes to accelerating PPD.

Assessment of variability is a prerequisite for crop improvement. Significant differences in PPD levels were found among the 150 cassava genotypes stored for 7 days. This finding was similar to studies by Ekanayake and Lyasse (2003) and Sánchez *et al.* (2006, 2013) where they found significant differences in the development and severity of physiological deterioration between cassava varieties. This study identified cassava genotypes with low reaction to PPD. Further studies could, therefore, be carried out on the identified cassava genotypes and subsequently be released to farmers. Correlation between PPD and root dry matter although positive and significant, was weak. This suggested that, genes that control PPD could also control dry matter content. The finding of this study was also reported by Morante *et al.* (2010) who also reported positive but weak correlation between PPD and dry

matter content in cassava. As a result, the genotypes with low PPD and high dry matter content identified from this study could be used as parents in PPD improvement.

Cassava roots in storage undergo considerable changes. Genotype, days of storage, and the genotype by storage interaction effects were highly significant for ash, carbohydrate, fibre, fat content and moisture. This suggested that there were differential response of the genotypes to the period of storage. Crude protein content was, however, not significant for all the sources of variation indicating that there were no genotypic differences among the cassava genotypes and period of storage had no effects on protein content. The genotypes used in this study showed similarity in some of the changes, whereas there were differences in other genotypes. This could be due to the influence of genotypic variation that exists between the genotypes. With the exception of protein content which peaked in all the genotypes on day seven, all other parameters were reduced. This reduction could be attributed to both physiological and microbial deterioration. The increase in crude protein content could be due to the presence of microbes which could have been processed along with the cassava storage roots or probably from active expression of genes resulting in PPD.

Swelling power was significant for all the sources of variation while with water binding capacity and pH, the variation was mainly due to the genotypic differences and the genotype by storage interaction. Variation in solubility was mainly due to the genotypic differences and period of storage. Similar studies have reported changes in the physicochemical and functional properties of cassava flour after days of storage (Lola *et al.*, 2012; Sanchez *et al.*, 2013).

These significant changes observed in the physicochemical and functional properties of cassava flour could have serious impact on the quality of products prepared from deteriorated cassava roots.

5.6 Conclusion

This study revealed that Booth's method was an easy and convenient method for estimating PPD. Hence for subsequent PPD evaluation, this method could be used. It also indicated that variability existed among cassava genotypes for PPD, although there were no cassava genotypes with complete tolerance or resistance to PPD. Genotypes with low reaction to PPD (K25, SW/00/064 and DMA00/031) were identified. These genotypes could be evaluated for other root quality traits and subsequently be released to farmers and processors. With the positive but weak correlation between PPD and root dry matter content, some genotypes with minimum deterioration and high root dry matter were also identified (DMA00/031 and SW/00/064). Variability also existed for other economic traits such as fresh root yield and dry matter content. These cassava genotypes could be selected and used in the improvement of farmer preferred accessions in the cassava breeding program in Ghana. Changes were also observed in the physicochemical and functional properties of cassava roots in storage. These changes could have adverse effects on the products prepared from deteriorated cassava roots.

CHAPTER SIX

6.0 GENOTYPE BY ENVIRONMENT ANALYSIS OF POSTHARVEST

PHYSIOLOGICAL DETERIORATION IN CASSAVA

The phenotype of an individual is determined by both the genotype and the environment. These two effects are not always additive because of the genotype by environment interaction (GEI). GEI is a result of differential response of genotypes across environments. The presence of genotype by environment interaction complicates selection of superior genotypes and thus, reduces the usefulness of subsequent inferences that would otherwise be valid. To overcome GEI challenges, breeders evaluate genotypes in several environments to ensure that they select genotypes with high and stable performance. It is, therefore, important to understand the environmental and genotypic interaction between varieties in order to make informed decision for a particular breeding program.

Cassava grows well in different environments, however, it exhibits different growth behaviors in different environments due to variation in climatic and soil conditions (Egesi *et al.*, 2007). Several studies on genotype by environment analysis on yield and yield components, diseases and other traits have been reported in cassava (Aina, 2007; Egesi *et al.*, 2007; Ssemakula and Dixon, 2007; Akinwale *et al.*, 2010). However, no genotype by environment analysis has been reported on postharvest physiological deterioration in cassava. Ntawuruhunga and Dixon (2010) observed highly significant interaction between location and season, environment and genotype upon evaluating ten cassava cultivars in three different locations in Uganda. Evaluating cassava genotypes for delayed PPD in different agro-ecologies will help in identifying genotypes that are best suitable for each agro-ecology as well as those that are stable across varying environments.

Several statistical methods are available to determine the effect of GE interaction on the response of varieties to environmental changes. In recent years, G x E interaction studies has been done with Additive Main Effect and Multiplicative Interaction (AMMI) method (Kumar *et al.*, 2009; Rea *et al.*, 2011). AMMI method integrates Analysis of Variance (ANOVA) and Principal Component Analysis (PCA) in a single approach to analyze multilocation trials (Crossa *et al.*, 1990; Gauch and Zobel, 1996). AMMI uses analysis of variance to study the main effects of genotypes and environments and a principal component analysis for the residual multiplicative interaction among genotypes and environments. In addition, AMMI quantifies the contribution of each genotype and environment to the interaction effects and provides a graphical interpretation of the results by the biplot technique to simultaneously classify genotypes and environments (Kempton, 1984; Zobel *et al.*, 1998).

Heritability is a measure of the proportion of phenotypic variance due to genetic effects. It informs the breeder on the feasibility of improving a trait through selection (Jones, 1986). Different methods have been used for estimating heritability, however, one of the commonly used is the analysis of variance method (Holland *et al.*, 2003). Heritability estimates provide the basis for selection on phenotypic performance. Estimation of the heritability for PPD would help breeders make informed decisions on the appropriate breeding method that could be used to effectively select for PPD in cassava.

6.1 Objectives

The objectives of this study were to:

- a. Determine the effects of genotype by environment interaction on postharvest physiological deterioration in cassava storage roots
- b. Estimate broad sense heritability for postharvest physiological deterioration

6.2 Materials and methods

6.2.1 Study area

The study was conducted in three locations Fumesua (277 m abs), Ejura (240 m abs) and Ohawu (197 m abs) for two growing seasons (2011/2012 and 2012/2013) in Ghana. The agro-ecological characteristics of the experimental sites are presented in Table 6.1. Characteristics of Fumesua are presented in section 5.2.1.1. Ejura is located in the Forest Savanna Transition zone characterized by rainfall ranging from 1000 - 1200 mm with temperature ranging from 21 - 34°C. Soils are dominated by the ferric lxisols type with sandy loamy texture. Ohawu is located in the Coastal Savanna Transition zone with rainfall ranging from 800 - 1100 mm and temperature ranging from 24 - 30°C. Soils are dominated by the lxisols type with sandy loamy texture.

6.2.2 Plant material and experimental design

Forty cassava genotypes used for this experiment were selected from Table 5.2 based on their PPD scores, dry matter content, yield and availability of planting materials. Healthy stem cuttings of 20 cm were planted in four row plots of eight plants per row at 1 m by 0.75 m spacing. Plots were separated by 1 m alleys. Materials were evaluated using a 5 x 8 alpha lattice design with three replicates, each block consisting of eight genotypes.

6.2.3 Data collection and root harvest

Roots were harvested 10 months after planting (MAP) and data were taken for the traits; storage root number per plant (SRN); fresh storage root yield (FRY); biomass yield (BY) and disease severity and incidence as described in section 5.2.1.3.

Table 6. 1 Agro-ecological characteristics of the experimental sites

Characteristics	Locations		
	Ohawu	Ejura	Fumesua
GPS coordinates	6 ⁰ 03N 0 ⁰ 49E	7 ⁰ 23N 1 ⁰ 21W	6 ⁰ 41N 1 ⁰ 28W
Agro-ecology	Coastal Savanna	Forest-savanna Transition	Humid Forest
Soil types	Lixisols Toje series with 20 cm thick top layer of loamy sandy soils with a sub-soil of red to brownish sandy loam	Ferric Lixisols Ejura series with 20-30 cm thick top layer of loamy soils. Soils are dark to brown to brown fine sandy loam.	Ferric Acrisol Asuani series, upper top soil consists of 5 cm greyish brown sandy loam over a lower top soil of dark brown gritty clay loam.
Slope*	2-6%	2-6%	2-6%
Temperature range (Min. - Max. °C)	24 - 30	21 - 34	22 - 31
Total annual rainfall (mm)	800 - 1100	1000 - 1200	1190 - 1650
Average annual rainfall	950	1108	1345

* Adu and Asiamah, 1992

6.2.4 Root quality analysis

Root processing for PPD evaluation, scoring and dry matter content determination were carried out as stated above in section 5.2.1.4. Roots were scored for PPD at each location where plants were grown.

6.3 Data analysis

Data were subjected to analysis of variance (ANOVA) using the GLM procedure of Statistical Analysis System (SAS) version 9.3 to determine the significance of the main effects and interactions. For the genotype by environment analysis, genotypes were considered as fixed effects whilst locations and years were considered as random effects (Steel and Torrie, 1960) to estimate the variance component of the traits measured. Separation of means was carried out using Fisher's Least Significance Difference (LSD) at 5% probability level. The ANOVA was computed according to the model statement:

$$x_i = P = G + (G \times L) + L + E$$

Where:

G = genotypic effect

L = location effect

GxL = genotype by location effect

E = experimental error

P = resulting phenotypic value

The corresponding mathematic linear model for an alpha lattice design is stated:

$$Y_{ijk} = \mu + e_j + r_{(ej)} + g_i + ge_{ij} + \varepsilon_{ijk}$$

Where;

μ = general mean

Y_{ijk} = the observed response of the experimental unit in the kth replicate within the jth location planted to the ith genotype

e_j = the main effect of the jth location

$r_{(ej)}$ = the effect of kth replicate within location j

g_i = the main effect of the ith genotype

ge_{ij} = the genotype x location interaction effect

ε_{ijk} = the error associated with ijth observation

Additive Main effect and Multiplicative Interaction (AMMI) analysis (Zobel *et al.* (1998) was carried out using the fixed effect model of GENSTAT version 12. The model equation is stated:

$$Y_{ger} = \mu + \alpha_g + \beta_e + \sum_n \lambda_n \gamma_{gn} \delta_{en} + \rho_{ge} + \varepsilon_{ger}$$

Where: Y_{ger} = yield for genotype (g) in environment (e) for replicate (r)

μ = the grand mean

α_g = genotype (g) deviation

β_e = the environment (e) deviation

λ_n = singular value for IPCA axis n

γ_{gn} = eigenvector value for genotype (g) of IPCA axis n

δ_{en} = eigenvector value for environment (e) of IPCA axis n

ρ_{ge} = the residual

ε_{ger} = error

GGE biplots (version 4.1) were used to do a graphical analysis to identify genotypes with broad or specific adaptation to target environments. Genotypic (σ^2_g) and phenotypic (σ^2_p) variances were estimated from the variance components using the mean squares from the analysis of variance table according to Comstock and Robinson (1952). Broad sense heritability (H^2) was estimated for the traits studied according to Falconer (1989) using the formula:

$$H^2 = \sigma^2_g / \sigma^2_p$$

Where:

σ^2_g = genotypic variance

σ^2_p = phenotypic variance

Phenotypic variance was estimated as:

$$\sigma^2_p = \sigma^2_{e/rly} + \sigma^2_{gly/ly} + \sigma^2_{gy/y} + \sigma^2_{gl/l} + \sigma^2_g$$

Phenotypic and Genotypic Coefficient of Variation were also estimated according to the formula proposed by Singh and Chaudhary (1985) as follows:

$$\text{GCV (\%)} = \sqrt{\sigma^2_{g/X}} * 100$$

$$\text{PCV (\%)} = \sqrt{\sigma^2_{p/X}} * 100$$

Where:

X = sample mean

6.4 Results

6.4.1 Mean performance for postharvest physiological deterioration

Mean postharvest physiological deterioration is presented in Table 6.2. Postharvest physiological deterioration varied significantly across locations. All genotypes expressed mild to moderate reaction to postharvest physiological deterioration. In all the locations, postharvest physiological deterioration was higher in year one than in year two. Mean PPD across locations ranged from 12.01% to 34.53%, with a grand mean of 22.99%. Genotype Debor (NK) recorded the lowest physiological deterioration while 01/1649-3 expressed the highest reaction. The highest mean for postharvest physiological deterioration was recorded in Fumesua-12 environment (14.01%) while Ejura-13 had the lowest reaction (11.82%).

Combined analysis of variance for postharvest physiological deterioration is presented in Table 6.3. The combined analysis of variance indicated that all main effects of genotype, location and year were significant ($P < 0.01$) and contributed 10.26%, 14.5% and 21.05% to the total sum of squares respectively. Genotype by location interaction was significant and contributed 16.28%, however, genotype by year and genotype by location by year interactions were not significant.

Table 6. 2 Mean postharvest physiological deterioration (%) of cassava genotypes evaluated across three locations for two years

Genotype		Ejura12	Ejura13	Fumesua12	Fumesua13	Ohawu12	Ohawu13	Genotypic mean
01/0131	G1	12.76	10.95	12.7	10.98	13.03	11.89	12.05
01/1224	G2	13.33	11.58	13.51	11.8	13.19	12.08	12.58
01/1235	G3	13.15	11.3	14.36	12.67	13.72	12.61	12.97
01/1371-2	G4	13.42	11.51	15.4	13.73	14.4	13.29	13.63
01/1412	G5	13.18	11.36	13.65	11.94	13.6	12.47	12.7
01/1413-1	G6	13.34	11.55	11.61	9.85	13.59	12.39	12.06
01/1442-2	G7	13.28	11.37	15.07	13.4	14.19	13.08	13.4
01/1646-2	G8	13.8	12.1	13.31	11.58	13.4	12.28	12.75
01/1649-2	G9	14.14	12.51	14.87	13.17	13.29	12.24	13.37
SW00/064	G10	14.80	13.25	15.46	13.76	13.32	12.30	13.82
94/0006-2	G11	13.69	11.93	14.62	12.92	13.65	12.56	13.23
95/0379-2	G12	14.04	12.18	14.03	12.31	14.75	13.58	13.48
ADW00/004	G13	13.43	11.63	14.09	12.39	13.74	12.62	12.98
Agbelifia	G14	13.46	11.62	16.3	14.65	13.94	12.89	13.81
Agric(M1)	G15	13.62	11.9	13.92	12.21	13.33	12.23	12.87
AkosuaTumtum	G16	16.09	14.8	13.27	11.48	13.03	11.98	13.44
AMW00/006	G17	13.6	11.79	15.03	13.35	13.93	12.84	13.42
Aworowa	G18	13.55	11.67	12.99	11.26	14.35	13.16	12.83
BD96/093	G19	13.74	11.84	13.39	11.67	14.74	13.55	13.16
BD96/134	G20	14.4	12.83	13.39	11.65	13.18	12.09	12.92
Debor(1)AF	G21	13.56	11.77	12.4	10.65	13.81	12.63	12.47
Debor(NK)	G22	13.46	11.75	11.67	9.91	13.21	12.04	12.01
DMA01/070	G23	13.4	11.61	15.78	14.13	13.49	12.44	13.48
IOO/0093	G24	13.27	11.52	14.96	13.28	13.12	12.06	13.04
KSI00/092	G25	13.26	11.51	13.21	11.5	13.24	12.11	12.47
KW00/095	G26	14.14	12.34	14.56	12.85	14.41	13.29	13.6
KW00/181	G27	13.49	11.75	13.14	11.41	13.35	12.22	12.56
Nkabom	G28	13.1	11.3	14.81	13.13	13.34	12.26	12.99
OFF 00/023	G29	13.36	11.61	13.87	12.17	13.3	12.19	12.75
Owuduro(1)NK	G30	13.41	11.67	13.49	11.78	13.28	12.17	12.63
Owuduro(NK)	G31	13.63	11.87	13.93	12.22	13.63	12.51	12.97
SW00/010	G32	13.19	11.37	13.21	11.49	13.57	12.43	12.54
01/1649-3	G33	34.82	33.28	37.61	35.96	33.22	32.28	34.53
SW00/152	G34	13.33	11.48	13.40	11.69	13.89	12.74	12.76
Tekbankye(WN)	G35	13.07	11.25	14.08	12.39	13.44	12.33	12.76
UCC(M1)	G36	13.21	11.51	13.17	11.45	12.77	11.67	12.30
UCC01/010	G37	13.66	11.68	13.47	11.75	15.15	13.94	13.28

Table 6.2 (cont'd) Mean postharvest physiological deterioration (%) of cassava genotypes evaluated across three locations for two years

Genotype		Ejura12	Ejura13	Fumesua12	Fumesua13	Ohawu12	Ohawu13	Genotypic mean
UCC01/218	G38	13.34	11.51	16.33	14.69	13.69	12.64	13.7
UCC01/249	G39	12.75	10.85	13.27	11.56	13.68	12.52	12.44
UCC01/250	G40	13.48	11.53	13.24	11.52	14.75	13.55	13.01
Environmental means		13.59	11.82	14.01	12.31	13.67	12.55	12.99
SE (genotype)		0.48						
SE (environment)		0.13						
LSD (5%) genotype		0.94						
LSD (5%) environment		0.26						
CV		10.2						

Table 6. 3 Combined analysis of variance for postharvest physiological deterioration of 40 cassava genotypes evaluated across three locations for two years

Source of variation	DF	Sum of Squares	Mean squares	Contribution to SS (%)
Rep/location	12	3.57	1.79NS	
Location	2	290.69	145.35**	14.50
Genotype	39	205.7	5.27**	10.26
Year	1	421.97	421.97**	21.05
GxL	78	326.51	4.19**	16.28
GxY	39	7.08	0.18NS	0.35
LxY	2	15.72	7.86*	0.78
GxLxY	78	11.45	0.15NS	0.57
Residual	478	233.38	0.49	
Total	719	2005.07		

*significant at 5% **significant at 1% NS: not significant

AMMI analysis of variance for postharvest physiological deterioration is presented in Table 6.4. The results showed that the GEI sum of squares was larger for genotypes, indicating that there were some differences in genotypic response across environments. The GEI effects were partitioned into two interaction principal components axes (IPCA 1 and IPCA 2). IPCA 1 of the interaction captured the largest variation of 69.43% of the interaction sum of squares in 51.13% of the interaction degrees of freedom. Similarly, IPCA 2 explained a further 14.31% of the GEI sum of squares. However, it was only the mean squares for IPCA 1 that was highly significant ($P < 0.001$). Interaction of the cassava genotypes with the environments was, therefore, best predicted by the first principal component of genotype and environment interaction.

Table 6. 4 AMMI analysis of variance for postharvest physiological deterioration of 40 cassava genotypes evaluated across six locations

Source of variation	DF	Sum of Squares	Mean squares	Contribution to SS (%)
Treatments	122	0.420	0.003***	
Genotypes	39	0.145	0.004***	
Environments	5	0.090	0.018*	
Block	6	0.031	0.005*	
Interactions	78	0.185	0.002***	
IPCA1	40	0.128	0.003***	69.431
IPCA2	38	0.026	0.001NS	14.309
Residuals	36	0.860	0.001	
Total	364	1.311	0.004	

*significant at 5% ***significant at 0.1% NS: not significant

The GGE biplot for mean performance of genotypes is presented in Figure 6.1. Genotypes that had PC1 scores > 0 were identified as high PPD genotypes and those that had PC1 scores < 0 were identified as low PPD genotypes. Genotype 01/1649-3 (G33) had the highest PPD value while genotype Debor (NK) (G22) had the lowest PPD value. The genotypes and

locations with large PC1 scores (positive or negative) indicated high interactions, while the genotypes with low or PC1 close to zero resulted in small interactions. In the case of PPD, the lower the PPD score the better hence, the focus was on genotypes with low PPD values that were stable across locations. Genotypes 01/0131 (G1) and KW00/181 (G27) had PC1 scores close to zero and therefore considered as stable for PPD. AMW00/006 (G17) and UCC01/218 (G38) had PC1 scores close to zero but with high PPD values. The genotypes associated with higher GxE interactions were SW00/064 (G10) and DMA01/070 (G23). Ejura with large and negative PC1 score was considered as an environment with lowest PPD score, Ohawu had large and positive PC1 score and was considered as an environment with highest PPD score while Fumesua with a PC1 near zero was considered to be a more stable environment for PPD. Genotypes with low PPD scores for the different locations were considered to perform well as a result, UCC01/249 (G39) performed better at Ejura whereas 01/1413-1 (G6) performed better at Fumesua while UCC (M1) (G36) performed better at Ohawu. Genotypes 01/0131 (G1), Tekbankye (G35), Nkabom (G28) and 01/1235 (G3) performed best at Ejura; genotypes Debor (NK) (G22), Debor(1)AF (G21) and 01/0131 (G1) also performed best at Fumesua; while, 01/0131 (G1), AkosuaTumtum (G16), IOO/0093 (G24) and Debor (NK) (G22) had best performance for PPD at Ohawu.

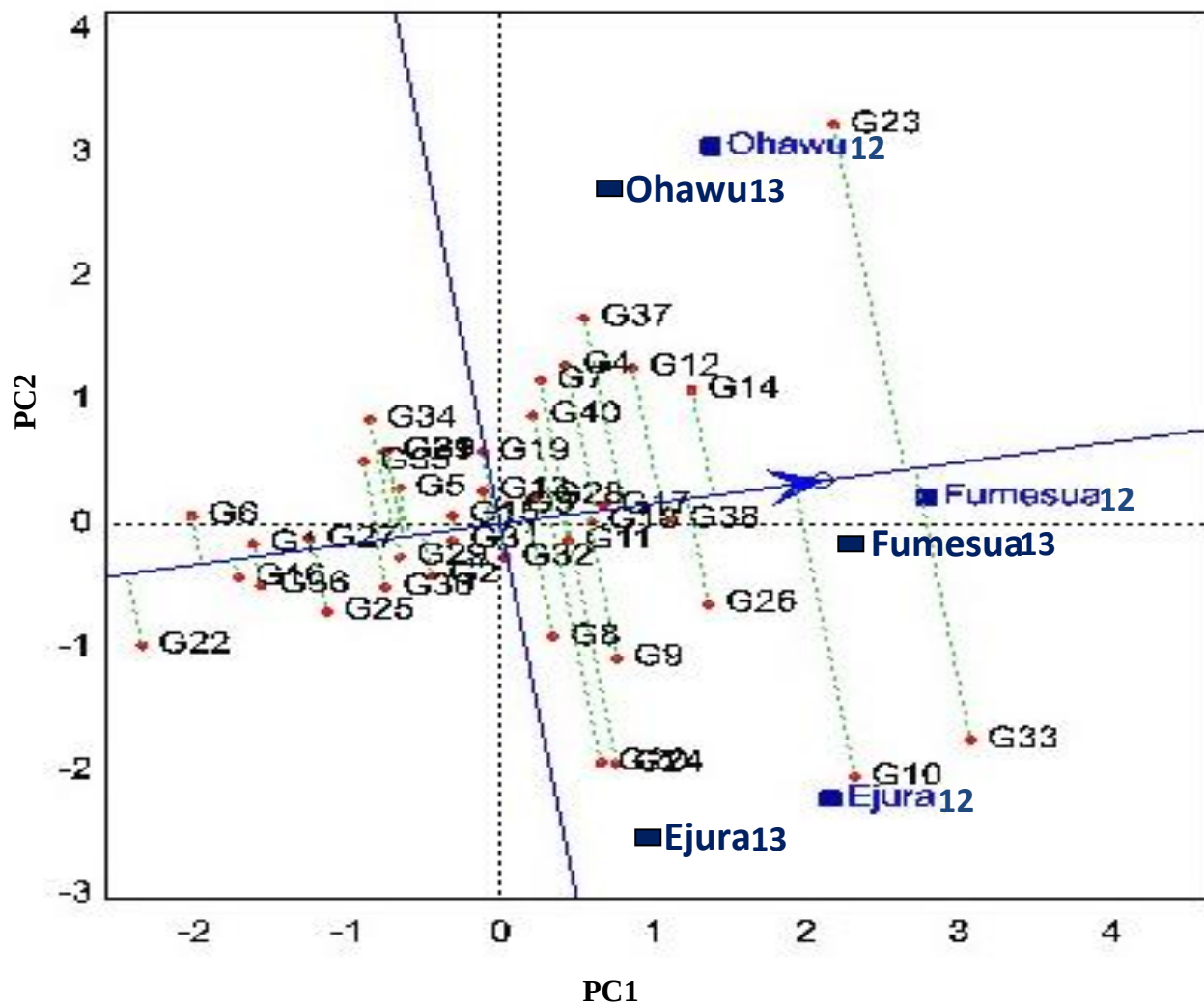


Fig. 6. 1 GGE biplot showing mean performance and stability for postharvest physiological deterioration of 40 cassava genotypes evaluated across three locations for two years

6.4.2 Mean performance for root dry matter content

Mean root dry matter content is presented in Table 6.5. The mean dry matter content across locations ranged from 24.27% to 35.39%, with a grand mean of 30.76%. Genotype IOO/093 recorded the lowest dry matter while genotype AMW00/006 recorded the highest. The highest mean was recorded in Ejura-12 environment (40.48%) while Ohawu-13 recorded the lowest dry matter content (17.73%).

Combined analysis of variance for root dry matter content is presented in Table 6.6. All the main effects of genotype, location and year were significant ($P < 0.01$) and contributed 24.71%, 10.41% and 15.79% to the total sum of squares respectively. Genotype by location interaction was significant and contributed 25.66%, however, genotype by year and genotype by location by year interactions were not significant.

AMMI analysis of variance for root dry matter content is presented in Table 6.7. GEI sum of squares was larger than that for genotypes, indicating that there were some differences in genotypic response across environments. IPCA 1 and IPCA 2 of the interaction captured 75.14% and 14.73% respectively of the GEI sum of squares. The mean squares for IPCA 1 and IPCA 2 were highly significant ($P < 0.001$), and they contributed 89.87% of the total GEI. Interaction of the cassava genotypes with the environments was best predicted by the two principal components of genotype and environment interaction.

Table 6. 5 Mean root dry matter content (%) of cassava genotypes evaluated across three locations for two years

Genotype		Ejura12	Ejura13	Fumesua12	Fumesua13	Ohawu12	Ohawu13	Genotypic means
01/0131	G1	40.03	34.9	31.35	26.09	30.66	26.04	31.51
01/1224	G2	27.37	22.43	22.83	18.07	35.29	30.76	26.13
01/1235	G3	21.33	16.7	24.97	19.66	35.73	31.29	24.95
01/1371-2	G4	28.43	23.63	27.42	22.27	36.54	32.04	28.39
01/1412	G5	28.48	23.63	26.55	21.31	32.94	28.43	26.89
01/1413-1	G6	27.09	22.54	33.58	27.5	33.45	29.01	28.86
01/1442-2	G7	24.84	20.14	26.41	21.23	37.53	33.07	27.2
01/1646-2	G8	36.36	31.23	27.15	22.36	34.3	29.71	30.19
01/1649-2	G9	27.9	23	24.23	19.38	35.91	31.39	26.97
01/1649-3	G10	21.07	16.52	27.1	21.41	33.48	29.05	24.77
94/0006-2	G11	28.35	23.5	26.37	21.25	34.96	30.45	27.48
95/0379-2	G12	33.65	28.6	26.34	21.52	34.93	30.36	29.23
ADW00/004	G13	36.87	32.05	36.36	30.63	35.51	30.99	33.74
Agbelifia	G14	34.04	28.96	25.85	21.11	34.99	30.42	29.23
Agric(M1)	G15	38.34	33.11	26.49	21.71	31.25	26.61	29.59
AkosuaTumtum	G16	29.3	24.58	31.32	25.39	29.37	24.87	27.47
Aworowa	G17	40.54	35.58	36.21	30.62	33.93	29.36	34.37
AMW00/006	G18	39.92	34.96	35.41	30.11	38.24	33.69	35.39
BD96/093	G19	40.27	35.21	32.95	27.92	37.76	33.18	34.55
BD96/134	G20	39.14	34.2	35.37	29.85	35.06	30.51	34.02
Debor(1)AF	G21	38.37	33.4	33.58	28.28	36.06	31.51	33.53
Debor(NK)	G22	38.65	33.44	27.91	22.72	26.49	21.85	28.51
DMA01/070	G23	38.93	33.97	34.76	29.27	34.52	29.96	33.57
IOO/0093	G24	33.69	28.5	23.36	18.22	23.24	18.62	24.27
KSI00/092	G25	34.7	30	37.4	31.44	35.73	31.25	33.42
KW00/095	G26	35.27	30.5	35.48	29.95	38.81	34.31	34.05
KW00/181	G27	37.08	32.02	29.57	24.63	35.97	31.4	31.78
Nkabom	G28	31.11	26.53	36.66	30.63	36.5	32.05	32.25
OFF 00/023	G29	40.59	35.56	34.33	29.2	38.51	33.94	35.36
Owuduro(1)NK	G30	41.37	36.18	31.11	25.98	31.24	26.61	32.08
Owuduro(NK)	G31	38.57	33.48	30.62	25.64	35.7	31.12	32.52
SW00/010	G32	37.26	32.36	34.15	28.86	38.65	34.11	34.23
SW00/064	G33	37.03	31.98	29.72	24.96	39.36	34.79	32.97
SW00/152	G34	35.03	30.13	31.67	26.43	36.69	32.15	32.02
Tekbankye(WN)	G35	37.49	32.5	31.94	26.76	35.88	31.32	32.65
UCC(M1)	G36	38.59	33.62	34.68	28.63	24.76	20.17	30.08
UCC01/010	G37	36.93	31.85	28.9	24.01	35.6	31.02	31.39

Table 6.5 (cont'd) Mean root dry matter content (%) of cassava genotypes evaluated across three locations for two years

Genotype		Ejura12	Ejura13	Fumesua12	Fumesua13	Ohawu12	Ohawu13	Genotypic means
UCC01/218	G38	36.05	31.09	31.3	26.21	37.56	33.02	32.54
UCC01/249	G39	38.53	33.5	32.42	27.14	33.86	29.28	32.46
UCC01/250	G40	40.48	35.25	29.13	23.98	27.79	23.15	29.96
Environment means		33.8	28.87	30.21	24.92	34.25	29.71	30.29
SE (genotype)		1.2						
SE (environment)		0.33						
LSD (5%) genotype		2.36						
LSD (5%) environment		0.65						
CV		12.8						

Table 6. 6 Combined analysis of variance for root dry matter content of 40 cassava genotypes evaluated across three locations for two years

Source of variation	DF	Sum of Squares	Mean squares	Contribution to SS (%)
Rep/location	12	178.42	89.21**	
Location	2	2870.73	1435.37**	10.41
Genotype	39	6816.35	174.78**	24.71
Year	1	4355.18	4355.18**	15.79
GxL	78	7078.27	90.75**	25.66
GxY	39	17.88	0.46NS	0.07
LxY	2	15.5	7.75NS	0.06
GxLxY	78	42.38	0.54NS	0.15
Residual	478	6209.84	12.99	
Total	719	27584.55		

**significant at 1% NS: not significant

Table 6. 7 AMMI analysis of variance for root dry matter content of 40 cassava genotypes evaluated across six locations

Source of variation	DF	Sum of Squares	Mean squares	Contribution to SS (%)
Treatments	122	5.53	0.04***	
Genotypes	39	1.40	0.04***	
Environments	5	2.15	0.43***	
Block	6	0.03	0.01NS	
Interactions	78	1.98	0.03***	
IPCA1	40	1.48	0.04***	75.14
IPCA2	38	0.29	0.01***	14.73
Residuals	36	1.62	0.04	
Total	364	7.18	0.01	

***significant at 0.1% NS: not significant

GGE biplot for mean performance and stability of genotypes for root dry matter content is presented in Figure 6.2. Genotype AMW00/006 (G18) recorded the highest dry matter content while genotype IOO/0093 (G24) recorded the lowest root dry matter. Genotypes DMA01/070 (G23) and UCC01/249 (G39) recorded high dry matter content and had PC1 scores close to zero and therefore considered as high and stable genotypes for root dry matter. 01/1235 (G3) and 94/0006-2 (G11) had PC1 scores close to zero but with low dry matter content. The genotypes associated with higher GxE interactions were IOO/0093 (G24) and UCC(M1) (G36). Ohawu with large and negative PC1 score was considered as an environment with low root dry matter, Fumesua and Ejura had large and positive PC1 scores and was considered as environments with high root dry matter, however, Ejura had a PC1 near zero was considered was therefore considered to be a more stable environment for root dry matter.

Genotypes that performed best at each location were identified. Owuduro (1)NK (G30), OFF/00/023 (G29), AMW00/006 (G18) and UCC01/0250 (G40) performed best at Ejura; genotypes KSI00/092 (G25), Nkabom (G28), ADW00/004 (G13) and AMW00/006 (G18) also performed best at Fumesua; while, SW00/064 (G33), KW00/095 (G26), SW00/010 (G32) and OFF00/023 (G29) had best performance for root dry matter at Ohawu.

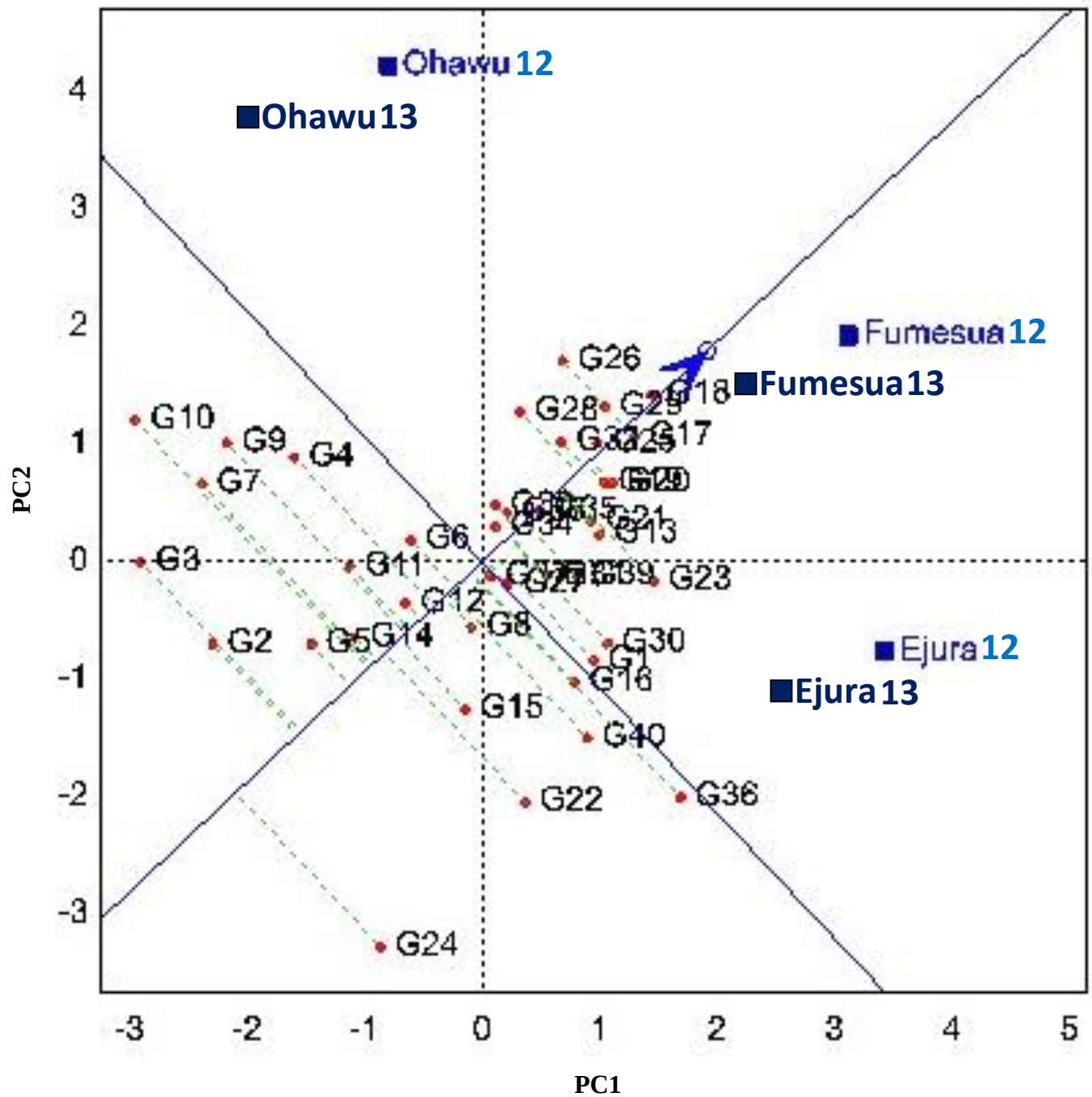


Fig. 6. 2 GGE biplot showing mean performance and stability for root dry matter of 40 cassava genotypes evaluated across three locations for two years

6.4.3 Mean performance for fresh root yield

Mean fresh root yield is presented in Table 6.8. The mean fresh root yield across locations ranged from 18.56t/ha to 38.81t/ha with a grand mean of 29.59t/ha. Genotype UCC (M1) recorded the highest yield while genotype UCC01/249 recorded the lowest yield. The highest mean was recorded in Ejura-12 environment of 38.83t/ha while Ohawu-13 recorded the lowest yield of 17.73t/ha.

Combined analysis of variance for fresh root yield is presented in Table 6.9. The results of the combined analysis indicated that all the main effects of genotype, location and year were significant ($P < 0.01$) and contributed 13.43%, 29.33% and 6.03% to the total sum of squares respectively. Genotype by location interaction was significant and contributed 29.6%. Genotype by year and genotype by location by year interactions were not significant.

AMMI analysis of variance for fresh root yield is presented in Table 6.10. GEI sum of squares was larger than that for genotypes, indicating that there were some differences in genotypic response across environments. IPCA 1 of the interaction captured the largest variation of 54.81% of the interaction sum of squares. Similarly, IPCA 2 explained a further 28.72% of the GEI sum of squares. The mean squares for IPCA 1 and IPCA 2 were highly significant ($P < 0.001$), and they contributed 83.53% of the total GEI. Interaction of the cassava genotypes with the environments was best predicted by the two principal components of genotype and environment interaction.

Table 6. 8 Mean fresh root yield (t/ha) of cassava genotypes evaluated across three locations for two years

Genotype		Ejura12	Ejura13	Fumesua12	Fumesua13	Ohawu12	Ohawu13	Genotypic means
01/0131	G1	35.79	31.12	37.28	28.85	19.82	15.97	28.14
01/1224	G2	35.94	31.53	38.57	30.52	26	21.71	30.71
01/1235	G3	45.36	40.48	39.35	32.17	32.05	27.49	36.15
01/1371-2	G4	42.4	37	30.87	23.72	21.1	16.88	28.66
01/1412	G5	44.47	38.82	42.65	32.62	5.41	3.29	27.88
01/1413-1	G6	21.19	17.78	38.08	29.1	22.49	18.15	24.47
01/1442-2	G7	38.29	32.83	27.79	20.28	14.46	10.51	24.03
01/1646-2	G8	27.03	22.97	38.61	29.29	16.43	12.76	24.52
01/1649-2	G9	33.48	28.75	43.09	32.61	6.69	4.26	24.81
UCC01/249	G10	9.2	6.47	31.05	22.56	23.61	18.48	18.56
UCC(M1)	G11	46.15	46.15	39.74	33.13	38.73	33.65	38.81
95/0379-2	G12	46.06	40.61	42.35	33.25	14.65	11.77	31.45
ADW00/004	G13	32.93	28.68	26.44	20.94	37.87	31.75	29.77
Agbelifia	G14	47.04	41.53	39.29	31	18.54	15.16	32.09
Agric(M1)	G15	43.82	38.89	37.81	30.51	29.16	24.72	34.15
AkosuaTumtum	G16	34.81	30.58	34.23	27.36	33.3	28.09	31.4
AMW00/006	G17	46.82	41.06	35.09	27.13	15.97	12.53	29.77
Aworowa	G18	45.35	39.37	30.35	22.61	11.95	8.53	26.36
BD96/093	G19	29.61	26.08	42.66	34.28	31.88	27.22	31.96
BD96/134	G20	40.45	35.48	45.91	35.82	11.79	9.26	29.79
Debor(1)AF	G21	38.98	34.15	37.02	29.03	22.7	18.66	30.09
Debor(NK)	G22	34.13	29.57	44.06	33.93	11.86	9.07	27.1
DMA01/070	G23	44.26	39.76	40.98	34.08	38.26	33.26	38.43
IOO/0093	G24	45.13	39.82	40.67	32.08	18.33	15.01	31.84
KSI00/092	G25	41.63	36.86	44.15	35.23	21.71	18.26	32.97
KW00/095	G26	41.46	41.46	36.18	28.49	23.46	19.35	30.9
KW00/181	G27	27.58	27.58	37.99	29.87	28.75	24.01	28.68
Nkabom	G28	31.37	31.37	30.41	23.26	26.2	21.28	26.59
OFF 00/023	G29	41.66	41.66	33.7	26.43	24.37	20.03	30.46
Owuduro(1)NK	G30	46.43	46.43	15.85	11.87	31.73	25.74	28.68
Owuduro(NK)	G31	32.82	32.82	44.29	34.57	17.51	14.23	28.67
SW00/010	G32	10.76	10.76	32.31	24.37	30.99	25.35	22
SW00/064	G33	33.44	33.44	28.72	21.57	22.43	17.76	25.44
SW00/152	G34	44.37	44.37	38.39	30.79	26.34	22.19	33.57
Tekbankye(WN)	G35	43.59	43.59	39.96	31.26	16.75	13.49	30.56
01/1649-3	G36	44.04	38.44	30.93	23.68	19.14	15.12	28.56
UCC01/010	G37	27.94	27.94	33.75	25.96	25.83	21.07	26.42
UCC01/218	G38	41.72	41.72	37.14	29.52	25.62	21.4	32.03

Table 6.8 (cont'd) Mean fresh root yield (t/ha) of cassava genotypes evaluated across three locations for two years

Genotype		Ejura12	Ejura13	Fumesua12	Fumesua13	Ohawu12	Ohawu13	Genotypic means
94/0006-2	G39	40.8	36.61	44.09	36.43	36.3	31.59	37.64
UCC01/250	G40	35.2	30.75	42.39	33.17	19.07	15.59	29.36
Environmental means		38.83	34	37.72	29.53	21.64	17.73	29.91
SE (genotype)		2.97						
SE (location)		3.64						
LSD (5%) genotype		4.13						
LSD (5%) location		1.13						
CV		43.3						

Table 6.9 Combined analysis of variance for fresh root yield of 40 cassava genotypes evaluated across three locations for two years

Source of variation	DF	Sum of Squares	Mean squares	Contribution to SS (%)
Rep/location	12	49.79	24.89NS	
Location	2	27562.47	13781.23**	29.33
Genotype	39	12622.83	323.66**	13.43
Year	1	5666.84	5666.84**	6.03
GxL	78	27813.63	356.59**	29.60
GxY	39	238.85	6.12NS	0.25
LxY	2	532.94	266.47**	0.57
GxLxY	78	450.00	5.77NS	0.48
Residual	478	19028.60	39.81	
Total	719	93965.94		

**significant at 1% NS: not significant

Table 6. 10 AMMI analysis of variance for fresh root yield of 40 cassava genotypes evaluated across six locations

Source of variation	DF	Sum of Squares	Mean squares	Contribution to SS (%)
Treatments	122	64.60	0.53***	
Genotypes	39	8.51	0.22***	
Environments	5	37.81	7.56*	
Block	6	0.48	0.08*	
Interactions	78	18.28	0.23***	
IPCA1	40	10.02	0.33***	54.81
IPCA2	38	5.25	0.14NS	28.72
Residuals	36	18.6	0.52	
Total	364	83.68	0.23	

*significant at 5% ***significant at 0.1% NS: not significant

GGE biplot for mean performance and stability of genotypes for fresh root yield is presented in Figure 6.3. Genotype UCC(M1) (G11) had the highest yield while genotype UCC01/249 (G10) had the lowest yield. Genotypes 01/0131 (G1) and KW00/095 (G26) had PC1 scores close to zero and therefore considered as stable for root dry matter across locations. Genotypes 01/1224 (G2) and BD96/134 (G20) were high yielding and had PC1 scores close to zero and therefore considered as high yielding and stable for root dry matter. 01/1413-1 (G6) and SW00/01 (G32) had PC1 scores close to zero but with low yield. The genotypes associated with higher GxE interactions were UCC01/249 (G10) and BD96/093 (G19). Fumesua was the highest yielding and unstable location; Ejura was high yielding and stable while Ohawu was low yielding and unstable. Genotypes that performed best at each location were identified. Agbelifia (G14), AMW00/006 (G17), Owuduro (1)NK (G30) and 01/1649-3 (G36) performed best at Ejura; genotypes BD96/134 (G20), Owuduro NK (G31), KSI00/092 (G25) and UCC(M1) (G11) also performed best at Fumesua; while, 01/1649-3 (G36),

DMA01/010 (G23), ADW00/004 (G13) and UCC (M1) (G11) had best performance for yield at Ohawu.

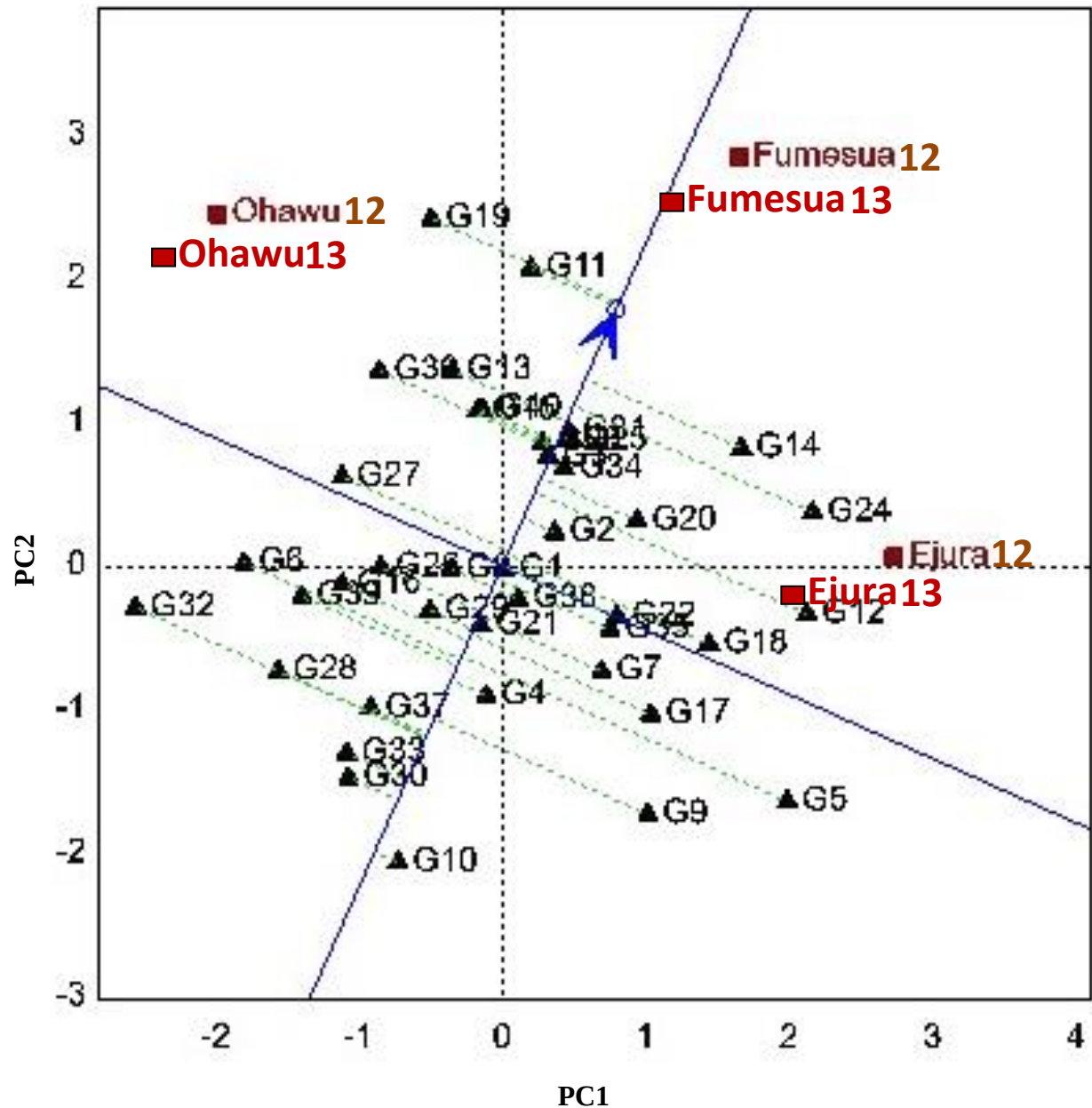


Fig. 6.3 GGE biplot showing mean performance and stability for fresh root yield of 40 cassava genotypes evaluated across three locations for two years

6.4.4 Association between PPD and dry matter across three locations for two years

Regression analysis of PPD and dry matter content across the three locations over two years is presented in Fig. 6.4. The analysis showed a weak linear relationship between PPD and dry matter content ($R^2 = 0.097$). For every one unit increase in dry matter content, you can expect PPD to increase by 0.345.

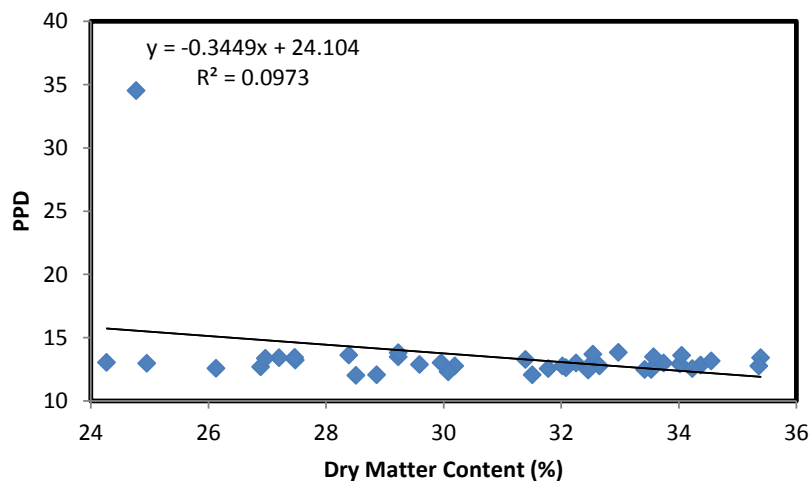


Fig. 6.4 Relationship between PPD and dry matter content across locations

6.4.5 Broad sense heritability for postharvest physiological deterioration, root dry matter and fresh root yield

Estimates for genetic variances, genetic parameters and broad sense heritability are presented in Table 6.11. The phenotypic coefficient of variation (PCV) for all the traits were higher than their corresponding genotypic coefficient of variation (GCV), indicating that the expression of the traits varied considerably with the environment. PCV and GCV were classified as per Sivasubramanian and Menon (1973) as low (0-10%), moderate (10%-20%) and high (> 20%). Phenotypic coefficients of variation ranged from 4.42 to 14.71% and the highest PCV was observed in fresh root yield and the lowest in postharvest physiological deterioration. The genotypic coefficient of variation also ranged from 1.84 to 7.03.

Heritability estimates were interpreted based on the classification by Bhateria *et al.* (2006) where > 0.50 values were high, $0.30 - 0.50$ values were medium and < 0.30 values were low. The heritability estimate indicated low to moderate heritability for all the traits measured. Root dry matter, fresh root yield and postharvest physiological deterioration had heritability estimates of 48.1, 11.51 and 17.63 respectively.

Table 6. 11 Genetic variances, genotypic and phenotypic coefficient of variation and broad sense heritability estimate for root dry matter, fresh root yield and PPD

Trait	Mean	σ^2_G	σ^2_{GL}	σ^2_{GY}	σ^2_{GLY}	σ^2_ϵ	σ^2_p	GCV	PCV	H ²
DMC	30.76	4.67	15.04	0	0	12.99	9.71	7.03	10.13	48.13
FRY	29.56	1.85	38.98	0.04	0	39.81	16.94	4.60	14.71	11.51
PPD	12.99	0.06	0.67	0.003	0	0.49	0.33	1.84	4.42	17.63

σ^2_G = genotypic variance; σ^2_{GxL} = variance due to genotype by location interaction effects; σ^2_{GxY} = variance due to genotype by year interaction effects; σ^2_{GxLY} = variance due to genotype by location by year interaction effects; σ^2_ϵ = residual/error variance; σ^2_p = phenotypic variance; GCV = genotypic coefficient of variance; PCV = phenotypic coefficient of variance; Hb = broad sense heritability

6.5 Discussion

The combined analyses revealed that main effects of genotypes, locations, years and interaction effects of genotype by location were significant for the traits measured. Findings of this study were similar to findings of previous studies by Akinwale *et al.* (2010) and Ntawuruhunga *et al.* (2010) who evaluated cassava genotypes across locations in Nigeria and Uganda respectively.

Partitioning of the variance components revealed that genotype by location interaction was the main source of variation for the interaction effects for root dry matter, fresh root yield and postharvest physiological deterioration, indicating that location contributed more than year effects to fluctuations in performance of the genotypes. This suggests the need to identify

genotypes with specific adaptation for the traits measured. The relatively high genotype effect on root dry matter suggests the possibility for the improvement of cassava for the trait using this germplasm though some genotypes may fail to respond because of the significant genotype by location interaction. Benesi *et al.* (2004) and Ssemakula and Dixon (2007) also reported higher genotype effects for root dry matter in cassava. In this current study, location effect was the major source of variation for fresh root yield. In a similar study by Aina (2007) and Akinwale *et al.* (2010), they also reported higher location effects for fresh root yield in cassava. In the case of postharvest physiological deterioration, year effects had the greatest impact on the variation of the trait, suggesting the need to evaluate genotypes for more than a year for reliable inferences to be made on genotype performance. This study is similar to an earlier study, where it was reported that, PPD is a multigenic trait with strong environmental influence (Rodríguez, 2001). Both fresh root yield and postharvest physiological deterioration are polygenic traits and therefore, subject to influence from the environment. The high environmental impact could, therefore, make future potential genetic gain in yield and delayed PPD difficult. This may require early testing of clones in multi-locations over multi-years so as to identify those with specific adaptation.

Due to the presence of genotype by location interactions, recommendations could not be made for a wide use of these genotypes that were used for the study. Consequently, AMMI analysis was used in identifying cassava genotypes that performed best at each specific location for the traits measured. Aina *et al.* (2009) and Akinwale *et al.* (2010) also used AMMI in identifying cassava genotypes for specific locations. Although a number of the genotypes performed best for each trait at each specific location, some genotypes performed best in more than one location. Genotype AMW00/006 performed best at Fumesua and Ejura while genotype OFF00/023 performed best at Ejura and Ohawu for root dry matter. For fresh

root yield, genotype 01/1649-3 performed best at Ejura and Ohawu. With PPD, genotype 01/0131 had the lowest score and therefore performed best across all locations. Further studies could be carried on them and subsequently released to farmers.

Among the locations, Fumesua was identified to be a high yielding and unstable location; Ejura was high yielding but stable while Ohawu was low yielding and unstable location for root dry matter and fresh root yield. This could be attributed to variations in the climatic conditions at these locations. This implies that Ejura could be used for wide adaptation studies while Fumesua could be useful for selecting specific adapted genotypes for these two traits. In the case of postharvest physiological deterioration, Ejura and Ohawu were unstable while Fumesua was more stable indicating that, Fumesua could be used for wide adaptation studies while Ejura and Ohawu could be useful for selecting specific adapted genotypes for PPD.

Genetic parameters estimated were low to moderate for all the traits studied. Phenotypic coefficient of variation was higher than corresponding genotypic coefficient of variation for all the traits measured. This was an indication that variation among the genotypes was not only genotypic but was also due to environmental influence. This observation was similar to earlier findings of Aina (2007) and Akinyele and Odiyi (2007).

The degree of success in selection however, depends on the magnitude of heritable variation determined through heritability estimates. Broad sense heritability estimates ranged from low to moderate for all the traits. PPD and fresh root yield with low heritability estimates suggested high environmental influence hence improving these traits through conventional breeding would be difficult. Dry root matter with moderate heritability indicated that this trait could have high genetic influence and hence can be improved through phenotypic selection.

In a study by Manu-Aduening *et al.* (2013), high heritability estimates for fresh root yield was reported. Ntawuruhunga and Dixon (2010) also reported high heritability estimates for fresh root yield and dry matter content in cassava. However, the estimate for this present study was lower than that reported by these two earlier studies. This could be due to genotypic differences among the cassava genotypes used for the study. Cortes *et al.* (2002) in a similar study reported a broad sense heritability of 60% for PPD. The findings of this study disagree with earlier studies by Cortes *et al.* (2002) where a higher broad sense heritability estimate was reported for PPD. This could be due to genotypic differences that existed among the cassava genotypes, environmental differences at the experimental sites and probably overestimation of heritability estimates.

6.6 Conclusion

This study revealed the effectiveness of using multi-locational experiments in assessing the response of genotype by environmental variations. Genotypes, locations and genotype by location interaction were significant and affected the performance of the genotypes for all the traits studied. The great contribution by environment effects on fresh root yield and postharvest physiological deterioration could make conventional breeding difficult.

The biplot provided an excellent graphical presentation and identified best genotypes in each location for all the traits studied. Genotypes with low PPD scores for the different locations were considered to perform well as a result, UCC01/249 (G39) performed better at Ejura whereas 01/1413-1 (G6) performed better at Fumesua while UCC (M1) (G36) performed better at Ohawu. Further studies could be carried out on these identified genotypes by cassava researchers for onward release to farmers. It also identified high yielding and stable locations. Ejura was identified as a high yielding and stable location; Fumesua was identified as high

yielding and unstable location while Ohawu was identified as a low yielding and unstable location for both fresh root yield and dry matter content, hence in subsequent studies Ejura could be used for wide adaptation studies while Fumesua and Ohawu could be used for specific adaptation studies for the two traits. For postharvest physiological deterioration, Fumesua was identified as a stable location.

This study has provided information on the heritability estimates for the traits studied. This would help cassava researchers in making informed decision on the feasibility of using phenotypic selection in improving these traits especially postharvest physiological deterioration. The low broad sense heritability recorded for postharvest physiological deterioration indicated non-additive gene action.

CHAPTER SEVEN

7.0 GENERAL DISCUSSION AND RECOMMENDATIONS

Postharvest Physiological Deterioration (PPD) is a major problem facing different cassava stakeholders along the cassava value chain. World postharvest losses due to PPD were estimated to be 5-30% (Zidenga *et al.*, 2012). Currently, there are limited reports on stakeholders perception on PPD in Ghana as what is available was carried out in 1992 by COSCA.

The PRA carried out classified stakeholders' constraints into four main groups: production, postharvest, financial and marketing constraints. Farmers, processors and marketers perceived PPD as a serious issue that have an adverse effect on cassava production and therefore, advocate for cassava varieties with delayed PPD. On the other hand, consumers did not envisage PPD as a serious issue. This could be attributed to consumers buying fresh cassava roots and using them immediately without storing. As a result, PPD becomes an issue for stakeholders who buy cassava roots in bulk and have to store. Stakeholders also indicated that there are no PPD tolerant varieties available in Ghana. In salvaging the situation, farmers have devised their own methods for root storage that could extend the shelf life of the roots. Validation of these methods indicated that polythene and jute sacks were the best storage method. Significant storage methods reported in this study implied that delaying PPD in cassava storage roots depended on the type of storage method used. It was observed that delay in physiological deterioration of the cassava roots were extended for only 3 - 5 days with minimum deterioration. It, therefore, necessitated finding another approach in solving the problem of PPD.

Knowledge of genetic variability for the trait under improvement is of paramount importance for the success of any plant breeding program. The follow-up diversity study of 150 cassava accessions using 19 qualitative traits and 100 SNP markers revealed a moderate to high genetic diversity among the cassava accessions. This supports similar findings by Raghu *et al.* (2007) and Mezette *et al.* (2013) who used morphological and molecular methods for diversity studies in cassava. SNP analysis with PowerMarker revealed a higher observed heterozygosity (H_o) than expected heterozygosity (H_e). This indicated heterogeneity between genotypes hence could be used for improvement of desirable traits including PPD.

PIC is a measure of the informativeness of a marker. Screening of these markers revealed that 96% of the SNP markers were polymorphic with a mean PIC value of 0.297. This suggested that only the polymorphic markers could be used for diversity assessment in cassava without necessarily screening all 195 markers used in this current study, thereby reducing the cost of optimizing larger number of markers. Based on the similarity matrices, a higher mean similarity coefficient was obtained for the morphological traits than was observed for SNP markers, indicating low diversity among the accessions based on qualitative traits than as observed in SNP markers. This could be explained by the influence of environment on the expression of the traits. Dendrograms generated from both morphological and molecular analyses clustered the genotypes into five and three main groups respectively although there were differences between them. Related accessions were grouped in same clusters while unrelated accessions were grouped in separate clusters. However, some accessions that appeared similar based on molecular analysis had morphologically different characteristics and were therefore clustered in different groups.

The use of molecular markers in genetic diversity studies may allow a more precise detection of variation than morphological characterization. Correlation coefficient based on Mantel test

observed between the two approaches was positive and significant but weak. This could be explained by the fact that morphological and molecular markers sample different regions of the genome. The weak correlation is, therefore, not unexpected since molecular markers cover a high proportion of the genome because of the high number of bands scored per analysis and such bands may probably fall in coding regions of the genome involved in morphological traits. Therefore, for an effective and efficient differentiation of accessions, an integrated approach using both markers is recommended. In this study, the high diversity within the cassava accessions identified could provide the genetic resource base for the improvement of economically important traits including PPD in Ghana.

Evaluation of these accessions for their reaction to PPD revealed the existence of genetic variability among the cassava accessions for PPD. This indicated that there were genotypic differences that could be selected for improving the trait. This finding confirmed farmers' assertion and was also similar to earlier reports by Ekanayake and Lyasse (2003) and Sánchez *et al.* (2006, 2013). Accessions with minimum deterioration identified were K25, SW/00/064 and DMA00/031. Assessing the relationship between traits indicated a significant but weak positive correlation between PPD and root dry matter content. This suggested that genes controlling these two traits could be linked or has pleiotropic effects. Sánchez *et al.* (2006) and Morante *et al.* (2010) made a similar observation when evaluating cassava for postharvest physiological deterioration. This significant but weak correlation between PPD and dry matter is an indication that, it is possible to find cassava genotypes with low PPD scores and high dry matter content. As a result, two genotypes with minimum deterioration but high root dry matter were identified (DMA00/031 and SW/00/064). Stored cassava roots undergo physiological changes that affect the root quality. Physicochemical and functional properties of deteriorated cassava roots indicated gradual but significant changes in all the

parameters considered with the exception of crude protein content. Similar findings were reported by Lola *et al.* (2012). These changes could have adverse effects on the final products suggesting that for any delayed PPD cassava genotype identified, the physicochemical and functional analysis are carried out to ensure that these roots could be stored for some period of time without much changes on their root qualities.

Despite the ability of cassava to thrive in different climatic conditions, its performance varies from one location to the other. Forty cassava genotypes selected and evaluated at three locations for two years indicated the effectiveness of multi-location experiments in determining effects of the environment on the studied traits. This study revealed that, genotype was more important for root dry matter than the other effects while location and years were more important for fresh root yield and postharvest physiological deterioration respectively. This suggested that evaluation for these traits must be multi-year and multi-locational before a reliable inference could be made. It also showed a significant genotype by location effects for all the studied traits. This indicated that genotypes could, therefore, be selected for specific adaptation and not for wide adaptation. The biplot identified best genotypes in each location for root dry matter, fresh root yield and PPD. Genotypes that performed best for root dry matter at each location were; Owuduro (1)NK (G30), OFF/00/023 (G29), AMW00/006 (G18) and UCC01/0250 (G40) performed best at Ejura; genotypes KSI00/092 (G25), Nkabom (G28), ADW00/004 (G13) and AMW00/006 (G18) also performed best at Fumesua; while, SW00/064 (G33), KW00/095 (G26), SW00/010 (G32) and OFF00/023 (G29) had best performance for root dry matter at Ohawu. Genotypes that performed best for yield at each location were; Agbelifia (G14), AMW00/006 (G17), Owuduro (1)NK (G30) and 01/1649-3 (G36) performed best at Ejura; genotypes BD96/134 (G20), Owuduro NK (G31), KSI00/092 (G25) and UCC(M1) (G11) also performed best at

Fumesua; while, 01/1649-3 (G36), DMA01/010 (G23), ADW00/004 (G13) and UCC (M1) (G11) had best performance for yield at Ohawu. Genotypes with low PPD scores for the different locations were considered to perform well as a result 01/0131 (G1), Tekbankye (G35), Nkabom (G28) and 01/1235 (G3) performed best at Ejura; genotypes Debor (NK) (G22), Debor(1)AF (G21) and 01/0131 (G1) also performed best at Fumesua; while, 01/0131 (G1), AkosuaTumtum (G16), IOO/0093 (G24) and Debor (NK) (G22) had best performance at Ohawu.

Breeding progress is primarily determined by the magnitude, nature and inter-relations of genotypic and phenotypic variations in the various characters. This necessitates partitioning of the overall variability into its heritable and non-heritable components with the use of suitable genetic parameters, such as genetic coefficient of variation and heritability estimates. Estimation of genetic parameters would inform cassava breeders on the feasibility of improving tolerance to PPD through selection. In the present study, phenotypic coefficients of variation values were higher than their corresponding genotypic coefficient of variation for PPD and the other traits measured. This indicated the considerable role of the environment on the expression of these traits. This supports previous observations by Akinwale *et al.* (2010) and Manu-Aduening *et al.* (2013) that the extent of environmental influence on any trait is indicated by the magnitude of the difference between the phenotypic coefficient of variation and genotypic coefficient of variation values. Heritability estimate is an important selection parameter. This current study revealed a low broad sense heritability estimate for PPD, suggesting that this trait could probably be under non additive gene action hence, improving tolerance to PPD using conventional breeding would be difficult. High heritability estimates for PPD was reported by Cortes *et al.* (2002) however, findings of this current study contradicts this report.

7.1 Recommendations

It is evident from this study that PPD is an important problem that needs to be addressed since postharvest losses due to PPD in cassava roots are inevitable. Solving this problem would open more opportunities for all the stakeholders in the cassava value chain. It is, therefore, recommended that;

- i. Further studies should be carried out on the cooking and eating qualities of delayed PPD cassava genotypes identified in this study.
- ii. Cassava genotypes with low PPD scores and high dry matter identified in this study could be used as parents for PPD improvement in Ghana.
- iii. Breeding for delayed PPD requires multidisciplinary and collaborative efforts from agricultural research institutions and universities, hence a holistic approach should be used in solving the problem.
- iv. It should also be a requirement that physicochemical and functional properties should be carried out on cassava genotypes identified to be PPD tolerant. This would ensure that, cassava genotypes with delayed PPD could be stored for some period of time without losing much of its root qualities.
- v. Since this study did not identify cassava genotypes with complete tolerance to PPD, there should be a continuous screening of cassava genotypes from all over the country and from introduced materials for additional and new sources of PPD tolerance.

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APPENDICES

Appendix 3.1 Questionnaire on stakeholders knowledge and perception on Postharvest Physiological Deterioration (PPD) in cassava

Date:

Region: District/Locality:

Name of community:

Respondent: A. Farmer B. Marketer C. Consumer D. Processor

Sex of Respondent: A. Male B. Female

Age of Respondent.....

1. Cassava Production Constraints (List and rank the constraints in order of importance)

2. Processing/Marketing of cassava

i. How often do you market/process/buy your produce?

A. Daily B. Weekly C. Monthly D. 2X/month E. 3X/month F. > 3X/month

ii. Is there a reason for this?.....

.....

.....

- iii. What is the distance (km) from your farm to market/processing centers?
.....
- iv. Do you use any method when transporting roots from farm gate to market?
A. Yes B. No
- v. If yes, what methods do you use? A. Packing in jute bags B. Packing in plastic bags
C. Others (specify)
- vi. Why do you use this (ese) method (s)?
.....
.....
- vii. What is the cost of this method?
- viii. If No, why don't you use any method?
- ix. Do you process/sell the roots immediately after harvesting? A. Yes B. No
- x. If Yes what is the reason?
.....
.....
- xi. If No what is the reason?
.....
.....
- xii. Do you process/sell everything at a go? A. Yes B. No
- xiii. If Yes what is the reason?
.....

.....

xiv. If No what is the reason?

.....

.....

3. Cassava consumption

i. How often do you consume cassava in a week?

A. Daily B. 1X/week C. 2X/week D. 3X/week E. > 3X/week

ii. If you consume cassava daily, what is the frequency?

A. Once B. Twice C. Thrice D. More than thrice

iii. Do you use deteriorated roots? A. Yes B. No

iv. If Yes, what is the reason? A. Has a unique taste

B. Others (specify)

v. If No, what is the reason?

.....

vi. What food products do you prepare from deteriorated cassava? A. Tapioca

B. Gari C. Fufu flour D. Dough E. Kokonte

F. Others (specify).....

i. Why do you use it for this (ese) product(s) and not others e. g. Fufu?

.....

ii. Do deteriorated roots have any effects on your products? A. Yes B. No

iii. If yes, what are they?

.....

.....

4. Basic knowledge on PPD

- i. Do you know what PPD is about? A. Yes B. No
- ii. If Yes, What is it about?.....
-
-
-
- iii. How do you call PPD in your local language?
- iv. Why that name?
-
-
- v. What are the causes of PPD?
-
-
- vi. What are the signs of PPD?
-
-
- vii. When do the signs of PPD appear?
- viii. Can PPD be controlled? A. Yes B. No
- ix. If Yes, how?.....

-
-
- x. If No, why?.....
- xi. Does PPD have any health implication? A. Yes B. No
- xii. What are they?.....
-
- xiii. What are the symptoms and how is it treated?
-
-
-
-

5. Storage methods

- i. Do you store cassava roots? A. Yes B. No
- ii. If Yes, what methods do you store?
-
-
- iii. If No, why don't you store the roots?
-
-
- iv. When do you use this method?

A. Immediately B. A day after C. Two days after

D. A week after E. Others (specify)

v. How long can they be stored before they begin to deteriorate?

A. 1 Day B. 2 Days C. 3 Days D. 1 week

E. Others (specify)

6. Identification of PPD tolerant materials

i. Which of your varieties are susceptible to PPD? List them

.....
.....

ii. How long can they be stored before they begin to deteriorate?

.....

iii. Are some of your varieties tolerant to PPD? A. Yes B. No

iv. If yes, list them

v. How long can they be kept before they begin to deteriorate?

A. 1 Day B. 2 Days C. 3 Days D. 1 week

E. Others (specify)

Perception of stakeholders on PPD in cassava

Indices	Score				
	-2	-1	0	1	2
Deteriorated roots taste better					
Deteriorated roots are difficult to pound					
PPD affects roots quality					
Cassava roots begin to deteriorate 2 to 3 days after harvest					
PPD occurs as a results of injury during harvesting					
PPD is caused by micro organisms					
PPD reduces the market value of roots					
PPD affected roots are easy to process					
Deteriorated roots can be used to prepare different dishes					
PPD can be prevented					

Key:

-2: strongly agree

-1: Agree

0: indifferent

1: Disagree

2: Strongly disagree

Appendix 4.1 SNP markers used to assess genetic diversity in cassava accessions

SNP	AlleleY	AlleleX	Sequence
7259-SNP	T	A	AGATCATGAAGATGG[A/T]AAATGTTCTTGACTC
7391-SNP	C	T	AAGAACAAATTTAAA[T/C]AACATTATAAGCCGT
7434-SNP	G	A	ATTTTTTGAAGTGA[A/G]CAAACCGGCTCTTCT
826-SNP	G	A	AAAAGTTTGCTTAAT[A/G]CACCATGGCTTTTTG
958-SNP	T	A	ATAAATTTTCAATTCTC[A/T]GGGTCCTACAAGAAA
Me_v4_MEF_c_1018	G	A	GATTAATGAACAGAC[A/G]GAAAATATGAAACAA
Me_v4_MEF_c_1047	T	A	AGAGCTACCCCTTT[A/T]CAAGTTTTAGGGCTA
Me_v4_MEF_c_1097	G	A	ACGTGGCACTGCACC[A/G]ATTGTTGATTTGCGC
Me_v4_MEF_c_1099	T	A	GGATACAATTACAGT[A/T]CCTGGCGGACCAAAA
Me_v4_MEF_c_1134	G	A	TGGTTGCTGCTTAGT[A/G]GTTTATCCACCTTTT
Me_v4_MEF_c_1154	G	A	AGAATTCAAGAGAAA[A/G]TTGGACCGTATGGTG
Me_v4_MEF_c_1175	C	T	TCAGCAGATGGCCAC[T/C]GATCAGCAGTACCAA
Me_v4_MEF_c_1183	C	T	GCACTGGACATGAAG[T/C]TGAGAGATGATTTGG
Me_v4_MEF_c_1187	C	A	AAGAATCAAAGGAAT[A/C]TAAGCAGTTGGAAAT
Me_v4_MEF_c_1220	C	T	CTCCTGTTTCATCGTT[T/C]TAAATCTACAACCTTA
Me_v4_MEF_c_1246	G	T	AGGAATGATTGTCAC[T/G]GGCATGGAAGTTGCA
Me_v4_MEF_c_1248	G	T	GTTGACAGAGTTGAA[T/G]AGCTCAAACCTTTGTT
Me_v4_MEF_c_1278	T	A	TAAATTTTATCTGAA[A/T]GATTTGTTTTGCAAT
Me_v4_MEF_c_1290	G	C	CAGAGGCTTGCATGG[C/G]TGTTCTTCGCTAGC
Me_v4_MEF_c_1307	T	A	AATACACACTCATTT[A/T]CTCCTTAATACAAGC
Me_v4_MEF_c_1314	C	T	AAAGACCATTCGTTA[T/C]GCTTCCCGCAAGGCG
Me_v4_MEF_c_1315	C	T	TCGTGATGTCTTTC[T/C]TATCGGACGCCCAAT
Me_v4_MEF_c_1320	C	A	AGTCTTAGACATTCT[A/C]GTAGATTTTCTAGTT
Me_v4_MEF_c_1337	G	C	CAGAGCTACAGTTGT[C/G]GCCGTGCAAAAAATG
Me_v4_MEF_c_1357	C	T	TTTCTTTGTTGTCCT[T/C]GAGCGCCCAGGTTAC
Me_v4_MEF_c_1360	C	T	AATGTTTCGTATCTCT[T/C]TCTAGAATCTGCAGG
Me_v4_MEF_c_1387	T	A	CAAGTGGTTATTGCG[A/T]ATTTGATGAACTTTT
Me_v4_MEF_c_1392	G	A	GTTTCTGCATCTGCC[A/G]TTGTTGCAGCTCAGA
Me_v4_MEF_c_1423	G	A	CGATACAAGCTTGGG[A/G]TATAATAGTTCTGTT
Me_v4_MEF_c_1443	G	T	TGGATCATCTTTTGC[T/G]CTTGATTCCATTTTG
Me_v4_MEF_c_1447	C	T	GCTATTTGTAATATT[T/C]TCATCATGGGGACTT
Me_v4_MEF_c_1450	G	A	ATTTTGAAGGAGAAG[A/G]GATATGAGGTGACAA
Me_v4_MEF_c_1566	C	T	CGTGAAGACTACTGA[T/C]AATTACACCTTGAGG
Me_v4_MEF_c_1615	G	C	TGCCGGAACACAGCT[C/G]GAGGACGGCCGCACA
Me_v4_MEF_c_1617	C	T	TTCAGCTATGGATAT[T/C]TTGATTTTAGTTGTT
Me_v4_MEF_c_1635	G	T	ATCCTTTCATTTTAG[T/G]CCTGGTCCCCCTATG
Me_v4_MEF_c_1637	T	A	ATGATCACGACATGA[A/T]TTTAGCYGGGATTTT
Me_v4_MEF_c_1645	C	T	TGCTGAGCAGCACTA[T/C]GCAGACCTCTCTGCA
Me_v4_MEF_c_1650	G	A	TTGAAAAGGACAAAA[A/G]GTAGTAGATTTGTAA
Me_v4_MEF_c_1679	C	A	CGACTCTTCTTTTTT[A/C]ATTTCTTTTTTGGGTC
Me_v4_MEF_c_1715	C	T	AGGTTGATGGATGMT[T/C]TGTTAATTGCTGCAT

Appendix 4.1 (cont'd) SNP markers used to assess genetic diversity in cassava accessions

SNP	AlleleY	AlleleX	Sequence
Me_v4_MEF_c_1730	G	A	ACCTCCTTCTCCACT[A/G]TCACCCTCCTCATCC
Me_v4_MEF_c_1828	G	C	GAAAGTCTTTGTAAC[C/G]GTGTCCGGTATCTCAT
Me_v4_MEF_c_1856	G	A	TGTTATAGATGATCT[A/G]TTGGCTAAAGGGATG
Me_v4_MEF_c_1876	C	T	ATTTAAGTCGATCCC[T/C]AGTATCATTGAGCTT
Me_v4_MEF_c_1877	G	T	ATGCCCTTACATTTT[T/G]ATGGGAGAATAAGAG
Me_v4_MEF_c_1892	G	A	GCAGTGGAAGGTTAC[A/G]TGAAGTGAATAAGTT
Me_v4_MEF_c_1919	C	T	AAAAGAATTGGATGC[T/C]CTCCTCTCAGATGAC
Me_v4_MEF_c_1940	C	A	TGTCAGATGCTGCAG[A/C]TTATGATGCTGGATA
Me_v4_MEF_c_1945	C	T	TGCTACTCTTACAAG[T/C]CATCTCAGATGATGA
Me_v4_MEF_c_1947	G	A	TGCAAACCTCAAGGT[A/G]GATGTATACACAAGG
Me_v4_MEF_c_1957	C	T	CAGGGGGTTTGCTCT[T/C]GAGTTGGATGGTTGG
Me_v4_MEF_c_1958	G	C	GAAGGTGGAGATGAC[C/G]AAGACGAAGGAGAAG
Me_v4_MEF_c_1960	G	A	GAGAAAGTACACTTG[A/G]CCGGATCTGAGATGA
Me_v4_MEF_c_1977	C	A	AGGGTGAGATGTTTA[A/C]TCGTCCTGGAAAAC
Me_v4_MEF_c_1990	G	A	TGGACTTACATCGCC[A/G]GGTGGTGCTTTGGAT
Me_v4_MEF_c_2034	G	A	TCGCATTAGTTCCCC[A/G]TTTCTTTATGGAATC
Me_v4_MEF_c_2043	G	T	GTGAGCTCTGTCATC[T/G]TTTTCCAGACGCCTG
Me_v4_MEF_c_2051	G	A	GTTTCCAAGCAATCC[A/G]ACTCCTCCACCACCA
Me_v4_MEF_c_2120	G	A	ATTCAGCTGATCCT[A/G]GCAATGCACTTCTGC
Me_v4_MEF_c_2124	G	A	ATGTGCTCAGAAACG[A/G]TGGAAGAGTGATGGA
Me_v4_MEF_c_2125	G	A	ATGCAAAGGAAATCA[A/G]GATCTGCAACTGAAA
Me_v4_MEF_c_2189	T	A	AGGACAAATCAGCTT[A/T]TGCATTGTGTACATT
Me_v4_MEF_c_2195	T	A	AGGCATATAGTTTCC[A/T]GTTTCTATGTTGAGT
Me_v4_MEF_c_2226	G	C	CTTTGTGGCCCGAGG[C/G]ACGGTGATATTGGCA
Me_v4_MEF_c_2236	T	A	CTCCAACCCTCTAAC[A/T]CCCATCAAGGCTACT
Me_v4_MEF_c_2248	C	T	GGAAGGAGTTGCTCA[T/C]TTCCTCAACCAGGAG
Me_v4_MEF_c_2259	G	A	AGAGAAGAAAATGGC[A/G]ACTTTGGGGTTGAGA
Me_v4_MEF_c_2267	C	T	TCTAGTTTAATATTA[T/C]GTCTGTAAGCTTGAA
Me_v4_MEF_c_2282	G	T	CGGATGCTACTTGTT[T/G]CGTTTGCTTAGTTGG
Me_v4_MEF_c_2283	G	T	CTTTGCTTGTTTGTG[T/G]TGTTTATTTCTTTTT
Me_v4_MEF_c_2286	C	A	ACTCTTGGCTGAATC[A/C]GACGACAAAGCGAGT
Me_v4_MEF_c_2304	G	T	GAGACGCCACAAAGT[T/G]TGATTGAGAAGAGAA
Me_v4_MEF_c_2317	G	T	TTATTATTCCCAATT[T/G]GACACTATGTGGTCC
Me_v4_MEF_c_2333	T	A	CTTCTGGGATGGGA[A/T]CATTACTTCATTGTA
Me_v4_MEF_c_2337	G	A	TCCATCTGTTGCCTG[A/G]AAAACGTGATCATGG
Me_v4_MEF_c_2346	G	C	TCCCTCTTATTTATC[C/G]TGGAACAGAACTCCT
Me_v4_MEF_c_2363	G	A	TGATGAATAGTAGTT[A/G]GGTTCTTGTTGTCGA
Me_v4_MEF_c_2366	G	A	CAAGTTGGTGGCTGA[A/G]GCAGCCAAAGCTTCA
Me_v4_MEF_c_2384	T	A	TGGTATCTTCGAAGC[A/T]GATGTTGAGCTGCT
Me_v4_MEF_c_2402	C	A	GACGGGAAGGATGGA[A/C]GGCCTAAATATCCAC
Me_v4_MEF_c_2409	G	T	ATGCAATGAACCAAA[T/G]CCCCAACCCATGGCA

Appendix 4.1 (cont'd) SNP markers used to assess genetic diversity in cassava accessions

SNP	AlleleY	AlleleX	Sequence
Me_v4_MEF_c_2419	G	T	ATCAAGTTGACGACM[T/G]CAAAGCTGGATGAGG
Me_v4_MEF_c_2437	C	T	TGGTCAGGGAAAGAC[T/C]CCTCTTGGAGCTCTT
Me_v4_MEF_c_2447	C	T	GGGACTTTACTCAAT[T/C]CTTCTCCTTCCTCTT
Me_v4_MEF_c_2448	G	T	CTTTCTGCAATGTTA[T/G]TGTTTTGAGCTTTAT
Me_v4_MEF_c_2456	C	T	ATACACGTATCAGAG[T/C]TGTTGGTGGAGTATC
Me_v4_MEF_c_2465	G	A	ACTCATGTTAATGGG[A/G]ATATTTGCTTGTTAA
Me_v4_MEF_c_2486	C	A	GAACTGATCGRTATA[A/C]TCGTGACCGGTCTGG
Me_v4_MEF_c_2494	C	A	TCAAAGGCTCTTCAC[A/C]AAGCTCCTACCCTCA
Me_v4_MEF_c_2497	C	T	GCCCGATAACACTAC[T/C]CGAATCCATACCCAC
Me_v4_MEF_c_2510	C	T	GCAAGTCGATGGACC[T/C]ACCACATCTAGTAAA
Me_v4_MEF_c_2512	C	A	AGATATTCCCAGATW[A/C]TGATCCAAAGTGGAA
Me_v4_MEF_c_2524	G	T	ATCATCAGGGTTATM[T/G]CTACCAGGAGCAAAG
Me_v4_MEF_c_2549	C	A	ATACCGAGACTGATC[A/C]AGCCAGTAAGCTAAG
Me_v4_MEF_c_2552	C	T	TTCTCTTCCATCTTC[T/C]TCTTCATCAGTTACC
Me_v4_MEF_c_2562	G	A	GAACTCCAAGGATTG[A/G]GCATAGAAGAGCTTC
Me_v4_MEF_c_2572	C	T	TGCATACTTGGCAAA[T/C]GAACGCATACTCTTA
Me_v4_MEF_c_2576	C	T	CACTTGGTCTGACTG[T/C]TGGTGGTCTTTGGAA
Me_v4_MEF_c_2653	G	A	TCTGTTTCATTGATA[A/G]CAGGACAATGTTCGC
Me_v4_MEF_c_2726	G	C	AACTTGCAGGAATCG[C/G]GGTTGTGGTAAACT
Me_v4_MEF_c_2748	G	A	AAAGGAGAATGGTGG[A/G]GCTGTTGTTCTTACT
Me_v4_MEF_c_2758	G	T	TGGGACTATTACTCK[T/G]GGGGTGTAGCTCCAT
Me_v4_MEF_c_2786	G	A	CTTGGCCTCACTGGG[A/G]GCGAAAATTGTCATT
Me_v4_MEF_c_2794	C	T	TGAAACTTTGTCTAC[T/C]ACCGGATTTCAACTT
Me_v4_MEF_c_2801	G	A	ACCAACTGATGAACC[A/G]GTGGTGACTAGCAGC
Me_v4_MEF_c_2815	G	T	ACATGAGATGTGGCC[T/G]CCACCTATAAAGATT
Me_v4_MEF_c_2821	T	A	AGTTTGATTTGAAAA[A/T]TTCTATTTCCCTTTA
Me_v4_MEF_c_2851	C	A	TCTCTTTAACTCAAA[A/C]TATGAGCTACTCCTC
Me_v4_MEF_c_2856	T	A	ACTGCAGACACAGGC[A/T]GCCCCAACAGTTTAGG
Me_v4_MEF_c_2873	G	C	GTCAAGTGTTAAGAA[C/G]GGAATCTCACATTGT
Me_v4_MEF_c_2874	G	C	TTTCTGTGTCTTTTG[C/G]GCAACCAAGCAAATT
Me_v4_MEF_c_2885	C	A	TGAATTTGTGGGAAC[A/C]GATGGGACCTGCGGA
Me_v4_MEF_c_2888	T	A	TCTTATTAGACCCCA[A/T]AAGCATCGCAGCAAA
Me_v4_MEF_c_2905	G	A	TCACACCAGGATACA[A/G]TGTTTAAAGGAGGTA
Me_v4_MEF_c_2909	C	T	TCATGTGGGTCATAT[T/C]CCGAATGAGCATGAT
Me_v4_MEF_c_2977	C	T	TCCTCATCTCATCAT[T/C]TCTCAGATGTGTAAT
Me_v4_MEF_c_2980	G	A	CTCTTCTCAGATTTT[C/A/G]CAAAAACCAAACACC
Me_v4_MEF_c_2990	C	A	TCGGGAGCATTTCATC[A/C]AGCAATCTGCATCCT
Me_v4_MEF_c_3024	G	A	TTAGCAAGATCTCTG[A/G]TATAGCCTTCCATGT
Me_v4_MEF_c_3039	T	A	TAGATGGAAGTAATA[A/T]AGAGGATGAAAAGGA
Me_v4_MEF_c_3055	C	T	GGGCTAAGTGTATTG[T/C]AAGGAATGGCTATTT
Me_v4_MEF_c_3057	G	A	CTGTTCAACAACTGC[A/G]CGATTAGGATCAAGG

Appendix 4.1 (cont'd) SNP markers used to assess genetic diversity in cassava accessions

SNP	AlleleY	AlleleX	Sequence
Me_v4_MEF_c_3070	C	T	ATGGTCAGGACCTGC[T/C]TGAGGATCCAAATGA
Me_v4_MEF_c_3072	T	A	GTAGCCTAAATAAGC[A/T]CAAACCTAGGTTTGTA
Me_v4_MEF_c_3081	C	T	GTTACTGGTGGGAAC[T/C]AAAACAAGCACCTAT
Me_v4_MEF_c_3092	G	C	TACAGTTGCATGAAG[C/G]TTTGGTGAGCGGCAA
Me_v4_MEF_c_3094	C	A	TGGATTAGGATTAAY[A/C]GTTTGCAACAGTGGA
Me_v4_MEF_c_3120	G	C	CAAGGAGGAGCTTCG[C/G]CGTGAAAGCAGAGCT
Me_v4_MEF_c_3131	G	A	CCGGTCGAGTGTGAG[A/G]GGTTTTATTGGGTTC
Me_v4_MEF_c_3137	T	A	AGACATTTCGTATAGG[A/T]TCAAAGACCGGTGCT
Me_v4_MEF_c_3142	C	T	GTAACGTAAGGTGTA[T/C]TAGTTCTGAGCAAAA
Me_v4_MEF_c_3155	T	A	GACAAAGGTTTTCTC[A/T]CAGACGTGCAGGTTA
Me_v4_MEF_c_3195	G	A	GAGTAAAGCTGTAA[A/G]ATGGAAGGACAGTGC
Me_v4_MEF_c_3197	C	T	CCATCTCTTGTCAAC[T/C]CTAATTATTATTATT
Me_v4_MEF_c_3200	G	A	TGACTGGAATTAATG[A/G]CCTGGTAGAACATGT
Me_v4_MEF_c_3207	G	A	TTGAGCGAGGCAATG[A/G]TAGCTGCTTTTTTTC
Me_v4_MEF_c_3214	G	A	ATGGGTATTTTATAT[A/G]GCAAAAGGAAGATAG
Me_v4_MEF_c_3227	C	T	TGGATCAATTCTACA[T/C]CCCTGTTTTTCTGAA
Me_v4_MEF_c_3236	G	A	TCTTGCAATTAAAAG[A/G]ACAATTGCAATGATT
Me_v4_MEF_c_3238	T	A	AATTGATCAAGACTC[A/T]GATCCTGATGCAACT
Me_v4_MEF_c_3242	T	A	CTGATTTCTGTGTTT[A/T]AAGATTATTGAATCC
Me_v4_MEF_c_3268	C	T	TGGGCTGGAGCTTGG[T/C]TTGTCACAGGATGGT
Me_v4_MEF_c_3310	C	T	GTTGTAAAGAAGGC[T/C]CAGGTGGAGGAGTTT
Me_v4_MEF_c_3338	C	T	ACGATAAGCAAATTA[T/C]TGGCAAGTTGAGTTA
Me_v4_MEF_c_3343	C	T	CATGGTTGGAGGCAT[T/C]GACGAGGTGATAGCC
Me_v4_MEF_c_3356	C	T	GGTTGCCGAAGCTGC[T/C]GAGTCTGGGGTTTCC
Me_v4_MEF_c_3361	C	T	TGTAGCTGTAAATGA[T/C]GTAGCTAGTAGTTTT
Me_v4_MEF_c_3376	C	T	CGAGGGTGGTCTTGA[T/C]TACTTGGGGAATCCA