GENETIC IMPROVEMENT OF ROOT YIELD AND NUTRITIONAL QUALITY OF CASSAVA (*Manihot esculenta* Crantz) IN SIERRA LEONE

By

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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

Cassava storage root is a major staple. However, the tuber is poor in nutrients especially micronutrients. The study was conducted to improve the nutritional status of cassava with farmers' preferred traits in Sierra Leone. The specific objectives were i) to assess adoption challenges, perception and preferences for provitamin A cassava among cassava value chain actors in Sierra Leone. ii) to estimate genetic diversity within provitamin A cassava germplasm using morphological, molecular tools and i-check device for establishing a collection in Sierra Leone. iii) to determine performance and stability of total carotene content and dry matter of selected provitamin A cassava accessions across environments.vi) to characterize F_1 progenies for total carotenoid, iron and zinc and protein content using biochemical tool. v) to estimate the combining ability of 12 cassava parents and their F_1 progenies for mealiness, dry matter, number of roots and fresh root yield. A participatory rural appraisal (PRA) was conducted in Bombali, Kailahun and Moyamba districts, to identify farmers' and consumers' adoption challenges, perceptions and preferences for provitamin. High production cost, low yield, scarcity of planting materials, high cost of fertilizers and agro-inputs, drudgery in peeling and processing and limited access to micro finance loan schemes, were identified as major challenges for provitamin A cassava adoption. The respondents show willingness to accept and adopt provitamin A cassava due to its perceived nutritional quality. A total of 188 cassava accessions cultivated in the southern part of Sierra Leone were assessed using molecular tools and the i-check device. The Cassava accessions were grouped into eight distinct clusters based on the morphological data while they grouped into nine distinct clusters based on the molecular analysis. A significant positive correlation was found between the morphological and molecular data sets ($r = 0.104$; $p < 0.034$) but the correlation was rather weak. Thirty provitamin A accessions with higher total

carotenoid contents were selected to form a collection. The collection evaluated in 3 environments for the GGE biplot analyses for dry matter content (DMC) and total carotenoid content (TCC) showed significant variation among the Genotypes, Environments and their interaction. Genotypes TR-1182 and TR-1313 had the highest performance for DMC and TCC. Njala was identified as an ideal environment for selecting superior genotype for total carotenoid content and Pendembu as ideal for dry matter content. The performance of 868 F_1 progenies (obtained from five crosses involving eight genetically diverse parents) was evaluated for selection of varieties with increased level of micro nutrients. F_1 progeny 13 and 33 from cross IITA-TMS-IBA 120004 x IITA-TMS-IBA 120003 recorded the highest (28.0 μg^{-1}) and lowest (6.0 μg^{-1}) values for total carotenoid content with a grand genotypic mean of 14.7 μg^{-1}. F_1 progeny 41 and 12 from cross IITA-TMS-IBA 088693x IITA-TMS-IBA 088747 recorded the highest (8.1%) and lowest (4.2%) crude protein content with a grand percentage mean of 5.4%. F_1 progeny from cross IITA-TMS-IBA 96/1165 x IITA –TMS-IBA 011368 recorded the iron content with a ranged 45.0 ppm to 59.2 ppm and harvested progeny with the grand mean 12.6 ppm. F_1 progeny from cross MM96/81791 x IITA-TMS-IBA 088747 had the highest zinc concentration ranging from 4.5 ppm to 17.7 ppm with a grand mean 8.5 ppm. Micro nutrients analysis on F_1 progenies revealed that there is variation for quality traits in cassava. In addition, a 12 x12 diallel study revealed highly significant differences for dry matter, number of roots and fresh root yield at $P < 0.01$, and 0.05 respectively. General combining ability (GCA) variance was significant for fresh root yield while estimates of specific combining ability (SCA) variance was highly significant for dry matter and number of storage roots. The significant difference observed for most of the traits for the parents and their F_1 progeny revealed that genetic diversity exists among the germplasm and Progenies from crosses IBA120004 x IBA961165, IBA 961165

x I088693 and IBA120004 x IBA961165 were the best combiners for number of storage roots, mealiness and fresh root yield.

DEDICATION

To the glory of God and to my beloved father (Hon) Dr. B.M. Kamanda. My late mother and grandmother of blessed memories Madam Aminata Sheriff and Haja Mrs. Ada Sheriff

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LIST OF ABBREVIATIONS

ACMV	African Cassava Mosaic Virus
AFLP	Amplified Fragment Length Polymorphism
ANOVA	Analysis of Variance
CBB	Cassava Bacterial Blight
CBSD	Cassava Brown Streak Disease
CIAT	International Centre for Tropical Agriculture
CMD	Cassava Mosaic Disease
CMGs	Cassava Mosaic Gemini viruses
DMC	Dry Matter Content
DNA	Deoxyribonucleic Acid
ESTs	Expressed Sequence Tags
FAO	Food and Agriculture Organization
FSRY	Fresh Storage Root Yield
GCA	General Combining Ability
GDP	Gross Domestic Product
GEI	Genotype and Environment Interaction
IFAD	International Fund for Agricultural Development
IITA	International Institute of Tropical Agriculture
MET	Multi Environmental Trials
NARC	Njala Agricultural Research Center
NGOs	Non-Governmental Organizations
PCA	Principal Component Analysis

QTL	Quantitative Trait Loci
PIC	Polymorphic Information Content
PRA	Participatory Rural Appraisal
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
PVAC	Provitamin A Cassava
SAS	Statistical Analysis System
SCA	Specific Combining Ability
SCARs	Sequence Characterized Amplified regions
SGD	Specific Gravity Determination
SLARI	Sierra Leone Agricultural Research Institute
SSR	Simple Sequence Repeats
SNP	Single Nucleotide Polymorphism
SWOT	Strengths, Weaknesses, Opportunities and Threats
TCC	Total Carotenoid Content
UNICEF	United Nations Children's Fund *(formerly United Nations International Children's Emergency Fund*)
WAAPP	West Africa Agricultural Productivity Program
WACCI	West Africa Centre Crop Improvement
WHO	World Health Organization
YFC	Yellow Flesh Cassava

CHAPTER ONE

1.0 GENERAL INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is an important food crop and source of calories for more than 900 million people in the tropics and sub tropics (FAO, 2014; Nassar, 2006). It is a staple crop for more than 300 million persons in sub-Saharan Africa (Ihemere *et al.,* 2011). The storage root of cassava is the most important source of dietary calories in the tropics after maize and rice.

Cassava plays a central role in food and economic security for small-holder farmers and holds an unrealized potential as a cash and food crop as it widely grown and well-adapted into the farming system. The crop is grown by most smallholder farmers due to its ability to yield better than other staple food crops under conditions of extended drought and poor soils (Esuma *et al*., 2016, Ceballos *et al.,* 2011 and El Sharkawy, 2007. It is a perennial crop native to South America and was among the first crops to be domesticated. It is believed to have been brought to Africa in the 17th century (Okigbo, 1980; Allem, 2002). Cassava cultivation is widespread in Africa, spanning across 40 countries. Generally, cassava serves five purposes: famine reserve crop, rural food staple, cash crop for urban consumption, industrial raw material, and foreign exchange earner (Nweke *et al.,* 2004).

In Sierra Leone cassava is the second major staple crop after rice. It has the potential of contributing to the achievement of the millennium development goal of poverty reduction as well as meeting requirement of the pillar II in the poverty reduction strategy paper in Sierra Leone (SLARI Strategic plan, 2012).

The agricultural sector in Sierra Leone employs more than 70% of the rural population and contributes 50% of the country's Gross Domestic Product (GDP) (SLARI Strategic plan, 2012).

All parts of the cassava plant are used either as food, animal feed or an industrial feedstock. However, the storage root portion of the plant is mostly used. Notably, the crop is deficient in essential micronutrients (vitamin A, iron, zinc and protein) hence presents a major health problem of nutritional insecurity in communities that heavily rely on cassava (Rice *et al.,* 2004; Gichuki *et al.*, 2010; and Esuma *et al.,* 2016).

Over seven billion people are afflicted with micronutrient malnutrition and the numbers are increasing (Mason and Garcia, 1993 and Welch *et al.,* 1997). In sub-Saharan Africa, micronutrient malnutrition is a major public health problem (WHO, 2002). This type of malnutrition results primarily from use of diets deficient in essential vitamins and minerals like iron and zinc. Known as hidden hunger, micronutrient malnutrition can exist even when poor people have enough to eat, but lacks essentials nutrients (FAO, 2003).

Diets poor in micronutrients cause illness, blindness, premature death, reduced productivity and impaired mental development. (UNICEF, 2004). The importance of micronutrients cannot be overemphasized, as micronutrient deficiencies remain a huge problem among young children and women in Africa and particularly in Sierra Leone. Micronutrients are essential for the normal functioning of the immune system, growth and development, maintenance of epithelial cellular integrity and for reproduction (Chavez *et al.,* 2007). In sub-Saharan Africa micronutrient deficiencies, are estimated to cause economic losses in productivity of more than U.S. $2.3 billion (UNICEF, 2004 and Nganga *et al.*, 2010). Although there have been efforts to alleviate

micronutrient malnutrition, the health problem remains highly prevalent because of inadequate or poor diet intake, which has triggered a call for concern.

Different strategies have been adopted in Sierra Leone to reduce micro nutrient deficiencies; these include dietary diversification, food fortification, and supplementation. These strategies have not always proven to be very successful remedies. Irrespective of this, the promising and sustainable strategy to address the micronutrient deficiencies is through food biofortification in Sierra Leone.

Biofortification is the process of breeding vitamins and nutrients into food crops, either by using natural breeding techniques or biofortification taking advantage of the genetic variability available in related species and varieties having specific nutrient traits that can be used for breeding (Welch and Graham, 2005; Chávez *et al.,* 2005 and Ceballos *et al.,* 2013). One approach towards the development, use and conservation of biofortified foods involves the screening of the existing plant genetic resources for germplasm with the required nutrient resources and desired quality traits (Harvest plus, 2002).

Screening efficiencies can be enhanced using molecular tools to identify genotypes having desirable traits (Njoku, 2012) without the confounding effect of the environment, reduce size of breeding populations, length of time and evaluation cost (FAO, 2004) required for breeding the desired traits.

Studies to evaluate the food quality, nutritional and genetic diversity of the cassava germplasm to ameliorate hunger and malnutrition have not previously been carried out in Sierra Leone. Characterization and evaluation of accessions within the cassava germplasm resources in Sierra Leone as well as screening for genetic variation in the germplasm is important in devising

optimum management strategies for sustainable utilization and conservation of the resource. As a starting point in breeding for higher micronutrient contents in cassava, with farmers preferred traits like dry matter and fresh root yield, the germplasm should be screened for useful quality traits that may be used or introduced into the breeding program for the development of breeding populations.

Regrettably, there is no evidence of adequate variability in protein, iron and zinc content of roots and leaves of cassava varieties in Sierra Leone which can serve as basis for improvement in the nutrient status of cassava

Thus, the main thrust of this study seeks to identify and select local and exotic cassava accession in Sierra Leone to establish a collection and develop superior cassava germplasm with improved yield and nutritional qualities. This formed the basis for the specific objectives which were;

1) To assess adoption challenges, perception and preferences for provitamin A cassava among cassava value chain actors in Sierra Leone.

2) To estimate genetic diversity within provitamin A cassava germplasm using morphological, molecular tools and iCheck device.

3) To evaluate the levels and stability of total carotene content and dry matter of selected provitamin A cassava accessions across environments.

4) To characterize F_1 progenies for total carotenoid, iron, zinc and protein contents.

5) To estimate the combining ability of 12 cassava parents and their F_1 progenies for mealiness, dry matter, number of roots and fresh root yield.

CHAPTER TWO

2.0 REVIEW OF LITERATURE

2.1 Importance of Cassava

Cassava is a major source of income for most farming households in Africa, creating youth employment opportunities and contributing to poverty alleviation (Njoku, 2012). It is consumed by over 600 million people in Africa, Asia and Latin America (Okoro, 2016). Among all staple crops in sub-Saharan Africa, cassava has been at the forefront as a 'crisis crop' since it can be left in the ground for well over one year and harvested when food shortages arise. The crop ranks third as source of calories in the tropics after maize and rice.

According to FAO (2014), Africa contributed 145.77 million tonnes to global cassava production, while Asia and Latin America recorded 89.03 and 30.64 million tonnes, respectively. Although Nigeria was the highest cassava producer between 2010 and 2014 using the largest land area, its average yields were relatively low among the top ten producing countries in Africa. Production in Ghana increased whilst that of Democratic Republic of Congo decreased during the period. Malawi recorded the highest average yields during the period followed by Ghana and Cameroon.

2.2 Cassava production in Sierra Leone

Cassava gained its importance after the civil war in Sierra Leone and has become a cash crop that generates income for many households and contributes positively to poverty alleviation. (Fomba *et al*, 2011). FAO (2014) reports that the average yield of cassava in Sierra Leone from 1990 to 2002 was below 2.0t/ha, followed by an increase to 7.9t/ha in 2004 and to 15.0t/ha in

2013. This tremendous progress in cassava productivity represents a positive relationship between yield and production which have grown to meet the rising demands for staple food and industrial applications. Cassava is the second most important staple after rice in Serra Leone. It constitutes an important portion of the diets of rural communities' and in competition with other traditional staples. As a food crop, cassava fits well into the farming systems of the smallholder farmers in Sierra Leone because it is available all year around, thereby improving household welfare and livelihood. Compared to grains, cassava is more tolerant to low soil fertility and more resistant to drought, pests and diseases.

It is largely cultivated by smallholder traditional subsistence farmers who make use of rain fed farming systems characterized by shifting cultivation practices, manual land preparation and inter-cropping systems (Jalloh and Dahniya, 1994). Cassava is grown in all regions of Sierra Leone (Eastern, Southern and Northern parts) with the northern region being the leading producer. The main cultivation period of cassava is from May through to April during the onset of rains. Since cassava is drought tolerant, there has not been any form of irrigation practices with its cultivation in Serra Leone. Varieties are grouped based on their texture, colour, taste, and mealiness (cooking ability). The widely-grown cassava varieties are the white root, which are extremely low in provitamin A. This has posed a nutritional challenge or alarming health issues in communities whose diets heavily rely on cassava.

2.3 Cassava production constraints in Sierra Leone

Despite the growing importance of cassava as a nutritional security and income generating crop for subsistence farmers in Sierra Leone, as well as its potential to support the national economic development, the crop's production output is constrained by a wide range of factors, some of

which include: the use of local unimproved low yielding cultivars with low nutritional value and lack of well adapted varieties. This indicates that farmers need better varieties. Furthermore, poor agronomic management practices, shortening fallow periods, declining soil fertility, poor access to market, cassava pests and diseases have limited cassava production in Sierra Leone. The development and yield stability of cassava rely on the quality of planting materials. The use of healthy plant materials is a very important factor in the attainment of good yields. Conversely, cuttings with low vigour which are infested/infected by pests and diseases limit cassava production. The long cropping cycle of cassava, harsh and unpredictable climatic conditions, lack of access to credit facilities and farm inputs also constrained cassava production in Sierra Leone (Spencer, 1997).

Poor Government policies and strategies, poor feeding and care practices, limited generation, and dissemination of technologies and preservation of Indigenous Technical Knowledge (ITK), has also resulted in low cycles in cassava production which has engendered chronic malnutrition within communities reliant on cassava and has triggered concerns in Sierra Leone (SLARI Strategic Plan, 2012). Improved cultivars enhanced with high beta carotene, iron, zinc, and protein should be made available to such communities to address the problem of malnutrition in Sierra Leone and regions in sub-Saharan Africa where cassava is consumed as a major staple.

2.4 Agricultural origin and spread of cassava in Africa

Historical and scientific evidence supports cassavas to have its origin as South America as the species is an ancient starchy root crop in the region (Allem, 2002). The progenitor of cassava (*M. esculenta* ssp. Flabellifolia) has been in existence and is adapted to forest and savanna ecozones of the Amazon basin.

The crop was domesticated around 4000 BC and has evolved as a food crop from the second and third millennium BC (Allem, 2002; Nassar and Ortiz, 2008). The domestication process initiated natural selection for cultivars with traits such as root size, growth habit and ability of clonal propagation through stem cuttings (Jennings, 1976 and Njoku, 2012). The Portuguese explorers introduced cassava to Africa during the 16th and 17th centuries through their trade within the West African coasts. Africans then spread cassava cultivation and is now found in almost all parts of tropical Africa. Early cultivation started in Fernando Po in the Gulf of Benin and around the Congo River in the 16th century and did not spread through West Africa until the 20th century (Hillocks, 2002; Njoku, 2012). Currently, Africa leads in the production of cassava with Nigeria as the largest producer in the world. Other top producing countries include Brazil, Thailand and Democratic Republic of Congo (FAO, 2010). Realizing the importance of cassava, the International Institute of Tropical Agriculture (IITA) established with its headquarters in Ibadan, Nigeria in 1967 under the guidance of the Consultative Group on International Agricultural Research (CGIAR) was mandated to oversee the development of the crop across Africa

2.4.1 Taxonomy of cassava

Cassava is placed in the Fructicosae section of the genus Manihot, which is a member of the Euphorbiaceous (Jennings and Iglesias, 2002: Parkes, 2011). About 98 species have already been identified in the Manihot genus (Rogers and Appan, 1973; Akuwa, 2016). The Fruticose section is made up of growing shrubs adapted to savanna, grassland or desert and is considered less primitive than Arboreae section of Euphorbiaceae, which are made up of tree species (Jennings and Iglesias, 2002). All the species of Manihot have 4x = 2n = 36 chromosomes and could be

regarded as polyploids with x = 9, n = 18. Although cassava is normally considered as a polyploid species (El-Sharkawy, 2003), 18 small and similar pairs of associated homologous chromosomes, or bivalents were reported during analyses conducted using diakinesis and metaphase I approach (Jennings and Iglesias, 2002 and Wang *et al.*, 2011). Cassava therefore, can be regarded as a functional diploid crop (Jennings, 1976; De Carvalho and Guerra, 2002; Nassar and Ortiz, 2008). Cassava classification is based on its qualitative characteristics such as leaf shape and size, plant height, stem colour, petiole length and colour, inflorescence and flower colour, storage root shape and colour, earliness, and content of cyanogenic glycosides (Nassar and Ortiz, 2006). Cultivars are classified as bitter and sweet based on cyanogenic glycoside. Onwueme (1987), confirmed against using the level of glycosides as a distinguishing characteristic for cultivars since the exact level of glycosides in a cultivar varies considerably according to the climatic conditions under which the plant is cultivated. Sweet cultivars are reported to have a short growing period with their storage roots maturing early (Nassar and Ortiz, 2006; Amenorpe *et al.*, 2007). It has been reported that the emergence of spontaneous species as well as other Manihot species have been required to occur naturally in Africa and Brazil (Nassar, 1994).

2.4.2 Root system

The root system of cassava is made up of feeder roots and storage roots. When propagated from stem cuttings it's developed adventitious root at the base of the cuttings within the first three weeks (Ekanayake *et al.*, 1997). The root system of cassava affects its establishment in the field. The adventitious roots subsequently develop into a fibrous root system, which absorb water and nutrients from the soil. The length of the fibrous root system reaches 200 cm or more in length (IITA, 1990). Formation of the storage roots begins about eight weeks after planting. Between

three to ten fibrous roots start to bulk and become storage roots (Alves, 2002). The fibrous roots that become storage roots lose their ability to absorb water and nutrients considerably. Cassava propagated from true botanical seeds develops the typical tap root system of dicot species within 30-60 days. Increase in roots diameter produces storage roots. Storage roots develop from the activity of cambium and starch accumulation (Alves, 2002). The shelf life of cassava storage roots is very short as compared to other major root crops (Ghosh *et al.,* 1988). Rapid post-harvest deterioration processes occur within 24-72 hours after harvest (Wheatley *et al.,* 1985; Wheatley and Chuzel, 1993).

2.4.3 Variability in colour and provitamin A of cassava storage root

Most breeding populations of cassava have white storage roots, although some yellow root landraces have attracted a lot of attention lately. Yellow pigmented cassava root is typically known to be cultivated in a limited way in Colombia, Philippines, Jamaica and some African countries (Oduro,1981), with some yellow landraces also identified in Amazonia in Brazil (Ferreira *et al.,* 2008; Nassar *et al.,* 2009). Wide variation exists in root pigmentation within the global yellow root germplasm. This varies from pale yellow through orange to pink (Nassar *et al.,* 2007). This variation in root pigmentation results from the wide variation in carotenoid contents within the global yellow cassava germplasm.

It is important to note that, previous studies have shown that yellow root cassava varieties tend to have low dry matter content (Vimala *et al.,* 2008; Akinwale *et al.,* 2010 and Njoku, 2012) which is associated with poor cooking quality (Vimala *et al.,* 2008). Yellow root cassava has high levels of provitamin A carotenoid and its consumption has been perceived as a sustainable approach for addressing Vitamin A deficiencies. In cassava, intensity of yellow pigment in roots

of some genotypes is strongly associated with β-carotene (Sanchez *et al.,* 2006). Enhanced content of β-carotene (provitamin A) in yellow flesh cassava (Chavez *et al.,* 2007 and Sanchez *et al.,* 2006) provides potential opportunity to effectively address vitamin A malnutrition through availability of provitamin A cassava varieties where the crop is largely consumed (Makokha and Tunze, 2005; Nassar and Ortiz, 2010, Esuma *et al*., 2016). It is interesting to note that global efforts towards breeding cassava for high β-carotene content, is a recent development with slow progress registered towards development and deployment of nutrient rich varieties to farmers (Hershey, 2012; Welch and Graham, 2004). Several attempts have been made to elevate the carotene levels of yellow cassava through the introgression of genes from existing genetic resources (Akuwa, 2016).

Carotenoids are a family of C40 isoprenoid pigments including approximately 600 identified structures in higher plants. The accumulation of intermediary carotenoids and their stable natural isomers (Z-*iso*) varies in accordance with plant species and plant organ types. In higher plants, carotenoids play the role of providing distinct yellow, orange, or red colors to certain organs, such as flowers, fruits, roots and tubers. Beta-carotene is probably the most well-known of the carotenoids; a phyto-nutrient family that represents the most widespread groups of naturally occurring pigments. ß-carotene known as provitamin A is one of approximately 50 carotenoids compounds which can be converted into retinol, an active form of vitamin A, in the body. β-carotene is not synthesized in animals but only in plants and micro-organisms. Carotenoids in plants are derived from the general isoprenoid biosynthesis pathway that takes place in chloroplasts of photosynthetic tissues and in chromoplasts of fruits and flowers. Synthesis of β-carotene is accomplished by insertion of only the missing genes necessary to complete or complement the biosynthetic pathway. Cervantes-Flores (2006) also confirmed the observation

of mRNA of genes in the carotenoid pathway in both orange mutant tissues and the 14-unpigmented wild-type tissues in a study conducted in *Brassica olearacea*. The molecular mechanism responsible for the increasing and variable levels of beta-carotene synthesis in the yellow flesh cassava (YFC) varieties compared with other differently colored varieties is still unknown.

2.5 Cassava growth and development

Studies on the growth and development of cassava are not so widespread (Connor *et al.,* 1981; Keating *et al.,* 1982 and Parkes, 2011). Seeds are known to take about 16 days to germinate. However, by carefully filing the sides of the seed coats at the radicle end and by temperature management, germination can be accelerated. Ellis *et al.*, (1982) reported required temperature for cassava seed germination when temperature exceeded 24°C and the best rates occurred at 30 to 35°C. Fourteen days' dry heat treatment at 60°C is beneficial for newly harvested seeds. Seeds should be stored at 50°C and at 60% relative humidity (IITA, 1978), as they tend to lose viability rapidly during a year storage at ambient temperatures (Kawano, 1978). In cassava, the initial growth phase after seed germination lasts about six weeks after which auxiliary shoots and adventitious roots regenerate. Photosynthesis commences as soon as the first leaves appear. Storage roots developments starts with the initiation of secondary thickening of the adventitious roots, a process observed as early as three weeks after planting (WAP), (IITA,1990). Onwueme (1978) and Vine (1979) reported that the thin root accomplishes the initial penetration through the soil, and the increase in girth or growth occurs afterwards. Soil physical properties and texture which affect storage root yield (Ntawuruhunga, 2000).

Cassava storage root differs arbitrarily thereby distinguishing one from another when their thickness surpasses 0.5cm, which is generally reached between one to four months after planting

(Veltkamp, 1986). Storage root bulking in cassava is affected by assimilate supply to the roots which is determined by shoot growth and hormonal changes (Williams, 1972). The number, shape, size and angle at which storage roots penetrate the soil, the colour of the outer cork, and internal tissues vary greatly among varieties. Usually there are three to ten storage roots per plant. Roots are cylindrical, 15 to 100cm long, 3 to 15cm in diameter, but could be occasionally branched. Storage root size is a function of root length and root diameter. Root size is genetically controlled in cassava (Dixon *et al.,* 1994a), and this depends on the extent of cell division and starch accumulation processes (Ekanayake *et al.,* 1997). Significant correlation observed between root diameter and yield (Okogbenin and Fregene, 2002) is an indication that diameter can be used to select for high storage root yield in cassava. The final yield is associated with the storage root number, diameter and size (Williams, 1972; Simwambana, 1988; Njoku, 2012).

2.6 Breeding for high beta-carotene content, iron, zinc and protein

Improvements in cassava yield and other traits are generally not geared towards the highest possible under favorable conditions but rather obtaining stable yields and durable traits under marginal conditions where cassava is grown at present and is likely to expand in future. (Cock, 1985; El-Sharkawy, 2003).

Outstanding yield is achieved first by selecting plants with good genetic structure which maximizes performance and secondly expressing potential resistance or tolerance to factors which limit yield (Ellis *et al.,* 1982). Heterozygosity is the main hybrid vigor requirement for the genetic structure of new varieties and is a major focus of breeding programmes (Nassar *et al.,* 2004).

Efforts have been undertaken over the years to determine the genetic potential for increasing the concentrations of bio-available Fe, Zn and provitamin A carotenoids in edible portions of several staple crops including rice, wheat, maize, beans and cassava (Graham and Welch, 1996).

Conventional cassava breeding and selection pipeline begins with artificial or open pollinated crossing to obtain botanical seeds, followed with the establishment of the seeds in the seedling nursery and then screening and advancing to different breeding stages; the clonal evaluation trials, preliminary yield trials, advanced yield trials, uniform yield trials, and finally the multi-locational testing and on farm testing towards release (Jennings and Iglesias, 2002; Kawano, 2003). This process lasts for a period of eight to ten years before a variety is released. During the past years, efforts have been made to circumvent the number of years it takes for conventional breeding of cassava varieties. Very recently, modern molecular tools are being employed to shorten the breeding cycle.

Iglesias *et al.* (1997) reported an increased level of carotene contents in roots from 632 clones and found a broad distribution of concentration from less than 0.1 to 2.4mg/100g of fresh roots. Chavez *et al.,* (2005) evaluated 2457 clones and found that carotene content in storage root ranged from 0.102 to 1.040 mg/100g. Jos *et al.,* (1990) screened 654 cassava stocks of indigenous and exotic origin for carotene, and found 21 clones with beta-carotene content ranging from 65I. µ /100g to 670 I. µ /100g. Recent progress has also been reported in the development of quick, inexpensive methods for screening for micronutrients with the use of molecular marker-assisted selection (Wong *et al.,* 2004). The process accelerates the introgression of increased micronutrients from exotic sources into locally adapted, elite varieties. However, this depends on bioavailability of the type of micronutrients and willingness of farmers

to adopt such varieties. Variability in carotene content among accessions of national germplasm collections have been reported in India (Moorthy *et al.*, 1990), in Brazil (Ortega-Flores, 1991), in Uganda (Esuma *et al*., 2016), in Nigeria (Njoku,2012) and in Ghana including iron and zinc (Baafi *et al*., 2016a). *Jos et al.* (1990), they however, demonstrated the potential to rapidly increase carotene content in cassava roots through cycles of recurrent selection. They increased the concentration from 4.2 mg/kg of fresh roots in the base population to 14 mg/kg after two cycles of selection and recombination.

Hierarchies of screening procedures have been exploited for selection for better nutritional quality traits in cassava. Dixon *et al.* (2000) reported a significant positive correlation between iron and zinc in cassava and concluded that the potential exists for developing cassava clones with higher levels in both iron and zinc. The broad genetic base combined with recurrent selection has been reported as the most appropriate procedure for improving base population (Bryne, 1984; Fregene *et al.*, 2006; Akuwa, 2016 and Baafi *et al.*, 2016a).

A study of 600 cassava clones revealed a zinc concentration of 2.6 and 37 mg/kg with an average of 7.5 mg/kg (Chavez *et al*., 2005) in storage roots. To supply the minimum daily zinc requirement for individuals consuming between 500 and 1000 kg of cassava would require varieties with at least six-fold zinc levels in the edible parts (Sayre *et al*., 2011). Through a transgenic approach, Ricachenevsky *et al*., (2013) used the endosperm-specific overexpression of *MTP1* proposed for zinc biofortification in rice to increase zinc concentration in the storage root of cassava.

2.6.1 Participatory plant breeding

Plant breeding exploits existing variability, gene manipulation and recombination of new genes into plant forms for human uses. Participatory plant breeding is the set of approaches that apply in situations where consumers are involved in the process of developing varieties that address their specific needs for different varietal traits. Early plant breeding was developed essentially as an art when farmers, who were though not educated, applied intuitive knowledge to skillfully and carefully select and retain plants with the most desirable features for crop improvement (Parkes, 2011). This informal breeding during the years have made farmers skillful in selection of varieties with their preferred traits. Most scientists now sourced farmers' indigenous knowledge on the cultivation of many crops (Francis, 1990; Dapaah *et al.*, 2003; Manu Aduening *et al.*, 2006) as it has been observed and documented that technologies that have been developed with little or no farmers' participation has gained little or no attention by farmers (Nweke, 2004; Manu-Aduening *et al.*, 2005; Zacarias *et al.*, 2004). This often occurs because farmers' perception priorities have been different from that of breeders (Manu-Aduening *et al*., 2005). Kizito *et al.,* (2007) and Parkes, 2011) indicated that farmers used some stable morphological traits like height at first apical branching, petiole colour and culinary attributes such as taste to differentiate and name varieties.

2.6.2 Progress in cassava improvement

Hahn *et al.* (1980), IITA (1982, 1993) and Ceballos *et al.* (2006a) have reported on yield increases in cassava through genetic improvement. However, despite the proven record of increasing in cassava improvement, there are still many challenges limiting the improvement of the crop. Lawson (1988) reported that cassava genotypes find optimum physiological expression

of their genetic potential within narrow ranges of biophysical conditions. Cock (1987) and El-Sharkawy (2003) also reported that only few cassava cultivars attain stability across multiple agro ecologies. A study of growth in cassava concluded that stability in productivity in cassava depends on several factors acting synergistically. These factors include abiotic factors (soils, temperature, photoperiod and latitude), biotic factors (diseases, pests and nematodes) and management practices (Allem and Hahn, 1991). Carter *et al*. (1992) concluded that 19% of cassava available in Africa is found in mid-altitudes where trends in socio-economic and physical environment favor cassava production.

This has provoked more interest in cassava production increment within this ecology since earlier research was focused on the lower altitudes of the tropics where cassava finds its most suitable growth environments (IITA, 1993; FAO, 1996). The variation in plant adaptation is linked with environmental factors that influence differential yield performance of genotypes (Cooper and Hammer, 1996). Understanding the factor that influence the environment is therefore a critical requirement for improving efficiency and effectiveness of a plant breeding programme.

2.7 Genetic diversity in cassava

Genetic diversity in plants depends on various evolutionary processes, which include mutation, hybridization, migration and polyploidy (Colombo *et al*., 2000). Studies have assessed and characterized the genetic diversity in cassava gene pools with the aim of aiding genetic resource conservation into breeding programs. One of the first attempts using molecular markers on a global scale looked at genetic diversity, for potential heterotic groupings in cassava (Fregene *et al.*, 2003; Ferguson *et al.*, 2011). Jennings (1963) suggested that a high genetic diversity of

cassava genotypes resulted from introduction of cassava genotypes by immigrants, followed by natural hybridization in the fields. Seeds from spontaneous crosses that occur in field establishes a field seed bank which eventually germinate and produce volunteer seedlings from which farmers make selection and add to their existing cultivars (Elias *et al*., 2001; Kizito *et al*., 2007; Manu-Aduening *et al.*, 2005; Mkumbira *et al.*, 2003; Nassar, 2006; Peroni *et al.*, 2007 and Pujol *et al*., 2002; 2007). The seed bank positively influences genetic diversity (Pujol *et al*., 2007) with patterns that suggest that the incorporation of volunteer seedlings accounts and supports the increases in intra-varietal genetic diversity (Elias *et al*., 2000 and Pujol *et al.*, 2007). Fregene *et al*. (2000) and Tumuhimbise (2013) indicate that the genetic diversity in the East African cassava is threatened by the adaptation to pests and diseases, agronomic practices, and post-harvest uses. Thompson. (2013) however, observed a moderate to high genetic diversity among the cassava accessions evaluated in Ghana.

Asante and Offei, (2003) suggested that even though the genetic diversity in *Manihot* spp is high in Ghana, a very low diversity is observed geographically in regions and is associated with the exchange of planting materials between farmers and selection for similar desired traits. It is believed that centuries of farmers' methods of selection accounted for the wide range of genetic diversity observed in crops (Jennings and Iglesias, 2002). Nassar (2004), however, indicates that the wide genetic diversity in cassava is due to natural hybridization between the wild *Manihot* spp and cultivated cassava. One of the first attempts at using molecular markers on a global scale looked at genetic diversity for potential heterotic groupings in cassava (Fregene *et al.*, 2003; Ferguson *et al.*, 2011)

Until 2014, only few relatively small-scale genetic diversity assessments of cassava in southern, eastern and central Africa had been exploited using a range of molecular tools (Benesi, 2005; Fregene *et al*., 2000; Kizito *et al.*, 2005 and Zacarias *et al.*, 2004).

2.8 Morphological and Molecular Markers in cassava

2.8.1 Morphological markers

Classification of cultivars and the study of their taxonomic status involving the use of measured qualitative and quantitative parameters of plants can be achieved using different plant breeding tools (Rogers and Appan, 1973; Parkes, 2011). The screening for cultivar identification traditionally depend on botanical traits (Stegemann, 1984). Breeders and geneticists have exploited qualitative characteristics such as leaf and flower attributes to study segregation of genes. Agro morphological traits are determined by more than one gene and are subject to varying degrees of environmental modifications and interactions.

2.8.2 Molecular markers

Molecular markers include isozyme and protein, biochemical and DNA markers. A molecular marker could be defined as DNA regions showing sequence polymorphism in different individuals in species (Liu, 1998). DNA fingerprinting is an approach which has been widely accepted and exploited to achieve inter and intra organism differentiation at genotypic species and subspecies levels and other levels (McClean *et al.*, 1994). The strategies have been used for cultivar identification, designed to detect the presence of specific DNA sequences or combination of sequences that uniquely identify the cultivar. Cultivar identification can be successfully attained more accurately through DNA fingerprinting data. The most closely related

cultivars are usually distinguished with the DNA fingerprinting methods (Beckman and Soller, 1986). The advantage of DNA fingerprinting over qualitative markers is the dominance of most DNA nucleus, absence of climatological and effects of tissue (Beeching *et al*., 1993). The application of DNA fingerprinting could be very important in the cultivar and species identification, and could help to create more efficient breeding programmes through the detection of genetic linkages between traits of interest. (Lin *et al*., 1993).

Advances in molecular biology have unveiled the potential of DNA approaches, markers for genetic improvement and identification of cultivar of food crops. Several DNA based markers have been developed for measuring similarity that reveal polymorphism at DNA level in agricultural crops (Kumar *et al.,* 2000). These markers have been proven to be an important tool in genetic variation assessment within and between populations and the elucidation of genetic relationships among cultivars (Lee, 1995; Karp *et al.,* 1996). DNA markers confirmed greater variation than isozymes. DNA composition is also consistent between tissues and this is not affected by environmental changes (Beeching *et al.,* 1993). DNA markers have been extensively used for the development of detailed genetic and physical chromosome maps in a variety of organisms. Molecular markers have been found very useful in conventional breeding for carrying out indirect selection in Quantitative Traits Loci (QTLs). In addition to these major applications, DNA markers can also be used in plant systems for germplasm characterization, genetic diagnosis, characterization of transformants, study of whole genome organization and phylogenetic analysis (Rafalski and Tingey, 1993). Although every marker system has some advantage and disadvantages, the choice of any marker system is dictated by the intended application, convenience, cost and time.

2.8.3 Single nucleotide polymorphism (SNP)

Single nucleotide polymorphisms (SNPs) are DNA variation in sequence that occur when a single nucleotide (A, T, C and G) in the genome sequence is altered. SNPs can occur in coding (genes) and non-coding regions (introns) of the genome but have been shown to be more commonly situated in introns regions (Rafalski, 2002; Tangphatsornruang, 2008). SNPs are classified into non-coding SNPs, coding SNPs, exonic SNPs, cDNA SNPs and candidate SNPs (Kahl *et al.*, 2004) with regards to their location. SNP markers could be defined as small insertions and deletions (indels) that represent the most available form of naturally occurring genetic variation within some populations. High density SNPs identification would dramatically facilitate progress in cassava genomics and breeding. SNPs are biallelic in nature (two alleles at a locus) meaning they are individually less informative than Simple Sequence Repeats (SSRs), which are generally multi-allelic meaning many alleles are found at a single locus (Ferguson *et al*., 2011). This drawback is complimented by the abundance and suitability of SNPs for ultra-high throughput genotyping strategies (Rafalski, 2002). SNP markers are extremely important in crop improvement. They are made up of a wide range of applications such as genetic diversity and phylogenetic studies, association mapping, marker diversity increase, QTL mapping, high-throughput MAS and evolutionary biology (Olsen, 2004; Lopez *et al.*, 2005; Njoku, 2012). In cassava, there are limited information on discovery and use of SNP markers. In a study to monitor genetic diversity in cassava, 26 SNPs were identified and characterized using direct sequencing of diverse cassava varieties and an estimated frequency of one SNP every 121 nucleotide was observed (Kawuki *et al.*, 2009). From this study nucleotide diversity varies from 7.8×10^{-4} to 8.6×10^{-3} and individual SNPs had lower polymorphic information content (PIC) values than haplotype based SNPs. SNPs have been used in cassava to understand the genome of

cassava origin and its phylogenetic relationship with wild relatives (Schaal *et al.*, 1997; Olsen, 2004). SNP markers are more abundant in the genome, more efficient and cost-effective than SSR genotyping (Thomspon, 2014).

2.8.4 Morphological and molecular characterization of cassava

Mathura *et al*., (1986) confirmed that phenotypic variance in cassava was higher than genotypic variance for traits of agronomic importance, such as weight of storage roots. However, all qualitative descriptors revolve around the three important parts of the cassava plant which can be classified into (a) the leaf characteristics (b) the stem characteristics and (c) the root characteristics (Alves, 2002; Fukuda *et al.*, 2010 and Akuwa, 2016). The *Manihot* gene pool ranges from a great variety of wild species to numerous domesticated species with very specific unique qualities. Past and present research work has used markers toward molecular linkage maps development to provide a better structural definition of the cassava genome (Fregene *et al.*, 1997 and Rabbi *et al.*, 2014)

2.9 Combining Ability in cassava breeding

Combining ability may be determined at two levels. GCA-General combining ability (GCA) and SCA-Specific combining ability (SCA). GCA measures the average performance of a specific inbred in a series of hybrid combinations, while SCA denotes the performance combination of specific inbred in a cross combination (Sprague and Tatium 1942). The importance of general combining ability (GCA) effects is to measure the comparative performance of parents in relation to one another and additive gene action (Byrne, 1984, Falconer and Mackay, 1996). Consequently, for genetic improvement of characters for a new population compared with the parental base population, SCA effects which measures the performance of distribution of crosses

in relation to GCA effects of parental combination becomes very essential (Singh, 2001). GCA and SCA effects show both the magnitude and direction of the genetic effects. According to Rajendran (1989), it is necessary to measure the combining abilities of parents before they are exploited in a hybridization program. This is because, selection of parents based on their direct performance may not always be dependable due to the type of gene action involved for expression of the trait and diverse genetic structure of the parents. The importance of mating designs in breeding is a two-way approach; firstly, to collate information for breeding population that can be exploited for selection and secondly, to develop outstanding varieties (Acquaah, 2009). In plant breeding, mating designs and arrangements are exploited by breeders to generate potential varieties. The kinds of mating techniques to use and its arrangements depend upon:

i. Predominant type of pollination (self or cross):
ii. Type of crossing used (artificial or natural)
iii. Type of pollen dissemination (wind or insect)
iv. Unique features, such as cytoplasmic or genetic sterility
v. Purpose of project and
vi. Size of population required.

Some commonly used mating designs include; top cross, polycross, North Carolina (i, ii and iii), diallel (1, 2, 3 & 4) and triallels. The information generated from any of these mating designs support scientists to determine appropriate breeding strategy, as well as to evaluate the improvement that can be expected for a given selection intensity (Akuwa, 2016). A complete diallel mating design refers to the one that allows the parents to be crossed in all possible combinations (Schlegel, 2010). This mating design scheme is required to successfully accomplish Hardy–Weinberg equilibrium in a population (Acquaah, 2012; Nduwumuremyi *et*

al., 2013). Different breeding approaches that give information regarding the choice of parents and elucidate the nature and magnitude of various types of gene action involved in the expression of quantitative traits are now being used. They include diallel analysis, line x tester analysis and recurrent selection as well as other procedures currently used in cassava breeding followed by phenotypic selection, as suggested by Kawano (1980) and Akuwa (2016).

2.10 Genotype, environment and genotype by environment Interaction

A phenotype (P), is known as the characteristic that is observed, depends on a combination of its genetic constitution, called the genotype (G), and the environment (E) and a component attributed to the interaction between genetic and ecological components (G×E). Since genes are expressed in an environment, the degree of expression of a heritable trait is impacted by its environment. This is usually expressed as: Phenotype = Genotype + Environment + G×E (Falconer and Mackay, 1996; Sleper and Poehlman, 2006; Tumuhimbise, 2014). The equation as stated below for phenotypic expression, it accommodates any variation observed in the phenotype due to variation in the factors resulting in the genotype. The relationship can be described as: VP = VG + VE + VGxE.

Where:

Where VP = Phenotypic variation

VG = Genotypic variation

Due VE = Variation because of the environment

And VG×E = variation due to genotype by environment interaction effects.

Acquaah (2009) explained genetic or heritable variation as the variation that can be attributed to genes that encode for some traits and can be expressed or seen from one traits to the other. Genotypic variation is separated into two components, namely additive and non-additive

components (Falconer and Mackay, 1996; Sleper and Poehlman, 2006). Additive variation is because of the cumulative effect of alleles on all gene loci influencing a trait, and is of most value in a crop improvement programme (Falconer and Mackay, 1996). Non-additive variation is separated into dominance variation, caused by the interaction of specific alleles at a gene locus, and epistatic variation, caused by the interaction among gene loci (Falconer and Mackay, 1996). The non-additive is variation normally given little attention since only the additive component of genetic variation determines the heritability of economic traits (Falconer and Mackay, 1996; Sleper and Poehlman, 2006). Genetic or heritable variation in nature originates from mutation, gene recombination, modifications in chromosome numbers, and structure and migration (Falconer and Mackay, 1996). The three phenomena namely mutation, gene recombination, and migration are manipulated more and more extensively to generate genetic variation for their breeding programmes (Acquaah, 2009).

Environmental variation is associated with ecological conditions of the site where the crops are grown (Annicchiarico and Perenzin, 1994). Some of these conditions, such as plant to plant competition and population density can be regulated by employing effective agronomic practices, while others, such as biotic and abiotic stresses, are truly uncontrollable. Environmental variation in crops is normally difficult to control because it is non-heritable. For example, when individuals from a clonal population (identical genotypes) are evaluated in a trial site, the plants will exhibit differences in the expression for some traits because of non-uniformity in the environment. Therefore, an understanding for the magnitude and nature of variation and diversity present in the gene pool for the traits of interest is of greatest importance (Akuwa, 2016). Cassava has been subjected to considerable genotype by environment interaction (GEI) studies (Kvitschal *et al*., 2006; Ssemakula and Dixon, 2007; Lebot, 2009; Thompson, 2013

and Esuma *et al*., 2016). Quantitative trait is especially influenced by environmental effects. Differences in genotypic values may increase or decrease from one environment to another which might cause genotypes to rank differently between environments. Investigation on different cassava genotypes evaluated in contrasting environments have proven that Fresh Storage Root Yield (FSRY) trait is subject to strong GEI (Ssemakula and Dixon, 2007; Aina *et al.,* 2009 and Esuma *et al*., 2016). Tan and Mak (1995) reported that GEI effects were significant for FSRY, commercial storage root number, harvest index, starch and cyanide content. Buerno (1986) revealed important genotype x location and genotype x location x-year interaction for FSRY when testing so many genotypes in the humid tropics of Brazil. To detect GEI cassava breeders evaluate advanced breeding lines in several environments. The concept of GEI enables plant breeders to determine the genotype among several genotypes that is high yielding and stable in multi-environmental trials (MET) i.e., across different environments (locations, years, growing seasons etc.). Breeding focuses on GEI as a typical requirement for to select the outstanding genotype for a target population of environments (Akinwale *et al.,* 2011 Maroya *et al*., 2012). Studies reported by (Akinwale *et al*., (2011), Tumuhimbise *et al*., (2014) and Agyeman *et al*., (2015) have indicated a very good variation in fresh root yield across multiple environmental conditions. Ssemakula and Dixon (2007) noted a low influence of GEI on carotenoid content in cassava roots at harvest, based on the analysis of 28 genotypes screened across five multiple environments over two growing seasons.

CHAPTER THREE

3.0 ADOPTION CHALLENGES, PERCEPTION AND PREFERENCES FOR PROVITAMIN A CASSAVA AMONG THE CASSAVA VALUE CHAIN ACTORS IN SIERRA LEONE

3.1 Introduction

Cultivated varieties of cassava in Sierra Leone either have white or cream/light yellow roots and high dry matter content but are deficient in vitamin A. The development of biofortified provitamin A cassava by IITA and research partners is a strategy to address the deficiency of vitamin A of white cassava root varieties. Biofortified yellow root cassava has β-carotene, a dietary precursor of vitamin A, which is known to be responsible for the yellow to orange colour of flesh storage roots (Rodriguez Amaya and Kimura, 2004; Njoku, 2012). Vitamin A is important for immune competence and good vision, as well as, cellular differentiation, growth, and reproduction. In cassava, β-carotene is the most abundant carotenoid which can be efficiently converted to vitamin A, E, ascorbic acid, enzymes and proteins; belongs to the biological antioxidant network converting highly reactive radicals (•OH) and free fatty peroxy radicals to less active species, thereby protecting the body against oxidative damage to cells (Packer, 1992). In human nutritional studies, vitamin A activity is expressed as retinol equivalent, and 6 μg of *all-trans*-β-carotene has the biological (vitamin A) activity of 1 μg retinol (Okaka *et al.,* 2001). The dietary allowance (RDA) of vitamin A for adults (men and women) and children (4 to 9 years) are 0.75 and 0.3 to 0.4 mg/day retinol activity equivalents (REjm A)/day, respectively (Okaka *et al.*,2001; Njoku, 2012). These recommended dietary requirements are not adequately supplied in diets, especially in children, pregnant women and the under privileged in most developing countries including Sierra Leone.

In a cassava biofortification breeding programme for vitamin A, iron, zinc and protein, the following factors must be considered: farmers' and consumers' acceptability of the new product and bioavailability of the vitamin and nutrients. In Sierra Leone, adoption rates for new or improved technologies have been rather low; this is because during the early stages of the breeding process, farmers and consumers are not involved. However, it has been recommended that to increase the acceptability and adoption rate for biofortified provitamin A cassava cultivars, farmers and consumers should be included in formulating research objectives and in the selection of varieties through participatory methods.

Participatory rural appraisal (PRA) is a technique that brings all stakeholders together with the aim of addressing a challenge and narrowing the communication gap between scientists and farmers. PRA forms the basis for stakeholders to work as a team to understand the target environment, identify the constraints and together formulate research agenda with plant breeders. This allows greater participation of farmers in the research process, leading to more effective and efficient information gathering and quick adoption of new research technologies. Adebo (2000) pointed out that, participatory approach is one of the ways that farmers' perceptions can be captured and accounted for as hard data; the people's resourcefulness and creativity can be challenged and captured and breeders may demonstrate respect for the insight and knowledge of the farmers in Sierra Leone. The present study aimed to solicit information from end-users in Sierra Leone regarding adoption challenges, perception and preferences for provitamin A cassava. This design allows the research team to source qualitative and quantitative data from study participants. This design supports a variety of analytical techniques including econometric and non-econometric analyses.

The objective was to i) assess perception and preferences for provitamin A yellow cassava roots and its gari among cassava value chain actors in Southern, Eastern and Northern region in Sierra Leone.

3.2. Materials and methods

3.2.1. Study area

The study was conducted in Sierra Leone a country located on the West Coast of Africa which lies between latitudes 6º55' and 10º00'N and longitudes 10º16' and 13º18'W, and covers a total area of 72 000 km^2. The climate is a monsoon humid tropical type with two distinct seasons: rainy season from May to October and dry season from November to April. Provisional results of a 2015 population census showed a total population of 7,075,641 people in Sierra Leone as opposed to 2004 of 4.5 million populations. Sierra Leone is divided into four regions: The Eastern, Western, Northern and Southern Regions. Each district is divided into districts of unequal sizes. Each district is divided into chiefdoms, which in turn are divided into districts and enumeration areas (EAs).

This study focused on three districts of three regions and fifteen chiefdoms in Sierra Leone. The three regions eastern, northern and southern were purposively selected based on the production, processing and distribution patterns of cassava or cassava products within the country. Each region is divided into districts of unequal sizes. The three districts namely Kaliahum, Bombali and Moyamba districts were randomly sampled from the eastern, northern and southern regions, respectively. (Figure 3.1). Five chiefdoms from each district were purposively selected based on the level of cassava production, processing and utilization.

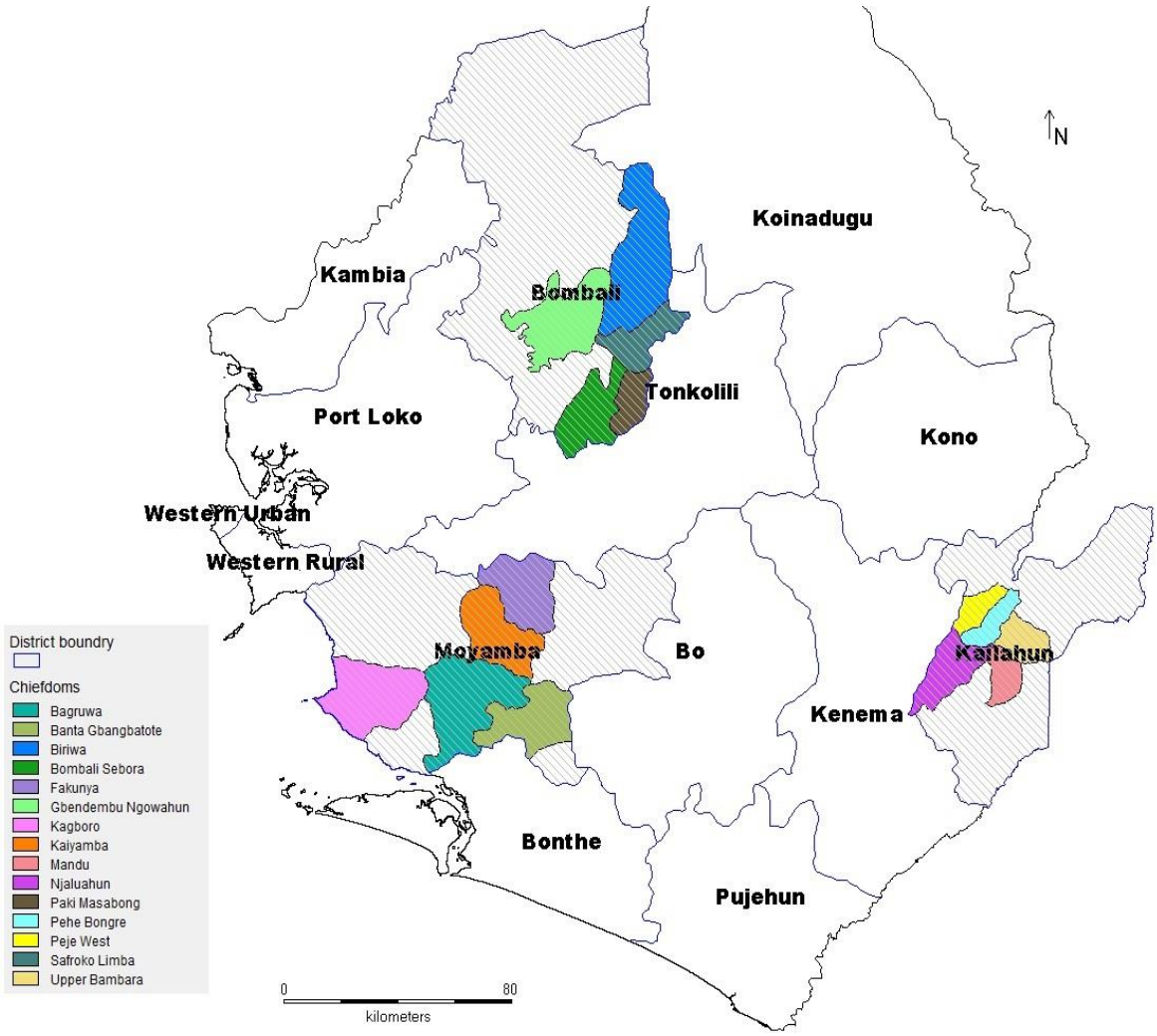


Figure 3.1 : Map of Sierra Leone showing surveyed regions

3.2.2 Sampling procedure

The mixed method research design as "qual quant" (qualitative and quantitative) approaches was exploited for this study. This approach is an integrated research paradigm that combines various schools of philosophy, such as positivism and realism, within the research design. Mixed-method design is normally used when researchers are interested in gaining a rich and deeper understanding of a research problem. A mixed-method research design was applied to the study .Mixed methods research is a methodology for conducting research that involves

collecting, analyzing and integrating quantitative (e.g., experiments, surveys) and qualitative (e.g., focus groups, interviews) research data from study participants to deepen understanding of a research problem. The qualitative methods exploited in the study include: focus group discussions and observations, while survey and documentary review were applied to gather quantitative information.

3.2.3 Sample size determination

Sampling refers to the procedure in which a sample is selected from an individual or a group of people of certain kind for research purpose. In sampling, the population is divided into a number of parts called sampling units.

For this study, a multistage sampling was used to arrive at 450 value chain actors that were then distributed among the cassava value chain actors, (producers, processors, traders and consumers). The 'value chain' actors are stakeholders who played important role in the structure and performance of the cassava value chain.

Anderson's (2007) sampling method was adopted for determining sample sizes using the formula sampling size:

$$n = \frac{z^2 pq}{d^2} = 1$$

Where n = the sample size, z =1.96, p = proportion of population (the proportion of cassava producers, processors, traders and consumers in the three regions of Sierra Leone), q = a weighting variable computed as 1-p and d = the margin of error.

Since the three regions in the country are known for agricultural production as a main livelihood activity, proportion of the population was estimated as 0.5 considering that the exact proportion

of the population was unknown. To ease identification among the cassava value chain actors in the sampled population, 8% was used as the margin of error for producers and consumers, while 11.32% was used as margin of error for traders and processors in the study.

The sample size for producer was computed using Anderson's (2007) formula below:

$$n = \frac{z^2 pq}{d^2} = 1$$

$$n = \frac{1.96^2(0.5)(0.5)}{0.0800^2} = 150$$

Since producers and consumers were households, sample size of producers was the same as that of consumers. A total of 150 producers and 150 consumers were sampled for all the three regions with each having 50 producers and 50 consumers. Traders and processors were not household. The sample size for traders and processor were determined by Anderson's formula as follows:

$$n = \frac{z^2 pq}{d^2} = 1$$

$$n = \frac{1.96^2(0.5)(0.5)}{0.11316^2} = 75$$

The sample size for processors and traders in all the three regions was 75 with each region having 25 traders and processors each (Table 3.1)

Table 3.1 Summary of systematic sampling methods used for the selection of respondents in the study areas.

Stage	Category	Sampling method	Sample size
Stage: 1	3 regions (East, North and South)	Purposive sampling technique	450 actors
Stage: 2	3 districts (Kaliahum, Bombali and Moyamba)	Simple random techniques	150 actors per district
Stage 3	15 chiefdoms	Simple random techniques	30 actors
Stage 4	*Chiefdom level*		
	Producers	Systematic sampling	10 Producers
	Consumers	Systematic sampling	10 Consumers
	Traders	Simple random techniques	5 Traders
	Processors	Simple random techniques	5 Processors

Note: Actors consist of consumers, producers, processors and traders

Community based information sharing, awareness creation and sensitization programs on the benefits of Provitamin A cassava in addressing malnutrition were undertaken across different districts before initiating the PRA study in the selected chiefdoms.

3.2.4 Data Collection

The following data were collected. The primary data set on provitamin A cassava was sourced from producer, processors, traders and consumers of cassava using pre-tested structured questionnaires for household interviews and semi- structured questionnaires for the focus group discussion. The household data set was collected through an electronic data capturing device using CSPro software, while, the focus group was exploited using charts and notepads. A total of 450 questionnaires were administered to respondents consisting 150 producers, 150 consumers, 75 processors and 75 traders. Qualitative and quantitative data sets were collected through focus group discussions with 4 groups of persons: youths, adult male and adult female and a pulled gender group. Efforts were made to capture gender issues within each category that consisted of 15 persons. Sixty persons were interviewed in total per chiefdom through group discussions. Secondary data were sourced from relevant journals, textbooks, internet, other related research projects, and extensive review of relevant literature on cassava value chain in Sierra Leone.

3.2.5 Data Analysis

Data collected were analyzed using the SAS 9.3, SPSS and Microsoft Office Excel 2010. Descriptive statistics such as frequency distribution tables, arithmetic means, standard deviation and percentage, were used to describe the socio-economic characteristics. SWOT variables of the various actors in the study area were ranked with the use of Kendall's coefficient of concordance.

3.3 Results

3.3.1 Socio economic characteristics of respondents

Results in Table 3.2 show the socio-economic characteristics of the respondents. 50% of farmers interviewed in Bombali district were adults between the ages of 36 to 60, while 38% and 46% of the farmers contacted in Moyamba and Kailahun, districts respectively, were youth below 36 years. The proportion of males was higher than female. Males constituted 86% in Bombali, 98% in Kailahun and 64% in Moyamba. The results also show those respondents with no education in Moyamba where the highest with 48% followed by Bombali 44% and Kailahun 38% and almost all respondents were married; 88% in Bombali, 88% in Kailahun and 76% in Moyamba. In Bombali and Kailahum districts, 90% and 52% of the respondents, respectively, did not belong to any organization. However, 56% in Moyamba district belonged to different organizations such as Agricultural Business Centers and Farmers Based Organizations. Although 60%, 54% and 57% of respondents in Bombali, Kailahum and Moyamba districts respectively revealed that labour exchange formed the major benefit derived from belonging to organizations, an additional 40% of respondents in Bombali revealed that they also benefit from loan facilities within their affiliated organizations.

Table 3.2: Socio economic characteristics of respondents across the three districts

Socio economic characteristics	Districts					
	Bombali (N = 50)		Kailahun (N = 50)		Moyamba (N = 50)	
	Freq	%	Freq	%	Freq	%
Age group						
Adults 36 - 60 years	25	50	19	38	23	46
Aged Above 60 years	4	8	4	8	2	4
Youths below 36 years	21	42	27	54	25	50
Total	**50**	**100**	**50**	**100**	**50**	**100**
Gender						
Female	7	14	1	2	18	36
Male	43	86	49	98	32	64
Total	**50**	**100**	**50**	**100**	**50**	**100**
Educational level						
None	22	44	19	38	24	48
Koranic	4	8	2	4	2	4
Primary	15	30	7	14	9	18
Junior secondary school	6	12	14	28	9	18
Senior secondary school	2	4	6	12	5	10
Tertiary	1	2	2	4	1	2
Total	**50**	**100**	**50**	**100**	**50**	**100**
Marital status						
Married	44	88	44	88	38	76
Single	3	6	5	10	7	14
Widow/widower	2	4	0	0	3	6
Divorced	1	2	0	0	0	0
Separated	0	0	1	2	2	4
Total	**50**	**100**	**50**	**100**	**50**	**100**
Membership in an organization						
Yes	5	10	24	48	28	56
No	45	90	26	52	22	44
Total	**50**	**100**	**50**	**100**	**50**	**100**
If yes, key benefits derived from organization						
Labour exchange	3	60	13	54	16	57
Loan facilities	2	40	2	8	6	21
Input	0	0	4	17	1	4
Marketing	0	0	2	8	4	14
Information sharing	0	0	3	13	1	4
Total	**5**	**100**	**24**	**100**	**28**	**100**

3.3.2 Average yearly farm size per household for cassava production

It was observed that 50%, 70% and 70% of the interviewees in Bombali, Kailahun and Moyamba districts, respectively, cultivate between 1 to 3 acres for cassava yearly (Figure 3.2).

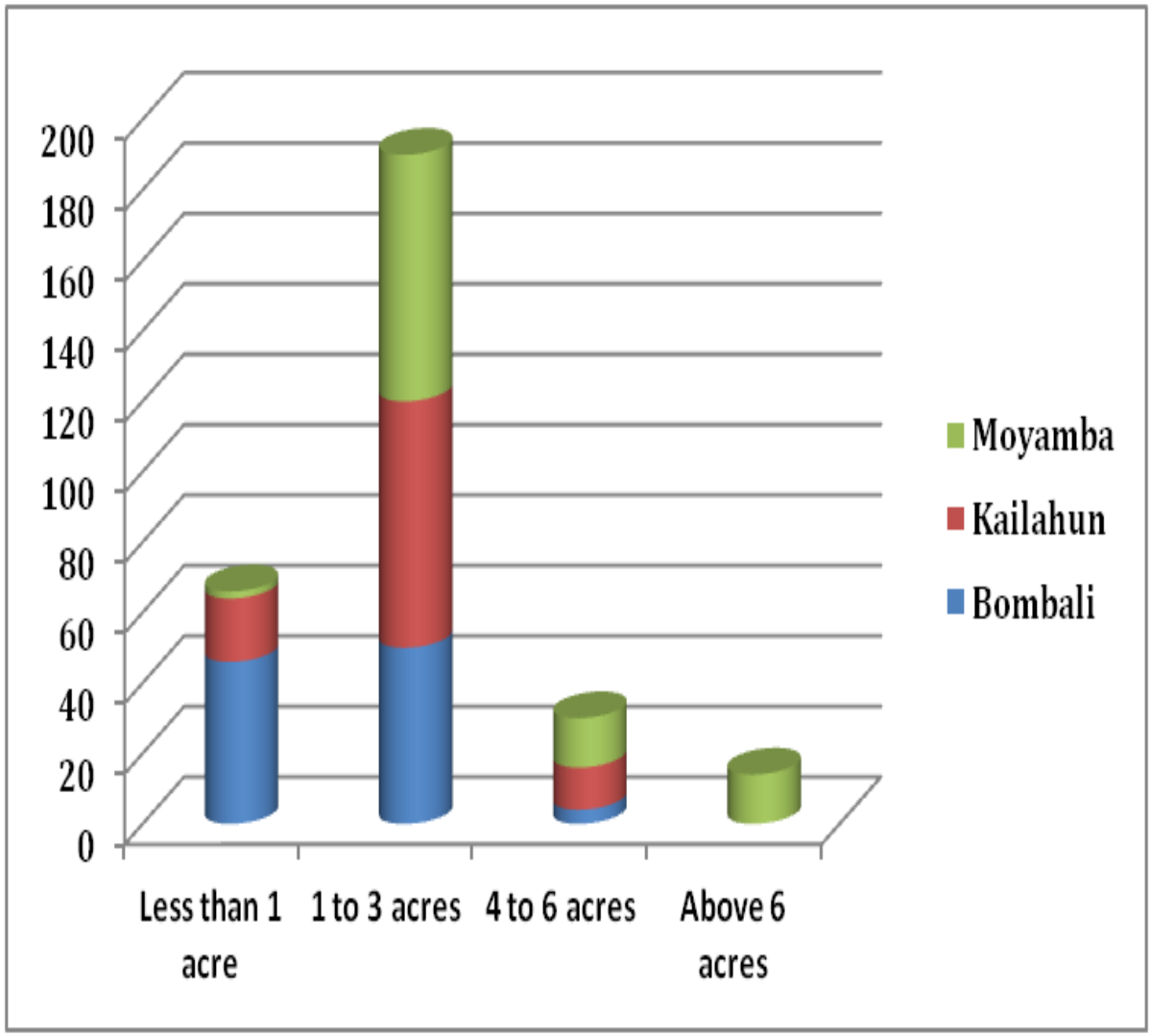


Figure 3.2 Average yearly farm size per household for cassava production

3.3.3 Cassava cultivation within 5 years in the study areas across the surveyed districts

Figure 3.3 show that all the respondents in Bombali, Kailahun and Moyamba districts have cultivated cassava for the past 5 years.

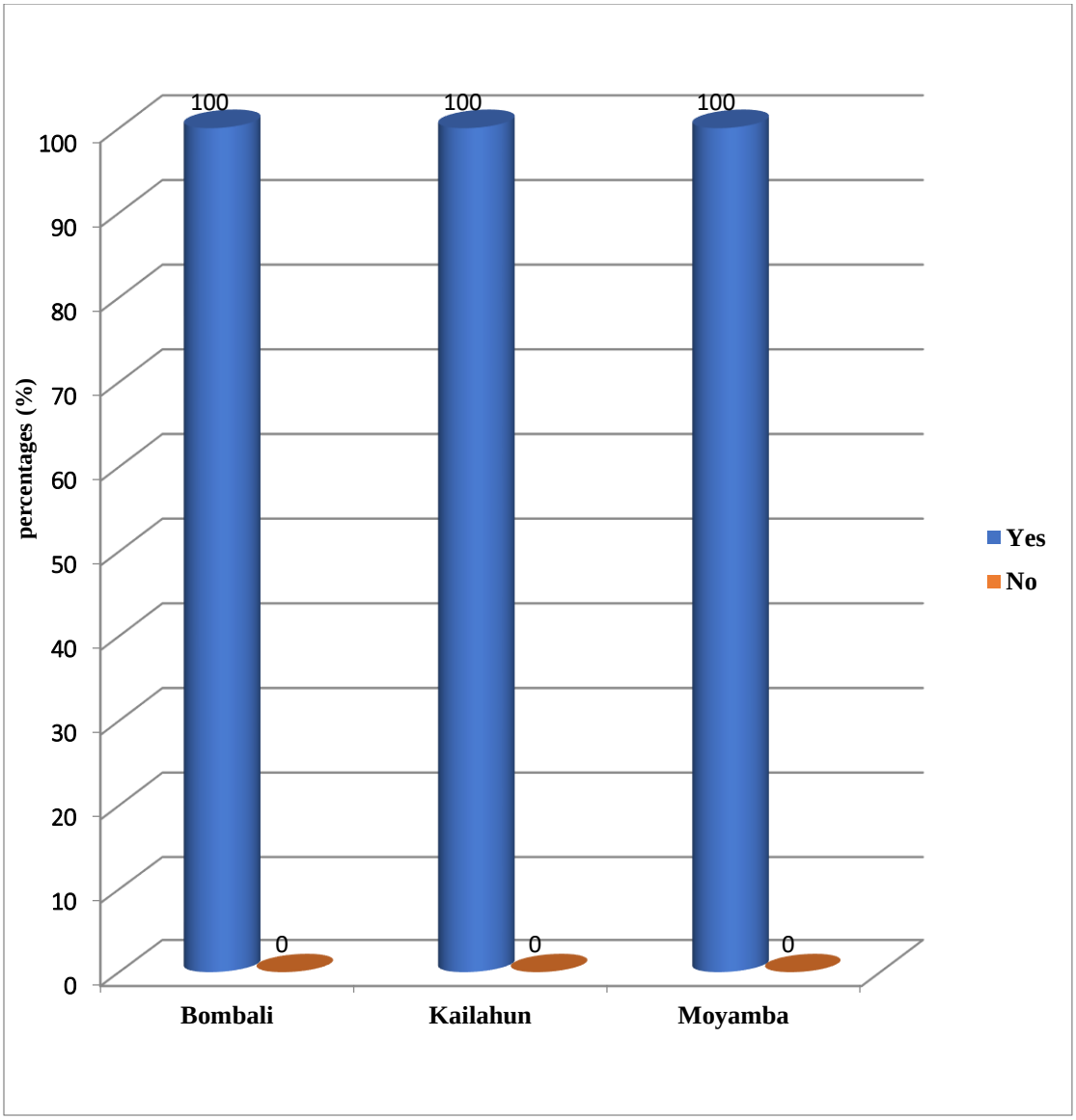


Figure 3.3: Cassava cultivation within 5 years in the study areas

3.3.4 Respondents' average income in the study areas

Most respondents earn less than Leones (SLL) 500,000 which is equivalent to US 67.67, annually (Figure 3.4)

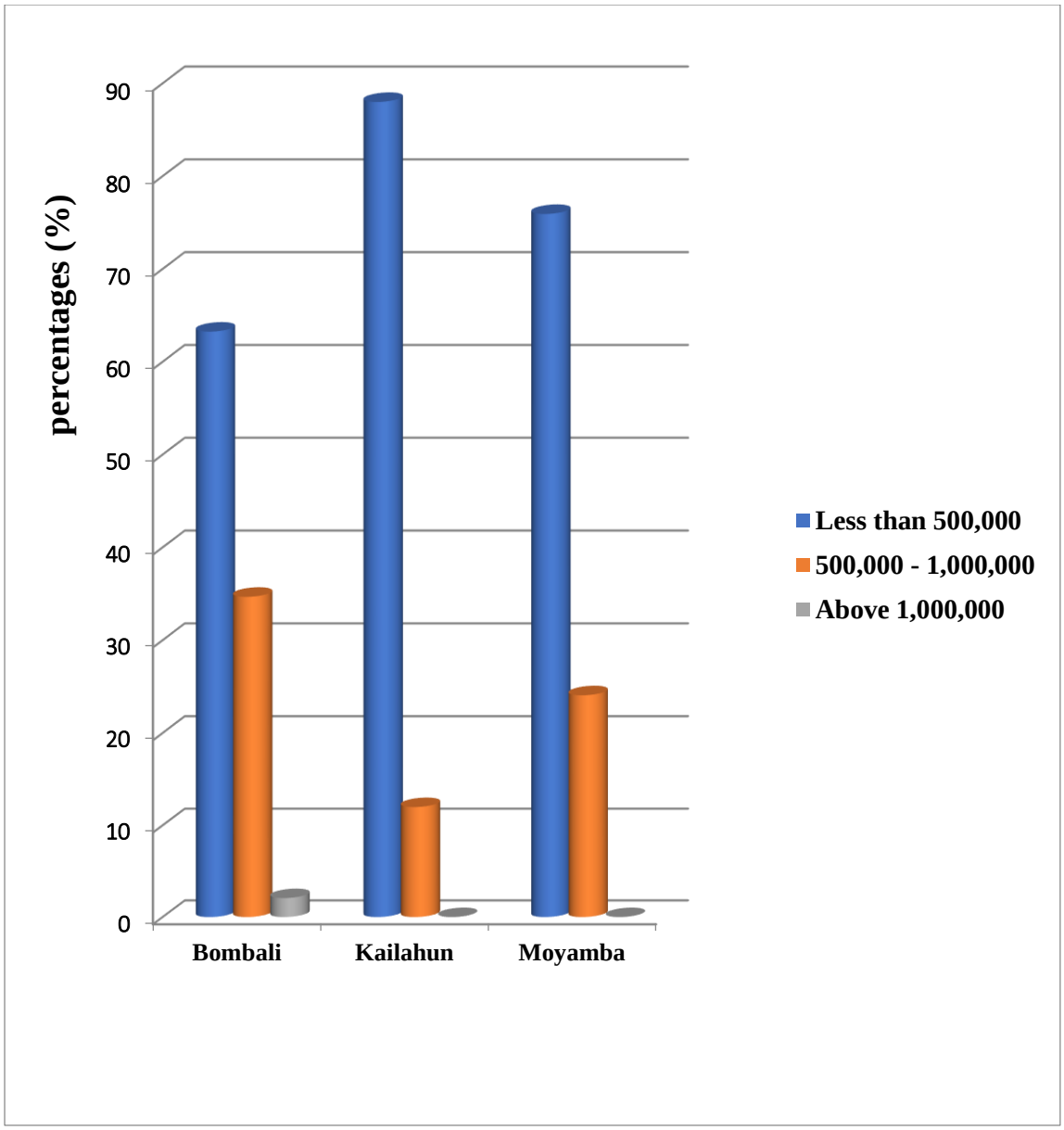


Figure 3.4: Respondents' average income in the study areas

3.3.5 Adoption challenges of producers for provitamin A cassava

Results in Table 3.3 indicate the importance attached to the different factors that determines the adoption of cassava cultivars by respondents in the three districts. Cultivar selections made by farmers across the surveyed areas Bombali, Kailahun and Moyamba were based on traits of preference ranked in order of their importance. The first four traits of utmost interest to the

farmers in Bombali district were; high yielding, early maturing, edible (mealiness) and root size. However, while in Kailahun the order of importance for the traits were high yielding, early maturing, root size and petiole colour. In the case of Moyamba, the four most important traits were high yield, early maturing, root size and mealiness. In all the three districts, high yielding and early maturing were the key traits for selection in that order.

Table 3.3: Varietal preferences of producers for provitamin A cassava across the surveyed districts

	Districts					
	Bombali (N = 50)		Kailahun (N = 50)		Moyamba (N = 50)	
Cassava traits	**Percent**	**Rank**	**Percent**	**Rank**	**Percent**	**Rank**
High Yield	92	1	98	1	58	1
Early maturing	72	2	94	2	48	2
Root size	52	4	46	3	42	3
Mealiness	70	3	4	7	38	4
Skin colour	4	6	34	5	24	6
Petiole colour	2	7	44	4	12	8
Plant size	2	7	4	7	28	5
Branching pattern	6	5	0	9	24	6
Root colour	0	9	8	6	16	8

Figures in brackets denote traits of preference ranked in order of importance for variety adoption by producers in each district; 1 = highest importance, 9 = lowest importance

3.3.6 Farmer's perception for provitamin A cassava across the survey districts

Results in Table 3.4 shows that 24% of respondents in Bombali, 6% in Kaliahum and 4% in Moyamba are aware of provitamin A cassava. However, 17%, 67% and 50% respondents in Bombali, Kaliahum and Moyamba are aware of yellow flesh cassava varieties. Also 66%, 33% and 50% of the respondent classified the variety SLICASS 11 (cream colour) as yellow fleshed. The study also reveal that only respondents in Bombali 67% have cultivated SLICASS 11 and

they source the planting material from NGOs. The presence and involvement of Village Hope, an NGO, in distributing planting materials and processing cassava in Bombali district coupled with the robust activities of SLARI's extension office has contributed greatly in influencing the availability and accessibility of the yellow flesh cassava across the district. The percentage of interviewees in Bombali, Kailahun and Moyamba districts willing to adopt provitamin A cassava varieties once available are 98%, 96% and 96%, respectively. Overall, more than 95% of the respondents across the three districts indicated willingness to adopt new varieties of provitamin A cassava when available as they get to appreciate and perceive their nutritional quality.

Table 3.4: Farmer's perception of provitamin A cassava across the surveyed districts

	Districts					
	Bombali (N = 50)		Kailahun (N = 50)		Moyamba (N = 50)	
Farmer's perception	**Freq**	**%**	**Freq**	**%**	**Freq**	**%**
Heard about yellow flesh cassava						
Yes	12	24	3	6	2	4
No	38	76	47	94	48	96
Total	**50**	**100**	**50**	**100**	**50**	**100**
Name of yellow flesh cassava variety						
SLICASS 11	8	66	1	33	1	50
Yellow flesh	2	17	2	67	1	50
Don't know	2	17	0	0	0	0
Total	**12**	**100**	**3**	**100**	**2**	**100**
Have you planted yellow flesh cassava						
Yes	8	67	0	0	0	0
No	4	33	3	100	2	100
Total	**12**	**100**	**3**	**100**	**2**	**100**
Source of planting of yellow flesh cassava variety						
MAFFS	1	13	0	0	0	0
NGOs	7	88	0	0	0	0
Total	**8**	**100**	**0**	**0**	**0**	**0**
Means of sourcing the yellow flesh cassava variety						
Purchasing	1	13	0	0	0	0
Gift	7	88	0	0	0	0
Total	**8**	**100**	**0**	**0**	**0**	**0**
Did you plant yellow flesh cassava last season						
Yes	7	88	0	0	0	0
No	1	13	0	0	0	0
Total	**8**	**100**	**0**	**0**	**0**	**0**
Proportion land area planted with yellow flesh						
0 - 25%	4	57	0	0	0	0
26 - 50%	2	29	0	0	0	0
Above 75%	1	14	0	0	0	0
Total	**7**	**100**	**0**	**0**	**0**	**0**
Purpose for growing yellow flesh in the HH						
Processing to other cassava products	7	100	0	0	0	0
Boil and eat	0	0	0	0	0	0
Total	**7**	**100**	**0**	**0**	**0**	**0**
Willingness to grow yellow cassava variety						
Yes	42	98	48	96	48	96
No	1	2	2	4	2	4
Total	**43**	**100**	**50**	**100**	**50**	**100**

3.3.7 Traders perception on cassava root trading across the surveyed districts

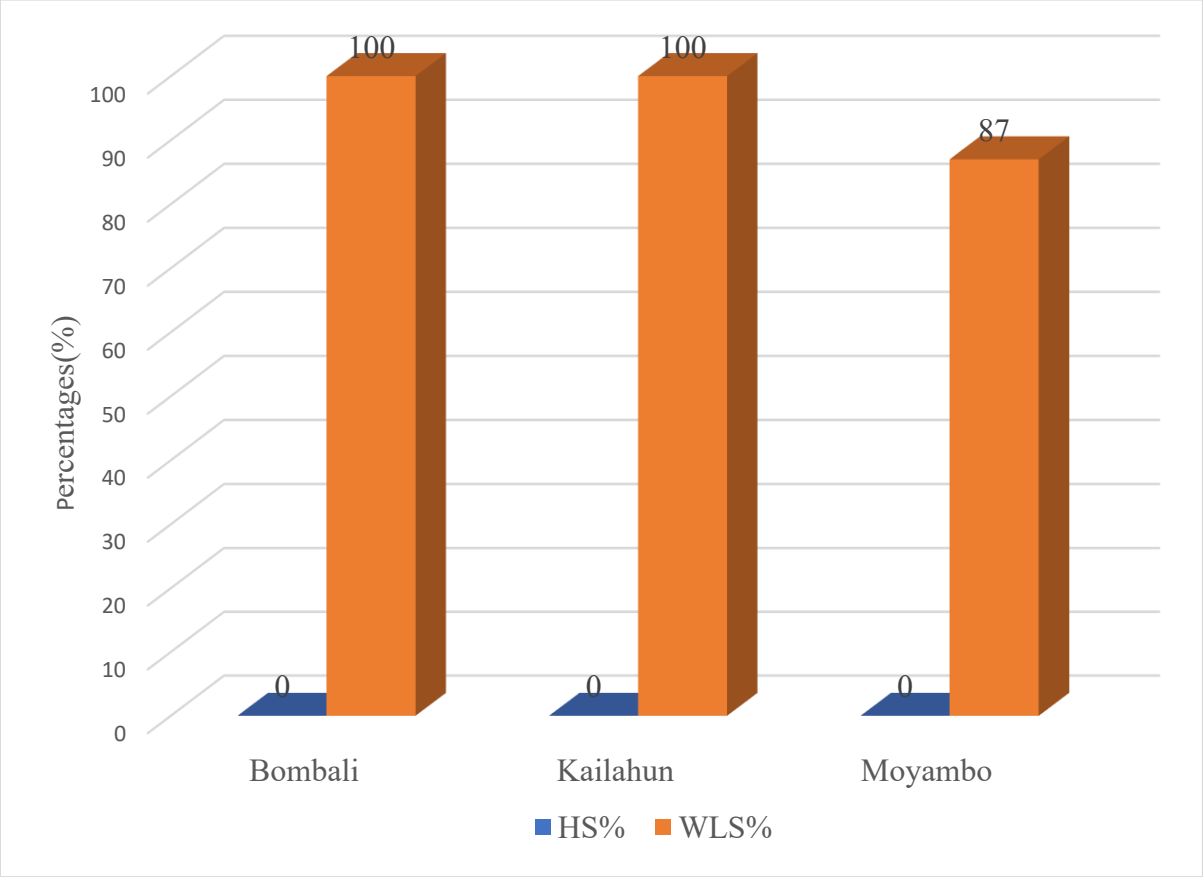
Almost all the traders in Bombali district sell creamy or white (non-provitamin A) cassava roots while 8% in Kailahun and 52% (Table 3.5) in Moyamba sell white roots. No yellow cassava root was sold in the study area. All respondents in Bombali and 50% and 53% of the respondents in Kailahun and Moyamba districts respectively, claimed that they source cassava roots from the periodic markets. One hundred percent and 50% of suppliers of cassava roots, in Bombali and Kailahun districts respectively, obtain their cassava roots from other traders, whereas, 47% in Moyamba obtained theirs directly from farmer's field. Hundred percent of respondents in Bombali and Kailahun reported that they sell cassava roots at the daily market, while less than 50% of respondents in Moyamba district sell their cassava roots through road side markets. Consumers are the most preferred customers of cassava roots across the three surveyed districts with 100% scores each in both Bombali and Kailahun and 67% in Moyamba.

Table 3.5: Traders perception on cassava root trading across the three districts

Cassava root trading	Districts					
	Bombali (N = 8)		Kailahun (N = 26)		Moyamba (N = 29)	
	Freq	%	Freq	%	Freq	%
Sell cassava roots						
Yes	7	88	2	8	15	52
No	1	12	24	92	14	48
Total	**8**	**100**	**26**	**100**	**29**	**100**
Flesh colour of cassava root sold						
White	0	100	1	50	11	73
Cream	7	0	1	50	4	27
Yellow	0	0	0	0	0	0
Total	**7**	**100**	**2**	**100**	**15**	**100**
Source of cassava roots						
Own production	0	0	0	0	7	47
Periodic market	7	100	1	50	8	53
Daily market	0	0	1	50	0	0
Road side market	0	0	0	0	0	0
Total	**7**	**100**	**2**	**100**	**15**	**100**
Suppliers of cassava roots						
Farmers	0	0	0	0	10	67
Wholesalers	2	29	0	0	5	33
Other traders	5	71	2	100	0	0
Total	**7**	**100**	**2**	**100**	**15**	**100**
Selling location of cassava roots						
Periodic market	0	0	0	0	5	33
Daily market	7	100	2	100	3	20
Road side market	0	0	0	0	7	44
Total	**7**	**100**	**2**	**100**	**15**	**100**
Preferred customers of cassava roots						
Consumers	7	100	2	100	10	67
Wholesalers	0	0	0	0	0	0
Other traders	0	0	0	0	5	33
Total	**7**	**100**	**2**	**100**	**15**	**100**

3.3.8 Provitamin A cassava root trading in the study areas

There's no evidence of trading or selling provitamin A cassava roots in the three districts (Figure 3.5). However, there is strong willingness among farmers in all the districts to sell provitamin A cassava roots when made available.

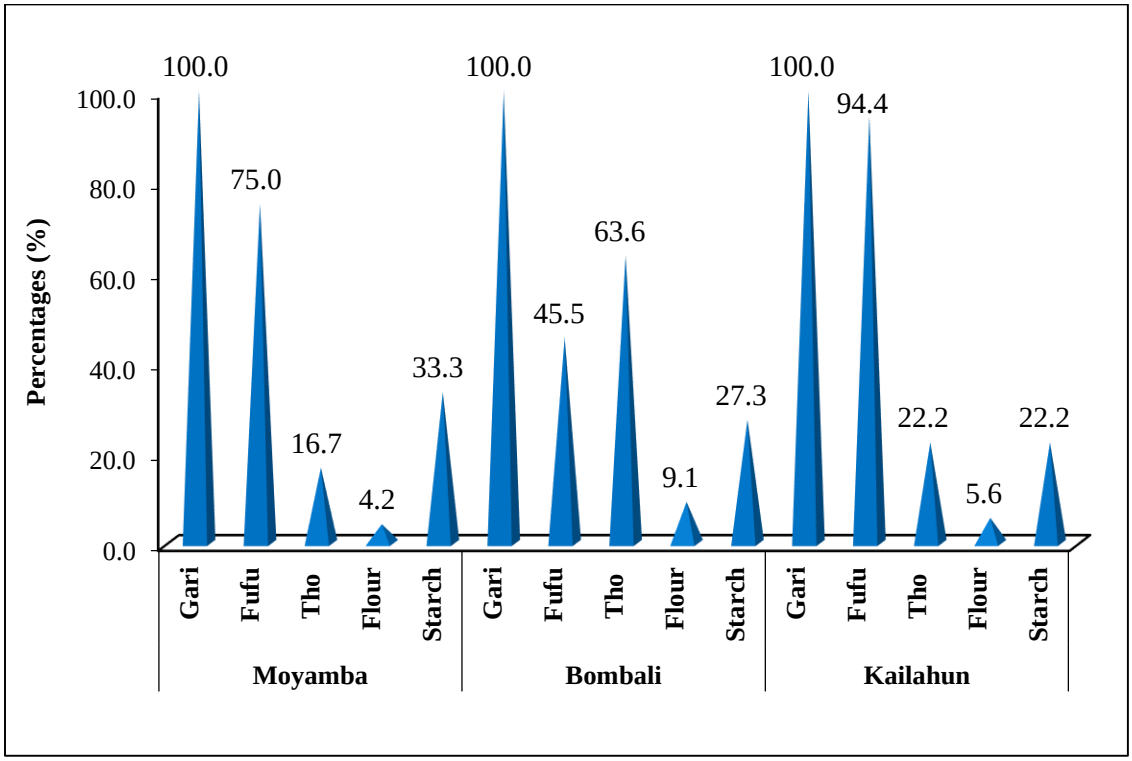


HS - Have sold yellow flesh cassava root, WLS – Would like to sell yellow flesh cassava root

Figure 3.5: Provitamin A cassava root trading in the study areas

3.3.9. Processors perception and preference for cassava roots processing and awareness across the different districts

Survey of respondents processing different cassava products in the study areas shows that Gari is the most important processed product in all the 3 districts, followed by Fufu in Moyamba and Kailahun and Tho in Bombali district (Fig 3.6). Starch processing had moderate importance in all 3 districts and accounted for around 30% of processing in Moyamba and Bombali and about 22% in Kailahun. Ninety-six percent of respondents in Moyamba, 100% (Bombali) and 78% (Kailahun) of the interviewees indicated willingness and preference for provitamin A gari when it is made available.

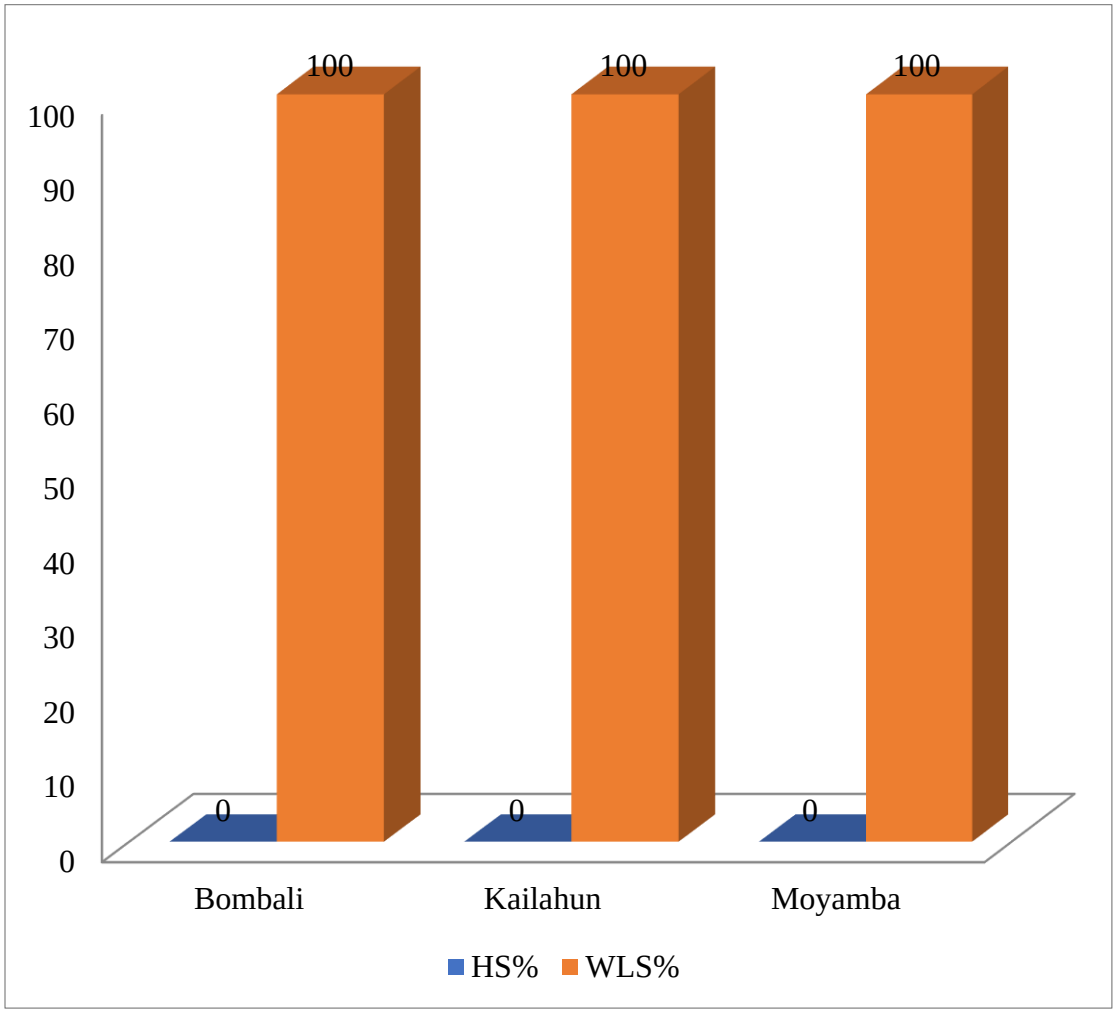


HS - Have sold yellow flesh gari, WLS – Would like to sell yellow flesh gari

Figure 3.6: Percentage of respondents processing different cassava products in the study area

3.3.10 Perception and preference for cassava roots processing/trading and awareness across the different districts

Figure 3.7 shows that 100% of respondents across all three districts indicated interest in trading in provitamin A cassava gari.



HS - Have sold yellow flesh gari, WLS – Would like to sell yellow flesh gari

Figure 3.7 Respondents interest in provitamin A cassava gari production/trading in the study areas

3.3.11 Perception and preference of consumers on consumption and awareness of provitamin-A cassava roots across the surveyed districts

Cassava roots consumption and awareness rates are presented in (Table 3.6). Over 90% of respondents across the three surveyed districts eat cassava. The widely consumed variety is the white flesh root with 97% Bombali, 100% Kailahun and 96% Moyamba of the respondents consuming it. There is very little knowledge on provitamin A cassava across the three surveyed districts with 14%, 6% and 6%, indicating awareness levels in Bombali, Kailahun and Moyamba respectively. Of the few respondents who indicated awareness of yellow cassava, only 50%, 33% and 0% consume the yellow cassava in Bombali, Kailahun and Moyamba respectively. However, all the respondents (100%) indicated interest in provitamin A cassava, and showed willingness to accept and introduce provitamin A cassava into family meals mainly because of its nutritional value. Poundability was least considered by all responded as a criterion for introducing provitamin A cassava to their families.

Table 3.6: Cassava roots consumption and awareness of consumers across the surveyed districts

Cassava root consumption	Districts					
	Bombali (N = 49)		Kailahun (N = 50)		Moyamba (N = 54)	
	Freq	%	Freq	%	Freq	%
Eat cassava roots						
Yes	48	97	50	100	52	96
No	1	3	0	0	2	4
Total	**49**	**100**	**50**	**100**	**54**	**100**
Roots flesh color						
White	44	91	47	94	51	98
Cream	0	0	3	6	1	2
Yellow	4	8	0	0	0	0
Total	**48**	**100**	**50**	**100**	**52**	**100**
Aware of YF cassava root						
Yes	7	14	3	6	3	6
No	41	86	47	94	49	94
Total	**44**	**100**	**50**	**100**	**52**	**100**
If yes, do you consume yellow flesh cassava root						
Yes	3	50	1	33	0	0
No	3	50	2	67	3	100
Total	**6**	**100**	**3**	**100**	**3**	**100**
If yes.do you prefer yellow flesh cassava roots						
Yes	3	100	1	100	0	0
No	0	0	0	0	0	0
Total	**3**	**100**	**1**	**100**	0	**0**
If yes, why?						
Nutritional values	3	100	1	100	0	100
Willingness to introduce YF cassava to family?						
Yes	41	100	49	100	54	100
No	0	0	0	0	0	0
Total	**49**	**100**	**50**	**100**	**54**	**100**
Reasons for willingness to introduce YF cassava to family						
Nutritional value	27	56	37	74	31	57
Taste	8	16	10	20	16	29
Flavor	1	2	3	6	3	6
Accessibility	12	24	0	0	3	6
Poundability	1	2	0	0	1	2
Total	49	**100**	**50**	**100**	54	**100**
Do you use cassava to wean infants?						
Yes	5	10	21	42	21	39
No	44	90	29	58	33	61
Total	**49**	**100**	**50**	**100**	**54**	**100**
Flesh colour of cassava root used						
White	5	100	21	100	20	95
Cream	0	0	0	0	1	5
Yellow	0	0	0	0	0	0
Total	**5**	**100**	**21**	**100**	21	**100**
Willing to use YF cassava root as weaning food?						
Yes	49	100	50	100	54	100
No	0	0	0	0	0	0
Total	**49**	**100**	**50**	**100**	**54**	**100**

Table 3.6: Cassava roots consumption and awareness of consumers across the surveyed districts (cont'd)

Cassava root consumption	Districts					
	Bombali (N = 49)		Bombali (N = 49)		Bombali (N = 49)	
	Freq	%	Freq	%	Freq	%
Price difference between YF cassava root and other roots						
Yes	0	0	22	44	12	22
No	6	12	28	56	5	9
Don't know	43	88	0	0	37	69
Total	**49**	**100**	**50**	**100**	54	**100**
Advantage of YF cassava roots						
Nutritional value	47	95	20	40	32	59
Good taste	2	4	16	32	7	13
Fine colour	0	0	14	28	15	28
Total	**49**	**100**	**50**	**100**	**54**	**100**
Disadvantage of YF cassava root						
Bitter taste	0	0	4	8	0	0
Not available	49	100	12	24	23	43
No awareness	0	0	34	68	31	57
Total	**49**	**100**	**50**	**100**	**54**	**100**

3.3.12 Perception and preferences of consumers for Gari consumption across the three districts among value chain actors

Table 3.7 shows that the respondents in Bombali 47%, Kailahun 50% and Moyamba 54% districts consumed gari. Virtually all the gari eaten in the 3 districts were white gari constituting 100%, 96% and 98% of the gari consumed in Bombali, Kailahun and Moyamba respectively. All the consumers interviewed had no knowledge of provitamin A gari, but all of them were willing to adopt it. Above 90% of the respondents prefer provitamin A gari, mainly because of its nutritional value. Respondents in Bombali 4%, Kailahun 26% and Moyamba 17% use white gari as weaning food. Over 90% of consumers interviewed across the three districts were willing to introduce provitamin A gari as weaning food.

Table 3.7 Gari consumption across the three districts

Gari consumption	Districts					
	Bombali (N = 49)		Kailahun (N = 50)		Moyamba (N = 54)	
	Freq	%	Freq	%	Freq	%
Eat gari						
Yes	23	47	25	50	29	54
No	26	53	25	50	25	46
Total	**49**	**100**	**50**	**100**	**54**	**100**
Color of gari						
White	49	100	48	96	53	98
Cream	0	0	2	4	1	2
Yellow	0	0	0	0	0	0
Total	**49**	**100**	**50**	**100**	**54**	**100**
Aware of YF gari						
Yes	0	0	0	0	0	0
No	49	100	50	100	54	100
Total	**49**	**100**	**50**	**100**	**54**	**100**
Adopt YF gari						
Yes	45	92	49	98	49	91
No	4	8	1	2	5	9
Total	**49**	**100**	**50**	**100**	**54**	**100**
Use gari to wean infants						
Yes	2	4	13	26	9	17
No	47	96	37	74	45	83
Total	**49**	**100**	**50**	**100**	**54**	**100**
If yes, colour of gari						
White	2	100	9	69	7	78
Cream	0	0	4	31	2	22
Total	**2**	**100**	**13**	**100**	**9**	**100**
Willing to use YF gari as weaning food						
Yes	45	92	47	94	50	93
No	4	8	3	6	4	7
Total	49	100	**50**	**100**	54	100
Advantage of YF gari						
Nutritional value	49	100	22	44	36	67
Good taste	0	0	15	30	16	30
Fine colour	0	0	3	6	2	3
Don't know	0	0.	10	20	0	0
Total	**49**	**100**	**50**	**100**	**54**	**100**
Disadvantage of YF gari						
Not available	49	100	19	38	13	24
No awareness	0	0	31	62	41	76
Total	**49**	**100**	**50**	**100**	**54**	**100**

3.3.13 Respondents' perception on the strengths, weakness, opportunities and threat for three districts among the cassava value chain actors

Table 3.8 shows the producers' strengths, weaknesses, opportunities and threats (SWOT) across the three surveyed districts. Access to land availability was ranked as the most important strength, limited access to credit finance for weakness, availability of improved varieties for opportunities and lack of external funding in Bombali and high cost of agricultural machinery for Kailahum and Moyamba for threats across the surveyed districts.

Table 3.8 Kendall SWOT ranking for cassava producers across three districts

SWOT	Bombali		Kailahun		Moyamba	
	Mean	Rank	Mean	Rank	Mean	Rank
Strengths						
Agricultural land	1.5	1	1.04	1	2.00	1
Labour	2.02	2	1.98	2	2.74	2
Improved planting material	2.88	3	3.04	3	4.02	3
Finance and credit	4.92	4	5.16	5	4.82	5
Member of FBO	5.5	5	5.58	6	4.82	5
Experience and knowledge	5.5	5	6.06	7	5.28	7
Have processing facilities	5.68	7	5.14	4	4.32	4
P value	<0.0001		<0.0001		<0.0001	
Kendall's W	0.71		0.83		0.31	
Weaknesses						
Limited access finance and credit facilities	2.04	1	1.22	1	2.45	1
High transportation cost of tubers	2.26	2	2.48	2	2.74	2
Limited access to market	3.08	3	3.14	3	3.09	3
Lack agricultural machines and equipment	3.5	4	3.82	4	3.4	5
Low agricultural productivity	4.12	5	4.34	5	3.32	4
P value	<0.0001		<0.0001		<0.064	
Kendall's W	0.30		0.59		0.01	
Opportunities						
Availability of improved varieties	1.82	1	1.08	1	2.38	1
Strong support from government and NGOs	2.9	2	3.08	3	3.82	5
Training on improved agronomic practices	3.02	3	3.76	4	3.3	4
Availability of markets	3.62	4	4.08	5	2.76	3
Availability of processing centres	3.64	5	3.00	2	2.74	2
P value	<0.0001		<0.0001		<0.0001	
Kendall's W	0.22		0.54		0.13	

Table 3.8: Kendall SWOT ranking for cassava producers across three districts (cont'd)

SWOT	Bombali		Kailahun		Moyamba	
	Mean	**Rank**	**Mean**		**Mean**	**Rank**
Threats						
Lack of external funding	2.00	1	4.00	5	3.02	5
High transport fares	2.92	2	3.62	4	3.14	3
High market competition	2.86	3	2.84	3	3.18	4
High interest rates on loan	3.46	4	2.40	2	2.94	2
High cost of agricultural machinery	3.76	5	2.14	1	2.72	1
P value	<0.0001		<0.0001		<0.611	
Kendall's W	0.18		0.25		0.01	

3.3.14 SWOT ranking for cassava processors across the three districts

Table 3.9 shows processors' strengths, weaknesses, opportunities and threats across the three surveyed districts. Knowledge and labour availability were ranked first and second for strength across the surveyed districts. Market and lack of finance and credit were observed as weaknesses across the three districts. Improved varieties and improved processing technologies were ranked as the most important opportunities in Bombali while for Kailahun and Moyamba improved varieties and strong linkages constituted the most important opportunities. High cost of processing technologies and high cost of labour were ranked as threats.

Table 3.9: Kendall SWOT ranking for cassava processors across the three districts

SWOT	Bombali		Kailahun		Moyamba	
	Mean	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**
Strengths						
Knowledge	2.18	2	1.22	1	1.50	1
Labour	2.00	1	2.61	2	2.19	2
Equipment	4.64	6	3.28	2	3.73	3
Finance and credit	4.14	5	4.83	6	4.69	6
Market and storage	4.00	3	4.50	4	4.65	5
Linkage with farmers	4.05	4	4.56	5	4.25	4
P value	<0.001		<0.0001		<0.0001	
Kendall's W	0.36		0.57		0.52	
Weaknesses						
Market	3.00	2	1.67	1	2.04	1
Finance and credit	1.45	1	2.31	2	2.38	2
Processing equipment	3.00	2	2.81	3	3.21	3
Training	4.09	3	3.67	4	4.19	4
High transport fares	4.18	4	4.75	5	4.12	5
Low production	5.27	5	5.81	6	5.06	6
P value	<0.0001		<0.0001		<0.0001	
Kendall's W	0.49		0.70		0.39	
Opportunities						
Improve varieties	2.85	2	2.03	1	2.23	1
Strong linkages	5.30	6	2.92	2	3.02	2
High demand for gari	3.35	4	3.47	4	3.60	3
Provision of training	4.15	5	4.17	5	4.44	6
Improved processing technologies	2.25	1	3.28	3	3.92	5
Processing centres with modern equipment	3.10	3	5.14	6	3.79	4
P value	<0.001		<0.0001		<0.001	
Kendall's W	0.04		0.35		0.18	
Threats						
High labour cost	2.45	2	2.06	1	2.19	2
High cost of processing technologies	1.64	1	2.97	2	2.17	1
High interest rates on loan	4.64	5	3.94	4	3.52	3
Market diversity and competition	4.05	4	4.08	5	3.71	4
Inadequate supply of raw materials	2.73	3	3.69	3	4.56	5
Theft	5.50	6	4.25	6	4.85	6
P value	<0.0001		<0.002		<0.0001	

3.3.15 SWOT ranking for cassava traders across the three districts

Table 3.10 reveals traders' strengths, weaknesses, opportunities and threat across the three survey districts. High demands for product, storage facilities and membership in traders' organization were ranked as the most important strengths in Bombali, Kailahun and Moyamba, respectively. Lack of access to finance and credit were the major weaknesses across the three districts (Bombali, Kailahun and Moyamba). Availability of financial institutions and availability of produce/products were ranked as the greatest opportunity for Bombali followed by Kailahun and Moyamba districts, while high transportation cost and no external funding were ranked as the most important threat across the three surveyed districts.

Table 3.10: Kendall SWOT ranking for cassava traders across the three districts

SWOT	Bombali Mean	Bombali Rank	Kailahun Mean	Kailahun Rank	Moyamba Mean	Moyamba Rank
Strengths	4.00	5	2.50	2	3.41	5
Access to market	2.51	2	2.21	1	2.81	2
Have storage facilities	3.43	3	3.21	3	2.74	1
Membership in trader's organisation	3.51	4	3.82	5	3.30	4
High demand of product	1.60	1	3.41	4	2.90	3
P-value	0.23		0.001		0.351	
Kendall's W	0.353		0.178		0.04	
Weaknesses						
Lack of finance and credit	1.51	1	2.40	1	2.30	1
Lack of storage facilities	3.40	3	2.61	2	3.10	3
Lack of market facilities	2.62	2	2.82	3	2.31	2
Low availability of cassava products	3.63	4	3.71	5	3.82	5
High market dues	3.91	5	3.61	4	3.61	4
P-value	0.019		0.004		0.001	
Kendall's W	0.369		0.342		0.192	
Opportunities						
Cassava product always available	2.31	2	1.90	1	1.90	1
High demand for cassava products	2.30	2	20	2	2.21	2
Availability of financial institutions	2.00	1	3.10	4	2.80	2
Availability of markets	3.52	4	3.42	3	3.10	4
P-value	0.275		< 0.001		0.002	
Kendall's W	0.086		0.255		0.176	
Threats						
High taxation	3.00	3	3.01	3	2.40	2
High transportation cost	1.51	1	2.40	2	2.11	1
No external funding	1.61	2	1.61	1	2.40	2
Market diversity and competition	3.94	4	3.23	3	3.01	4
P-value	< 0.001		< 0.001		0.089	
Kendall's W	0.781		0.255		0.078	

3.4 Discussion

The PRA approaches used in this study yielded meaningful results as cassava value chain actors willingly shared their experiences and knowledge. Youth below 36 years in the surveyed areas constituted more than 50 percent of the cassava farmers interviewed in Moyamba, Kailahun and Bombali. This implies that the youth were actively involved in cassava cultivation in Moyamba and Kailahun. However, in Bombali the adult population was highest. Age is said to be a primary latent characteristic in adoption decisions. This agrees with Okoye (2004), who reported that young people adopt innovations faster than old people. Nwaru (2004) reported that the ability of a farmer to break risk is innovative and starts from 30 years, but decreases with age. Eighty-six percent, 98% and 64% of respondents in Bombali, Kailahun and Moyamba were male indicating the predominance of males in cassava farming. The very high percentage of men compared to women in cassava farming in the surveyed districts could possibly be due to lack of mechanization facilities, labour intensiveness and task demands which may discourage women from undertaking cassava cultivation and production independently (SLARI Cassava Value Chain Report unpublished). This was contrary to findings of Adebayor and Salahu (2007), Oyegbami *et al*. (2010) and Thompson (2013) who reported of higher percentage of female (women) in cassava cultivation and production.

Cassava farming is a gender-friendly occupation where men and women play active roles. Cassava cultivation activities which range from brushing, burning, ploughing and harvesting were mainly undertaken by men, while women participated in activities like planting, weeding and transportation of harvested storage roots. Although women spend more time in agricultural activities, unfortunately, most women have less access to information technology (FAO, 1988).

Education has been reported to be very important as it helps to refine a person's perceptions of issues and help him/her to make reasonable decisions based on available information. Low level of formal education of about two-third of the farmers in all three districts contributed to the slowdown of the adoption of released technologies (improved varieties). Ajibefun and Aderinola (2004) observed that education facilitates adoption of new varieties; hence the low level of education among farmers in all the three districts sampled could influence the selection and adoption of introduced improved yellow root cassava varieties and provitamin A gari.

Creating awareness and promoting their nutritional value and benefits in a dissemination program will accelerate adoption. Eighty-eight percent of respondents in both Bombali and Kailahun and 76% in Moyamba which makes up the farming population in the three districts were legally married implying that the land on which cassava is being produced is owned by family, rented by family or secured as a family property. The average land holdings in the surveyed districts ranged between 0.5 to 6 hectares. This implies that only a small parcel of land is allocated to cassava production and that subsistence agriculture predominates. This does not provide enough motivation to try innovations. Ochola, (2006) found that adoption of new technologies may be affected by the land sizes used by the farmers for agricultural purposes.

Studies on adoption have shown that farm size positively correlates with the adoption of an introduced technology (FAO, 2002; Njoku *et al*., 2012). Forty percent of the farmers in Moyamba district, became members of an organization for them to have access to loan opportunities while farmers in the other two districts (Kailahun and Bombali) are members of organization mainly because of labour exchange. This shows that access to loan and labour are the most important resource required for cassava production in the surveyed area as majority of the farmers are poorly resourced. The survey conducted confirms that most of the cassava

produced in all the districts is processed into gari for sale locally and for family consumption. Majority of the farmers grow variety SLICASS 4 (non-provitamin A) because of its high percentage dry matter content. Dry matter content is a quality trait responsible for dry texture in cassava products, and this is preferred by most farmers especially in gari processing. There is a great demand for gari and a well-structured market outlet (Gbangbatok) where traders purchase bags of gari to sell as retailers. White root varieties are largely consumed in all the surveyed districts because of the low awareness of provitamin A varieties. SLICASS 11 is cream coloured which does not qualify it to be called a biofortified variety, as biofortified cassava variety is a cassava which should have been developed through hybridization with an increased level of total carotenoid content (provitamin A). Its nutritional status would have been increased.

In the present study, respondents showed a high level of willingness to adopt and promote the use of provitamin-A or yellow cassava roots and products, due to its perceived nutritional benefits. This agrees with the results of Nkonya and Featherstone (2001), who found that varieties with farmers' preferred traits were easily adopted. Farmers' personal experience influenced decisions on what varieties they grew. There is no available market outlet where yellow cassava or provitamin A storage roots/products can be obtained across the three surveyed districts. The major constraints identified in this study including high cost of transportation impeding the growth and expansion of cassava production in the districts surveyed. These finding agrees with Parkes, (2011) who reported that high cost of transportation negatively affects cassava production.

3.5 Conclusion

Farmers appreciate new technologies that have an added advantage over their current existing technologies. There is high prospect for this new technology (provitamin A cassava) to be adopted in Sierra Leone as revealed in this study. Consumers' preference for provitamin A cassava root is linked to the crop's nutritional benefits as informed through the awareness and sensitization campaigns conducted before the data collection period across the districts. Provitamin A cassava has been proven to improve nutrition especially among the vulnerable group (children less than five years, pregnant and lactating women). All consumers interviewed had no prior knowledge about provitamin A gari, but were willing to adopt it. Above 90% of the respondents preferred provitamin A gari, after the sensitization and awareness campaign during the survey about its nutritional value. This underscores the importance of creating awareness of nutritional value of provitamin A cassava genotypes/accessions in Sierra Leone. Cassava value-chain actors' (Producers, consumers, Traders and Processors) preferences to further improve production and adoption of provitamin A cassava is understood. The study confirms that Moyamba district should serves as a hub for gari processing and marketing in Sierra Leone. A reliable database on provitamin-A cassava has been developed from this study.

CHAPTER FOUR

4.0 DIVERSITY STUDIES OF PROVITAMIN A CASSAVA (*Manihot esculenta* Crantz) IN SIERRA LEONE.

4.1 Introduction

Genetic diversity provides species with the ability to adapt to changing environments. Several studies have been reported on the use of morphological descriptors to determine the genetic diversity among cassava genotypes (Rimoldi *et al.*, 2010 and Asare *et al.*, 2011 and Thompson, 2013). Recent advances in molecular biology techniques have led to the development of important tools for genetic studies in several plant species. The accuracy in accession characterization may therefore, be enhanced/achieved with the use of molecular markers associated with morphological traits. A lot of research has been undertaken in plant genetic diversity using molecular markers including DNA (Ferreira *et al.*, 2008 and Rimoldi *et al.*, 2010), such as amplified fragment length polymorphism (Benesi *et al.*, 2010), simple sequence repeats (Alves *et al.*, 2011; Parkes, 2009; Oliveira *et al.*, 2012 and Costa *et al.*, 2013) and single nucleotide polymorphism (Kizito *et al.*, 2005; Tangphatsornruang *et al*., 2008; Ferguson *et al.*, 2011; Thompson, 2013 and Rabbi, 2015). With recent advances in high throughput genotyping technologies, single nucleotide polymorphic markers (SNPs) are increasingly becoming markers of preference for plant genetic studies and breeding. SNPs are the most common type of genetic variation among species, involving just a change in a single nucleotide. Many Expressed Sequence Tags (ESTs) have been exploited to explain 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) and Rabbi *et al*., (2014; 2015) have also reported on SNPs detection from ESTs in cassava.

Cassava being an outbreeding and highly heterogeneous crop, possesses an extreme level of phenotypic plasticity and thereby, lacks the potential for unified classification system for cultivars (Kawano *et al.*, 1978). Consequently, characterization of agronomic traits becomes a challenge (Carvalho and Schaal, 2010). To conduct diversity studies on cassava germplasm in Sierra Leone, there is need to augment the existing collection with cassava germplasm collection. This engenders the need for assessing the existing collection to identify gaps that need to be filled.

A collection was exploited to quantify the diversity of provitamin A cassava germplasm in Sierra Leone. One hundred and eighty-three provitamin A cassava accessions and five released varieties selected from clonal trials established at Taiama in 2014 (Personal communication with Dr. J.B.A. Whyte). The objectives of the present study were to:

i) characterize the 183 provitamin A cassava germplasm and 5 released varieties using;
    a) morphological traits
    b) total-carotenoid content and
    c) SNP markers

(ii) develop a collection for conservation.

4.2 Materials and Methods

4.2.1 Land preparation

The land preparation was undertaken to control early weeds emergence and to ensure better crop establishment. The trial was conducted without supplemental irrigation and was weeded regularly until canopy closure.

4.2.2 Germplasm sources

A total of 183 provitamin A cassava accessions with varying yellow colour were selected from the Sierra Leone's germplasm development program (Table 4.1). Selections were made based on their performance in terms of storage root yields, dry matter content, and pest and disease tolerance/resistance, plant architecture, nutritional quality and flowering ability. Cocoa, SLICASS 4, SLICASS 6, SLICASS 7 and SLICASS 11 (cream fleshed) varieties were used as checks.

4.2.3 Experimental Design

The trial was laid out in an Alpha lattice design with two replications at the Njala Agricultural Research Institute (NARC), Foya crop site, Njala, representing the transitional rain forest agro climatic zone (Van Vuure *et al*., 1972; Odell *et al*., 1974). Each replication had four blocks with 47 entries per block. The blocks were separated by 1m and 2m alleys between and within blocks to reduce intra and inter block plant competition respectively. Each entry was grown on 10m row ridge at a spacing of 1m x 1m between and within ridges, respectively.

Cassava cuttings of 20-25 cm length were obtained from healthy stem cuttings and horizontally planted. The established trial was evaluated for one cropping season (2015-2016).

Table 4.1 Germplasm/accession and their pedigree

Accession Name	Pedigree	Accession Name	Pedigree	Accession Name	Pedigree	Accession Name	Pedigree
TR 1563	IBA 082708	TR 0334	IBA 070675	TR 1389	IBA 083724	TR 1361	IBA 070557
TR 1337	IBA 011368	TR 1610	IBA 30572	TR 1259	IBA 070738	TR 0189	IBA 990313
TR 0421	IBA 051652	TR 0631	MM 090564	TR 1182	SM 3374	TR 1269	IBA 070593
TR 1207	SM 3374	TR 1233	SM 3374	TR 1543	IBA 102429	TR 1533	IBA 102429
TR 0267	IBA 961439	TR 0998	SM 3666	TR 0975	GM 3594 - 12	TR 1762	IBA 070749
TR 0626	MM 050626	TR 1744	IBA 070749	TR 1155	IBA 101438	TR 0015	IBA051740
TR 0431	IBA 011735	TR 1153	IBA 101438	TR 1404	IBA 083724	TR 0018	IBA051740
TR 0085	IBA 050311	TR 0886	IBA 102480	TR 1202	SM 3374	TR 1073	IBA 100224
TR 1295	IBA 011412	TR 0446	IBA 070620	TR 0955	IBA 101645	TR 0890	IBA 102480
TR 1627	TMEB 693	TR 0974	GM 3594	TR 0520	IBA 071313	TR 0316	IBA 050099
TR 0224	IBA 000351	TR 1565	IBA 082708	TR 1208	SM 3374	TR 1199	SM 3374
TR 1578	BA 011371	TR 0785	IBA 011206	TR 0843	SM 3444	TR 1144	IBA 100198
TR 0222	IBA 020134	TR 1569	IBA 082708	TR 1113	IBA 982101	TR 0982	GM 3594 -
TR 1755	IBA 070749	TR 0713	SM 3434	TR 0893	IBA 102480	TR 1244	IBA 070738
TR 0854	KIBAHA	TR 0423	IBA 071393	TR 1316	IBA 070520	TR 1279	IBA 070593
TR 1051	IBA 961089A	TR 0887	IBA 102480	TR 0693	IBA 102286	TR 1008	SM 3666
TR 0261	IBA 961439	TR 1785	IBA 980505	TR 1593	IBA 30572	TR 0861	KIBAHA
TR 1201	SM 3374	TR 0025	Z 960012	TR 1598	IBA 30572	TR 0983	GM 3594
TR 0894	IBA 102710	TR 1374	IBA 070557	TR 0282	IBA 050303	TR 1031	IBA 100403
TR 0232	BA 010169	TR 1562	IBA 082708	TR 1350	IBA 083849	TR 0683	IBA 102286
TR 1302	IBA 070520	TR 1236	IBA 082708	TR 0957	IBA 101645	TR 0772	IBA I011086
TR 1128	IBA 100198	TR 0838	SM 3444	TR 1422	IBA 102612	TR 1229	SM 3374
TR 1808	IBA 070539	TR 0688	IBA 102286	TR 0932	IBA 070337	TR 0118	IBA 970219
TR 1556	IBA 082708	TR 1480	IBA 980581	TR 1689	TMEB 2026	TR 0840	SM 3444

Table 4.1 Germplasm/accession and their pedigree (con't)

Accession Name	Pedigree	Accession Name	Pedigree	Accession Name	Pedigree	Accession Name	Pedigree
TR 0172	IBA 010732	TR 0937	IBA 101040	TR 1349	IBA 083849	TR 0396	IBA 070525
TR 0382	BA 011404	TR 0743	IBA 101094	TR 0927	IBA 070337	TR 1788	IBA 980505
TR 0384	IBA 011404	TR 1540	IBA 102429	TR 0810	IBA 011206	TR 0485	IBA 051654
TR 1688	TME B2026	TR 0747	IBA 100252	TR 0718	IBA 100449	TR 1152	IBA 101438
TR 1437	IBA 102612	TR 1348	IBA 083849	TR 0907	SM 3434	TR 0990	SM 3666
TR 0696	IBA 102286	TR 1438	IBA 102612	TR 0335	IBA 030007	TR 1004	SM 3664
TR 0033	IBA 050327	TR 1477	IBA 980581	TR 1327	IBA 070520	TR 0679	KALESO
TR 1034	SM 3444- 2	TR 1243	IBA 070738	TR 1666	IBA 070703	TR 1515	IBA 102429
TR 0700	IBA 102286	TR 0807	BA 011206	TR 1748	IBA 070749	TR 1735	IBA 070749
TR 1463	IBA 980581	TR 0707	SM 3434	TR 0856	KIBAHA	TR 1448	IBA 980581
TR 0365	IBA 011663	TR 1007	SM 3666	TR 1359	IBA 070557	TR 1322	IBA 070520
TR 1620	TMEB 693	TR 0299	IBA 051625	TR 0744	IBA 101094	TR 0399	IBA 070525
TR 0289	IBA 961632	TR 1289	IBA 011412	TR 0881	IBA 102480	TR 1525	IBA 102429
TR 1603	IBA 30572	TR 0851	SM 3444	TR 1405	IBA 083724	TR 1753	IBA 070749 -
TR 1505	IBA 102429	TR 0295	IBA 051625	TR 0385	IBA 961551	TR 1501	IBA 102429
TR 1849	TME B778 -	TR 1590	IBA 30572	TR 1223	SM 3374	TR 0019	IBA961039
TR 0031	IBA 050311	TR 0918	IBA 101803	TR 0868	KIBAHA	TR 0296	IBA 051625
TR 0319	IBA 050099	TR 1133	IBA 100198	TR 1313	IBA 070520	TR 1502	IBA 102429
TR 1198	SM 3374	TR 1331	IBA 070520	TR 0480	BA 051654	Cocoa	Local Cultivar
TR 1256	IBA 070738	TR 0461	IBA 051654	TR 1266	IBA 070738	SLICASS 4	Released Variety
TR 1557	IBA 082708	TR 1419	IBA 102612	TR 1071	IBA 100649	SLICASS 6	Released Variety
TR 0535	IBA 020091	TR 0368	IBA 011663	TR 0703	SM 3434	SLICASS 7	Released Variety
TR 1360	IBA 070557	TR 1527	IBA 102429	TR 0560	MM 980747	SLICASS 11	Released Variety

4.2.4 Molecular Characterization

The Dellaporta method of DNA extraction (Dellaporta *et al*., 1983) was adopted at the Institute of Tropical Agriculture (IITA), Nigeria. Genomic DNA was extracted from young fresh leaves of the 188 cassava genotypes. 200 mg of young leaves tissue were harvested into a genogrinder tube with two steel balls inside. The tubes together with the leaves were freeze-dried inside a container of liquid nitrogen and later genogrinded at 5,000 rpm for 2min. 400 µl of DNA

extraction buffer was added and incubated at 65^{o}C for 20min with 2min intermittent mixing. After incubation, 250µl of chilled 5M potassium acetate was added and iced for 20min. The 188 samples were removed from ice and centrifuged at 10,000 rpm for 10 min. The supernatant solution was transferred into a fresh tube, 450 µl cold isopropanol was added and mixed by inversion four to five times. The content stayed at -20^{o}C for 5 min and later centrifuged at 10,000 rpm for 10 min. The retained pellets were allowed to dry for about 30 min at room temperature and later dissolved in 300 µl sterile distilled water. 300 µl of chloroform-isoamyalcohol was added, mixed and centrifuged at 10,000 rpm for 10 min. The supernatant solution was carefully transferred into another tube and 25 µl of 3M sodium acetate and 500 µl of ethanol were added. The tube was mixed by inversion and kept at -20^{o}C for 1 hr. The samples were centrifuged at 10,000 rpm for 10 min. DNA pellets were washed twice in 70% solution and air dried at room temperature for 30 min. The DNA pellet in each genotype sample was re-dissolved in 250 µl sterile distilled water and kept in -20^{o}C. After preparation of the samples plate and ID, 2µl of the DNA samples were loaded into the Nanodrop on a nucleic acid file for quality assessment. For genotyping-by-sequencing library preparation. the ApekI restriction enzyme (recognition site: G|CWCG) that produces less variable distributions of read depth and therefore a larger number of scorable SNPs in cassava (Hamblin and Rabbi, 2014) was used. Two 96-plex GBS libraries were constructed as described by Elshire *et al*., (2011) and sequenced at the Institute of Genomic Diversity at Cornell University using the Illumina HiSeq2500. Raw read sequences were processed through cassava GBS production pipelines developed using TASSEL 5.0 V2. The GBS-derived SNPs were further filtered using the TASSEL software (Bradbury *et al*., 2007) to retain only polymorphic SNPs. Initially filtered for minor allele frequency (MAF<0.05), the generated 5,634 SNPs were processed under the Next Generation Cassava project. The resulting

SNP dataset was used for the diversity analysis study among the 188 cassava accessions already phenotyped and analyzed. Results from both the phenotype and genotype analyses were compared to check the correspondence between the two.

4.2.5 Data Collection

Data collection was undertaken at 1, 3, 6 and 9 months after planting (MAP), on the parameters listed below using the IITA cassava descriptor (Fakuda *et al*., 2010):

Table 4.2 Parameters evaluated at 1, 3, 6 and 9 MAP

Traits	Parameter
Leaf color	Color of stem epidermis
Number of leaf lobes	Color of stem cortex
Length of leaf lobe	Growth habit of stem
Width of leaf lobe	Prominence of foliar scars
Lobe margin	Leaf retention
Pubescence of apical leaves	Level of branching
Color of apical leaves	Height at 1st branching
Orientation of petiole	Height at 2nd branching
Petiole color	Height at 3rd branching
Leaf area	Color of end branches of adult plant
Length of stipule	Percentage sprout
Stipule margin	African Cassava Mosaic Disease
Stem color	Cassava Green Mite
Stem diameter base	Cassava Anthracnose Disease
Stem diameter-1foot below	

Harvesting was done at 12 MAP (August – September). The following parameters were taken at harvest: number of marketable roots (no), number of non-marketable roots (no), total number of storage roots (no), roots weight/tuber (kg), inner skin color, and outer skin color, ease of peel, root shape, marketable weight (kg), and non- marketable weight (kg). Dry matter content

expressed as a percentage was determined by selecting three representative storage roots. These were bulked, washed, peeled and sliced using knives. Slices were randomly selected and weighed to obtain a 100g fresh mass sample per genotype before being dried for 48hours in an oven at 80°C. The dried samples were then re-weighed to obtain the dry mass.

4.2.6 Total carotene determination

The 188 provitamin-A cassava accessions were screened at harvest using a scale of 1 – 6 in the color chat .75 harvested provitamin-A accessions were selected from the field screening based on color chat. Five storage roots samples were taken to the nutritional laboratory for analysis. The fresh roots were washed with tap water to eliminate sand and other substances that could serve as contaminants. The roots were then peeled and placed on a chopping board, and divided into 4 equal longitudinal parts. The two opposite parts were pulled together. A portion was used for the iCheck analysis to determine total carotenoid content levels whilst the remaining was used for dry matter determination. The selected portion for the iCheck analyses were chopped into cubes mixed together and divided into 4 equal parts. The two opposite parts were again pulled together, mixed and divided as described above until a composite sample was obtained. The samples were processed further for iCheck analysis as follows: Five grams of each sample was weighed into a medium mortar and ground with 20ml of distilled water as the solvent. Each resultant solution was poured into a well labeled falcon tube. The falcon tubes were shaken vigorously to obtain homogenous slurry and 0.4ml of the suspension was injected into the iCheck reagent vials. The vials were shaken vigorously for 10sec and allowed to stand for 5min. The suspension inside the vial separated into two distinct phases (a clear upper phase and a turbid lower phase). Reading was done using the iCheck device.

4.2.7 Data Analysis

Data sets from these trials were subjected to selected statistical packages for analysis. Analytical procedures comprised the following: descriptive statistics using XLSTAT (2010) and MINITAB 15 programs, multiple regressions analysis to identify variables that best discriminate among the classes, Principal Component Analysis (PCA) to examine the structure of the correlations between the variables. Correlation matrixes were used to visualize associations among variables and parameters (Daulfrey, 1976). Cluster analyses were performed to group observations together using the method of Ward's minimum variance distance with SAS 9.4. A dendrogram was plotted from the computed similarity values to show the relationship among the accessions. The accessions were grouped based on the varying levels of total carotenoid content.

Basic diversity indices for the population of the 188 cassava accessions were calculated using Power marker (Liu & Muse, 2005) and GenAlex version 6.41 (Peakall and Smouse, 2006). The Power maker software was used to generate the following statistics: number of alleles per locus, major allele frequency, observed heterozygosity (Ho), expected heterozygosity (He) and polymorphic information content (PIC) (Bostein and White, 1980). PIC values were calculated with the equation:

$$PIC=1-\Sigma P^2i-\Sigma\ 2P^2i$$

Where: ΣP^2i = sum of each squared ith haplotype frequency.

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. The pairwise genetic distance (identity-by-state, IBS) matrix was calculated among all individuals using PLINK (Purcell *et al*., 2007). A Ward's minimum variance hierarchical cluster

dendrogram was built from the IBS matrix using the analyses of phylogenetic and evolution (ape) package in R.

4.3 Results

4.3.1 Descriptive statistics for the 188 cassava accessions

Significant differences were observed among the 188 accessions for all the measured traits. Severity scores for African Cassava Mosaic Disease, Cassava Bacteria Blight and Cassava Green Mite variably ranged from 1 to 4 in the studied population. Yield per hectare ranged from 0.2 to 42.5t/ha while dry matter content ranged from 4.0 to 44.5% (Table 4.3)

Table 4.3 Descriptive statistics of some morpho-agronomic traits of 188 cassava accessions

Trait	Descriptive statistics				
	Time of data collection (MAP)	Minimum	Maximum	Mean	Standard Deviation
Sprouting (%)	1	6.5	10	9.56	0.6
ACMD Incidence (%)	1, 3, 6 and 9	0	4.25	0.08	0.42
ACMD Severity (score)	1, 3, 6 and 9	0.75	2	1.04	0.13
CAD Incidence (%)	1, 3, 6 and 9	0	2.75	0.11	0.41
CAD Severity (score)	1, 3, 6 and 9	0.5	2.75	1.05	0.23
CBB Incidence (%)	1, 3, 6 and 9	0	4	0.41	0.6
CBB Severity (score)	1, 3, 6 and 9	0.5	4.5	1.15	0.34
Mealybug incidence (%)	9	0	9	3.22	2.17
Mealybug severity (score)	9	1	6.5	2.54	0.84
CGM Incidence (%)	9	2	8	5.27	1.66
CGM Severity (score)	9	2	9	3.31	0.72
Colour of apical lobe (score)	3	3	9	6.8	1.61
Colour of apical lobe (score)	9	0	9	6.71	1.74
Plant height (cm)	6	65.5	284.5	155.69	26.12
Height of branching (cm)	6	37	196.5	85.83	29.38
Stem diameter base (cm)	6	1.07	3.94	1.51	0.26
Stem diameter (mid height) (cm)	6	1.03	2.25	1.53	0.2
Leaf area (cm^2)	6	10.24	73.93	34.13	11.04
Leaf retention (score)	6	1.75	4.5	2.87	0.5
Shape of central leaflet (score)	6	1.75	6.25	3.13	0.94

Table 4.3 Descriptive statistics of some morpho-agronomic traits of 188 cassava accessions (con't)

Trait	Descriptive statistics				
	Time of data collection (MAP)	Minimum	Maximum	Mean	Standard Deviation
Petiole colour (score)	6	0.5	7	1.94	1.48
Petiole colour (score)	9	1	8	3.2	1.54
Leaf colour (score)	6	1.5	5	3.69	0.87
Leaf colour (score)	9	3	6	3.94	0.77
Colour of leave vein (score)	6	3	18.75	3.85	1.73
Petiole length (cm)	6	3	32.95	14.79	6.09
Orientation of petiole (score)	6	0.5	7	2.55	1.13
Number of leaf lobes (no)	6	3.75	8	6.18	0.89
Length of leaf lobe (cm)	6	3.13	15.15	11.15	1.61
Width of leaf lobe (cm)	6	1.08	7.05	3.05	0.81
Lobe margin (score)	6	1.5	8	4.38	1.87
Length of stipules (cm)	9	1	4	2.97	0.22
Stipule margin (score)	9	1	5	1.31	0.59
Prominence of foliar scars colour (score)	9	3	6	4.93	0.39
Stem colour (score)	6	4	8	6.47	0.79
Colour of stem exterior (score)	9	1	7	2.55	0.71
Colour of stem epidermis (score)	9	4	8.5	6.52	1.1
Colour of end branches of adult plants (score)	9	1	32.5	4.62	2.47
Stem colour (score)	6	4	8	6.47	0.79
Color of stem exterior (score)	9	1	7	2.55	0.71
Color of stem epidermis (score)	9	4	8.5	6.52	1.1
Color of end branches of adult plants (score)	9	1	32.5	4.62	2.47
Mean number of storage root (no)	12	7.5	88	44.83	14.21
Yield (t/ha)	12	0.24	42.5	12.09	5.69
Mean weight per storage root (kg)	12	0.09	28	0.47	2.62
Dry matter content (%)	12	4	44.5	29.56	6
Root size (score)	12	2	7	4.93	1.07
Root shape (score)	12	1	5	2.76	0.62
Outer root colour (score)	12	1	4	3.4	0.72
Inner root colour (score)	12	1	3	1.9	0.36
Pulp colour (score)	12	1	3	2.01	0.19
Ease of peeling (score)	12	2	7	2.83	0.53
Biomass (kg)	12	2.5	13.5	9.99	1.91
Field carotene (score)	12	1	4.5	3.34	0.72
Total carotene level (µg/g fresh root weight)	12	3.6	13.7	7.6	2.74

MAP= Month after Planting, ACMD=African cassava mosaic Disease, CAD= Cassava Anthracnose Disease, CBB-Cassava Bacterial Blight, CGM-Cassava Green Mite

4.3.2 Multiple Regression of yield on agro-morphological traits

Marketable (MWET) and non-marketable weights (NMWET) were the most discriminating characters among the accessions with a very high b value of 1.43 (Table 4.4).

Table 4.4 Multiple regression coefficients of yield on some agro-morphological traits of 188 cassava accessions

Traits	**b± Se**
ACMDI (1MAP)	0.003±0.001
ACMDI (3MAP)	0.000±0.001
LA (9MAP)	-0.000±0.000
MROT	0.000±0.000
NMROT	0.000±0.000
TSR	0.000±0.000
MWET	1.429±0.000**
NMWET	1.43±0.000**
TWET	0.000±0.000
WSROT	0.002±0.003
DM	-0.000±0.000

****Significant at 1%,** ACMDI=African cassava mosaic disease incidence, LA=Leaf area, MROT=Marketable roots, NMROT=Non-marketable roots, TSR= Total no of storage roots, MWET=marketable weight, NMWET=Non- marketable weight, WSROT=Storage root weight, DM=dry matter

4.3.3. Correlations among morphological traits of 188 cassava accessions

Table 4.5 shows 49 Pearson's correlation coefficients among some measured traits. Yield was positively associated with storage root weight (r=0.51**) and root size (r=0.45**). Correlation among yield and all other traits were not significant. Significant correlation coefficients were however observed between INCOL with BIOMASS (0.32*) and WSROT with BIOMASS (0.48*).

Table 4.5 Correlations among morphological traits of 188 cassava accessions

Variables	YLD	WSROT	DMC	RZ	RS	OCOL	INCOL	PCOL	EPEEL
WSROT	0.51**								
DMC	0.12	0.09							
RZ	0.45**	0.16	0.04						
RS	0.00	0.04	-0.01	-0.19					
OCOL	-0.03	0.08	0.11	-0.07	-0.00				
INCOL	-0.06	0.24	-0.06	-0.15	0.04	0.05			
PCOL	0.14	0.11	0.01	0.20	0.08	0.02	-0.3		
EPEEL	0.06	0.04	0.01	0.18	0.05	-0.02	0.09	-0.05	
BIOMASS	-0.08	0.48**	-0.12	0.02	-0.08	-0.05	0.32*	0.01	0.05

YLD=Yield; WSROT=Storage root weight; DM=Dry matter; RZ=Root size; RS= Root shape; OCOL=Outer color; INCOL=Inner color; PCOL= Petiole color; EPEEL= Ease of peel; Biomass, **=significant at1% level

4.3.4. Principal component analysis of yield and yield related traits of 188 cassava accessions

The first four PCs together accounted for 51.50 % of the total phenotypic variation among the 188 accessions (Table 4.6). PC1 axis had an eigenvalue of 1.92 and acounted for 17.5%, of the total variation whereas PC2, PC3 and PC4 axes had eigenvalues of 1.43, 1.20 and 1.11, and acounted for 13.00%, 10.54% and 10.10% of the total variation respectively. Storage root weight, root size and yield had postive loadings on PC1, whereas root shape had negative loading. Dry matter content and root shape had positive loadings on PC2 , while RCOL had a negative loading. Storage root weight, root size, root shape, outer skin colour and pulp colour had positive loadings in PC3. PC4 axis contained negative loadings for dry matter, root size and outer color with positive loadings for root shape, pulp color and ease of peel.

Table 4.6 Principal component analysis of yield and yield related traits of 188 cassava accessions

Variables	Principal Component Analysis			
	PC1	PC2	PC3	PC4
YLD	**0.6**	0.03	0.06	0.03
WSROT	0.46	-0.02	0.36	-0.06
DMC	0.19	0.31	0.09	-0.43
RZ	**0.50**	-0.17	0.32	-0.45
RS	**-0.50**	0.14	0.48	0.37
OCOL	0.03	0.04	0.49	-0.53
PCOL	-0.12	-0.12	0.38	0.35
EPEEL	0.23	-0.11	0.20	0.43
BIOMASS	0.15	-0.26	-0.03	0.04
RCOL	-0.06	-0.62	0.07	0.02
Eigenvalue	**1.92**	**1.43**	**1.20**	**1.11**
Variance %	**17.50**	**13.00**	**10.90**	**10.10**
Cumulative %	**17.50**	**30.5**	**41.40**	**51.50**

YLD=Yield; WSROT=Storage root weight; DMC=Dry Matter; matter content RZ=Root size; RS= Root shape; OCOL=Outer color; INCOL=Inner color; PCOL= Petiole color; EPEEL= Ease of Peel; Biomass; RCOL= Root color

4.3.5. Cluster analysis of the accessions based on Ward's minimum variance and total carotenoid content

Figure 4.1 revealed that the semi partial R – squared derived from the cluster analysis ranged from 0.00 to 0.15. At 0.04 eight clusters were observed suggesting a moderate to high diversity among the accession. Table 4.7 showed the 188 accessions grouped into eight clusters. The number of accessions per cluster varied from 1 in Cluster C to 56 in cluster D. Clusters A, B, E, F and G had 2, 43, 51, 5 and 28 accessions respectively. Only 1 out of the initial 2 accessions in Cluster H was identified as a carotenoid accession whereas, 11, 34, 27, 2 and 2 accessions in clusters B, D, E, G and H had higher levels of total carotenoid content. Cluster F did not have any accession with measurable total carotenoid content. The one accession in cluster C could be considered as an outlier or a unique accession. Thirty provitamin A accessions with higher levels of total carotenoid were selected from five out of the eight clusters. These included 3 from Cluster B, 13 from cluster D, 9 from E, 4 from G and 1 from H. Accessions TR-0399, TR-0707,

TR-0222, TR-1337, TR-1569, TR-1313, TR-0998, TR-1755, and TR-1557 had higher total carotenoid content levels of 11.1, 10.9, 13.1, 11.8, 10.3, 11.7, 13.7, 10.7, and 11.2 mg/100g^{-1} .

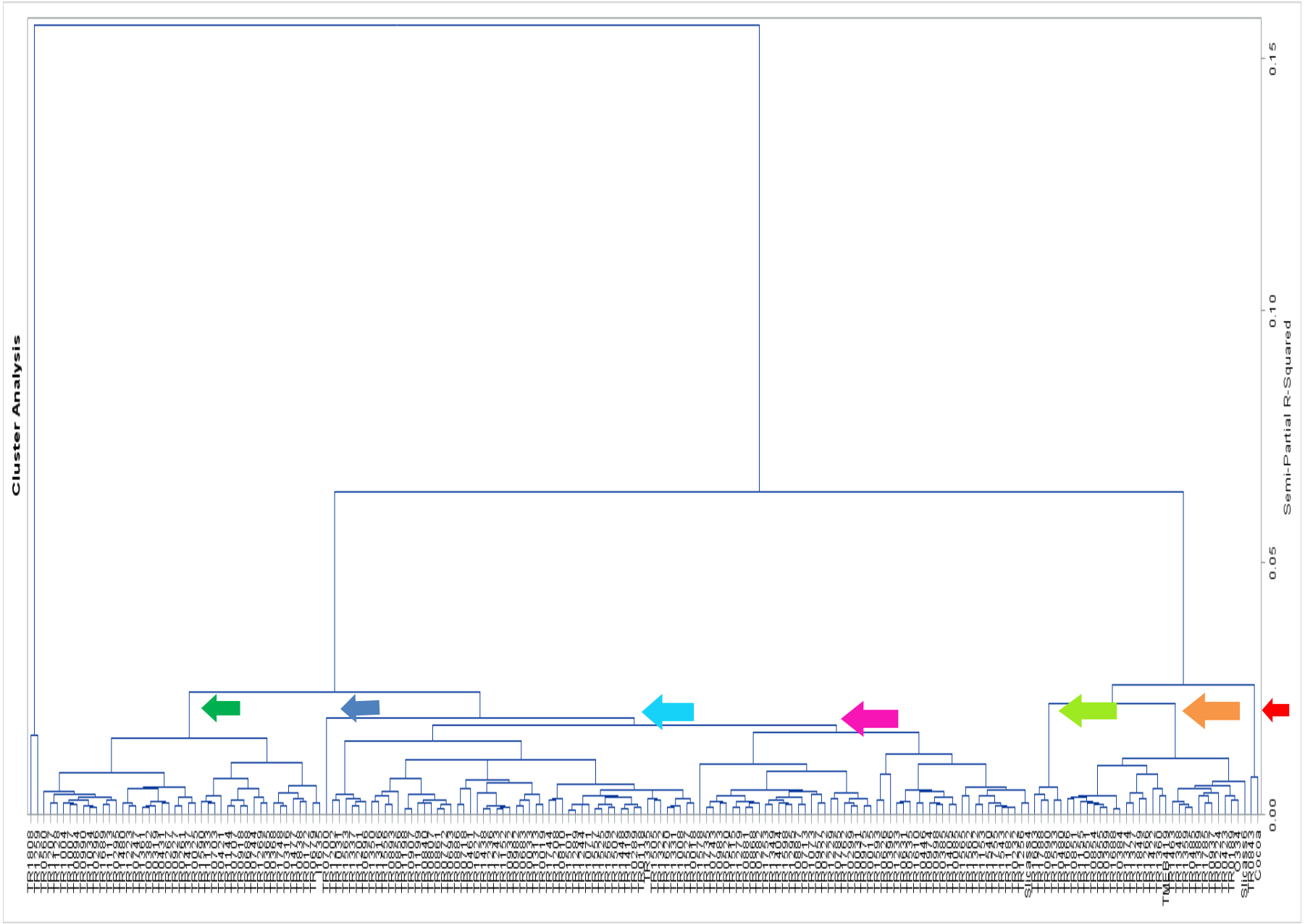


Figure 4.1 Dendrogram of 188 cassava accessions based on morpho-agronomic traits using Ward's minimum Variance

Table 4.7 188 cassava accessions grouped into 8 clusters based on total carotenoid content

C=A	C=B	C=B	C=C	C=D	C=D	C=E	C=E	C=F	C=G	C=H
TR 1259	TR 0382	TR 0703	TR 0700	TR 0982	TR 1289	TR 0868	TR 0957	TR 0480	TR 0384	Cocoa
TR 1808	TR 1361	TR 1133		TR 0693	TR 1501	TR 0881	TR 0520	TR 1533	TR 1688	TR 0843
	TR 0296	SLICASS 11		TR 1244	TR 1525	TR 1279	TR 0983	TR 0890	TR 0399	
	TR 1034	TR 0679		TR 0893	TR 1557	TR 1515	TR 1540	TR 1788	TR 0955	
	TR 0707	TR 0025		TR 1556	TR 1152	TR 0232	SLICASS 4	TR 1603	TR 0485	
	TR 1144	TR 1437		TR 0019	TR 1243	TR 1182	TR 1256		TR 1359	
	TR 0990	TR 0368		TR 1313	TR 1419	TR 1543	TR 0446		TR 0854	
	TR 0261	TR 0267		TR 1071	TR 1448	TR 0743	TR 1610		TR 1348	
	TR 0927	TR 0431		TR 0718	TR 0907	TR 1735	TR 1762		TR 1051	
	TR 0316	TR 1128		TR 0886	TR 1198	TR 0031	TR 0535		TR 1327	
	TR 1748	TR 1207		TR 0807	TR 1236	TR 0299	TR 0626		TR 1374	
	TR 0688	TR 1004		TR 0840	TR 1438	TR 1755	TR 1593		TR 0937	
	TR 0918	TR 1480		TR 1199	TR 0772	TR 0015	TR 0785		TR 1785	
	TR 0319	TR 0365		TR 0018	TR 0861	TR 0975	TR 1229		TR 1463	
	TR 0747	TR 1269		TR 1527	TR 1502	TR 0385	TR 1562		TR 1155	
	TR 1223	TR 0172		TR 1316	TR 1569	TR 0998	TR 1565		TR 0334	
	TR 0894	TR 0838		TR 1350	TR 0118	TR 0713	TR 1073		TR 0189	
	TR 1007	TR 1590		TR 0696	TR 0289	TR 0887	TR 0396		TR 1266	
	TR 1113	TR 0295		TR 1322	TR 0932	TR 1295	TR 1578		TR 1849	
	TR 1689	TR 1477		TR 1505	TR 1331	TR 1598	TR 0974		TR 0851	
		TR 0744		TR 0033	TR 1620	TR 0085	TR 0631		TR 1666	
		TR 0421		TR 1208	TR 1201	TR 1405	TR 1233		TR 1389	
		TR 0560		TR 1744	TR 1337	TR 1349	TR 1404		SLICASS 7	
				TR 1031	TR 1008	TR 1753	TR 0282		TR 1360	
				TR 1202	TR 0683	TR 1153	TR 0222		TR 0423	
				TR 0810	TR 1627	TR 1302			TR 0224	
				TR 0335	TR 0856				TR 1422	
				TR 1563	TR 0461				SLICASS 6	

4.3.6 Clusters mean and standard deviation of provitamin A cassava accessions

Results in Table 4.8 shows the different cluster groups for provitamin A containing cassava accessions with their means and standard deviations. Cluster D has the highest number of total carotenoid containing accessions with a mean and standard deviation of (7.3±2.9), while two clusters G and H had the lowest number of total carotenoid accessions with a mean and standard deviation of 10±00 and 9.3± 2.6, respectively. Clusters A, C and F did not have carotenoid cassava accessions.

Table 4.8 Number of accession/clusters, mean and standard deviation for provitamin A content

Cluster Name	Accessions	TCC Mean and Standard Deviation
D	34	7.3±2.9
E	27	8.1±2.6
B	11	7.2±2.3
G	2	10±00
H	2	9.3±2.6

4.3.7 Summary statistics of genetic variation of the accessions using SNP markers

Summary statistics for number of alleles observed, expected heterozygosity and polymorphic information content are presented in Appendix 2. An average of 2 alleles per locus was observed from the analysis. The expected heterozygosity was lowest for TR 1233 (0.15) and SLICASS 6 (0.15) and highest in TR 1525 (0.23) with a mean of 0.36. The observed heterozygosity per individual observation ranged from 0.30 (TR 1233) to 0.47 (TR 1525) with a mean of 0.38. The mean of observed heterozygosity was higher than the expected heterozygosity.

This substantiates the heterozygous nature of most of the accessions and the fact that cassava is largely cross-pollinated. However, the major allele frequency of all the markers used in the observations was generally below 0.95, indicating that they were all polymorphic. PIC values

ranged from 0.11 in TR 1233 to 0.18 in TR 1199 and TR 1525. The mean PIC is 0.28. The higher the PIC value the more informative is the marker.

4.3.8. Cluster groupings of the 188 cassava accessions based on SNP markers.

The 188 accessions were grouped into 9 clusters based on the 5,643 SNP markers (Figure 4.2). Clusters A, B, C, D, E, had 21, 7, 10, 16 and 11 accessions, while cluster F, G, H and I consisted of 50, 44, 12 and 7 accessions. Clusters A, B, D, E, F, G, H and I had 5, 3, 7, 6, 20, 23, 5 and 3 accessions with varying levels of total carotenoid content. Cluster C did not have any carotenoid accessions. A comparison of the two dendrogram based on Mantel matrix test showed a significant positive but weak correlation between the morphological and molecular data sets ($r = 0.104; P<0.034$)

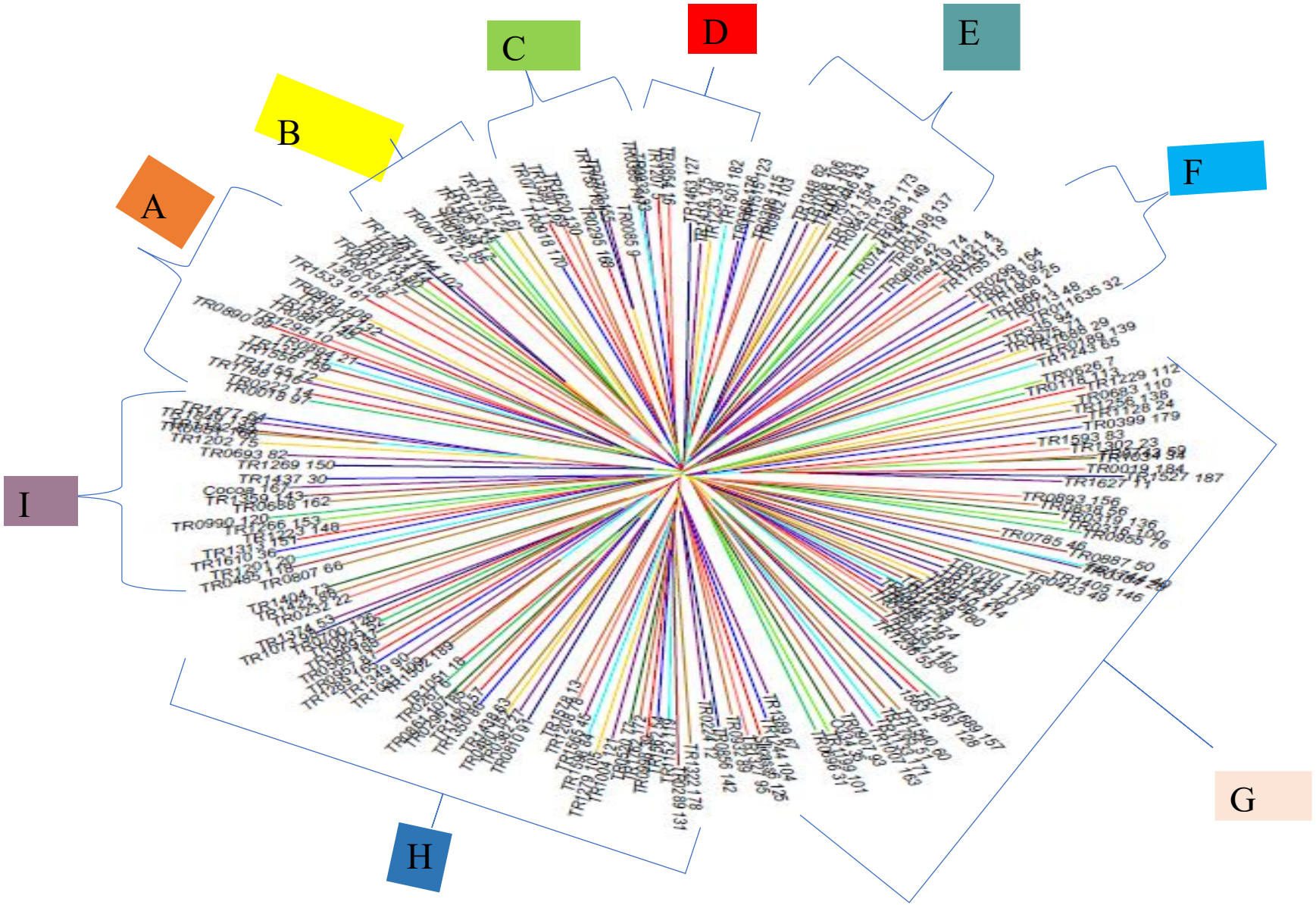


Figure 4.2 Dendrogram of 188 cassava accessions based on SNP Markers

Table 4.9 Cluster groupings of the 188 cassava accessions based on SNP markers

Cluster A	Cluster B	Cluster C	Cluster D	Cluster E	Cluster F	Cluster F	Cluster G	Cluster G	Cluster H	Cluster I
TR 0018	TR 0679	TR 0918	TR 1755	TR 0299	TR 0626	TR 0172	TR 1389	TR 0267	TR 0907	TR 1269
TR 0222	TR 0282	TR 0772	TR 1337	TR 0718	TR 0118	TR 0840	TR 1244	TR 1051	TR 0485	TR 0693
TR 1788	SLICASS 4	TR 1590	TR 0421	TR 1808	TR 1229	TR 1259	SLICASS 6	TR 1502	TR 1201	TR 1202
TR 1155	TR 1505	TR 1620	SLICASS 7	TR 1661	TR 0683	TR 1525	TR 1327	TR 1031	TR 1610	TR 0851
TR 1556	TR 1534	TR 0295	TR 0886	TR 0713	TR 1256	TR 0937	TR 0932	TR 1349	TR 1313	TR 1182
TR 3168	TR 1735	TR 1753	TR 0261	01/1635	TR 1128	TR 0431	TR 0856	TR 1289	TR 1223	TR 1849
TR 0894	TR 0747	TR 0703	TR 1198	TR 0335	TR 0399	TR 0461	TR 0224	TR 0957	TR 1266	TR 1477
TR 0890		TR 0085	TR 0744	TR 0975	TR 1593	TR 0365	TR 1322	TR 0560	TR 0990	
TR 1296		TR 0033	TR 0868	TR 1688	TR 1302	TR 0535	TR 0289	TR 1569	TR 0688	
TR 0881			TR 1331	TR 0189	TR 0743	TR 0927	TR 1152	TR 0025	TR 1359	
TR 1557			TR 0843	TR 1243	TR 1034	TR 1236	TR 1562	TR 0700	Cocoa	
TR 1603			TR 1071		TR 0019	TR 1689	TR 0998	TR 1073	TR 1437	
TR 0983			TR 0446		TR 1527	TR 1361	TR 1762	TR 1374		
TR 1533			TR 0015		TR 1627	TR 1563	TR 0520	TR 0232		
TR 1360			TR 1008		TR 0893	TR 1540	TR 1004	TR 1422		
TR 0631			TR 1348		TR 0838	TR 1785	TR 1279	TR 1405		
TR 0031					TR 0319	TR 1133	TR 1598	TR 0480		
TR 1113					TR 0316	TR 1007	TR 1565	TR 1438		
TR 0974					TR 0955	TR 0907	TR 1208	TR 1350		
TR 1748					TR 0795	TR 0334	TR 1578	TR 1480		
TR 1144					TR 0887	TR 1199	TR 0810	TR 0296		
					TR 1744	TR 0696	TR 0382	TR 0861		
					TR 0384	TR 0747				
					TR 1405	TR 1448				
					TR 0423	TR 1543				

4.3.9 Thirty provitamin-A cassava accessions with varying levels of total carotenoid, yield and dry matter content based on morphological and molecular clustering Analyses.

Thirty provitamin A accessions with higher levels of total carotenoid were selected from five out of the eight and eight out of the nine clusters derived from morphological and molecular (SNPs) clustering analyses with some showing appreciable high dry matter content and yield for the multilocational testing across the three environments (Table 4.10). Although TR-1208, TR-1152 and TR-0713 were selected and formed part of the carotenoid accessions, they had the lowest levels of total carotenoid content of 8.9, 9.9 and 8.2 respectively.

4.10 Thirty provitamin-A cassava accessions with varying levels of total carotenoid, yield and dry matter content

Accession	Phenotypic cluster name	Genotypic cluster name	Yield	Dry matter content	Total carotenoid content (µg-^{1})
TR 0747	B	B	4.3	29.5	10.9
TR 0365	B	F	2.3	25.5	7
TR 0560	B	G	7.5	25.5	9.7
TR 1208	D	G	7.5	39.5	8.9
TR 0461	D	F	2	23	11.5
TR 1337	D	D	14.6	25.5	11.8
TR 1569	D	G	21.8	26.5	10.3
TR 0683	D	F	5	28.5	10.2
TR 1198	D	D	7	28.5	10.8
TR 1313	D	H	11	35	11.7
TR 0696	D	F	6.5	12.5	11.1
TR 1322	D	G	13	29.5	9.9
TR 1350	D	G	8	29.5	9
TR 0907	D	H	6	31.5	9.1
TR 1557	D	A	10.6	18	11.2
TR 1152	D	G	4.8	33	8.08
TR 0232	E	G	22.8	27	9.9
TR 1279	E	G	6.3	35.6	9.1
TR 0031	E	A	6.9	29.5	10.3
TR 0222	E	A	7.8	37	13.1
TR 0998	E	H	2.8	38.1	13.7
TR 1755	E	D	5.3	24	10.7
TR 1182	E	I	10.8	24	10.4
TR 1753	E	B	16.8	35	8.6
TR 0713	E	E	7.5	28	8.2
TR 0423	G	F	6.5	25.5	8.7
TR 0384	G	F	5.5	27	10.6
TR 1327	G	G	4.5	21	11.1
TR 0399	G	F	11.8	25.5	11.1

4.4 Discussion

Significant variation observed among the economically important traits such as African cassava mosaic disease, yield and dry matter content (DMC) among the 188 accessions studied offers a prospect for progress in cassava breeding program in Sierra Leone. The marketable tuber weight which is positively associated with yield and commonly preferred by farmers to increase their income and enhance their livelihood showed the highest variability which can be used for yield improvement. Similarly, the non-significant very low negative correlation (-0.06) between dry

matter content and inner color (INCOL) in the current study does appear to be contrary to findings from Latin American germplasm evaluated at CIAT (Esuma *et al*., 2016). It is worth noting that combined selection for both total carotenoid content (INCOL) and dry matter in Latin America has been underway much longer than in Africa and probably explains why the Latin American yellow cassava also have high dry matter content. Thus, such negative correlations could be lost during the several cycles of recombination (Ceballos *et al*., 2013 and Esuma *et al*., 2016). Diversity studies of cassava germplasm has been widely undertaken worldwide (Bolanos, 2001; Chavez *et al.,* 2005; Morillo *et al.*, 2009; Fregene, 2007; Parkes, 2011; Njoku, 2012 and Thompson, 2013) with little or no attention in Sierra Leone. In the present study, descriptive analysis of the cassava accessions based on selected traits showed the existence of high variability among the accessions. These findings were in confirmation with the findings of Carvalho and Schaal (2001) who reported a high degree of variability among 94 cassava accessions of Brazilian origin. Raghu *et al*. (2007) in a similar study also identified a high level of diversity among 58 cassava accessions based on 29 morphological traits. Lyimo *et al*. (2012) reported significant variability among 39 cassava accessions of Tanzanian origin using 14 morphological traits. Thompson (2013) observed a moderate to high diversity among 150 accessions using 25 morphological traits in Ghana. In a similar study, Raghu *et al*. (2011) mentioned that 24 morphological traits out of 28, contributed to the total variation observed. In the present study, clustering based on similarity index of both qualitative and quantitative traits grouped the 188 cassava accessions into 8 and 9 distinct clusters based on morphological and molecular analyses respectively. In a similar study, Carvalho and Schaal (2001) identified 22 distinct clusters using 94 cassava accessions. 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 or controlled by the environment are preferable in genetic diversity studies (Kaemmer *et al.*, 1992; Gepts, 1993; Njoku, 2012 and Thompson, 2013). The study by Kawuki *et al*. (2009) was the first published report where SNPs were used for diversity studies in cassava. They identified, characterized some SNP markers and assessed their utilization in cassava diversity assessment. The present study seems to be first reported case in Sierra Leone where SNP markers have been exploited in cassava diversity study on provitamin A cassava accessions. Using the 5,634 SNP markers, 95% of them were polymorphic. The informativeness of a genetic marker is measured by the polymorphic information content (PIC). The Mean PIC value observed for this study was 0.28. Kawuki *et al*. (2009) reported a PIC value of 0.29 in 74 cassava accessions using 26 SNP while Thompson (2013) also reported PIC value of 0.29 using 150 cassava accessions. 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.

Although morphological and SNP data grouped the accessions into eight and nine distinct clusters respectively, some similarities were observed. Related accessions were grouped in the same cluster while unrelated accessions were grouped in separate clusters. Accessions TR 1337, TR 1198, TR 0747, TR 0713 and TR 1327 were both morphologically and genetically similar. The significant positive but low correlation ($r = 0.104$; $p < 0.034$) observed between the two dendrograms revealed by the Mantel matrix test could be attributed to the relatedness of the accessions within the studied population. This could explain why the morphological and molecular analysis showed similar accessions between the two clusters.

4.5 Conclusion

The present morphological and molecular assessment studies showed that provitamin A cassava accessions in Sierra Leone have moderate to high diversity based on total carotenoid content, based on morphological and molecular assessment studies. The results obtained will serve as a guide and basis of germplasm management and improvement for total carotenoid content, yield and African cassava mosaic disease resistance. The diversity of the provitamin A cassava accessions was sufficient to enable the creation of a collection of 30 provitamin A cassava accession with diverse genetic background from the different cluster groups.

CHAPTER FIVE

5.0 GENOTYPE BY ENVIRONMENT INTERACTION ANALYSIS OF PROVITAMIN A CASSAVA IN SIERRA LEONE

5.1 Introduction

Plant breeders have established that the expression of quantitative traits such as crop yield is controlled not only by the genetic make-up of the variety but also the environment (Carpena *et al.,* 1982). Dixon and Nukenine (2000) defined genotype by environment interaction (GEI) as the change in a cultivar's behavior over environments, from differential response of the cultivar, to various edaphic, climatic and biotic factors. Assessment of GEI effects for a given trait is therefore useful in understanding varietal stability (Acquaah, 2012). A significant GEI limits the usefulness of superior genotypes. Breeders address the GEI challenge by evaluating genotypes in multiple environments to select better adapted genotypes with high and stable performance in different environments (Fakuda *et al.*, 2002, Nassar and Ortiz, 2006, Esuma *et al.*, 2016). The GGE biplot is a decision-making tool that allows identification of stable and good performing genotypes in test environments towards subsequent release (Farshadfar *et al.*, 2013 and Rao *et al.*, 2011).

GGE biplot is a data visualization tool, which graphically displays GEI in a two-way table (Yan, 2001). GGE biplot is an efficient tool for mega –environment analysis (e.g. which won where), thereby specific genotypes can be recommended to a special mega –environments (Yan and Tinker, 2006); genotype screening (the mean performance and stability), and environmental assessment provides the power to discriminate among genotypes in preferred environments). GGE biplot analysis is increasingly being used in GEI data analysis in agriculture (Yan, 2001;

Crossa *et al*., 2010; Yan and Hunt ,2002; Yan and Tinker, 2005; Samonte *et al*., 2005; Dehghani *et al*., 2006; Yan and Tinker, 2006).

Winning genotypes and mega-environments in a polygon-view of a GGE biplot is the most effective approach in identifying winning genotypes and mega environments. A polygon is drawn by joining the genotypes that are located farthest from the biplot origin, while all other genotypes are contained in the polygon. Mean performance and stability of genotypes can be visualized on a GGE biplot by drawing an average environment coordinate (AEC) on genotype-focused biplot. The AEC is decomposed into two axes, which are perpendicular to each other, the abscissa and the ordinate. Evaluation of test locations is done by defining three parameters, namely: the ability to discriminate between genotypes (discrimination ability), the ability to represent the target environment (representativeness) and the biplot distance from an ideal location (desirability index) (Xu *et al*., 2013).

Provitamin A cassava genotypes have featured so distinctly in biofortification because they have an increasing level of micronutrients, such as carotenoids (Iglesias *et al.*, 1997; Chávez *et al.*, 2005; Ssemakula *et al.*, 2007 and Esuma *et al*., 2016). However, the acceptance of micronutrient biofortified genotypes largely depends on their agronomic qualities eg, including, dry matter content, fresh root yield, resistance to major pests and diseases, and the stability of these traits over time and space. Though cassava is widely adapted to a variety of environmental conditions, usually the adaptability of most white flesh varieties is narrow with large GEI effects (Dixon *et al*, 1994b; Dixon and Nukenine, 1997). Due to poor social infrastructure and high poverty levels, food fortification and supplementation have been less impacting (Boy *et al.*, 2009, Mayer *et al.*, 2008, Thompson and Amoroso, 2011). The national cassava breeding programme in Sierra Leone has initiated new strategies geared towards developing and advancing provitamin A

cassava that incorporates farmer preferred traits, especially high dry matter content and fresh storage root yield. It is envisioned that this initiative will culminate in deployment of provitamin A cassava varieties for purposes of improving the nutritional status of populations that depend upon cassava as a major staple.

The objectives of this study were to i) determine the GEI among selected provitamin A accessions. ii) identify and select stable genotypes with high carotenoid levels and dry matter.

5.2 Materials and Methods

5.2.1 Experimental materials

Genotypes used for the study are the 30 selected provitamin A cassava of high carotenoid content selected from the collection established in chapter 4.

5.2.2 Experimental sites and design

Trials were planted for one season beginning in October, 2016 to September 2017, at three locations in three different agroecological zones namely; Kambia (north savannah grassland), Njala (Transitional Rain Forest) and Pendembu (Forest Zone). The experiment and climatic information are showed in Figure 5.1 and Table 5.1 respectively. The trials were laid out in a randomized complete block design using three rows of 8m per plot with three replications.

5.2.3 Planting

Planting was done at a spacing of 1 × 1 m, giving a density of 10,000 plants ha^{-1}. To increase the chances of sprouting and uniform plant establishment, all stakes used for planting were taken

from the middle portions of mature stems. Adjacent plots were separated by 2 m alleys. Weeding was done as necessary.

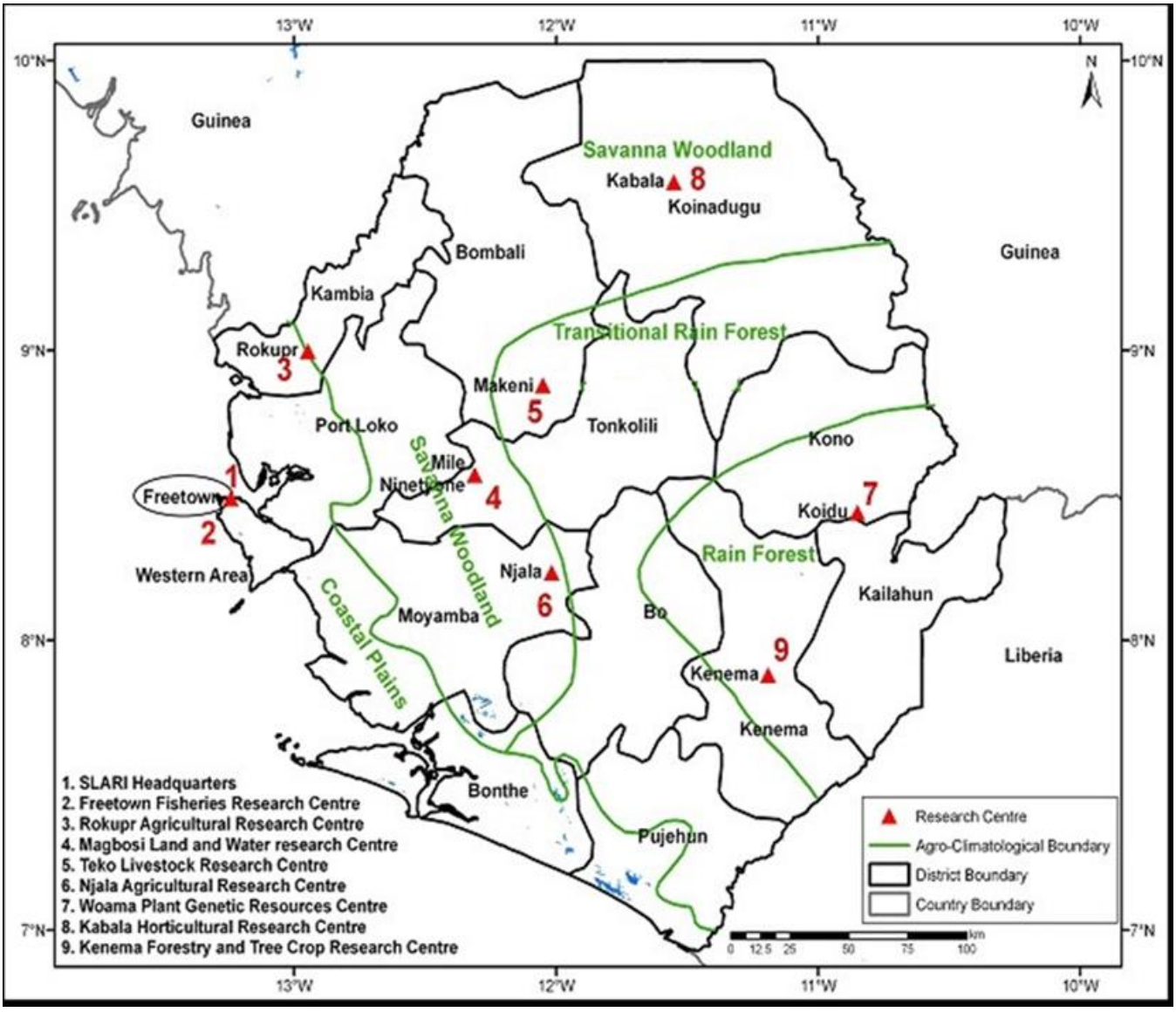


Figure 5.1 Map of Sierra Leone; showing trial sites

Table 5.1 Climatic data of the three experimental sites

Characteristics	Agro-climatic Regions		
	Kambia	**Njala**	**Pendembu**
Mean temperature. (^{O}C)	28.2	28.5	28.6
Rainfall (mm)	2280	2730	2660
Altitude (m)	150 – 300	150 - 300	300 – 600
Dominant vegetation	Lophira savannah, savannah woodland, mixed tree savannah, upland grassland, and forest re- growth.	Savannah woodland, montane grassland, and forest re-growth.	Forest and forest re-growth.
Dominant land form	Drainage depressions, undulating plains, low plateau, and hills.	Plateau with undulating high lying plains, and rolling hills.	Plateau with undulating plains, rolling plains, and hills.
Average length of growing period (days)	255 ± 10	270 – 300	314 ± 9

5.2.3 Data collection

Data were collected at various crop growth stages on morphological parameters using the IITA Cassava descriptor by Fakuda *et al*., (2010).

At harvest (11 months after planting), the following data were taken, number of marketable roots (MKR), non-marketable roots (NMKR) marketable weight (MKW), nonmarketable weight (NMKW), total carotenoid content (TCC), fresh root yield (FRYD) and dry matter content (DMC). Data were taken on eight plants from the inner rows of each experimental plot. Samples were taken from five roots out of the eight harvested plants to measure dry matter content (DMC) as total carotenoid content (TCC).

5.2.4 Data Analysis

Data were subjected to statistical analyses. GGE Biplot analysis was used to determine GGE interaction and stability performance (Yan and Hunt, 2002). The GGE biplot model equation is as follows;

$$\boldsymbol{Y_{ij} - y_j = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \varepsilon_{ij}}$$

where y_{ij} = is the mean yield of genotype,

i = in environment j,

y_j = is the mean of genotypes in environment j,

λ_1 and λ_2 = are the eigenvalues for PC1 and PC2,

ξ_{i1} and ξ_{i2} = are the scores of genotype i,

η_{j1} and η_{j2} = are the scores for environment j,

ε_{ij}= is the residual term related to the mean of genotype i in environment j.

GGE biplots (version 4.1) was used for graphical analysis to identify genotypes with broad or specific adaptation to target environments.

5.3. Results

5.3.1. Analysis of variance for multilocational trials across 3 locations for dry matter and total carotenoid contents.

The results of the analysis of variance are shown in Table 5.2. Differences in DMC and TCC were not significant among the genotypes. However, significant environmental differences were observed among the three sites for both traits. Genotype x environment interaction was significant for only DMC.

Table 5.2 Analysis of variance dry matter and total carotenoid contents

Source	Df	Dry matter content	Total carotenoid content
Genotype (G)	29	15.09	3.04
Environment (E)	2	767.08**	52.21**
G*E	58	45.45*	3.3
Error	161	32.49	3.2

5.3.2 Performance of 30 provitamin A cassava evaluated across 3 test environments for dry matter content and total carotenoid content

Results show significant differences among genotypes across the 3 environments for TCC but not for DMC (Table 5.3). Generally dry matter content varied between 35% and 54% across the three sites. Mean dry matter content above 50% were recorded for 13 genotypes across the 3 sites. TR 0384 (54.39%), TR 0232(53.26%) and TR 0998(42.63%) had the highest dry matter content in Pendembu, Njala and Kambia, respectively. Although TR 0998 had the highest dry matter in Kambia, it had very low values in Njala and Pendembu. Total carotenoid content for the genotypes for all the 3 sites varied between 5 and 13 μg^{-1} of fresh weight. TR 1313 had the highest level of carotenoid content with a mean of 10.6 μg^{-1} of fresh weight across 3 locations followed by TR 0683 and TR 1152 with 10.28 μg^{-1} of fresh weight and 10.20 µg/g of fresh weight, respectively. The accessions with the lowest levels of total carotenoid in each location were TR 0423 (Pendembu), TR 1337 (Njala) and TR 1279 (Kambia). Accession with highest total carotenoid content were recorded for TR 0998 (Pendembu), TR 0222 (Njala) and TR 0907 (Kambia) with total carotenoid content of 13.02, 11.34 and 11.24 μg^{-1} of fresh weight, respectively.

Table 5.3 Mean performance of 30 provitamin A cassava accessions evaluated across three environments

Entry		Dry matter content (%)				Total carotenoid content (μg^{-1})			
No	**Genotype**	**Kambia**	**Njala**	**Pendembu**	**Mean**	**Kambia**	**Njala**	**Pendembu**	**Mean**
1	TR 0747	43.98	42.20	44.51	43.56	10.65	9.61	10.07	10.11
2	TR 0365	42.73	44.22	45.84	44.26	7.69	10.79	11.60	10.03
3	TR 0560	39.39	48.44	48.20	45.34	9.95	7.85	9.03	8.94
4	TR 1208	45.79	51.51	44.47	47.26	9.54	10.37	10.13	10.01
5	TR 0461	40.23	46.67	45.19	44.03	7.07	9.72	10.64	9.14
6	TR 1337	39.55	50.24	47.01	45.60	9.76	6.59	10.13	8.83
7	TR 1569	43.39	47.81	42.87	44.69	7.66	8.97	10.62	9.08
8	TR 0683	40.74	45.93	48.91	45.19	9.04	10.37	11.44	10.28
9	TR 1198	47.55	45.78	46.59	46.64	5.77	8.22	10.79	8.26
10	TR 1313	39.39	45.55	51.10	45.35	9.47	9.98	12.40	10.62
11	TR 0696	41.21	47.84	44.14	44.40	8.53	9.24	10.36	9.38
12	TR 1322	41.50	45.98	45.03	44.17	5.82	9.44	11.17	8.81
13	TR 1350	44.84	41.47	44.05	43.45	7.83	10.43	10.48	9.58
14	TR 0843	38.04	44.29	51.17	44.50	8.23	8.66	10.17	9.02
15	TR 0907	43.16	44.99	51.15	46.43	11.24	9.50	9.16	9.97
16	TR 1557	42.35	44.54	48.12	45.00	7.83	7.63	9.54	8.33
17	TR 1152	39.94	46.38	42.59	42.97	10.02	9.93	10.64	10.20
18	TR 0232	43.72	53.26	44.11	47.03	8.27	9.26	9.34	8.96
19	TR 1279	39.14	48.34	50.80	46.09	4.85	9.04	11.15	8.35
20	TR 0031	38.72	49.10	45.72	44.51	10.07	8.23	11.00	9.77
21	TR 0222	37.15	47.27	46.74	43.72	7.93	11.34	9.11	9.46
22	TR 0998	46.48	39.61	41.80	42.63	9.00	8.03	13.02	10.02
23	TR 1755	38.45	52.73	46.55	45.91	8.82	8.64	11.09	9.52
24	TR 1182	34.93	53.03	46.25	44.74	9.06	10.52	9.30	9.63
25	TR 1753	41.15	50.07	49.53	46.92	9.57	7.91	10.37	9.28
26	TR 0713	39.98	50.93	45.08	45.33	8.41	8.29	10.31	9.00
27	TR 0423	39.50	41.05	48.48	43.01	8.62	9.39	7.39	8.47
28	TR 0384	46.32	39.12	54.39	46.61	9.06	8.88	9.58	9.17
29	TR 1327	43.50	41.68	44.89	43.36	8.27	10.40	10.33	9.67
30	TR 0399	44.58	39.68	51.69	45.32	4.90	8.41	9.11	7.47
	Mean	**41.58**	**46.32**	**46.90**	**44.93**	**8.46**	**9.19**	**10.31**	**9.32**
	SE	**3.82**	**5.1**	**3.81**	**2.70**	**0.28**	**0.19**	**0.04**	**1.00**
	LSD (5%)	**7.69**	**10.23**	**7.65**	**5.30**	**0.58**	**0.39**	**0.09**	**2.10**
	CV %	**11.3**	**13.5**	**9.9**	**12.70**	**18.44**	**11.86**	**10.67**	**19.10**

5.3.3 Polygon view of GGE biplot for dry matter content (%)

The PC1 and PC2 axes together explained 87.5% of the total variation observed for dry matter content (Figure 5.2). The polygon view of the GGE biplots revealed that the three locations fell into 3 mega environments. The three mega environments were Pendembu (Pen) Forest zone environment 1, Kambia (Kam) North savanna grassland environment 2 and Njala (Nja) Transitional rain forest environment 3. In Pendembu (Environment 1) the vertex/best performing genotype was TR 0384 (28). Other accessions in the Pen mega environment were TR 0907 (15), TR 0423 (27), TR 1557(16) and TR 0399 (30). TR 0998 (22) was the vertex genotype in mega environment 2 (Kam). Other entries within the Kam mega environment were TR 0747 (1) TR 1327 (29), and TR 1350 (13). TR 1182 (24) was the vertex genotype in mega environment 3 (Nja) which also had these entries TR 0031 (20), TR 0222 (21) and TR 1755 (23).

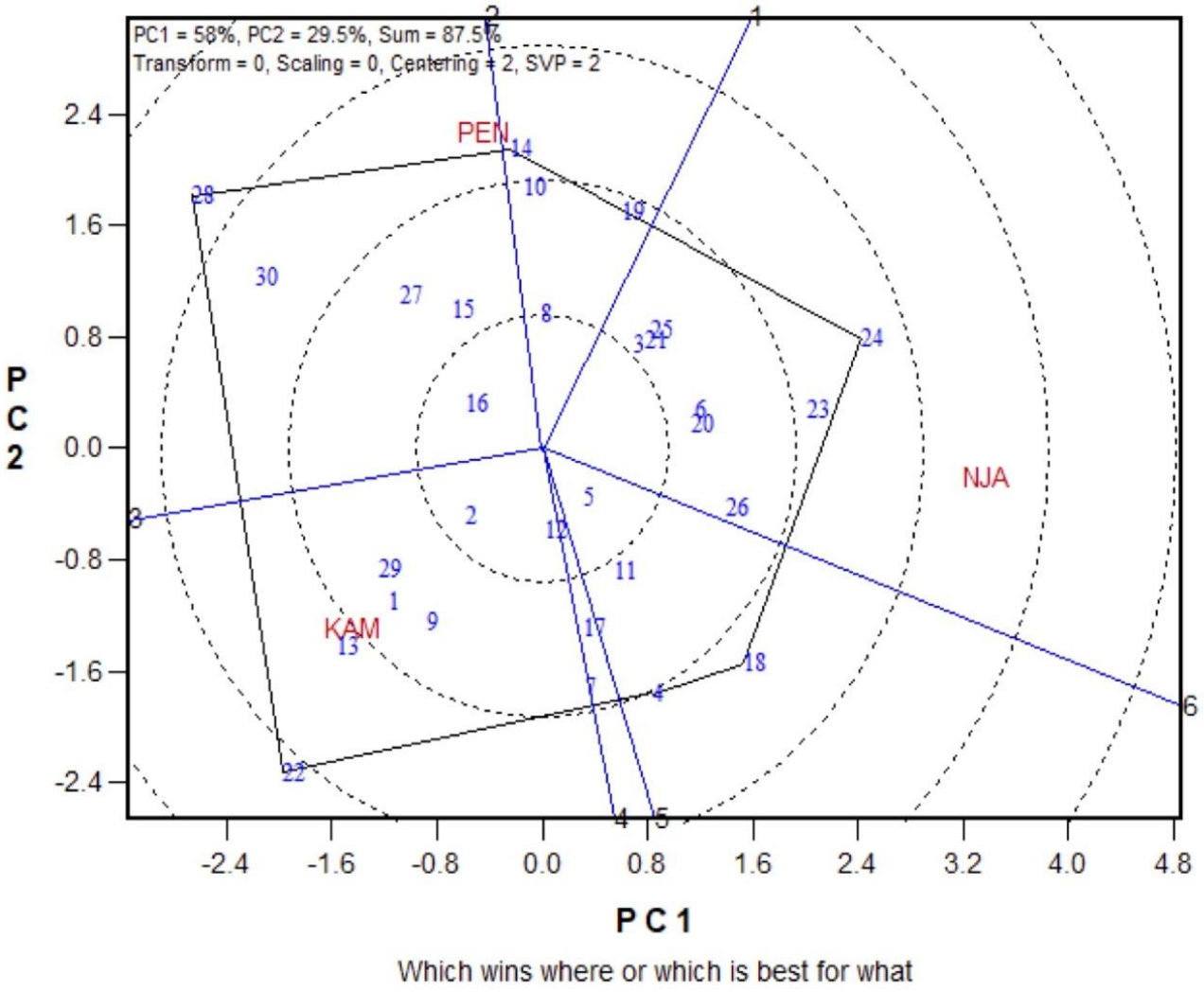


Figure 5.2: GGE biplot showing the best genotype for each of the 3 mega environments for dry matter content (%)
Key: 1-TR0747, 2-TR0365, 3-TR0560, 4-TR1208, 5-TR0461, 6-TR1337, 7-TR1569, 8-TR0683, 9-TR1198, 10-TR1313, 11-TR0696, 12-TR1322, 13-TR1350, 14-TR0843, 15-TR0907, 16-TR1557, 17-TR1152, 18-TR0232, 19-TR1279, 20 -TR0031, 21 - TR0222, 22 - TR0998, 23 - TR1755, 24 - TR1182, 25 - TR1753,26-TR0713,27-TR0423,28-TR0384, 29-TR1327, 30-TR0399, PEN-Pendembu, KAM-Kambia, NJA-Njala

5.3.4 Mean performance and stability of genotypes for dry matter content (%) across the 3 environments

Figure 5.3 shows the stability for dry matter content of the 30 genotypes across the 3 mega environments. Thus, TR 1182 (24) was observed as the genotype with the highest dry matter content across test environments followed by 4 entries TR 1755 (23), TR 0843 (14), TR 1279 (19) and TR 1753 (25) constituted the top. The projection on the AEC ordinate, depending on the length measures the stability of the genotypes across test environments. The shorter the length of the projection, the more stable is the high dry matter content for the associated genotype. Consequently, the most stable entries were TR 0365 (2), TR 1279 (19) and TR 0033 (21). The arrow on the AEC abscissa delineates the ideal genotypes and entry TR 1182 (24) is the closest followed by TR1753 (25) and TR1279 (19) respectively.

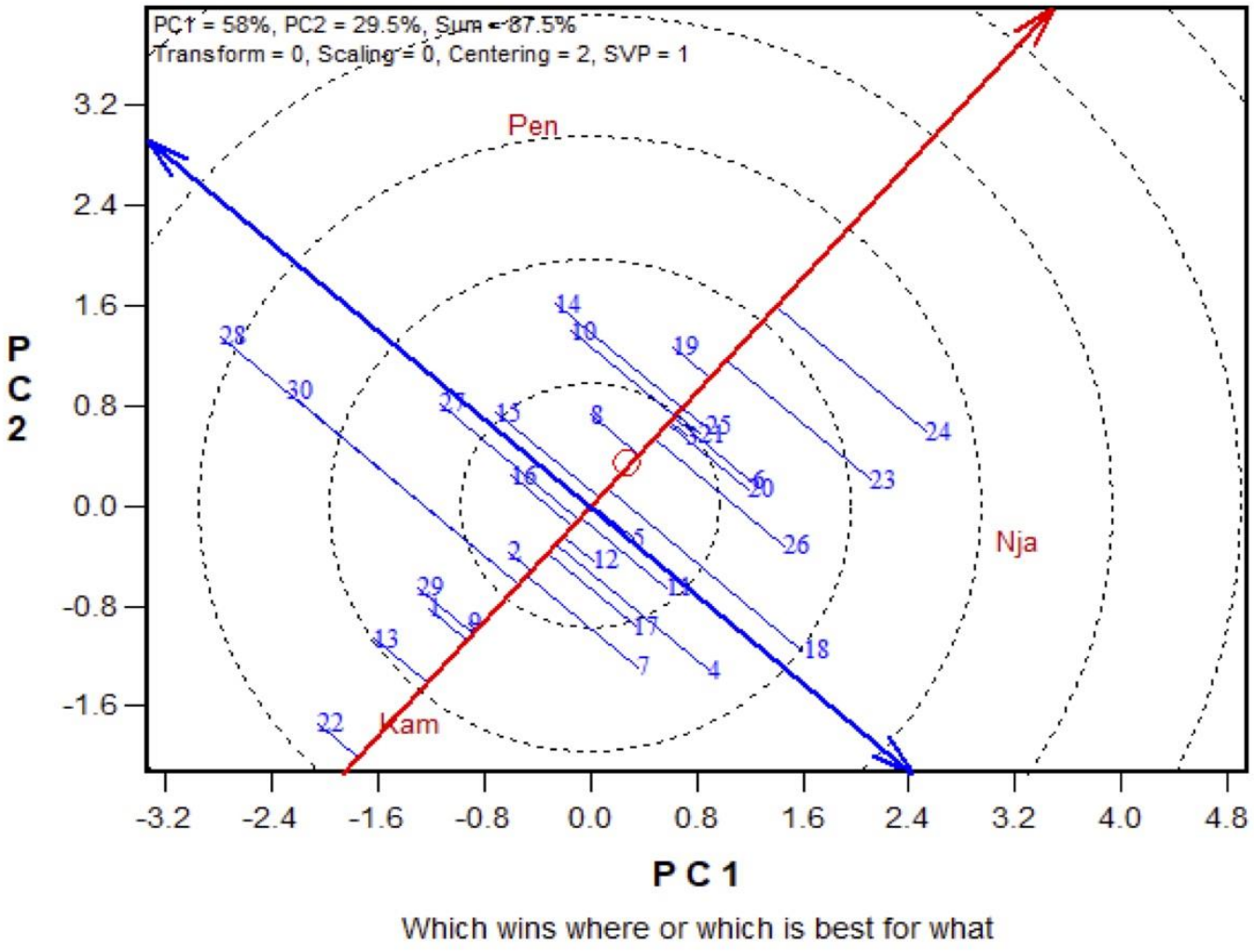


Figure 5.3: Stability of dry matter content (%) of 30 Provitamin A cassava genotypes evaluated across three environments

Key: 1-TR0747, 2-TR0365, 3-TR0560, 4-TR1208, 5-TR0461, 6-TR1337, 7-TR1569, 8-TR0683, 9-TR1198, 10-TR1313, 11-TR0696, 12-TR1322, 13-TR1350, 14-TR0843, 15-TR0907, 16-TR1557, 17-TR1152, 18-TR0232, 19-TR1279, 20 -TR0031, 21 - TR0222, 22 - TR0998, 23 - TR1755, 24 - TR1182, 25 - TR1753,26-TR0713,27-TR0423,28-TR0384, 29-TR1327, 30-TR0399, Pen-Pendembu, Kam-Kambia, Nja-Njala

5.3.5 Discriminativeness vs representativeness for dry matter content (%)across the 3 environments

Figure 5.4 shows the discriminativeness vs representativeness for dry matter content (%) of the 3 environments. The biplot identified Njala having the longest vector, as the most discriminatory followed by Pendembu while Kambia was the least discriminatory (Figure 5.4). Kambia was identified as the most representative of the test environments. The arrow on the AEC abscissa delineates the ideal environments. Among the test environments Pendembu had its vector closest to the arrow, it was therefore considered the ideal environment for dry matter content production.

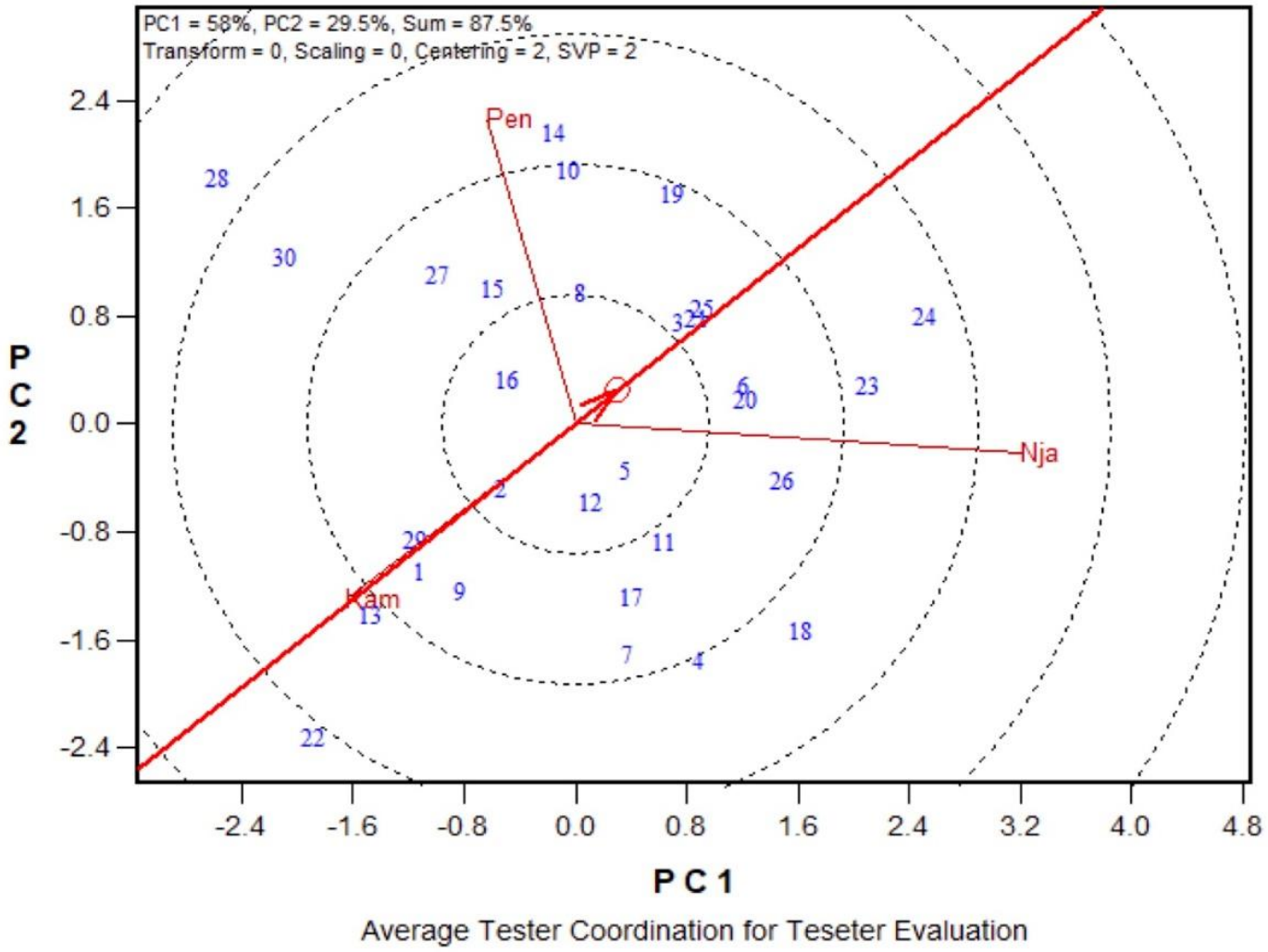


Figure 5.4: Discriminativeness and representativeness of the three environments for dry matter content (%) determination of 30 Provitamin A cassava genotypes

Key: 1-TR0747, 2-TR0365, 3-TR0560, 4-TR1208, 5-TR0461, 6-TR1337, 7-TR1569, 8-TR0683, 9-TR1198, 10-TR1313, 11-TR0696, 12-TR1322, 13-TR1350, 14-TR0843, 15-TR0907, 16-TR1557, 17-TR1152, 18-TR0232, 19-TR1279, 20 -TR0031, 21 - TR0222, 22 - TR0998, 23 - TR1755, 24 - TR1182, 25 - TR1753,26-TR0713,27-TR0423,28-TR0384, 29-TR1327, 30-TR0399, Pen-Pendembu, Kam-Kambia, Nja-Njala

5.3.6 Winning Genotype and Mega Environment GGE biplot for Total Carotenoid Content

The GGE biplot show the response of the 30 evaluated pro vitamin A cassava genotypes for total carotene content across the three-test environment. The polygon view of biplot (Figure 5.5), showed that all the test environments fell into 3 of the 7 sectors on the biplot and thereby identified the 3 mega environments namely Pendembu (Pen), Njala (Nja) and Kambia (Kam). In the Pendembu environment TR 0365 (2) was the vertex genotype with entries TR1350 (13) and TR 0222 (21) TR 0747 (1) being the other members. The vertex genotype in environment 2 (Njala) was TR 1313 (10) followed by TR 0683 (8), and TR 0998 (22), while TR 0907 (15) was the vertex genotype in Kambia, the third mega environment.

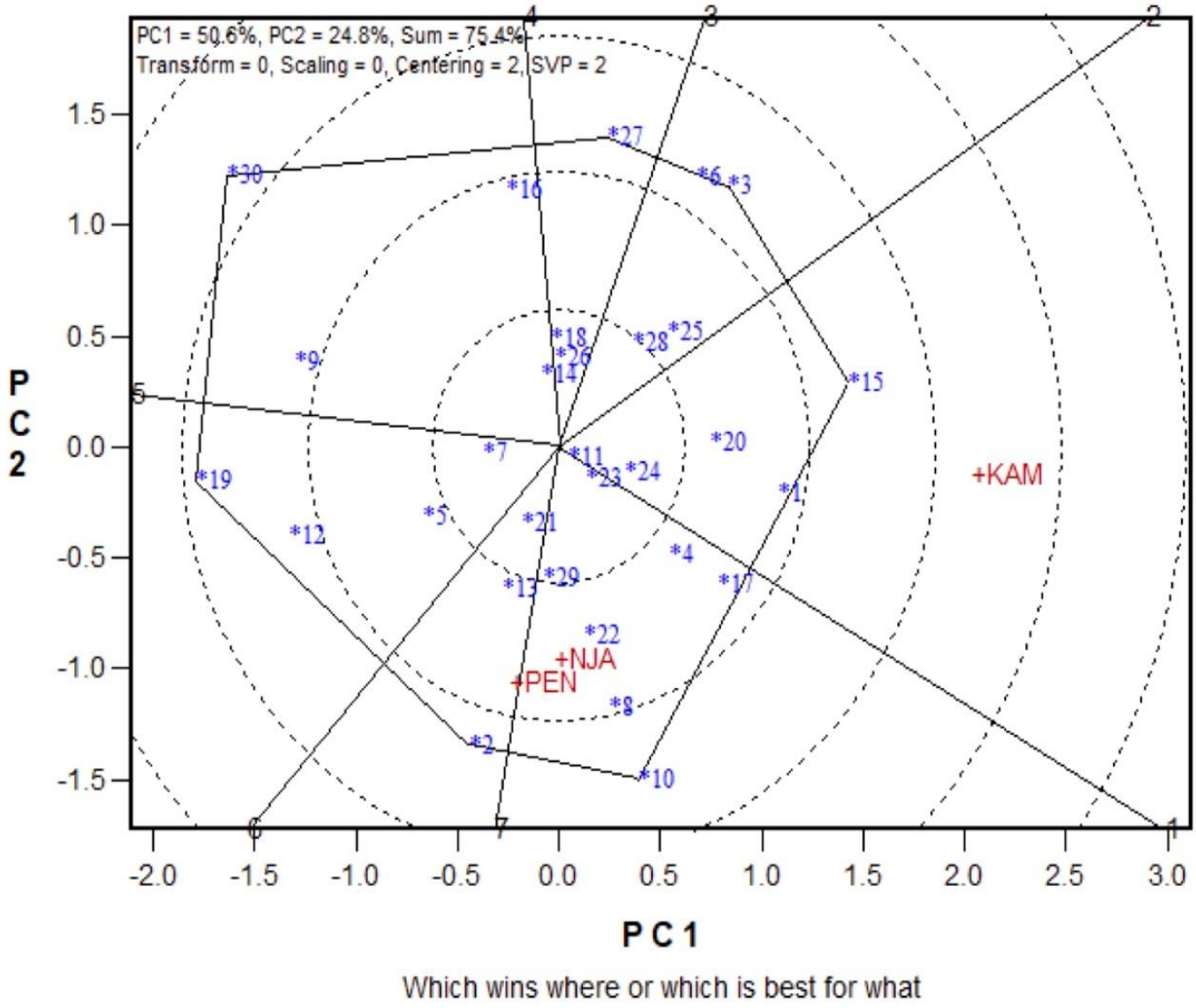


Figure 5.5: GGE biplot for total carotenoid content showing the best genotype among the 30 Provitamin A cassava genotypes in each Mega Environment

Key: 1-TR0747, 2-TR0365, 3-TR0560, 4-TR1208, 5-TR0461, 6-TR1337, 7-TR1569, 8-TR0683, 9-TR1198, 10-TR1313, 11-TR0696, 12-TR1322, 13-TR1350, 14-TR0843, 15-TR0907, 16-TR1557, 17-TR1152, 18-TR0232, 19-TR1279, 20 -TR0031, 21 - TR0222, 22 - TR0998, 23 - TR1755, 24 - TR1182, 25 - TR1753,26-TR0713,27-TR0423,28-TR0384, 29-TR1327, 30-TR0399, PEN-Pendembu, KAM-Kambia, NJA-Njala

5.3.7 Mean Performance and Stability for Total Carotenoid Content across three Environments.

Stability of total carotenoid/carotene content across the three environments is shown in Figure 5.6. TR 1313 (10) had the highest total carotenoid content followed by TR 1152 (17) and TR 0683 (8) and the genotype with the lowest total carotenoid content was TR 0399 (30). Other low performers included TR 1198 (9), TR 0423 (27) and TR 1557 (16). Of the 30 Pro-vitamin-A cassava genotypes evaluated for total carotenoid content,15 had values above the mean of the population.TR 1557 (16), TR 1152 (17), TR 1198 (9), TR 0843 (14) and TR 0461 (6) but neither of them were the highest performer for carotenoid content. Therefore, among the top ten performers TR 1313 (10), TR 0683 (8), and TR 0998 (22) were observed as being the most stable genotypes. The arrow on the AEC abscissa delineates the ideal genotypes and entry TR1313 (10) was the closest followed by TR 0683 (8) and TR 1152 (17).

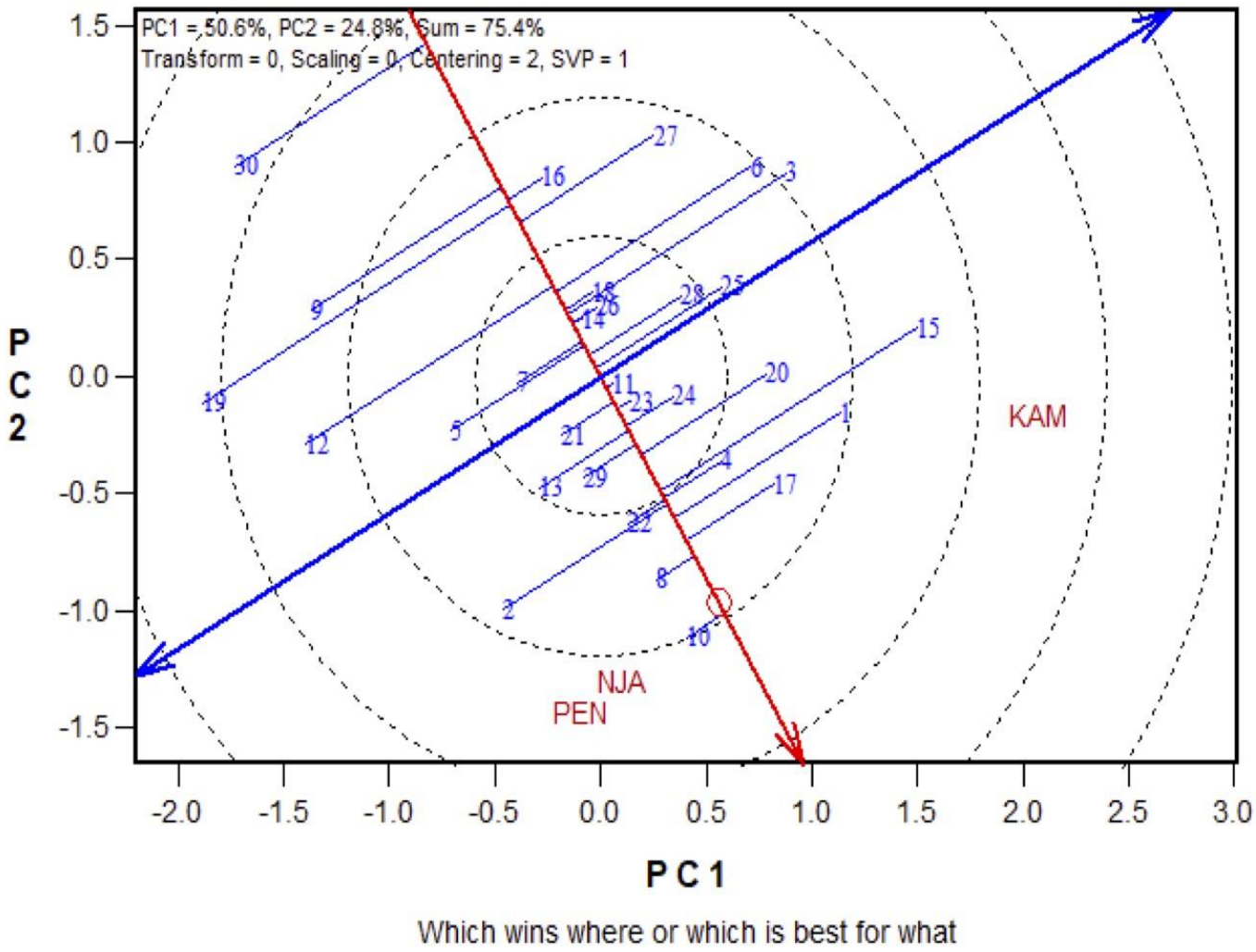


Figure 5.6 : Stability for total carotene content of 30 Provitamin- A cassava genotypes evaluated across three Environment.

Key: 1-TR0747, 2-TR0365, 3-TR0560, 4-TR1208, 5-TR0461, 6-TR1337, 7-TR1569, 8-TR0683, 9-TR1198, 10-TR1313, 11-TR0696, 12-TR1322, 13-TR1350, 14-TR0843, 15-TR0907, 16-TR1557, 17-TR1152, 18-TR0232, 19-TR1279, 20 -TR0031, 21 - TR0222, 22 - TR0998, 23 - TR1755, 24 - TR1182, 25 - TR1753,26-TR0713,27-TR0423,28-TR0384, 29-TR1327, 30-TR0399, PEN-Pendembu, KAM-Kambia, NJA-Njala

5.3.8 Discriminativeness vs Representativeness for Total Carotene Content across three Environments.

Figure 5.7 shows the discriminatory ability and representativeness of the test environments for total carotene production. The biplot identified Kambia with the longest vector, as the most discriminating while Pendembu and Njala did not differ much. Njala formed the smallest angle with the AEC abscissa and was therefore identified as the most representative of the test 3 environments.

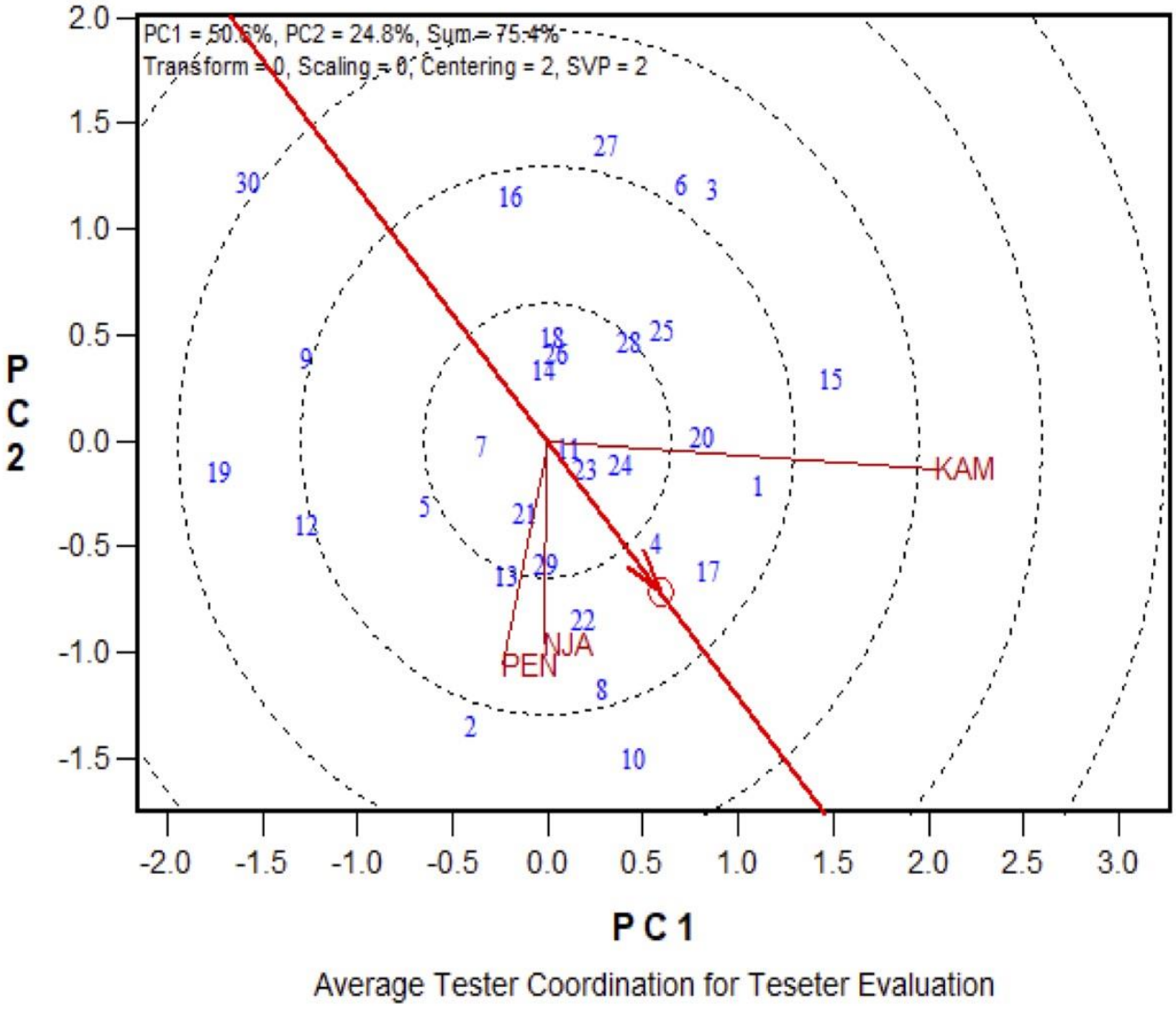


Figure 5.7: Discriminativeness vs representativeness for total carotenoid content of the 30 accessions in the 3 environments

Key: 1-TR0747, 2-TR0365, 3-TR0560, 4-TR1208, 5-TR0461, 6-TR1337, 7-TR1569, 8-TR0683, 9-TR1198, 10-TR1313, 11-TR0696, 12-TR1322, 13-TR1350, 14-TR0843, 15-TR0907, 16-TR1557, 17-TR1152, 18-TR0232, 19-TR1279, 20 -TR0031, 21 - TR0222, 22 - TR0998, 23 - TR1755, 24 - TR1182, 25 - TR1753,26-TR0713,27-TR0423,28-TR0384, 29-TR1327, 30-TR0399, PEN-Pendembu, KAM-Kambia, NJA-Njala

5.4 Discussion

The performance of cassava is subject to strong influence of genotype, environment and genotype*environment interaction (Aina *et al.,* (2007); Aina *et al*., (2009); Ntawuruhunga *et al.*, (2010). It is a routine practice for plant breeders to evaluate different genotypes in multi-locational trials to be able to compute the stability and performance of a genotype. The significant variability observed in the dry matter content (DMC) among the cassava genotypes presents improvement opportunities for the crop in Sierra Leone. The significance of G x E interactions for the dry matter content (DMC) revealed that genotypes responded differently across the tested environments.

An obvious deduction from the low environmental effect on TCC is that selection for the trait can effectively be achieved by evaluating target genotypes in one location.). Genotypic by environment was observed over the different environments as indicated by crossover performances for some of the genotypes. This led to variations in the mean ranks of the genotypes in the different environments (Dixon *et al*., (2000) and Marlosetti *et al.,* (2009). This implies different adaptation by the different genotypes suggesting the need to identify and select location specific genotypes for different environments

The GGE biplot permits identification of outstanding genotypes in each mega-environment "which-won-where" (Yan et al., 2000; Yan, 2001, 2002; Yan and Tinker, 2006. In this study the high percentage of total variation explained by the first and second principal component axes PC1 =57.99 and PC2=29.49, PC1 = 50.62 and PC2= 24.85 for both dry matter content and caroteniod content captured the largest variation while component 2 contributed below 30% of the observed variation.

Different genotypes excelled for dry matter and total carotenoid production in the three mega-environments, - Njala, Pendembu and Kambia. Genotypes TR 0365 (2), TR 1313 (10) and TR 0907 (15) were the vertex/best performing for total carotenoid content while TR 0384 (28), TR 0998 (22) and TR 1182 (24) were the best genotypes for dry matter content across the 3 mega environments. However, they can be recommended for specific environments where they performed well.

On the other hand, TR 0560 (3), TR 1337 (6), TR 1152 (17), TR1279 (19) and TR 0399 (30), fell into sectors that contained none of the tested locations for total carotenoid content while TR1208 (4), TR 1313 (10), TR 0696 (11), TR 0232 (18), and TR 1279 (19) fell into sectors that did not belong to any of the 3-test environment for dry matter content. They were therefore not the best in any of the 3-test locations and were probably the poorest in some of the locations tested for total carotenoid content and dry matter content respectively (Yan, 2001).

Entry TR 1182 (24) and entry TR 1313 (10) were identified as the best yielders while entry TR 0461 and TR 0399 were the lowest performing genotypes for total carotenoid content and dry matter content respectively. Genotypes TR 0560 and TR 0461 showed stability for dry matter predicting that these genotypes possess the ability to prevent substantial fluctuation in dry matter over a range of environmental conditions. TR 1208 (4) and TR 1337 (6) and TR 0461(5) and TR 1350(13) were the most stable genotypes for dry matter content. TR 1313 (10) and TR 1182 (24) were the most ideal for total carotenoid content and dry matter content. These genotypes were the most outstanding and responsive at the mega-environmental levels for total carotenoid content and dry matter content, respectively. From the study, Njala was ideal for selecting superior genotype for total carotenoid content while Pendembu seems to be the ideal environment for dry matter content. This finding agrees with (Olayiwola *et al.*, 2013) who

reported ideal genotypes from his studied population. Nonetheless, the best performing genotypes identified in this study could form the material for such genetic improvement through hybridization. DMC in the 30 genotypes studied here was less than that in varieties commonly grown by farmers in Sierra Leone. Typically, in a cassava variety selection scheme, screening involves no less than five candidate genotypes for total carotenoid content and dry matter content that are key drivers of variety adoption (Fakuda *et al.,* 2002, Owusu and Donkor, 2012, Abdoulaye *et al.*, 2014 and Esuma *et al.*, 2016). Therefore, a breeding programme targeting development and advancement of provitamin A rich cassava varieties could exploit on-station trial evaluation or assessment in identifying carotene-rich genotypes that can be subjected to multi-locational evaluations where focus shifts to other traits highly influenced by environmental effects. This strategy would save costs while increasing precision to identify best performers for root yield, carotenoid content and dry matter content.

5.5 Conclusion

The environment and genotype x environment interaction for the two studied traits (dry matter and total carotenoid content) of the 30 provitamin A genotypes showed significant differences ($P < 0.001$). To realize gains in translating investments in cassava bio-fortification research into impact on human nutrition, breeding efforts will need to focus on advancing varieties that combine high levels of both DMC and TCC alongside high-yielding genetic backgrounds. Nonetheless, the best performing genotypes identified in this study could form the material for such genetic improvement through hybridization in Sierra Leone.

DMC value in the 30 provitamin A genotypes were less than that in the local cultivar (Cocoa) commonly grown by farmers in Sierra Leone. With respect to each trait studied, genotypes

showed differences in performance in specific environments. Genotypes TR 1182 and TR 1313, were the highest performers for dry matter and total carotenoid content. For the dry matter content alone genotypes TR 0683, TR 1152, TR 1208, TR 0365, and TR 1182 were the best. The identified superior genotypes could be used as parents for breeders to improve other accessions for increased carotenoid concentration and dry matter contents in provitamin A cassava varieties in Sierra Leone.

CHAPTER SIX

6.0 PERFORMANCE OF F_1 CROSSES FOR TOTAL CAROTENOID, PROTEIN, IRON AND ZINC CONTENTS OF STORAGE ROOTS

6.1 Introduction

Cassava is primarily a carbohydrate source that is often considered a low-quality food, when compared to most cereals (Nganga, 2010), and of some major traditional staples (Chavez *et al.,* 2004). This is probably attributable to the low vitamins, protein and other nutrients and micronutrients content of its storage roots. Micronutrients which include folic acid, vitamin A, iodine, iron and zinc are required in minute quantities for human health, growth and development (Baafi *et al.,* 2016b). They play crucial roles in metabolism and maintenance of tissue function (Shenkin, 2006 and Baafi *et al.*, 2016b). Micronutrient deficiencies are chronic deprivation of these nutrients and constituted an alarming public health problem adversely affecting one third of the population globally (Darnton-Hill *et al.*, 2005 and Simon *et al.,* 2013). Micronutrient deficiencies have been estimated to cost sub- Saharan African economies more than $2.3 billion in lost productivity (UNICEF, 2004). WHO (2009) reported that almost 100 million preschool children suffer from vitamin A deficiency worldwide. Vitamin A deficiency affects about one-third of preschool children globally, of which 1% develop of these results to night blindness (West, 2002; Howe and Tanumihardjo, 2006; and WHO, 2009), while anemia, caused by inadequate intake of iron, and has affected 1.6 billion people globally (De Benoist *et al.,* 2008; WHO, 2014). Severe and extensive deficiencies also prevail for zinc (Baafi *et al.,* 2016b). These nutrient deficiencies have been of greatest global health concern.

According to the WHO (2009), three possible interventions to reduce nutritional deficiencies in humans have been established: (1) improving vitamin A, protein and nutrients availability through a diversified diet, (2) increasing the consumption levels of micronutrients by biofortification of staple foods, and (3) supplementation through vaccines. The use of vaccine is not sustainable and can hardly reach all affected persons especially those in rural communities where cassava is consumed as a major staple. However, sustainable solutions to malnutrition can be developed through linking agriculture, nutrition and health. Cassava can be improved through biofortification to increase its provitamin A, protein and micronutrient contents (HarvestPlus, 2002). This is an impressive step in developing a food systems approach to reducing malnutrition. This approach provides solution to the root causes of micronutrient malnutrition, targeting the marginalized people, involves built-in delivery mechanisms, and is scientifically feasible and cost-effective (HarvestPlus, 2002). As part of an integrated food systems approach, biofortification represents the best means for enabling rural households to improve family health and nutrition in sustainable ways (HarvestPlus, 2002).

Cassava is a vegetatively propagated crop however the crop that undergoes natural outcrossing and is amenable to controlled crossing for genetic improvement. Improving vitamin- A, protein, iron and zinc content in cassava cultivars with traits preferred by farmers and consumers through biofortification (Dixon *et al.*, 2005), would go a long way towards preventing vitamin-A, protein, iron and zinc deficiencies in Sierra Leone where cassava is largely consumed as the second major staple after rice. The development of biofortified cassava storage root varieties provides a cheap source of provitamin- A precursors (total carotenoid content), protein and minerals that are essential for the supply of quality food for better nutrition. This will properly address vitamin A and other nutritional deficiencies and ensure food security.

Therefore, the objective was to evaluate the levels of total carotenoid content, protein, iron and zinc contents in F_1 cassava progeny to identify elite clones and potential sources of germplasm for nutritional improvement in cassava.

6.2 Materials and Methods

Table 6.1 Pedigree and characteristics of breeding lines

Genotype	Sex	Pedigree	Characteristics
IITA-TMS-IBA011368	F	940561 X 940263	Vitamin A, early bulking, high root yield
IITA-TMS-IBA 120004	F	IBA011224 X IBA011368	Vitamin A, early bulking, high root yield
IITA-TMS-IBA 91/00416	F	91934 X 63397	Zinc, high dry matter, resistant to ACMD, high root yield
IITA-TMS-IBA088693	F	05/0303 X 06/0657	Protein, resistant to ACMD, high root yield
IITA-TMS-UBJ 120003	M	Not Available	Vitamin A, early bulking, high root yield
IITA-TMS-IBA 96/1165	M	O88/00210 HS	Iron, high dry matter, resistant to ACMD, high root yield
IITA-TMS-IBA088747	M	FBA-1 X 05/0561	Protein, resistant to ACMD, high root yield
MM96/81791	M	Not Available	Zinc, high dry matter, resistant to ACMD, high root yield

6.2.1. Development of F_1 progenies

Three breeding lines with high beta-carotene, two with high protein and zinc content and 1 with high iron contents were intercrossed to generate genetic materials (Table 6.1). The crossing block was established at Ubiaja, Nigeria in May 2015. The soil within this location comprises sandy, clay loam. Ubiaja is characterized by a bimodal rainfall pattern, with two distinct rainy seasons and dry seasons of nearly equal length. Ubiaja is an ideal location for cassava breeding program as most cassava genotypes flower at the location. The crossing block trial was laid out at the randomized complete block design in two replications with ten stands per genotypes been planted.

Controlled pollination using diallel mating design was undertaken starting three months after planting for period of 2-months according to the standard procedure described by Kawano (1980). Pollination bags were used to enclose flowers about to open to prevent uncontrolled pollination. Pollen was collected in the morning (7 to 8 am) and pollination was done later in the day between (11 am to 2 pm). Pollinated flowers were tagged and labelled. After pollination, the female flowers were covered with the pollination bags for one week. Harvesting and collection of seeds were undertaken two months after pollination. Seeds were sorted, labeled and stored till the time of seedling nursery establishment.

Table 6.2 Number of F_1 seeds generated, planted and (*germinated*) per family

		Female (♀)			
		IBA120004	IBA91/00416	IBA011368	IBA088693
Male (♂)	UBJ120003	300 (*230*)		40 (*8*)	
	MM96/81791		78 (*62*)		
	IBA96/1165			200 (*200*)	
	IBA088747				250 (*167*)

NB: Values in brackets are the number of seeds that germinated in each cross combination

6.2.2 Establishment of seedlings F_1 progenies Nursery evaluation

A total of 868 seeds obtained from parental cross combinations were sown directly into a well-prepared soil following a flotation test in June 2016 for one cropping season (2016-2017) (Table 6.2). At Foya crop site Njala, in Sierra Leone under a transitional rain forest agro climatic zone seeds were sown at a spacing of 0.30 m x 1 m using serpentine arrangement in an RCBD in two replications. An alley of 2m was established between blocks to reduce inter-block plant competition. Cocoa, a local variety was planted around the trial to ensure uniform exposure to African Cassava Mosaic Disease. NPK 20-10-10 fertilizer, was applied eight weeks after planting at the recommended rate of 450 kg/ha. The trial was conducted under rain fed

conditions. Field maintenance was done as required. Harvesting was done at 12 MAP per individual cross combination.

6.2.3 Data Collection

Percentage fruit sets were determined by dividing total number of fruits collected with total number of flowers pollinated (Table 6.1). Number of seeds generated per cross are presented in Table 6.2. Cassava is highly heterozygous which makes each cross between two different parent plants genetically distinct with large variation in the ensuing F_1 families. Families comprised $868F_1$ progenies from five crosses. The field was maintained by hand weeding as and when necessary.

At harvest, data was collected on a set of traits according to the standard procedure outlined by Fukuda *et al*. (2010). During harvesting data were collected on the following parameters number of roots per plant, pulp color of root, inner color, outer color and root size. Storage root samples were selected form harvested plant stand with storage roots. Storage root samples with different sizes were randomly selected for determination of total carotenoid, protein, iron and zinc contents.

Selected storage root samples with roots from 3 cm or more in diameter, without cracks, insect damage or rotten parts were sent to the food science and nutrition laboratory of Njala Agricultural Research Centre Njala, for sample preparation for total carotenoid content analysis. For protein, iron and zinc analyses, the harvested storage roots samples were shipped to crop utilization laboratory IITA, Ibadan for analysis. About 200g of the dried storage root samples were ground into fine powder using a benzene 750 watts' electric blender for analysis. Crude

percent Protein analysis was done at the crop utilization laboratory IITA, Ibadan, while samples for iron and zinc determination were shipped to the Microchem laboratory, Pretoria, South Africa for analysis.

6.2.4 Total carotenoid determination

Total carotenoids content was estimated following IITA standard operating laboratory procedure using iCheck device. Procedure for estimation of total carotenoids in cassava using iCheck device as described in Section 4.2.7 (page 68).

6.2.5. Determination of Crude Protein

Procedure for estimating percent crude protein followed the IITA standard operating laboratory procedure in the food science laboratory using Kjeldahl procedure. Two grams of dried milled cassava flour was weighed into a digestion tube, one tablet of selenium catalyst, 5ml of conc 80 % sulphuric acid and 5ml of conc hydrogen peroxide as antifoaming agent were added and placed in a digestion block preheated at 420^0C in a fume cupboard. After digestion, the tube was cooled and transferred to the Automated Kjeldahl distillation apparatus (Kjeltec 8400). The digest was diluted with distilled water and equal volume of 40% NaOH was added and thereafter steam distilled into a receiver flask containing 4% boric acid solution, 3 drops of methyl red indicator and bromocresol green. A total of 50ml distillate was collected and titrated with normal hydrochloric acid solution using colorimetric end-point detection for estimation of the total Nitrogen. Samples were analyzed in duplicate. Three random samples were selected from the total batch of samples and re-analyzed to check on the accuracy and reproducibility of the method. The average values were recorded. The Nitrogen content was calculated using the formula

$$\% \text{ Nitrogen} = \frac{(\text{Va} - \text{Vb}) * \text{ Normality of HCL } x \text{ } 1.4007 \text{ } x100}{\text{Sample Weight}}$$

Where

V_a = Volume, in mL, of standard HCl required for sample

V_b = Volume, in mL, of standard HCl required for blank

% Crude Protein (CP) = % Nitrogen *x* 6.2

6.2.6 Iron and Zinc determination

These were carried out at the Microchem laboratory, Pretoria, South Africa. Being a private company, protocols for their analyses were not provided.

6.2.5 Data Analysis

Descriptive statistics were computed using XLSTAT (2010) and Genstat.v12.

6.3 Results

6.3.2 Descriptive analysis of F_1 progenies

Table 6.3 shows the performance of the 667 germinated F_1 progenies evaluated in the seedling nursery trial from five crosses. The summary analysis for the F_1 progenies showed differences in mean values of the quality traits measured. The coefficient of variation (CV) ranged from 14.8 – 58.6 %, for the quality traits.

Table 6.3 Descriptive statistics for nutritional quality of F_1 progenies of root quality

Summary Statistics	Traits			
F1 progenies assessed	Total Carotenoid Content (µg $^{-1}$)	Protein (%)	Iron (PPM)	Zinc (PPM)
Mean	14.7	5.4	12.6	8.5
Minimum	6.0	4.2	4.5	4.5
Maximum	28.0	8.1	59.2	17.7
Standard error of mean	0.5	0.1	0.6	0.5
Coefficient of variation (%)	34.7	14.8	58.6	37.5

6.3.3 Variation in total carotenoid content in roots of F_1 progeny

The performance on total carotenoid content of the F_1 progeny is presented in (Table 6.4). Progenies 13 and 33 from cross IITA-TMS-IBA120004 x IITA-TMS-IBA120003 recorded the highest 28.0 μg^{-1} and lowest 6.0 μg^{-1} values of total carotenoid content with a grand mean of 14.7 μg^{-1}. Ten progeny had total carotenoid values higher than the mean.

Table 6.4 Variation total carotenoid content of F_1 progenies using the color chart and i-check device

F_1 progeny No (IITA-TMS-IBA120004 x IITA-TMS-IBA120003)	**TCC (μg^{-1})**	**F_1 progeny No (IITA-TMS-IBA120004 x IITA-TMS-IBA120003)**	**TCC (μg^{-1})**
1	13.5	31	12.7
2	12.6	32	12.7
3	14.9	33	6.0
4	12.3	34	12.8
5	13.8	35	7.5
6	13.0	36	15.0
7	14.9	37	16.9
8	16.9	38	6.6
9	18.2	39	6.4
10	13.6	40	7.4
11	12.5	41	15.7
12	13.3	42	11.8
13	28.0	43	7.6
14	21.2	44	7.1
15	14.2	45	7.3
16	12.9	46	6.2
17	13.3	47	7.1
18	13.0	48	6.7
19	19.5	49	9.1
20	13.6	50	10.4
21	10.9	51	10.0
22	13.1	52	6.4
23	14.2	53	7.9
24	14.8	54	8.6
25	14.1	55	9.1
26	14.4	56	7.3
27	12.7	57	8.8
28	13.2	58	7.1
29	14.4	59	16.4
30	14.8	60	9.4
		Grand Mean	**14.7**

6.3.4 Mean protein content (%) in roots of F_1 progeny

Progeny numbers 41 and 12 from cross IITA-TMS-IBA 088693 x IITA-TMS-IBA 088747 recorded the highest 8.1% and lowest 4.2% percent protein respectively with a grand mean of 5.4% (Table 6.5). Twenty four out of the 58 harvested progeny had levels higher than the mean with values ranging from 5.5 to 8.1%.

Table 6.5 Mean percent protein of F_1 progeny (evaluated in seedling nursery trial in Njala)

F_1 progeny No (IITA-TMS-IBA 088693 x IITA-TMS-IBA 088747)	**Protein (%)**	**F_1 progeny No (IITA-TMS-IBA 088693 x IITA-TMS-IBA 088747)**	**Protein (%)**
1	6.0	31	4.9
2	4.5	32	8.1
3	7.3	33	6.3
4	4.5	34	4.9
5	5.5	35	5.6
6	6.9	36	4.9
7	5.8	37	4.4
8	4.2	38	4.2
9	5.4	39	5.4
10	4.8	40	6.1
11	4.8	41	4.3
12	5.2	42	4.2
13	6.0	43	5.5
14	5.6	44	4.6
15	5.0	45	6.1
16	5.4	46	5.2
17	5.5	47	6.4
18	6.0	48	5.4
19	5.4	49	4.8
20	6.1	50	5.0
21	4.3	51	5.3
22	4.9	52	4.8
23	6.3	53	5.5
24	6.1	54	5.8
25	4.9	55	4.8
26	5.9	56	5.2
27	6.6	57	5.9
28	4.3	58	5.5
29	5.1		
30	5.1		
		Grand Mean	**5.4**

6.3.5 Mean Iron content (ppm) in storage roots of F_1 Progeny

Iron concentration of progeny from cross IITA-TMS-IBA 96/1165 x IITA –TMS-IBA 011368 ranged from 45.0 ppm to 59.2 ppm for harvested progeny (Table 6.6). Sixty-one progeny had higher levels than the grand mean (12.6 ppm), of which 22 had levels above 20.0 ppm.

Table 6.6 Mean iron concentration of F_1 progeny

F_1 progeny No (IITA-TMS-IBA 96/1165 x IITA – TMS-IBA 011368)	**Concentration (ppm)**	**$F_{1\ progeny}$ No (IITA-TMS-IBA 96/1165 x IITA – TMS-IBA 011368)**	**Concentration (ppm)**	**F_1 progeny No (IITA-TMS-IBA 96/1165 x IITA – TMS-IBA 011368)**	**Concentration (ppm)**	**F_1 progeny No (IITA-TMS-IBA 96/1165 x IITA –TMS-IBA 011368)**	**Concentration (ppm)**
1	9.9	26	8.4	51	32.2	76	13.1
2	7.8	27	7.2	52	18.8	77	11.8
3	4.5	28	16.7	53	17.8	78	7.2
4	12.4	29	11.2	54	17.2	79	14.4
5	4.7	30	12.4	55	7.5	80	15.8
6	20.3	31	18	56	15	81	10.4
7	9	32	25.6	57	7.3	82	15.6
8	21.9	33	13.9	58	8.7	83	11.3
9	11.2	34	17.9	59	14.9	84	8.2
10	11.6	35	9.5	60	7.6	85	4.7
11	16.2	36	23.7	61	9.2	86	10.4
12	5.8	37	5.8	62	10.2	87	9.9
13	9	38	14.7	63	5.9	88	28.9
14	6.9	39	16.8	64	13.9	89	13.2
15	7.6	40	12.9	65	9	90	10
16	10.9	41	12.7	66	7	91	17.6
17	7.2	42	21.4	67	7.8	92	20.4
18	8.8	43	14.6	68	7.5	93	25.1
19	6.4	44	9.7	69	23.7	94	7.3
20	8	45	7.9	70	12.2	95	16.8
21	9.1	46	6.1	71	14.6	96	15.5
22	7.4	47	17.5	72	23.1	97	12.4
23	16.3	48	24.6	73	30.1	98	38.5
24	11.1	49	17.4	74	6.2	99	11.7
25	7.5	50	11.7	75	10.6	100	17.7
101	10.9	117	45	133	7.6	149	17.3
102	14.2	118	16.3	134	8.9	150	10.4

Table 6.7 Mean iron concentration of F_1 progeny (cont'd)

F_1 progeny No (IITA-TMS-IBA 96/1165 x IITA – TMS-IBA 011368)	**Concentration (ppm)**	**F_1 progeny No (IITA-TMS-IBA 96/1165 x IITA – TMS-IBA 011368)**	**Concentration (ppm)**	**F_1 progeny No (IITA-TMS-IBA 96/1165 x IITA – TMS-IBA 011368)**	**Concentration (ppm)**	**F_1 progeny No (IITA-TMS-IBA 96/1165 x IITA –TMS-IBA 011368)**	**Concentration (ppm)**
103	7.8	119	24.2	135	7.8	151	9.8
104	6.8	120	29.9	136	10	152	11.2
105	15.6	121	59.2	137	9.1	153	8.1
106	9.2	122	22.2	138	6.9	154	13.1
107	11.6	123	18	139	7.7	155	7.7
108	10.8	124	15.9	140	9.8	156	8.9
109	9.7	125	34.2	141	8.9	157	8.7
110	8.7	126	33.8	142	8.5	158	9.1
111	12.4	127	16	143	9.3	159	6.6
112	7.7	128	7.8	144	17.2	160	10.5
113	11.3	129	18.4	145	9.6	161	8.4
114	20.4	130	30.6	146	7.1	162	9.3
115	22.4	131	13	147	8.6		
116	29.8	132	10.9	148	7.2		
						Grand Mean	**12.6**

6.3.6 Mean zinc content (ppm) in storage roots of F_1 progeny

The zinc content of F_1 progeny from cross MM96/81791 x IITA-TMS-IBA 088747 ranged from 4.5 ppm to 17.7 ppm (Table 6.7). Fourteen progeny recorded higher values than the grand genotypic mean (8.5 ppm) while 20 progeny recorded values that were lower than the grand mean.

Table 6.7: Mean zinc concentration of F_1 progeny

F_1 progeny No (MM96/81791 x IITA-TMS-IBA 088747)	**Concentration (ppm)**
1	14.9
2	10.9
3	8.8
4	6.8
5	15.4
6	9.6
7	4.8
8	9.5
9	7.3
10	11.1
11	8.0
12	10.3
13	4.5
14	7.1
15	9.3
16	5.4
17	5.8
18	5.3
19	6.0
20	6.6
21	8.6
22	12.1
23	5.9
24	9.2
25	7.1
26	8.2
27	6.2
28	8.1
29	6.7
30	7.2
31	10.3
32	4.5
33	17.7
34	5.6
Grand Mean	**8.5**

6.4. Discussion

Performance of the F_1 progenies from different crosses showed a high variation in root nutrient which offers range of genetic variations. Similar findings were reported by Nganga (2010), Micheal *et al*. (2015) and Akuwa. (2016). Biochemical analysis on the F_1 progenies revealed a high variation in the root nutrient quality traits (total carotenoid, protein, iron and zinc contents) for which genotypic selection can be applied. In addition, it is the performance of the individual F_1 or clone that cassava breeders are most interested in because, it forms the basis for plant breeders to build efficient breeding programme in a crop like cassava (Tumuhimbise *et al.*, 2014; Micheal *et al.*, 2015; Akuwa, 2016 and Esuma *et al*., 2016).

Total carotenoid content in the F_1 progeny ranged from 6.0 μg^{-1} to 28.0 μg^{-1} with mean of 14.7μg^{-1}. The observed mean of the total carotenoid content of the F_1 progenies was comparable to the mean 14.7 $\mu g\ g^{-1}$ reported for populations developed at CIAT (Ceballos *et al.*, 2013). The high level of total carotenoid content revealed in cassava breeding populations at CIAT is a result of several years of cyclic selection processes exploited to primarily improve the total carotenoid levels in cassava to levels above 15 μg^{-1}. IITA's cassava breeding program has benefitted from the effective germplasm and seed exchange program with CIAT, thus benefited from clones of carotenoid levels above 15 μg^{-1}. There is a high probability that parents used in hybridization are related to the CIAT germplasm. The mean carotenoid content was higher than findings of Maroya *et al.* (2012) and Ssemakula and Dixon. (2007), who reported 3.6 μg^{-1} and 5.0 μg^{-1} for cassava breeding populations evaluated at IITA and that of Esuma *et al*. (2016 where a mean of 3.8 μg^{-1} was obtained for population in Uganda.

There were progenies with high levels of crude protein that were above the 2% threshold value (Nganga, 2010). These high levels of crude protein content have also been reported in some landraces and improved varieties of cassava in CIAT (Chavez *et al.,* 2005). The authors suggested that the variation in crude protein content are high genetic in nature which could be exploited for improving the protein content of cassava. In another study in CIAT by Ceballos, (2006b) large differences were observed in crude protein content of cassava roots ranging from 0.95% to 6.42%. He suggested that a considerable proportion of these differences are genetic in nature and therefore are excellent possibilities for exploiting these differences and further increasing them by traditional breeding methods. However, I'm suggesting the use of a different analytical method which is not based on the use of nitrogen since the cassava samples that are analysed in most studies even though dry, still have locked nitrogen in hydrogen cyanide that gives false high levels of protein. Nganga (2010) also reported a similar trend of high levels of percent crude protein ranging from 1.35% to 3.45%.

Some of the F_1 progeny from the cross combination IITA-TMS-IBA 91/00416 x MM96/81791 had high zinc content (8.4 ppm), but lower than the findings of Nganga (2010) who reported above 64.0 ppm from one of his test sites in Kenya. CIAT (2006) reported that higher levels of zinc positively correlated with the beta carotene conversion to vitamin A when high carotenoid cassava cultivars are consumed. Chavez *et al*., (2005) have also reported that there is genotypic variation for zinc content in cassava.

Also, some of the F_1 progeny from cross IITA-TMS-IBA 96/1165 x IITA–TMS-IBA 011368 gave higher levels of iron but less than values observed in cassava collections from Meso America (Chavez *et al.*,2005), for collections from Nigeria (Dixon *et al.,* 2005) and for Kenyan collections (Nganga 2010).

Dixon *et al.*, (2000) reported values ranging from 4 to 19 ppm for iron and 4 to 8 ppm for zinc that were similar to the ranges observed in the present study as they also observed a significant positive correlation between iron and zinc. Baafi (2016a) reported strong positive genotypic association among total carotenoid content, iron and zinc indicating sufficient variability for these traits in sweet potato. The observation from this study showed that F_1 progenies developed from this study could also serve as useful parents or source in any biofortification programmes.

6.5 Conclusion

Genetic variation existed among the cassava F_1 progenies for total carotenoid content, protein, iron and zinc contents. The observed values for total carotenoid, protein, iron and zinc contents in the F_1 progenies indicate their potential for improving the nutritive value of cassava in Sierra Leone. Higher levels of micronutrients which are favorable for the human diet were recorded for some progenies evaluated within this population. An important aspect of enhancing micronutrient levels in cassava roots is maintaining a good agronomic background of micro nutrient-rich genotypes (Dixon *et al.*, 2005).

CHAPTER SEVEN

7.0. GENETIC STUDIES ON MEALINESS, DRY MATTER, ROOT NUMBER AND FRESH ROOT YIELD, IN CASSAVA (*Manihot esculenta* Crantz)

7.1. Introduction

Breeding for increased mealiness, number of roots, dry matter content and good fresh root yield enhancing the production of cassava to meet consumer acceptance for cassava storage root and its products has become the next challenge in cassava breeding in Sierra Leone. These traits are essential for cassava production as they are the most preferred traits for end-use consumption. Although other breeding mating designs had been successfully used for cassava, they are limited in that, they do not provide information for estimation of specific combining ability (SCA), which is important in the inheritance of key traits such as number of storage roots and fresh root yield (Ceballos *et al.*,2004, 2015; Crossa *et al.,* 2010). Subsequent shifts in cassava breeding schemes have seen an increased production of full-sib progenies (Ceballos *et al.,* 2012; Nassar and Ortiz, 2006). The selection of suitable parents and good mating designs are keys to successful breeding schemes.

The importance of mating designs in cassava breeding cannot be neglected as it provides information on the genetic control of the character under investigation, generates a breeding population to be used as a basis for the selection and development of potential varieties, estimating the genetic gain and provides information for evaluating the parents used in breeding program (Acquaah, 2012).The full-sib crossing schemes employ controlled pollinations, where selected mating designs are used to generate families from specific parental combinations,

facilitating genetic studies alongside production of breeding populations with traits of interest (Esuma *et al*., 2016, Nduwumuremyi *et al.*, 2013).

The diallel mating design, particularly, has become popular for cassava breeding simply because it facilitates generation of useful information on genetics of key agronomic traits and allows identification of parents with superior combining ability for developing breeding populations (Kulembeka *et al.*, 2012; Parkes *et al.*, 2011; Tumuhimbise *et al.*, 2014; Zacarias and Labuschagne. 2010 and Akuwa, 2016). It is this genetic information that guides breeders to deploy appropriate strategies for crop improvement (Acquaah, 2012; Nduwumuremyi *et al.*, 2013).

Knowledge of general combining ability (GCA) of parental lines is particularly helpful for predicting genetic gains in a breeding program (Falconer and Mackay, 1996). Hayman (1954) elaborated on the procedure for statistical analyses based on diallel data, which partitions total variation into GCA of the parents and SCA of crosses. Combining ability could be described as the relative potentials of an inbred line or a clone, when allowed to mate with another inbred line or clone, to transmit desirable traits or specific trait to the next generation (Chaudhari, 1971). It facilitates the prediction of the behavior of a line when exploited as a progenitor in a hybrid and compliments the selection of superior breeding lines for hybrid combination and for studying the nature of genetic variation). Griffing (1956) reported a method of analyzing combining abilities using the genetic estimates of the parent and hybrid components of diallel analysis through general and specific combining abilities. Falconer and Mackay (1996) described general combining ability (GCA) as the mean performance of the progenitors in all its crosses and it is expressed as a deviation from the mean of all crosses. This average behavior of parents in

crosses (GCA), calculates the breeding value of a given genotype because of additive gene effects (Ceballos *et al.,* 2004; Micheal *et al*., 2015).

Specific combining ability (SCA) is described as the deviation of individual crosses from the average performance of parents, because of the presence of dominance effects. Understanding the expression of gene action would be important for formulating breeding techniques to generate and develop desired traits. Therefore, information on combining abilities are required to identify suitable and superior progenitors and genotypes which can be hybridized for the development of elite cultivars and progenies varieties that would ultimately ensure sustained production and productivity by subsistence farmers in Sierra Leone.

The cassava breeding programme in Sierra Leone has been evaluating half sib seeds and clones sourced from the cassava breeding unit in IITA. Ibadan. In Cassava breeding, various mating designs and arrangements are exploited by breeder to generate improved cassava genotypes or varieties. The present study is probably the first breeding research undertaken for Sierra Leone. Currently cassava varieties released in Sierra Leone are white or cream fleshed with little amount of high dry matter, mealiness and increased yield. To employ suitable breeding strategies for genetic improvement of economically important traits which are quantitatively inherited, diallel experimental design was used for this study.

The objectives of the study were to:

i) estimate the combining abilities of twelve cassava genotypes for mealiness, dry matter content, number of roots and fresh root yield.

ii) determine the gene action for mealiness, dry matter content, number of roots and fresh root yield, and

iii) identify and select superior families for the development of elite clones.

7.2. Materials and Methods

7.2 1 Establishment of crossing block

The crossing block was established in May 2015 in Ubiaja, Edo state, Nigeria. The soil within this location comprises sandy clay loam. Ubiaja has a bimodal rainfall pattern, with two distinct rainy seasons and dry seasons of nearly equal length. The peak rainfall occurs between April to May and September to November. Twelve cassava parents (Table 7.1) were selected based on mealiness, dry matter content, pest and disease resistance, plant architecture, flowering ability and fresh root yield from the IITA genetic gain as well as landraces from Ghana. The 12 genotypes (Table 7.1) were crossed in a 12 x 12 diallel mating design without reciprocals and selfs. Controlled pollinations were undertaken three months after planting. Hand weeding was performed as required. Controlled hand pollination was carried out according to the standard procedure described by Kawano (1980) (See Section 6.2.1). Total seeds obtained was 5,382 (Table 7.2)

Table 7.1 Twelve cassava genotypes used as parents for F_1 progenies, their pedigree and important traits

Genotype	Pedigree	Traits
IITA-TMS-IBA 120003		Vitamin A, early bulking, high root yield
UCC 20012 (246)		High dry matter/Mealy, resistant to ACMD, high root yield
IITA-TMS-IBA 693		High dry matter/Mealy, resistant to ACMD, high root yield
TMEB 419		High dry matter/Mealy, resistant to ACMD, high root yield
TMEB 1		High dry matter/Mealy, resistant to ACMD
MM96/8179		Zinc, high dry matter, resistant to ACMD, high root yield
IITA-TMS-IBA 120004	IBA011224 X IBA011368	Vitamin A, early bulking, high root yield
96/1165	O88/00210 HS	Iron, high dry matter, resistant to ACMD, high root yield
91/00416	91934 X 63397	Zinc, high dry matter, resistant to ACMD, high root yield
IITA-TMS-IBA088747	FBA-1 X 05/0561	Protein, resistant to ACMD, high root yield
IITA-TMS-IBA088693	05/0303 X 06/0657	Protein, resistant to ACMD, high root yield
IITA-TMS-IBA011368	940561 X 940263	Vitamin A, early bulking, high root yield
IBA - Ibadan; IITA-International Institute of Tropical Agriculture; TMS - Tropical Manihot Selection;		

NB- Parent1-UBJ120003: Parent2- UCC2001 (246): Parent3- TMEB693:Parent4- TMEB419: Parent5-TMEB1: Parent6- MM961781: Parent7-IBA120004: Parent8- I961165: Parent9- I9100416: Parent10 – I088747: Parent11-I088693: Parent12- I011368

Table 7.2: Seeds obtained from 12 x 12 half diallel cross of cassava genotypes and number of seeds produced

Female	UB120003	UCC2001(246)	TMEB693	TMEB419	TMEB 1	MM961871	IBA120004	IBA961165	I9100416	I088747	I088693	I011368
Male												
UBJ120003		77	71	148	20	46		96	54	75	100	100
UCC2001(246)			13	148	10	29	93	150	95	100	150	151
TMEB693				151	30	76	78	59	44	150	90	75
TMEB419					21	29	75	70	35	98	75	7
TMEB1						75	23	64	38	100	250	86
MM961871							60	114	72	100	88	100
IBA120004								150	56	83	106	100
I961165									67	100	84	150
I9100416										21	45	18
I088747											143	200
I088693												124
I011368												

7.2.2 Seedling Nursery evaluated at Foya crop site

A total of 40 seeds were randomly selected after germination test from each of the 65 F_1 families or cross combination. Seeds were planted at a spacing of 0.30 cm x 1 m in June, 2016 in two replications at Foya crop site Njala, representing the transitional rain forest agro climatic zone in Sierra Leone (Van Vuure *et al*., 1974; Odell *et al*., 1974) for one cropping season (2016--2017). The trial was established in a randomize complete block design using serpentine design within the 2 replications in three blocks each. The 'Cocoa' cassava variety (an ACMV susceptible variety) was planted around the trial to ensure screening for ACMD. Fertilizer, NPK 20-10-10 was applied 8 weeks after planting at the recommended rate of 450 kg/ha. The blocks were separated by 2m alleys. The trial was conducted without supplementary irrigation, and field maintenance was undertaken throughout the period of evaluation as necessary.

7.2.3 Data Collection

At harvest data were collected on some yield related (Table 7.3) traits per the standard procedure outlined by Fukuda *et al*. (2010). Evaluation was done on 40 plants per family.

Table 7.3 Phenotypic traits used in the characterization of cassava F_1 Progeny

Traits	Procedure	Time of evaluation	Mode of Scoring	Remarks (phenotypic class)
Number of harvested roots per family	Record the number of roots harvested per plot of each clone.	12 MAP (at harvest)	count	Number of stand harvested and counted
Number of storage root	Record the number of plant stand harvested	12 MAP (at harvest	Count	Number of roots harvested and counted
Number of root per plant	The most frequent occurrence was observed and recorded.	12 MAP (at harvest)	Count	3 = small sized roots, 5= medium sized roots 7 = large sized roots
Fresh storage root weight	Roots were placed in a clean synthetic bag and weighed using a spring balance scale set to zero using the empty bag.	12 MAP (at harvest)	Weighing	Shoot fresh weight (stem and leaves)
Shoot weight (kg)	Shoots were tied with a twig and weighed using a spring balance scale set to zero.	12 MAP (at harvest)	Weighing	Harvested samples
Mealiness	Root were cooked for 25 minutes and a scale of 1,2, and 3 was used to screened the cooked samples	12 MAP (at harvest)	Hand screening	Harvested samples

MAP = Month After Planting

Dry matter content (DMC) expressed as a percentage was determined by selecting at least 3 storage roots from a bulk of storage roots of each plant. The roots were washed, peeled and sliced into piece. The sliced of the six fresh samples were weighed to obtain 100grams before drying for 48hours in an oven at 80°C. The dried samples were then reweighed to obtain the dry mass and the DMC was calculated as;

$$\text{DMC (\%)} = (\text{Oven dried root weight} / \text{Fresh root weight}) \times 100$$

Fresh root yield (FYLD in t ha^{-1})

Fresh root yield was estimated as from fresh storage root weight (FRW) for each family;

$$FYLD\ (t\ ha^{-1}) = Fresh\ root\ weight \times 10\ 000$$

Mealiness

Mealiness is an important trait in breeding for quality assessment, it is a method used in assessing the cooking quality of mealy cassava genotypes. Mealiness was estimated following IITA standard laboratory procedure. Cassava stands with at least three roots were randomly selected from cross combinations. Samples were prepared by washing each root with clean water to remove all soil. The roots were peeled and washed again and then dried with paper towel. Each root was sliced into four equal parts. The sliced samples were placed into a pot when the temperature of the boiling water attained 100^{o}C. The samples were cooked for 25 min on a gas cooker. A scale of 1 to 3 was used to assess mealiness, where 1 = 10-30% mealy, 2 = 40-60% mealy, and 3 = 70-100 mealy.

7.2.4 Data analysis

Data were subjected to statistical analyses using SAS version 9.3. Analytical tools employed included general analysis of variance and diallel analysis of variance for combining abilities and estimates.

The general analysis of variance was used to test if the sources of variation had any significant influence on the character. Diallel analysis of variance for combining ability was performed using mean values, following Griffing‘s Model I Method IV (1956).

$$\mathbf{Y_{ijkl} = \mu + R_i + G_j + G_k + S_{jk} + E_{ijkl}}$$

Where;

Y_{ijkl} = is the l-th observation of the i-th replication for the jk-th cross;

μ = is the overall mean;

R_i = is the fixed effect of the i-th replication, i=1 to b;

G_j or G_k = is the random general combining ability (GCA) effect of the j-th female or the k-th male ~Normally and Independently Distributed (NID) (0, σ^2G), j, k=1 to p and j<k;

S_{jk} = is the random specific combining ability (SCA) effect of the j-th and the k-th parents (j≠ k) ~NID (0, σ^2S);

E_{ijkl} = is the random within plot error term ~NID (0, σ^2E)

Test of significance for combining ability effects:

The t-test was applied to examine the effects of GCA and SCA at 0.05, 0.01 and 0.001 levels of probability. T statistic calculated for GCA effects and SCA effects are as follows:

GCA effects = GCA / S.E

SCA effects = SCA/ S.E

The distribution of crosses in relation to GCA and SCA effects were determined by denoting significant positive combining ability effects as high, non-significant as average and significant negative as low for characters studied (mealiness, dry matter content (DMC), number of storage roots and fresh root weight ha^{-1}) following the method described by Michael *et al.* (2015) and Akuwa (2016).

7.3. Results

7.3.1 Mean performances of crosses for number of storage roots per plant and fresh root yield.

Results in Table 7.4 shows mean performances of crosses for number of storage roots per plant and fresh root yield. The mean performance for number of roots per plant ranged from 4.85 to 2.50 for crosses IBA 120004 x I088693 and IBA120004 x TMEB1 with a grand mean of 3.59, while the mean performance for fresh root yield ranged from a highest of 0.80kg/plant, I9100416 x I088693 to 0.23kg/plant UBJ120003 x IBA 120004.

Table 7.4 Mean performances of crosses for number of roots per plant and fresh root yield

Cross	Number of storage roots	Fresh root Yield (kg/plant)	Cross	Number of storage roots	Fresh Root Yield (kg/plant)
1x2	3.80	0.38	**4x8**	4.00	0.60
1x3	3.00	0.26	**4x9**	3.70	0.43
1x4	4.45	0.63	**4x10**	3.70	0.55
1x5	3.85	0.38	**4x11**	3.65	0.43
1x6	3.70	0.34	**4x12**	3.20	0.41
1x7	2.65	0.23	**5x6**	3.15	0.31
1x8	4.20	0.61	**5x7**	3.50	0.32
1x9	3.25	0.31	**5x8**	2.85	0.39
1x10	3.60	0.33	**5x9**	4.00	0.27
1x11	4.20	0.58	**5x10**	3.65	0.31
1x12	3.65	0.37	**5x11**	4.25	0.58
2x3	3.85	0.58	**5x12**	3.50	0.36
2x4	3.90	0.39	**6x7**	3.65	0.54
2x5	3.95	0.54	**6x8**	3.25	0.37
2x6	3.75	0.38	**6x9**	2.85	0.31
2X7	3.75	0.64	**6x10**	3.05	0.28
2x8	3.75	0.31	**6x11**	2.85	0.34
2x9	3.75	0.35	**6x12**	3.15	0.35
2x10	4.25	0.54	**7x8**	3.30	0.80
2x11	3.20	0.38	**7x9**	4.85	0.40
2x12	3.75	0.39	**7x10**	3.15	0.34
3x4	3.80	0.56	**7x11**	2.50	0.27
3x5	3.80	0.56	**7x12**	3.30	0.37
3x6	3.75	0.55	**8x9**	3.20	0.33
3x7	3.75	0.55	**8x10**	3.15	0.36
3x8	3.75	0.55	**8x11**	3.45	0.40
3x9	3.75	0.55	**8x12**	3.40	0.38
3x10	3.80	0.57	**9x10**	3.30	0.36
3x11	3.75	0.54	**9x11**	4.30	0.80
3x12	3.80	0.57	**9x12**	3.15	0.35
4x5	3.75	0.55	**10x11**	4.15	0.60
4x6	3.75	0.55	**10x12**	3.70	0.43
4x7	3.05	0.30	**11x12**	3.85	0.56
Grand mean				**3.59**	**0.44**
SE				**0.41**	**0.14**
LSD				**0.82**	**0.28**
CV				**11.5**	**31.9**

NB- Parent1-UBJ120003: Parent2- UCC2001 (246): Parent3- TMEB693:Parent4- TMEB419: Parent5-TMEB1: Parent6- MM961781: Parent7-IBA120004: Parent8- I961165: Parent9- I9100416: Parent10 – I088747: Parent11- I088693: Parent12- I011368

7.3.2 Mean performances of crosses for mealiness and dry matter content.

Results in Table 7.5 shows mean performances of crosses for mealiness and dry matter content. The highest and lowest mean performance for dry matter content were recorded from crosses UBJ120003 x TME693 and UBJ120003 x TME419 respectively ranging from 51.60% to 23.55%, while the mean performance for mealiness ranged from 2.55 in IBA 120004 x I088747 to the lowest of 1.55 in IBA 961165 x I9100416.

Table 7.5 Mean performances of crosses for mealiness and dry matter content.

Cross	Dry Matter content (%)	Mealiness	Cross	Dry Matter Content (%)	Mealiness
1x2	48.80	1.90	**4x8**	46.65	2.10
1x3	23.55	1.85	**4x9**	46.10	1.85
1x4	51.60	2.20	**4x10**	47.50	2.15
1x5	47.75	2.15	**4x11**	46.05	2.00
1x6	48.50	2.00	**4x12**	41.65	2.20
1x7	46.10	2.30	**5x6**	45.95	2.10
1x8	47.60	2.15	**5x7**	44.85	2.05
1x9	47.35	2.00	**5x8**	46.25	2.30
1x10	46.05	2.15	**5x9**	47.15	2.00
1x11	37.60	1.80	**5x10**	48.95	2.15
1x12	45.25	2.10	**5x11**	46.45	2.15
2x3	45.80	2.15	**5x12**	45.90	2.10
2x4	46.40	2.55	**6x7**	48.35	2.15
2x5	43.50	2.15	**6x8**	46.50	1.90
2x6	44.55	2.10	**6x9**	39.00	2.45
2X7	45.85	2.10	**6x10**	45.80	2.30
2x8	46.35	1.90	**6x11**	44.40	2.05
2x9	44.65	2.10	**6x12**	45.75	2.15
2x10	45.00	2.05	**7x8**	49.15	1.95
2x11	44.80	2.10	**7x9**	45.70	2.15
2x12	45.20	2.15	**7x10**	43.25	2.55
3x4	45.20	2.15	**7x11**	40.90	2.25
3x5	45.30	2.10	**7x12**	44.90	2.20
3x6	45.05	2.05	**8x9**	48.70	1.55
3x7	45.20	2.10	**8x10**	47.00	2.00
3x8	45.25	2.10	**8x11**	45.45	2.45
3x9	45.25	2.10	**8x12**	41.15	1.95
3x10	45.20	2.10	**9x10**	49.35	2.05
3x11	45.15	2.10	**9x11**	49.40	2.10
3x12	45.20	2.10	**9x12**	47.65	1.80
4x5	45.20	2.10	**10x11**	48.40	1.80
4x6	45.20	2.10	**10x12**	47.65	2.00
4x7	49.70	1.95	**11x12**	46.75	1.90
Grand mean				**45.57**	**2.09**
SE				**2.34**	**0.21**
LSD				**4.67**	**0.41**
CV				**5.1**	**9.9**

NB- Parent1-UBJ120003: Parent2- UCC2001 (246): Parent3- TMEB693:Parent4- TMEB419: Parent5-TMEB1: Parent6- MM961781: Parent7-IBA120004: Parent8- I961165: Parent9- I9100416: Parent10 – I088747: Parent11-I088693: Parent12- I011368

7.3.3 Estimates of general combining effect (GCA) and specific combining effect (SCA)

Significant differences were observed among the 65 F_1 crosses for dry matter ($P < 0.01$); number of storage roots per family and fresh root yield per family at $P < 0.05$. General combining ability (GCA) variance was significant only for fresh root yield per family at $P < 0.05$ whereas specific combining ability (SCA) variance were significant for dry matter ($P < 0.05$) and number of storage roots at $P < 0.01$ and fresh root yield at $P < 0.05$ (Table 7.6).

Table 7.6 Mean squares of 12 x 12 diallel of measured traits in Njala

Source	df	Dry Matter Content	Number of Storage Roots	Mealiness	Fresh Root Yield
Rep	1	60.01**	0.88	0.40**	1.17**
Cross	65	26.23**	0.39*	0.06	0.03*
GCA	11	11.63	0.21	0.05	0.04*
SCA	54	13.42*	0.19**	0.06	0.03*
Error	65	2.73	0.09	0.04	0.01

***, **: Significant at P <0.05 and P<0.01 respectively**

7.3.4 Estimates of general combining ability (GCA) effects

Both significant positive and negative GCA effects were observed for the measured traits (Table 7.7). For dry matter content, eight (8) out of the 12 parents showed significant effect. Parent I088747 gave the highest positive and significant GCA effect (1.28) at $p < 0.01$. Of the remaining six parents with negative GCA effects for dry matter, parents (UBJ120003 and TMEB693) gave the highest negative but significant GCA effects -1.12 ($P <0.05$) and -2.51 ($P < 0.01$)). For number of storage roots, significant GCA effects were recorded in four parents UCC2001 (246), TMEB693 MM961871 and I9100416). Parents UCC2001 (246) and TMEB693 showed positive significant effects while MM961871 and I9100416 had significant negative GCA effects at $p < 0.05$. For mealiness, only two parents (IBA120004, and I9100416) gave significant GCA effects with values of 0.08 and -0.08 at $p < 0.05$. For fresh root yield, three

parents (UBJ120003, I088747 and I011368) with parent I088747 showed highly significant (p <0.01) GCA effect. Two parents (TMEB693 and I088747), had positive significant values (0.10 and 0.06) at p < 0.05 and 0.01 respectively.

Table 7.7 Estimate of General combining Ability of 12 cassava genotypes evaluated in Njala

Parent	Dry Matter Content	Number of Storage Roots	Mealiness	Fresh Root Yield
UBJ120003	-1.12*	0.08	-0.04	-0.05*
UCC2001(246)	-0.04*	0.22*	0.03	0.00
TMEB693	-2.51**	0.22*	-0.01	0.10
TMEB419	0.99*	0.14	0.04	0.03
TMEB1	0.59**	-0.02	0.04	0.00
MM961871	-0.27	-0.23*	0.04	-0.05
IBA120004	0.38	-0.13	0.08*	0.00
I961165	0.87*	-0.02	-0.06	0.01
I9100416	0.9*	-0.16*	-0.08*	-0.04
I088747	1.28**	-0.07	0.03	-0.01
I088693	-0.60	0.15	-0.03	0.06**
I011368	-0.40	-0.09	-0.03	-0.04*
Se	**0.52**	**0.08**	**0.03**	**0.02**

*, **: Significant at P <0.05 and P<0.01 respectively, NB: Parent1-UBJ120003: Parent2-UCC2001 (246): Parent3-TMEB693:Parent4- TMEB419: Parent5-TMEB1: Parent6- MM961781: Parent7- IBA120004: Parent8-I961165: Parent9- I9100416: Parent10 – I088747: Parent11-I088693: Parent12- I011368.

7.3.5 Estimates of Specific combining ability (SCA) effects

Table 7.8a and b shows the estimates of SCA effects. Some significant positive and negative SCA effects were obtained for measured traits. For dry matter content, SCA for hybrids from eleven crosses 1x7, 1x4, 1x6, 1x11, 3x10, 4x 12, 6x9, 7x10, 7x11,8x11, 8x12 and 9x11were significant. Progenies from cross 1x 4 had the highest positive significant SCA value (6.14), while progenies from cross 1x3 had the highest significant negative value (-18.39) at p < 0.01 respectively. For number of storage roots, eleven cross progenies (1x2, 1x4, 1x7, 1x8, 2x7, 2x8,

4x7, 5x8, 7x8, 7x12 and 9x11) had SCA effects. F_1 progeny from 7x8 had the highest positive significant value (1.41), while progeny from cross 7x11 had the highest negative significant value (-1.11) at $p < 0.01$ respectively. For mealiness, six progenies 2x4, 6x9, 7x10, 8x9, 8x11 and 10x11 recorded positive or negative significant effects, with F_1 progeny of cross 8x11 having the highest positive significant value (0.45) and progeny cross of 8x9 having the highest negative significant value (-0.39) at $p < 0.05$ and 0.01 respectively. In the case of fresh root yield, eight crosses (1x3, 1x4, 1x 8, 2x7, 5x8, 7 x8, 7x11 and 9x11) showed significant SCA effects. The progeny from cross 7x8 gave the highest positive significant value (0.35), while F_1 progeny from cross 7x11 gave the lowest negative significant value (-0.23) at $p < 0.05$.

Table 7.8a Estimate of SCA for no of roots and fresh root yield of 65 diallel crosses evaluated in Njala

Cross	No of Storage Roots	Fresh Root Yield	Cross	No of Storage Roots	Fresh Root Yield
1x2	-0.1	-0.02	**4x8**	0.28	0.12
1x3	-0.80*	-0.23*	**4x9**	0.13	-0.01
1x4	0.63*	0.20*	**4x10**	0.03	-0.03
1x5	0.19	-0.02	**4x11**	-0.24	-0.12
1x6	0.26	0.12	**4x12**	-0.45	-0.12
1x7	-0.89**	-0.17	**5x6**	-0.19	-0.06
1x8	0.54*	0.20*	**5x7**	0.06	-0.04
1x9	-0.26	-0.05	**5x8**	-0.70*	-0.18*
1x10	-0.01	-0.06	**5x9**	-0.36	-0.09
1x11	0.37	0.12	**5x10**	0.15	0.12
1 x12	0.06	0.02	**5x11**	0.53	0.09
2x3	-0.09	0.04	**5 x 12**	0.02	-0.03
2x4	-0.06	-0.08	**6x7**	0.42	0.16
2x5	0.16	0.10	**6x8**	-0.09	-0.03
2x6	0.17	-0.01	**6x9**	-0.34	-0.04
2X7	0.57*	0.20*	**6x10**	-0.44	-0.1
2x8	-0.59*	-0.15	**6x11**	-0.36	-0.11
2x9	0.1	-0.05	**6 x12**	0.03	0.01
2x 10	0.01	0.11	**7x8**	1.41**	0.35**
2 x11	-0.21	-0.13	**7x9**	0.25	0.07
2x12	0.03	-0.01	**7x10**	-0.24	-0.09
3x4	-0.07	-0.01	**7x11**	-1.11**	-0.23*
3x5	0.09	0.03	**7x12**	-0.07	-0.03
3x6	0.26	0.07	**8x9**	-0.21	-0.09
3x7	0.16	0.02	**8x10**	-0.35	-0.09
3x8	0.05	0.11	**8x11**	-0.27	-0.11
3x9	0.19	0.04	**8x12**	-0.08	-0.03
3x10	0.15	0.04	**9x10**	-0.06	-0.04
3x11	-0.12	-0.06	**9x11**	0.72*	0.34**
3x12	0.17	0.07	**9x12**	-0.19	-0.01
4x5	0.03	0.09	**10x11**	0.48	0.11
4x6	0.25	0.13	**10x12**	0.27	0.04
4x7	-0.56*	-0.17	**11x12**	0.2	0.1
SE	**0.27**	**0.09**	**SE**	**0.27**	**0.09**

*, ** **Significant at P<0.005and P< 0.001 respectively**

NB- Parent1-UBJ120003: Parent2- UCC2001 (246): Parent3- TMEB693:Parent4- TMEB419: Parent5-TMEB1: Parent6- MM961781: Parent7-IBA120004: Parent8- I961165: Parent9- I9100416: Parent10 – I088747: Parent11- I088693: Parent12- I011368

Table 7.8b Estimate of SCA for dry matter content and mealiness of 65 diallel crosses evaluated in Njala

Cross	Dry Matter	Mealiness	Cross	Dry Matter	Mealiness
1x2	4.38*	-0.18	**4x8**	-0.79	0.04
1x3	-18.39**	-0.19	**4x9**	-1.36	-0.19
1x4	6.14**	0.11	**4x10**	-0.35	-0.01
1x5	2.69	0.06	**4x11**	0.07	-0.1
1x6	4.26*	-0.09	**4x12**	-4.49**	0.11
1x7	1.37	0.17	**5x6**	0.32	-0.06
1x8	2.26	0.16	**5x7**	-1.58	-0.15
1x9	1.99	0.03	**5x8**	-0.79	0.24
1x10	0.3	0.07	**5x9**	0.08	-0.04
1x11	-6.26**	-0.22	**5x10**	1.49	-0.01
1 x12	1.21	0.08	**5x11**	0.87	0.05
2x3	2.78	0.04	**5 x 12**	0.15	0.01
2x4	-0.12	0.39**	**6x7**	2.73	-0.05
2x5	-2.62	0	**6x8**	0.27	-0.16
2x6	-0.75	-0.05	**6x9**	-7.24**	0.41**
2X7	0.05	-0.09	**6x10**	-0.83	0.14
2x8	-0.05	-0.15	**6x11**	-0.35	-0.05
2x9	-1.78	0.07	**6 x12**	0.82	0.06
2x 10	-1.81	-0.1	**7x8**	2.44	-0.15
2 x11	-0.13	0.01	**7x9**	-1.03	0.07
2x12	0.09	0.07	**7x10**	-3.87*	0.35*
3x4	1.14	0.03	**7x11**	-4.34*	0.11
3x5	1.64	-0.02	**7x12**	-0.51	0.07
3x6	2.21	-0.07	**8x9**	1.35	-0.39**
3x7	1.87	-0.06	**8x10**	-0.73	-0.06
3x8	1.31	0.08	**8x11**	-0.4	0.45**
3x9	1.29	0.1	**8x12**	-4.87**	-0.04
3x10	0.85	-0.01	**9x10**	1.59	0.01
3x11	2.68	0.05	**9x11**	3.52*	0.12
3x12	2.56	0.05	**9x12**	1.6	-0.17
4x5	-1.96	-0.06	**10x11**	2.13	-0.29*
4x6	-1.14	-0.06	**10x12**	1.21	-0.09
4x7	2.86	-0.25	**11x12**	2.19	-0.13
SE	**1.49**	**0.13**	**SE**	**1.49**	**0.13**

*, ** **Significant at P<0.005and P< 0.001 respectively**

NB- Parent1-UBJ120003: Parent2- UCC2001 (246): Parent3- TMEB693:Parent4- TMEB419: Parent5-TMEB1: Parent6- MM961781: Parent7-IBA120004: Parent8- I961165: Parent9- I9100416: Parent10 – I088747: Parent11- I088693: Parent12- I011368

7.4.Discussion

Diallel analysis for the twelve parents and their F_1 progenies evaluated in a seedling nursery trial confirmed that the progenies were significantly different for all the traits revealing the genetic diversity existing among the parents and hence F_1 progenies. This predicts the opportunity to make progress with selection. A segregating cassava population comprising of 65 families from a 12 x 12 diallel without reciprocals was tested in the study.

High variability for different traits had also been reported in cassava (Parkes *et al.*, 2013 and Akuwa, 2016). The mean FRSY and SRN at the seedling evaluation stage in this study were lower than those reported by Ojulong *et al*., (2010), Mtunda (2009) and Tumuhimbise *et al*., (2014). The higher values for these two traits reported by the previous studies cited by the two researchers above may be attributed to the technique that was used for germinating botanical their seeds. Their botanical seeds were germinated in plastic bags and the resulting seedlings with undamaged roots were transplanted to the field. Ceballos *et al*., (2004) indicated that cassava seeds germinated in seedling containers and later transplanted as seedlings to the field often do not develop taproot and that the mature plants that develop from such seedlings are like the plants derived from stem-cuttings in storage root formation. Generally, it was observed from this study that there were significant GCA and SCA effects for the traits studied revealing that the inheritance of these traits had both additive and non-additive gene actions conferring the inheritance of these studied traits.

This is an indication that breeding strategies for the improvement of fresh root yield may exploit the advantage of both additive and non-additive gene action. SCA variance was slightly higher than GCA variance for dry matter but higher magnitude of GCA variance was observed for fresh

root yield and number of roots. Contrasting findings were reported from Akuwa, (2016) for which he concluded that the propondence on the inheritance of fresh root yield based on non-additive gene action.

Fresh root yield had significant GCA effects, suggesting that the inheritance of this trait is largely under the control of additive gene action hence in making selection from such crosses, larger portion of the improvement would be expected to come from the GCA with relatively small contribution from the SCA. This disagrees with findings reported by Calle *et al*., (2005). Zacarias and Labuschagne. (2010), Kulembeka *et al.,* (2012) and Esuma *et al.* (2016). The contrasting findings from this study and from other reports may be attributed to the differences in the genotypes used in the crosses as well as differences in the environment under which the different studies were conducted.

General combining ability effects measure the performance of parents in relation to one another (Akuwa, 2016). Significant positive values were denoted as high GCA; non-significant as average while significant negative effect was regarded as low for the traits evaluated. Highest positive and significant GCA in Parent 10 (I088747) for dry matter means that this genotype was the best general combiner and a superior genotype as presented for dry matter while, Parents 1 (UBJ120003) and 3 (TMEB693) which had negative but significant GCA effects may be regarded as very poor general combiners for dry matter because of their low performance among the set of parents. For number of roots, Parents 2 (UCC2001 (246) and 3 (TMEB693) were best general combiners; Parent 7 (IBA120004) was the best combiner for mealiness; and for fresh root yield, Parent 11 (I088693) was observed as the best general combiner.

The specific combining ability (SCA) effects measures distributive performance of crosses based

on their parental GCA effect (Akuwa, 2016). Estimates of the SCA effects also varied both in magnitude and direction. Analysis of SCA showed that families developed from parents with contrasting GCA effects for traits, quite often had correspondingly high and significant SCA effects, suggesting that specific combinations of alleles were important in controlling traits or that there could be some interlocus gene interaction.

Highest magnitude of positive and significant SCA effects was shown by F_1 progenies from crosses UBJ120003 x UCC2001(246), UBJ120003 x TMEB693, UBJ120003 x TMEB 419, UBJ120003 x MM961871, UBJ120003 x I088693, TMEB419 x I088747, TMEB419 x I011368, MM961871 x I9100416, IBA120004 x I088747, IBA 120004 x I088693, IBA961165 x I088693, IBA 961165 x I088693, and I9100416 x I088693.

For dry matter, it confirmed that UBJ120003 x TMEB 419 F_I cross was the best specific combiner and superior genotype for dry matter while, progeny from cross MM961871 x I9100416 which gave the highest negative but significant SCA effect is considered as a very poor specific combiner for dry matter. Progeny from crosses with positive significant effects can be selected for development of elite clones for dry matter. For number of roots, F_1 progeny from cross IBA120004 x IBA 961165 was the best combiner, for mealiness, while progenies from crosses IBA120004 x IBA961165 and IBA 961165 x I088693 had the best SCA for fresh root yield.

Combining ability analysis for storage root related traits at this early stage was possible because of the high SRN produced by the seedling plants ranging from 1 to 23 storage roots plant−1. This study clearly demonstrates that it is possible to conduct combining ability analysis for storage root related traits at the seedling stage of cassava breeding. This high SRN was attributed to the

method that was used in raising seedlings combined with good growing conditions in Sierra Leone

However, in some cases, parents with best GCA effects did not necessarily produce best progeny for the respective traits, implying that the GCA of a given progenitor did not predict well the performance of a given progeny. Combining ability analysis at the seedling stage cannot be undertaken in areas where storage root development by seedlings is poor and in fields where variability is high. Findings of this study also demonstrated that it is possible to simultaneously select for yield and quality traits, such as DMC at the seedling stage using simple statistical methods. It was also revealed that breeding strategies for crop improvement should exploit the advantage of both additive and non-additive gene action to be able to achieve the different levels of stages in improving most of these studied traits.

7.5 Conclusion

A high degree of variation was detected among individual genotypes and families for all traits studied, indicating potential for recurrent selection and improvement. The highest general combiners were Parent 10 (I088747), Parent 2 (UCC2001 (246)), Parent7 (IBA120004) and Parent 11 (I088693) for dry matter content, number of storage roots, mealiness and fresh root yield respectively whereas the lowest specific combiners were UBJ120003 x I088747, UBJ120003 x MM961781, UCC2001 (246) x IBA120004 and UCC2001 (246) x TMEB1 for number of storage roots, fresh root yield, dry matter content and mealiness. For dry matter and number of storage roots recurrent selection method would be efficient. Following hybridization of the selected parents, the non-additive gene effects that predominate in some traits could be "captured" or fixed in subsequent generations by exploiting the vegetatively propagatable nature

of cassava.

This study could be utilized as a model for reducing the potential loss of useful genetic data and breeding material, subsequently improving the effectiveness and efficiency of the standard cassava breeding cycle. We, however, acknowledge the limitation of one-year data in this study, and therefore emphasize that these results might not be very conclusive. In view of enhancing or maximizing the potential of cassava production and cassava products, significant progress in increasing these farmer preferred traits which include mealiness, dry matter, and fresh root yield can become successful through appropriate breeding techniques. This result however confirmed that among the cross, families UBJ120003 x TMEB419, IBA 120004 x IBA 961165, IBA961165 x I088693 and IBA120004 x IBA961165 show high potential for exploitation in breeding for multiple important economic traits.

CHAPTER EIGHT

8.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

8.1 GENERAL CONCLUSIONS

The present study constitutes the first steps in cassava provitamin-A and nutritional quality traits improvement in Sierra Leone. The participatory rural appraisal study revealed that youth and adults within the ages of 36-60 years within the surveyed areas were mainly involved in cassava cultivation and production. The youth population forms a large proportion of the farming group across the three districts. Most of the farmers in Bombali, Kailahun and Moyamba were male indicating the predominance of males in farming. The very high percentage of men compared to women in cassava farming revealed in the surveyed districts, could possibly be due to the drudgery of non-mechanized cassava production which may scare women from independently undertaking production. Cassava cultivation activities which range from brushing, burning, ploughing and harvesting were mainly undertaken by men, while women participated in activities like planting, weeding and transportation of harvested storage roots. Although women have been found to spend more time in agricultural activities, unfortunately, most women have less access to information technology.

The low level of formal education of about two-thirds of the farmers in all three districts contributes to the reduced adoption of released technologies specifically improved varieties. Farmers with very low levels or without any formal education are less receptive to improved farming techniques or new products. The low level of education among farmers in all the three districts sampled could have implications on the selection and adoption of improved provitamin

A cassava varieties when introduced to them. Acceptance of provitamin A cassava by farmers is contingent upon awareness of its superior nutritional quality, root size, yield, earliness, consumer acceptance/mealiness and potential for high financial return. White root varieties are largely consumed in all the surveyed districts due of the low awareness of provitamin-A cassava and no market outlet exists where provitamin-A cassava storage roots/products can be obtained across the surveyed districts. However, producers, processors, traders and consumers expressed willingness to accept the provitamin-A cassava along the cassava value chain due to its perceived nutritional value.

Three approaches, agro-morphological, total carotenoid content and molecular characterizations, were used to analyze the diversity among 183 pro-vitamin-A accessions and 5 released varieties as checks. The agro-morphological data set revealed moderate to high diversity for agro-morphological characters, while the SNP-based characterization indicated moderate diversity with a PIC value 0.28. Clustering based on similarity index grouped the 188 cassava accessions into 8 and 9 distinct clusters based on morphological and molecular analyses, respectively. Cassava accessions clustered differently based on morphological and molecular datasets. A significant positive correlation was found between the morphological and molecular data sets ($r = 0.104$; $p < 0.034$) even though weak. This could perhaps explain why the agro-morphological and molecular analysis showed different accessions for both lowest and highest similarities. The variation among the accessions was moderate to high indicating that the germplasm can be used as source of parents for population improvement. The discriminative agro-morphological descriptors such as African cassava mosaic disease incidence, leaf area, marketable roots, non-marketable roots, total no of storage roots, marketable weight, non- marketable weight, storage

root weight and dry matter content identified in this study can be used to distinguish provitamin-A cassava accessions in future studies.

The genotype by environment interaction for total carotenoid content indicated that most of the variation in total carotenoid content was due to genotype effects and hence, evaluation of this trait may be successfully done in a single environment. Dry matter content, however, was subjected to genotype by environment interaction effects and to genotype effects. Genotypes TR 1569, TR-1313, TR-1327 were specific across the three test environments while genotypes TR 1182 (24), TR-1279 (19) and TR-1337 (6) were observed as stable genotypes across three test environments for dry matter content. The datasets from the genotypes by environment interaction for dry matter and total carotenoid contents of the 30 provitamin-A genotypes in 3 environments showed significant differences ($P < 0.001$) among G, E, and GE. With respect to each trait, genotypes showed differences in performance in specific environment. Genotypes TR 1313 and TR 1182, had the highest performance for total carotenoid contents and dry matter, respectively. This study revealed genotypes TR 1313 and TR 1182 as good performers and best genotypes for food technologists and nutritionists to use in their nutritional studies to combat vitamin A deficiencies.

Despite cross incompatibility among several parents, local parents were successfully crossed to exotic provitamin-A, protein, iron and zinc parents to generate different progenies populations. The performance in total carotenoid content from the generated F_1 progeny revealed that F_1progeny 13 and 33 from cross IITA-TMS-IBA 120004 x IITA-TMS-IBA 120003 recorded the

highest 28.0 μg^{-1} and lowest 6.0 μg^{-1} values of total carotenoid content respectively with a grand mean of 14.70 μg^{-1}.

F_1 progeny 41 and 12 from cross IITA-TMS-IBA 088693 x IITA-TMS-IBA 088747 recorded the highest (8.1%) and lowest (4.2%) crude protein contents respectively with a grand mean of 5.4%.

Iron concentration from cross IITA-TMS-IBA 96/1165 x IITA –TMS-IBA 011368 ranged from 4.5 ppm to 5.9 ppm in harvested F_1 progeny. 61 progeny recorded higher values than the grand genotypic mean of 12.6 ppm. Zinc concentration ranged from 4.5 ppm to 17.7 ppm for the progenies from cross MM96/81791 x IITA-TMS-IBA 088747.

Results obtained from this study confirmed that there is great variation in the nutrient quality parameters studied (total carotenoid content, protein, iron and zinc). Observed values for total carotenoid content, protein and micronutrient contents predict the potential for improving the nutritive value of cassava in Sierra Leone.

Diallel analysis from the crosses confirmed highly significant differences for dry matter, number of roots and fresh root yield at $P < 0.01$, and 0.05 respectively. General combining ability (GCA) variance was significant for fresh root yield only at $P < 0.05$, while estimates of specific combining ability (SCA) variance were highly significant for dry matter, number of storage roots and fresh root yield at $P < 0.05$. The results revealed that among the crosses, cross families UBJ120003 x TMEB419, IBA 120004 x IBA 961165, IBA961165 x I088693 and IBA120004 x IBA961165 had high potential which can be exploited in breeding for the traits studied.

8.2 RECOMMENDATIONS

- The food culture and cultural belief of the people about a variety of cassava largely influences what they produce and market. In view of this finding; Agriculture extension services are needed to enlighten, empower and encourage the farmers on what food quality is, and the need to gear their production towards planting for food quality (nutritional, commercial, industrial, and utilization) rather than for subsistence use only. Researchers need to involve farmers in nutrition and food quality studies as a form of holistic approach in achieving improved food and nutrition security.

- This type of qualitative study can also be applied to different types of crops and food products in Sierra Leone and elsewhere. All the farmers, processors, consumers and market sellers interviewed desired provitamin A gari with good appearance and of high dry matter content.

- Empowerment of Sierra Leonean farmers through timely provision of credit, good market places, storage facilities, equipped processing centers and improved planting materials at affordable prices would improve the adoption of cassava technologies as in this case the provitamin A cassava varieties when developed. Participatory involvement of farmers at an early stage of the breeding programme could significantly reduce the years of multi locational evaluation and should be encouraged by all breeders in national research systems that are involved in crop improvement and variety development.

- Cassava breeders in Sierra Leone should focus their attention on breeding for high pro-vitamin A, protein, iron and zinc until the Harvestplus benchmark of 20-25 ug $^{-1}$, 7-8 %, 2.0-5.0 ppm and 4.0-20.0 ppm are obtained.

- The results of agro-morphological study and sequencing by genotyping (SNP markers) showed moderate to high diversity.

- There is need for further studies and advancement of these progenies in multiple trials across multiple environments to confirm the results of the present study.

- Additional genome wide association analysis or QTL mapping to find more genomic regions that influence total carotenoid content, protein, iron and zinc inheritance in yellow cassava germplasm in Africa is warranted.

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APPENDIX

Appendix 1: Questionnaire for Participatory Rural Appraisal (PRA)

Market Traders for Cassava/Cassava Products

Q1. Questionnaire Identification	/___/___/___/___/___/___/___/___/___/___/

Location Information

No.	NAME 0F LOCATION		CODES	
A1	Region Name _____		/___/	
A2	District Name _____		/___/___/	
A3	Chiefdom Name _____		/___/___/___/___/	
A4	Village/town Name: _____			
A5	Name of Enumerator_____		/___/___/	
A6	Market name			
A7	Date: /___/___/ /___/___/ /___/___/___/___/			
A8	Start time: /___/___/ /___/___/			
A9	GPS coordinates of Industry/Processing unit	**N(S)**	**E(W)**	**Altitude/height**
		/___/___/./___/___/	/___/___/./___/___/	/___/___/___/

Trader's Information

B1	Name of respondent		
B2	Age	*Years*	/___/___/
B3	Gender	*0= female* *1=male*	/___/
B4	Level of Education	*0=none ; 1= Koranic ; 2=primary, 3=junior secondary school, 4=senior secondary school, 5=tertiary*	/___/

B5	Marital status	*1=married, 2=single, 3=widow/widower, 4=divorced ; 5=Separated*	/___/
B6	Are you the owner of this business?	*1= Yes,* *2= No*	/___/
B7	Family member		
B8	How many family members are involved in this cassava/cassava products trading?	*Total number of members in this business*	/___/___/
B9	How many Males	*Total number of males including youths*	/___/___/
	How many female	*Total number of males including youths*	/___/___/
B10	Children	*People with age 8 and below*	/___/___/
B11	Are you a member of any cassava trading organization?	*1= Yes, 2= No>>Next Section*	/___/
B12	If yes, what is the name of the organization?		
B13	Years of membership in the organisation		
B14	The **KEY** purpose of the organization?	, 1= Loans facilities, 2= Welfare 3= Market Information 4= Other specify	

Enumerator*: **Before asking below questions, please use the introductory leaflet to explain to the respondent in details about the purpose of the PRA study***.

Cassava Products Trading

C1.0	Do you sell cassava/cassava products	*1=yes* *2=no*	/___/
C1.1	Have you or any of your member sell the following cassava/cassava products within the last 5 years?	*1=cassava roots* *2=gari* *3=starch* *4 fufu* *5 flour* *6 tho*	**MULTIPLE RESPONSE**
C1.2	Do you or any of your member sell the following cassava/cassava products as at now?	*1=cassava roots* *2=gari* *3=starch* *4 fufu* *5 flour* *6 tho*	**MULTIPLE RESPONSE**
C2	Is cassava/cassava product trading your main economic activity	*1=yes>>C3* *2=no*	/___/

C3	If no, what is your main economic activity?	1= other agricultural products 2= Non-agricultural product 3= farming 4= Teaching 5= Driving 6=Other specify	
C4	Average operating capital/stock for this business		
Cassava Roots Trading			
C5	Do you sell Cassava Roots	*1=yes* *2=no>>C5*	/___/
C5.1	What is the flesh colour of the most commonly sold cassava root?	1 =white 2= yellow 3= cream	/___/
C5.2	What is your main buying location?	*1= Periodic markets 2= Daily village/town markets 3= Road side markets 4= Street markets 5= own farm 6=farm gate*	/___/
C5.3	Main suppliers	*1=farmer 2=wholesaler 3=trader 4=own farm 5= collectors*	/___/
C5.4	Main selling location	*1= Periodic markets 2= Daily village/town markets 3= Road side markets 4= Street markets 5= own farm 6= hawking*	/___/
C5.5	Main preferred buyer	*1=consumers 2=wholesaler 3=other retailers 4=processor 5= Government institutions 6= Hotels*	/___/
C5.6.1	Have you ever planted, bought or sold any yellow flesh cassava root	*1=yes* *2=no>>C4.7*	/___/
	What is your major source of yellow flesh cassava root you sell	*1= own production 2= Farmers 3=Wholesaler 5= Retailer*	/___/
C5.6.2	If yes, main buyers for the product?	*1=consumers 2=wholesaler 3=other retailers 4=processor 5= Government institutions 6= Hotels*	/___/
C5.6.3	If yes, how is it compared to the other flesh color in terms of PRICE	*1=higher* *2=same* *3=lower*	/___/
C5.6.4	If yes, how is it compared to the other flesh color in terms of MARKET DEMAND	*1=high* *2=same* *3=low*	/___/
C5.7	If no, would you prefer it as opposed to the others if made available in the	*1=yes* *2=no*	/___/

	market?		
Gari Trading			
C6.0	Have you ever bought or sold any gari	*1=yes* *2=no>>Next Section*	/___/
6.1	What is your major source of gari you sell	*1= own production 2= Farmers 3=Wholesaler 5= Retailer*	/___/
C6.2	What is the flesh colour of the most commonly sold gari?	1 =white 2= yellow 3= cream	
C6.3	Main buying location for gari	*1= Periodic markets* *2= Daily village/town markets* *3= processing Centre 4= Street markets 5= own product 6=Others*	/___/
C6.4	Main suppliers for gari	*1=Processors 2=wholesaler 3=trader 4=own product*	/___/
C6.5	Main selling location for gari	*1= Periodic markets 2= Daily village/town markets* *3= processing Centre 4= Street markets 5= own product 6=Others*	/___/
C6.6	Main buyer type for gari	*1=consumers 2=wholesaler 3=other traders 4=Others*	/___/
C5.6	Have you ever bought and sold any yellow color Gari	*1=yes* *2=no>>C5.7*	/___/
C6.7.1	If yes, main suppliers for the product?	*1=Processor 2=wholesaler 3=trader 4=own product*	/___/
C6.7.2	If yes, main buyers for the product?	*1=consumers 2=wholesaler 3=other traders 4=Others*	/___/
C6.7.3	If yes, how is it compared to the other color gari products in terms of PRICE	*1=higher* *2=same* *3=lower*	/___/
C6.7.4	If yes, how is it compared to the other color gari products in terms of MARKET DEMAND	*1=high* *2=same* *3=low*	/___/
C6.8	If no, would you like to sell yellow flesh gari?	*1=yes* *2=no*	/___/

Access to Credit and Products on Cassava Cultivation

Credit			
D1.1	Do your HH have access to formal credit facilities for cassava processing?	*1=yes* *2=no*	/___/
D1.2	**Did you access last season?**	*1=yes* *2=no*	
D1.3	**If yes, how much?**		
D1.4	If yes, which institution is available?	*1=Community Banks 2=FSA's 3=Micro-finance 4= commercial banks*	/___/
D1.5	Name of the INSTITUTION		
D1.6	If no, why?	1= No credit facility 2= Lack of collateral 3= High interest rate 4= Not needed 5= Lack of trust	
Inputs			
E3.0	Do your HH have access to major inputs for yellow flesh cassava processing?	*1=yes* *2=no*	/___/
E3.1	If yes, which type of inputs is easier to access in this community?	1= Transportation 2= Labour 3= Market information 4= sales ground 5= Storage facility	

SWOT Analysis

No.	SWOT	Options	Tick	Rank
G1	What are your STREINGTH			
G2	What are your WEAKNESS			
G3	What are your OPPORTUNITIES			

G4	What are your THREATS			

Processor Interview

Q1. Questionnaire Identification	/___/___/___/___/___/___/___/___/___/

Location Information

No.	**NAME 0F LOCATION**		**CODES**	
A1	Region Name _____		/___/	
A2	District Name _____		/___/___/	
A3	Chiefdom Name _____		/___/___/___/___/	
A4	Village/town Name: _____			
A5	Name of Enumerator_____		/___/___/	
A6	Date: /___/___/ /___/___/ /___/___/___/___/			
A7	Start time: /___/___/ /___/___/			
A8	GPS coordinates of Industry/Processing unit	**N(S)**	**E(W)**	**Altitude/height**
		/___/___/./___/___/	/___/___/./___/___/	/___/___/___/

Respondent Information

B1	Name of respondent		
B2	Age	*Years*	/___/___/
B3	Gender	*0= female* *1=male*	/___/

B4	Education Level	*0=none ; 1= Koranic ; 2=primary, 3=junior secondary school, 4=senior secondary school, 5=tertiary*	/___/
B5	Marital status	*1=married, 2=single, 3=widow/widower, 4=divorced ; 5=Separated;*	/___/
B6	Are you the head of the processing unit	*1= Yes, 2= No*	/___/
B7	How long have you been processing gari		/___/___/
B8	How many workers do you have in this unit?		/___/___/
B9	How many Females?		/___/___/
B10	How many males?		
B11	Is your enterprise registered?	*1= Yes, 2= No*	/___/
B12	Are you a member of a processors' organization	*1= Yes, 2= No*	/___/
B13	**If yes, what is the name of the organization?**		
B14	**Years of membership**		
B15	**Main benefit derive from it**	1= Labour exchange, 2= Loans facilities, 3= inputs, 4= Marketing, 5= Welfare 6= Information 7= Other specify	

Enumerator*: **Before asking below questions, please use the introductory leaflet to explain to the respondent in details about the purpose of the PRA study**.*

Processing and Processing Conditions

C1.0	**Do you produce cassava base product?**	*1= Yes, 2= No*	/___/
C1.1	**If yes, what are the cassava based products you produce**	1=Gari 2= fufu 3=tho 4= Flour 5= Starch	Multiple responses
C1.2	**What is the main cassava based products you produce**	1=Gari 2= fufu 3=tho 4= Flour 5= Starch	/___/
C1.3	**How many kg of the main product do you produce in a month?**		
C1.4	**To make 50kg of the main product, from your experience, what will it cost?**		
C1.5	**For the first product how much is 50kg of normal sold?**		
C1.6	**Do you have varietal preference for each of the product you produce**	*1= Yes* *2= No*	/___/
C1.7	**If yes, why do you have preference**		
C2.1	**What is the name of the most suitable cassava variety for main product?**	Variety Name:	
C2.2	**Is this variety local or improved?**	1= Local 2=Improve	/___/
C2.3	**What is the color of its flesh?**	*1= white flesh roots* *2= yellow flesh roots* *3= Cream*	/___/
C2.4	**Why do you prefer this variety for processing?**		
C2.5	**If gari is the main product, do you**		
C2.6	If gari is the main product**,** do you add palm oil during frying/roasting?	*1= Yes* *2= No*	/___/
C2.6.1	If yes, why		
C2.6.2	If no, Why		

C2.6.3	**What processing facilities do you use?**	*1=Traditional (specify) 2=Mechanical (specify) 3. both*		
C2.6.4	**If mechanical what type of material is the equipment made of?**	*1= Stainless Steel 2= mild steel*		
C2.6.5	**If both, which of the equipment are mechanical**	*1=Peeler 2=Greater 3= Washer 4= Dryer 5 =Presser 6=Fryer*	*Multiple responses*	
C3.0	**Have you ever processed yellow flesh cassava?**	*1= Yes,* *2= No>>*		/___/
C3.1	**If yes, what are the products?**	*1= gari*	***Enumerator:*** *please enter* *1=yes* *2= no* *For each of the boxes*	/___/
		2= fufu		/___/
		3= tho		/___/
		4= starch		/___/
		5= Flour		/___/
C3.2	**Do you have any complaint on it from your customer**	*1= Yes,* *2= No>>*		
C3.3	**If yes, list them here**	*1= Product quality* *2= Price* *3= Availabilty*		
C4	**Reasons for choosing the products**	*1= Add value before sale 2= Improve cassava quality 3= Reduce cassava loses 4= For better and longer storage 5=Other(specify)*		/___/
C5	Where do you source your yellow fleshed cassava roots?	*1= own farm 2= from other farmers 3= from market 4=Others specify*		/___/
C6	**What do you do with the processed products got from yellow fleshed cassava?**	*1= home consumption,* *2= sales, 3= both*		/___/
C7	**If no, would you like to process yellow flesh cassava roots into other products ?**	*1=yes* *2=No*		/___/
C8	**How much is 1kg of normal gari sold?**			/___/
C9	**How much is 1kg of yellow gari sold?(from palm oil)**			/___/

C10	**How much is 1kg of YF sold?**		/___/

Access to Trainings, Credit and Inputs on Cassava Cultivation

Trainings			
D1.1	Has your HH received any training on cassava Processing for the last 24 months?	*1=yes* *2=no>>E2*	/___/
D1.2	If yes, from whom?	*1=Research Institution personnel 2=MAFFS Extension Officers 3=NGOs*	/___/
D1.3	Name of the INSTITUTION		
Credit			
D2.1	Do your HH have access to formal credit facilities for cassava processing?	*1=yes* *2=no*	/___/
D2.2	**Did you access last season?**	*1=yes* *2=no*	
D2.3	**If yes, how much?**		
D2.4	If yes, which institution is available?	*1=Community Banks 2=FSA's 3=Micro-finance 4= commercial banks*	/___/
D2.5	Name of the INSTITUTION		
D2.6	If no, why?	1= No credit facility 2= Lack of collateral 3= High interest rate 4= Not needed 5= Lack of trust	
Inputs			
E3.0	Do your HH have access to major inputs for yellow flesh cassava processing?	*1=yes* *2=no*	/___/
E3.1	If yes, which type of inputs is easier to access in this community?	1= Equipment 2= Labour 3= Raw material	

SWOT Analysis

No.	SWOT	Options	Tick	Rank

F1	What are your STRENGHT			
F2	What are your WEAKNESS			
F3	What are your OPPORTUNITIES			
F4	What are your THREATS			

THANK YOU!!!

Household Cassava Producers

Q1. Questionnaire Identification	/___/___/___/___/___/___/___/___/___/___/

Location Information

No.	**NAME 0F LOCATION**	**CODES**
A1	Region Name _____	/___/
A2	District Name _____	/___/___/
A3	Chiefdom Name _____	/___/___/___/___/___/
A4	Village/town Name: _____	
A5	Name of Enumerator_____	/___/___/
A6	Date: /___/___/ /___/___/ /___/___/___/___/	
A7	Start time: /___/___/ /___/___/	

A8	GPS coordinates of Industry/Processing unit	**N(S)**	**E(W)**	**Altitude/height**
		/___/___/./___/___/	/___/___/./___/___/	/___/___/___/

Respondent Information

B1	Name of respondent		
B2	Age	*Years*	/___/___/
B3	Gender	*0= female* *1=male*	/___/
B4	Level of Education	*0=none ; 1= Koranic ; 2=primary, 3=junior secondary school, 4=senior secondary school, 5=tertiary*	/___/
B5	Marital status	*1=married, 2=single, 3=widow/widower, 4=divorced ; 5=Separated;*	/___/
B6	Are you the head of the household?	*1= Yes,* *2= No*	/___/
B7	Household size	*Total number of house hold size*	/___/___/
B8	How many Male	*Total number of males including youths in HH*	/___/___/
B9	How many female	*Total number of males including youths in HH*	/___/___/
B10	Children	*People with age 8 and below*	/___/___/
B11	Are you a member of any Agricultural organization?	*1= Yes, 2= No>>Next Section*	/___/
B12	If yes, what is the name of the organization?		
B13	Main purpose of the organization?	1= Labour exchange, 2= Loans facilities, 3= inputs, 4= Marketing, 5= Welfare 6= Information 7= Other specify	

Enumerator: ***Before asking below questions, please use the introductory leaflet to explain to the respondent in details about the purpose of the PRA study***.

Household Yellow Flesh Cassava Cultivation

C1	Have you or any of your	*1=yes*	/___/

	HH member cultivated cassava within the last 5 years?	*2=no>>>END OF INTERVIEW*	
C2	Is cassava your main crop of cultivation within this HH?	*1=yes* *2=no*	/___/
C3	What is the average yearly farm size for cassava farm for this HH?	*1=< 1 acre; 2=1-3 acres; 3=4-6 acres; 4=7-9 acres* *5= >9 acres*	/___/
C4	Have you heard of YF cassava before?	*1=yes* *2=no*	/___/
C5	If yes, the name of the variety		
C6	Have you ever planted it?	*1=yes* *2=no*	/___/
C7	What is the main source of planting materials for your YF cassava?	*1=Personal farms; 2=Other farmers; 3= Research station; 4=Commercial farms; 5=MAFFS; 6=NGOs*	/___/
C8	What is the major source of acquiring YF planting materials in this HH?	*1=own materials 2=Purchasing 3=Gift 3=Exchanging*	/___/
C9	Did you plant it last season?	*1=yes* *2=no*	/___/
C10	If yes, of the area stated above, what is the proportion given to YF cassava, if planted by your HH?	*1=<25%; 2= 25%-50%; 3= 51%-75%; 4= 76%-100%;*	
C11	If growing the yellow flesh, what's the purpose for growing it with in this HH?	*1=HH consumption; 2= selling; 3=processing to other cassava products 4=Consumption & Selling*	/___/
C12	Name (s) and colour of other varieties of cassava mostly grown within this HH?	1=Variety Name_____	Flesh colour :1=cream 2= white
		2=Variety Name_____	Flesh colour : 1=cream 2= white
C13	What is the main purpose for growing these varieties within this HH?	*1=HH consumption; 2= selling; 3=processing to other cassava products 4=Consumption & Selling*	/___/
C14	If not growing yellow flesh root, will you be willing to adopt it for cultivation within this HH?	*1=yes* *2=no*	/___/

C15	Where do you source your planting materials for your cassava cultivation?	*1=Personal farms; 2=Other farmers; 3= Research station; 4=Commercial farms; 5=MAFFS; 6=NGOs*	/___/
C16	How do you acquire your planting materials in this HH?	*1=own materials 2=Purchasing 3=Gift 3=Exchanging*	/___/
C17	Do your HH have special cassava variety for feeding weaning children?	*1=yes* *2=no>>Next Section*	/___/
C18	If yes, what is the name and flesh color of that variety?	*Variety Name*_____	Flesh colour : 1= yellow; 2= Cream; 3= White
C19	What is the main reason for choosing that type of variety?	1= Nutritional value; 2=Taste; 3= flavour; 4= Texture/Poundability 5=Accessibility;	

Access to Trainings, Credit and Inputs on Cassava Cultivation

Trainings			
D1.1	Has your HH received any training on cassava cultivation for the last 24 months?	*1=yes* *2=no>>E2*	/___/
D1.2	If yes, from whom?	*1=Research Institution personnel 2=MAFFS Extension Officers 3=NGOs*	/___/
D1.3	Name of the INSTITUTION		
Credit			
D2.1	Do your HH have access to formal credit facilities for cassava cultivation?	*1=yes* *2=no*	/___/
D2.2	**Did you access last season?**	*1=yes* *2=no*	
D2.3	**If yes, how much?**		
D2.4	If yes, which institution is available?	*1=Community Banks 2=FSA's 3=Micro-finance 4= commercial banks*	/___/
D2.5	Name of the INSTITUTION		
D2.6	If no, why?	1= No credit facility 2= Lack of collateral 3= High interest rate 4= Not needed 5= Lack of trust	

D2.7	Do your HH have access to credit facilities for yellow flesh cassava cultivation	*1=yes* *2=no*	
Inputs			
E3.0	Do your HH have access to major inputs for yellow flesh cassava cultivation?	*1=yes* *2=no*	/___/
E3.1	If yes, which type of inputs is easier to access in this community?	1= Agro-chemical 2= Labour 3= Planting materials 4= Tools/instruments	

F1	**FARMERS' PERCEPTION SELECTION OF CASSAVA GENOTYPES**									
Variety name	**Plant size**	**Branching pattern**	**Root size**	**Root color**	**Root water content**	**Gen. Assess**	**Early maturing**	**Edibility like yam**	**High Yield**	**Starch content**

SWOT Analysis

No.	**SWOT**	**Options**	**Tick**	**Rank**
G1	What are your STRENGHT			
G2	What are your WEAKNESS			
G3	What are your OPPORTUNITIES			

G4	What are your THREATS			

End time: /___/___/ /___/___/

THANK YOU!!!

Household Consumers

Questionnaire Identification: /___/___/___/___/___/___/

SECTION A: Location Information

No.	**NAME 0F LOCATION**		**CODES**	
A1	Region Name _____		/___/	
A2	District Name _____		/___/___/	
A3	Chiefdom Name _____		/___/___/___/___/	
A4	Village/town Name: _____			
A5	Name of Enumerator_____		/___/___/	
A6	Date: /___/___/ /___/___/ /___/___/___/___/			
A7	Start time: /___/___/ /___/___/			
A8	GPS coordinates of Industry/Processing unit	**N(S)**	**E(W)**	**Altitude/height**
		/___/___/./___/___/	/___/___/./___/___/	/___/___/___/

SECTION B: Respondent Information

B1	Name of respondent		
B2	Age	*Years*	/___/___/
B3	Gender	*0= female* *1=male*	/___/
B4	Level of Education	*0=none ; 1= Koranic ; 2=primary, 3=junior secondary school, 4=senior secondary school, 5=tertiary*	/___/

B5	Marital status	*1=married, 2=single, 3=widow/widower, 4=divorced ; 5=Minor (not in age) ; 6=other*	/___/
B6	Are you the head of the household?	*1= Yes,* *2= No*	/___/
B7	Household size	*Total number of house hold size*	/___/___/
B8	How many Males	*Total number of males including youths in HH*	/___/___/
B9	Youths/children's	*People with age 8 and below*	/___/___/
B10	Are you a member of any organization?	*1= Yes, 2= No>>**B11***	/___/
B11	If yes, what is the name of the organization?		
B12	Years of Membership	No. in years	/___/___/Yrs.
B13	The **KEY** purpose/benefit of the organization?		
B14	**OTHER** purpose/benefit of the organization?		
B15	Average Monthly Income	Le:	

Respondent Information

C1	Name of respondent		
C2	Age	*Years*	/___/___/
C3	Gender	*0= female* *1=male*	/___/

C4	Level of Education	*0=none ; 1=literate; 2=primary, 3=junior high school, 4=senior high school, 5=tertiary, 6=/Koranic/religious education 7. other (specify)*	/___/
C5	Marital status	*11=married, 2=single, 3=widow/widower, 4=divorced ; 5=Separated;*	/___/
56	Are you the head of the household?	*1= Yes,* *2= No*	/___/
C7	Household size	*Total number of house hold size*	/___/___/
C8	How many Males	*Total number of males including youths in HH*	/___/___/
C9	Youths/children's	*People with age 8 and below*	/___/___/
C10	Are you a member of any organization?	*1= Yes, 2= No>>**Next Section***	/___/
C11	Income per annum		
C12	If yes, what is the name of the organization?		
C13	The **KEY** purpose of the organization?		

Enumerator*: **Before asking below questions, please use the introductory leaflet to explain to the respondent in details about the purpose of the PRA study***.

SECTION C: Household Cassava Tubers									
D1.0	Do you or any of your HH member eaten cassava roots without converting them to other cassava products?						*1=yes 2=no>>**D1***	/___/	
D1.1 HH disaggregation	**D1.2** Which category of your family eat cassava	**D1.3** If yes, which flesh color roots do each category prefer?	**D1.4** Are you aware of FY cassava?	**D1.5** If yes, do you consume it in your HH	**D1.6** Does this family category prefer yellow cassava roots	**D1.6.1** Why preferred?	**D1.6.2** If no to C1.5, would you be willing to introduce it to any member of your	**D1.6.3.** If yes , what is the major reason for your willingness to introduce it to	**D1.6.4** If no, Why ?

							HH	this category of family	
Family members categories	*1=yes 2=no>>* ***Next row & C2***	*1=white 2= yellow* ***>>C1.6.3*** *3=cream 4=not applicable*	*1=yes 2=no>>* ***C1_6_2***	*1=yes 2=no>>* ***C1_6_2*** *3=Don't know>>* ***C1_6_2***	*1=yes 2=no>>* ***C1_6_2*** *3=Not app>>* ***C1_6_2***	*Key Reason* ***>>Next row & C2***	*1=yes 2=no>>* ***C1_6_4***	***>>Next row & C2***	*Key Reason*
Self	/___/	/___/	/___/		/___/		/___/	/___/	
Spouse	/___/	/___/	/___/		/___/		/___/	/___/	
Infant Male	/___/	/___/	/___/		/___/		/___/	/___/	
Infant female	/___/	/___/	/___/		/___/		/___/	/___/	
Adolescence male	/___/	/___/	/___/		/___/		/___/	/___/	
Adolescence female	/___/	/___/	/___/		/___/		/___/	/___/	
Adult	/___/	/___/	/___/		/___/		/___/	/___/	

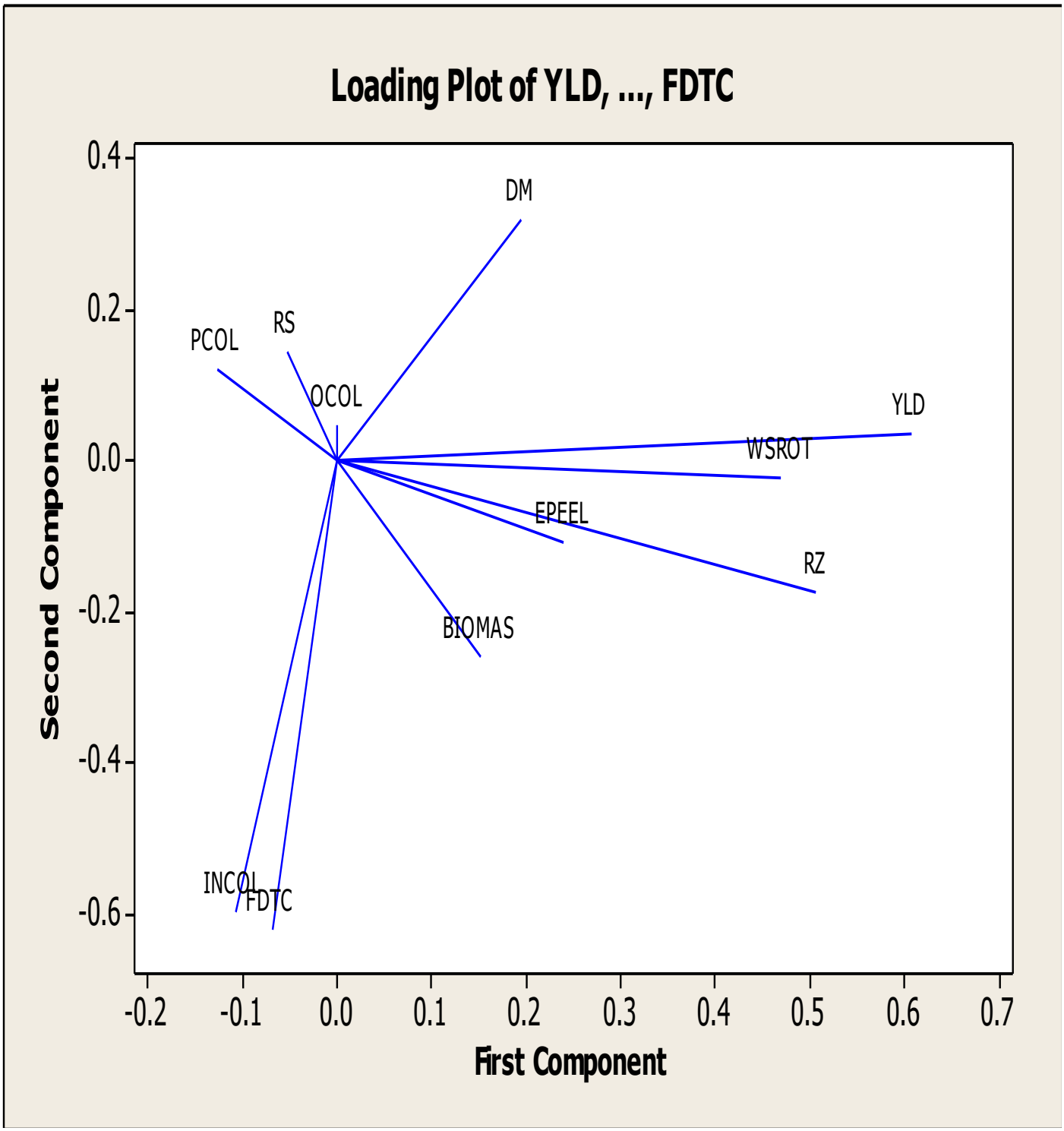
D2	Do you use cassava to wean infants within this HH?	*1=yes 2=no>>* ***C4***	/___/
D3	If yes, which color type?	*1=white 2=yellow>>***C5** 3=cream *4=Other*	/___/
D4.0	If No, would you be prepare to use the yellow flesh as a weaning food?	*1=yes >>***C5** *2=no*	/___/
D4.1	If no, Why (Key Reason)	***Key Reason >>C7***	
D5	Is there any price difference between yellow root and other color root?	*1=yes 2=no>>***C7** *3= Don't Know >>***C7**	/___/
D6	If yes, what is the average price difference?	Le	
D7	What advantage(s) do you think yellow flesh root has over the other color roots?		
D8	What disadvantage(s) do you think yellow flesh root has over the other color roots?		

SECTION D: Household Gari Consumption							
E1.0 Do you or any of your HH member consume gari or any cassava products					*1=yes 2=no>>**End Time***		/___/
E1a If yes, list the cassava products?							
E1.1 HH disaggregation	**E1.2** This category of your family consume cassava products	**E1.3** If yes, this category of your family consume gari	**E1.4** **If** yes, which color type gari is mostly preferred?	**E1.4.1** Why preferred?	**E1.5** If no yellow gari, would you be willing to introduce it to this family category?	**E1.5.1** If yes , what is the major reason for your willingness to introduce it to this category of family	**E1.5.2** Why?
Family members categories	*1=yes 2=no>>**Next row & D2***	*1=yes 2=no>>**Next row & D2***	*1=White 2=Yellow>>**D1.5.1*** 3=cream *4= both*	*Key Reason*	*1=yes 2=no >>**D1_5_2***	*>>Next row **& D2***	*Key Reason*
Self	/___/	/___/	/___/		/___/	/___/	
Spouse	/___/	/___/	/___/		/___/	/___/	
Infant Male	/___/	/___/	/___/		/___/	/___/	
Infant female	/___/	/___/	/___/		/___/	/___/	
Adolescence male	/___/	/___/	/___/		/___/	/___/	
Adolescence female	/___/	/___/	/___/		/___/	/___/	
Adult	/___/	/___/	/___/		/___/	/___/	
SECTION D: Household Gari Consumption							
E1.0 Do you or any of your HH member consume gari or any cassava products					*1=yes 2=no>>**End Time***		/___/
E1a If yes, list the cassava products?							

E1.1 HH disaggregation	**E1.2** This category of your family consume cassava products	**E1.3** If yes, this category of your family consume gari	**E1.4** **If** yes, which color type gari is mostly preferred?	**E1.4.1** Why preferred?	**E1.5** If no yellow gari, would you be willing to introduce it to this family category?	**E1.5.1** If yes , what is the major reason for your willingness to introduce it to this category of family	**E1.5.2** Why?
Family members categories	*1=yes 2=no>>**Next row & D2***	*1=yes 2=no>>**Next row & D2***	*1=White 2=Yellow>>**D 1.5.1*** 3=cream *4= both*	*Key Reason*	*1=yes 2=no >>**D1_5_2***	*>>Next row **& D2***	*Key Reason*
Self	/___/	/___/	/___/		/___/	/___/	
Spouse	/___/	/___/	/___/		/___/	/___/	
Infant Male	/___/	/___/	/___/		/___/	/___/	
Infant female	/___/	/___/	/___/		/___/	/___/	
Adolescence male	/___/	/___/	/___/		/___/	/___/	
Adolescence female	/___/	/___/	/___/		/___/	/___/	
Adult	/___/	/___/	/___/		/___/	/___/	

E2	The yellow color gari you normally consume**,** Is it processed from yellow root or palm oil during roasting?	*1=yellow root 2=palm oil >>**D5*** *3=No yellow color consumed >>**D5***	/___/
E3	If option one, how yellow it is?	*1=Very deep yellow 2=Moderate yellow 3=Deep yellow 4=Light yellow*	/___/
E4	How yellow color gari compared with the white color in terms of		
E4.1	Taste	*1=better 2=same 3=lesser*	/___/
E4.2	Quality	*1=better 2=same 3=lesser*	/___/
E4.3	Price	*1=higher 2=same>>**D4.4*** *3=lower*	/___/
E4.3.1	If option one, what is the price difference?	Le	
E4.4	Demand/consumption	*1=high 2=same 3=low*	/___/
E5	Would you be willing to shift from palm oil yellow gari and white color to yellow flesh?	*1=yes* *2=no*	/___/
E6	Is there any gari specifically used in feeding weaned children within this HH?	*1=yes* *2=no>>**D6.2***	/___/
E6.1	If yes, which color type?	*1=white* *2=yellow>>**D7*** *3=Brown*	/___/
E6.2	If No, would you be prepare to use the yellow gari as a weaning food	*1=yes* *2=no*	/___/
E7	What advantage(s) do you think yellow flesh root possesses over the other color roots?		
E8	What disadvantage(s) do you think yellow flesh root possesses over the other color roots?		

Appendix 2: Score plot of the principal components with their Eigenvalue and the proportion of variance explained of 188 provitamin A accessions



Appendix. 2: Summary statistics of genetic variation using 5,643 SNP markers among 188 cassava accessions

No of entry	MAF	No of Allele	H_e	H_o	PIC
TR 1563	0.83	1.34	0.17	0.34	0.13
TR 1337	0.82	1.36	0.18	0.36	0.14
TR 0421	0.82	1.36	0.18	0.36	0.14
TR 1207	0.82	1.36	0.18	0.36	0.13
TR 0267	0.77	1.45	0.23	0.45	0.17
TR 0626	0.79	1.42	0.21	0.42	0.16
TR 0431	0.82	1.36	0.18	0.36	0.13
TR 0085	0.82	1.36	0.18	0.36	0.13
TR 1295	0.82	1.35	0.18	0.35	0.13
TR 1627	0.82	1.37	0.18	0.37	0.14
TR 0224	0.82	1.37	0.19	0.37	0.14
TR 1578	0.81	1.38	0.19	0.38	0.14
TR 0222	0.82	1.36	0.18	0.36	0.14
TR 1755	0.81	1.38	0.19	0.38	0.14
TR 0854	0.82	1.36	0.18	0.36	0.14
SLICASS 4	0.82	1.35	0.18	0.35	0.13
TR 1051	0.82	1.36	0.18	0.36	0.14
TR 0261	0.80	1.39	0.20	0.39	0.15
TR 1201	0.81	1.38	0.19	0.38	0.14
TR 0894	0.82	1.36	0.18	0.36	0.14
TR 0232	0.82	1.37	0.18	0.37	0.14
TR 1302	0.83	1.35	0.18	0.35	0.13
TR 1128	0.81	1.38	0.19	0.38	0.14
TR 1808	0.82	1.36	0.18	0.36	0.14
TR 0172	0.83	1.35	0.17	0.35	0.13
TR 0382	0.82	1.37	0.19	0.37	0.14
TR 0384	0.81	1.37	0.19	0.37	0.14
TR 1688	0.82	1.35	0.18	0.35	0.13
TR 1437	0.82	1.37	0.18	0.37	0.14
TR 0696	0.81	1.37	0.19	0.37	0.14
SLICASS 11	0.84	1.32	0.16	0.32	0.12
TR 0033	0.83	1.35	0.17	0.35	0.13
TR 1034	0.81	1.38	0.19	0.38	0.14
TR 0334	0.78	1.43	0.22	0.43	0.16
TR 1610	0.83	1.34	0.17	0.34	0.13
TR 0631	0.81	1.37	0.19	0.37	0.14
TR 1233	0.85	1.30	0.15	0.30	0.11
TR 0998	0.82	1.37	0.18	0.37	0.14
TR 1744	0.81	1.38	0.19	0.38	0.14
TR 1153	0.82	1.36	0.18	0.36	0.14

No of entry	MAF	No of Allele	H_e	H_o	PIC
TR 0886	0.82	1.37	0.18	0.37	0.14
TR 0446	0.82	1.37	0.19	0.37	0.14
TR 0974	0.82	1.36	0.18	0.36	0.14
TR 1565	0.81	1.38	0.19	0.38	0.14
TR 0785	0.83	1.34	0.17	0.34	0.13
TR 1569	0.81	1.39	0.19	0.39	0.14
TR 0713	0.83	1.34	0.17	0.34	0.13
TR 0423	0.80	1.40	0.20	0.40	0.15
TR 0887	0.81	1.38	0.19	0.38	0.14
TR 1785	0.81	1.38	0.19	0.38	0.14
TR 0025	0.82	1.36	0.18	0.36	0.14
TR 1374	0.82	1.35	0.18	0.35	0.13
TR 1562	0.81	1.38	0.19	0.38	0.14
TR 1236	0.84	1.33	0.17	0.33	0.12
TR 0838	0.77	1.46	0.23	0.46	0.17
TR 1480	0.83	1.34	0.17	0.34	0.13
TR 0937	0.81	1.39	0.19	0.39	0.15
TR 0743	0.81	1.38	0.19	0.38	0.14
TR 1540	0.81	1.37	0.19	0.37	0.14
TR 0747	0.82	1.37	0.18	0.37	0.14
TR 1348	0.81	1.39	0.19	0.39	0.15
TR 1438	0.80	1.39	0.20	0.39	0.15
TR 1477	0.80	1.39	0.20	0.39	0.15
TR 1243	0.81	1.39	0.19	0.39	0.15
TR 0807	0.82	1.37	0.18	0.37	0.14
TR 1389	0.80	1.40	0.20	0.40	0.15
TR 1259	0.82	1.37	0.18	0.37	0.14
TR 1182	0.81	1.37	0.19	0.37	0.14
TR 1543	0.80	1.39	0.20	0.39	0.15
TR 0975	0.82	1.36	0.18	0.36	0.13
TR 1155	0.81	1.38	0.19	0.38	0.14
TR 1404	0.82	1.37	0.19	0.37	0.14
SLICASS 7	0.81	1.38	0.19	0.38	0.14
TR 1202	0.82	1.37	0.18	0.37	0.14
TR 0955	0.81	1.37	0.19	0.37	0.14
TR 0520	0.81	1.39	0.19	0.39	0.15
TR 1208	0.81	1.39	0.19	0.39	0.15
TR 0843	0.82	1.37	0.18	0.37	0.14
TR 1113	0.82	1.37	0.18	0.37	0.14
TR 1316	0.80	1.40	0.20	0.40	0.15
TR 0693	0.82	1.36	0.18	0.36	0.14
TR 1593	0.82	1.36	0.18	0.36	0.14
TR 1598	0.83	1.35	0.17	0.35	0.13
TR 0282	0.79	1.42	0.21	0.42	0.16
TR 1350	0.80	1.41	0.20	0.41	0.15
TR 0957	0.82	1.36	0.18	0.36	0.13
TR 1422	0.79	1.42	0.21	0.42	0.16
TR 0932	0.83	1.35	0.17	0.35	0.13
TR 1349	0.82	1.36	0.18	0.36	0.14
TR 0810	0.80	1.41	0.20	0.41	0.15
TR 0718	0.79	1.41	0.21	0.41	0.16
TR 0907	0.82	1.36	0.18	0.36	0.14
TR 0335	0.83	1.34	0.17	0.34	0.13
TR 1327	0.82	1.36	0.18	0.36	0.13
TR 1666	0.81	1.38	0.19	0.38	0.14

No of entry	MAF	No of Allele	H_e	H_o	PIC
TR 1748	0.82	1.37	0.19	0.37	0.14
TR 1361	0.80	1.39	0.20	0.39	0.15
TR 0189	0.81	1.38	0.19	0.38	0.14
TR 1269	0.84	1.31	0.16	0.31	0.12
TR 1533	0.81	1.38	0.19	0.38	0.14
TR 1762	0.81	1.39	0.19	0.39	0.15
TR 0015	0.79	1.41	0.21	0.41	0.15
TR 0018	0.81	1.38	0.19	0.38	0.14
TR 1073	0.81	1.38	0.19	0.38	0.14
TR 0890	0.82	1.37	0.18	0.37	0.14
TR 0316	0.82	1.36	0.18	0.36	0.13
TR 1199	0.77	1.47	0.23	0.47	0.18
TR 1144	0.81	1.39	0.20	0.39	0.15
TR 0982	0.81	1.39	0.19	0.39	0.14
TR 1244	0.83	1.35	0.18	0.35	0.13
TR 1279	0.80	1.40	0.20	0.40	0.15
TR 1008	0.81	1.39	0.19	0.39	0.15
TR 0861	0.82	1.36	0.18	0.36	0.14
TR 0983	0.80	1.40	0.20	0.40	0.15
TR 1031	0.81	1.39	0.19	0.39	0.15
TR 0683	0.81	1.38	0.19	0.38	0.14
TR 0772	0.82	1.36	0.18	0.36	0.14
TR 1229	0.81	1.38	0.19	0.38	0.14
TR 0118	0.81	1.38	0.19	0.38	0.14
TR 0840	0.81	1.38	0.19	0.38	0.14
TR 0396	0.81	1.39	0.20	0.39	0.15
TR 1788	0.80	1.39	0.20	0.39	0.15
TR 0485	0.77	1.45	0.23	0.45	0.17
TR 1152	0.82	1.36	0.18	0.36	0.14
TR 0990	0.81	1.38	0.19	0.38	0.14
TR 1004	0.81	1.39	0.19	0.39	0.14
TR 0679	0.82	1.37	0.18	0.37	0.14
TR 1515	0.81	1.38	0.19	0.38	0.14
TR 1735	0.80	1.40	0.20	0.40	0.15
SLICASS 6	0.85	1.31	0.15	0.31	0.12
TR 0700	0.82	1.36	0.18	0.36	0.14
TR 1463	0.82	1.37	0.18	0.37	0.14
TR 0365	0.83	1.35	0.17	0.35	0.13
TR 1620	0.84	1.32	0.16	0.32	0.12
TR 0289	0.81	1.38	0.19	0.38	0.14
TR 1603	0.81	1.37	0.19	0.37	0.14
TR 1505	0.80	1.40	0.20	0.40	0.15
TR 1849	0.79	1.43	0.21	0.43	0.16
TR 0031	0.81	1.38	0.19	0.38	0.14
TR 0319	0.78	1.44	0.22	0.44	0.17
TR 1198	0.81	1.39	0.19	0.39	0.14
TR 1256	0.82	1.36	0.18	0.36	0.14
TR 1557	0.81	1.39	0.20	0.39	0.15
TR 0535	0.81	1.38	0.19	0.38	0.14
TR 0856	0.82	1.36	0.18	0.36	0.13
TR 1359	0.82	1.37	0.19	0.37	0.14
TR 0744	0.82	1.36	0.18	0.36	0.13
TR 0881	0.82	1.37	0.18	0.37	0.14
TR 1405	0.81	1.39	0.19	0.39	0.15
TR 0385	0.81	1.38	0.19	0.38	0.14

No of entry	MAF	No of Allele	H_e	H_o	PIC
TR 1223	0.78	1.43	0.22	0.43	0.16
TR 0868	0.79	1.42	0.21	0.42	0.16
TR 1313	0.81	1.38	0.19	0.38	0.14
TR 0480	0.82	1.37	0.18	0.37	0.14
TR 1266	0.80	1.40	0.20	0.40	0.15
TR 1071	0.82	1.37	0.18	0.37	0.14
TR 0703	0.81	1.37	0.19	0.37	0.14
TR 0893	0.84	1.33	0.16	0.33	0.12
TR 1689	0.83	1.34	0.17	0.34	0.13
TR 0707	0.82	1.37	0.18	0.37	0.14
TR 1556	0.81	1.37	0.19	0.37	0.14
TR 0927	0.81	1.37	0.19	0.37	0.14
TR 0688	0.81	1.38	0.19	0.38	0.14
TR 1007	0.82	1.37	0.18	0.37	0.14
TR 0299	0.82	1.37	0.19	0.37	0.14
TR 1289	0.79	1.42	0.21	0.42	0.16
TR 0851	0.80	1.40	0.20	0.40	0.15
Cocoa	0.82	1.35	0.18	0.35	0.13
TR 0295	0.81	1.38	0.19	0.38	0.14
TR 1590	0.78	1.44	0.22	0.44	0.16
TR 0918	0.81	1.39	0.19	0.39	0.14
TR 1133	0.82	1.37	0.18	0.37	0.14
TR 1331	0.79	1.42	0.21	0.42	0.16
TR 0461	0.81	1.38	0.19	0.38	0.14
TR 1419	0.83	1.34	0.17	0.34	0.13
TR 0368	0.83	1.35	0.17	0.35	0.13
TR 1448	0.83	1.35	0.17	0.35	0.13
TR 1322	0.81	1.38	0.19	0.38	0.14
TR 0399	0.83	1.35	0.17	0.35	0.13
TR 1525	0.77	1.47	0.23	0.47	0.18
TR 1753	0.81	1.38	0.19	0.38	0.14
TR 1501	0.81	1.38	0.19	0.38	0.14
TR 0019	0.80	1.40	0.20	0.40	0.15
TR 0296	0.79	1.42	0.21	0.42	0.16
TR 1360	0.81	1.39	0.19	0.39	0.15
TR 1527	0.81	1.38	0.19	0.38	0.14
TR 0560	0.82	1.36	0.18	0.36	0.14
TR 1502	0.81	1.38	0.19	0.38	0.14
Mean	**0.71**	**2.00**	**0.36**	**0.38**	**0.28**

MAF: major allele frequency; NA number of alleles; He: expected heterozygosity; Ho: observed heterozygosity; PIC: Polymorphic information content.

Appendix 3 : Provitamin A cassava accessions with varying levels of total carotenoid content using color chat and i-check device

Accession	Total Carotene content	Pulp color	Field TC
TR 1259	11.2	2	4
TR 1808	7.4	2	4
TR 1208	8.9	2	4
TR 0015	8.5	2	4
TR 0461	11.5	2	3
TR 1543	7.2	2	4
TR 335	7.6	2	4
TR 0932	4.3	2	2
TR 1279	9.1	2	4
TR 0560	9.7	2	4
TR 0019	3.6	2	4
TR 1590	8.4	2	4
TR 0520	3.9	2	3
TR 1590	3.4	2	4
TR 1337	11.8	2	4
TR 1505	8.4	2	4
TR 1525	4.0	2	3
TR 1034	8.3	2	4
TR 0974	3.9	2	3
TR 1569	10.3	2	4
TR 0998	13.7	2	4
TR 1569	3.7	2	4
TR 1477	3.9	2	4
TR 0843	10.0	2	4
TR 1540	9.4	2	4
TR 0887	9.1	2	4
TR 1598	7.4	2	4
TR 0718	7.0	2	4
TR 1593	7.7	2	4
TR 1031	7.2	2	4
TR 0982	7.3	2	4
TR 0683	10.2	2	4
TR 1008	9.3	2	4
TR 0031	10.3	2	4
TR 1198	10.8	2	4
TR 1405	9.2	2	4
TR 1313	11.7	2	4
TR 0881	9.7	2	4
TR 0299	4.2	2	4
TR 1448	4.0	2	4
TR 1331	4.2	4	4
TR 0296	4.3	2	4
TR 1562	7.4	2	3
TR 1437	6.8	2	3
TR 1565	9.4	2	4
TR 0696	11.1	2	4
TR 1755	10.7	2	4
TR 0033	9.0	2	4
TR 0747	10.9	2	4

Accession	Total Carotene content	Pulp color	Field TC
TR 0807	8.2	2	4
TR 1182	10.4	2	4
TR 1349	7.5	2	4
TR 0990	4.1	2	3
TR 1229	3.9	2	4
TR 0700	7.7	2	4
TR 1243	4.2	2	3
TR 1502	4.2	2	3
TR 1419	4.3	2	3
TR 1762	4.4	2	3
TR 1071	4.1	2	3
TR 0957	3.8	2	4
TR 0707	8.0	2	5
TR 1557	11.2	2	3
TR 1322	9.9	2	4
TR 1302	8.1	2	4
TR 1350	9.0	2	4
TR 0907	9.1	2	4
TR 1236	4.1	2	4
TR 1199	8.0	2	4
TR 1152	4.0	2	4
TR 0365	7.0	2	4
TR 0851	3.6	2	4
TR 0423	4.4	2	4
TR 1753	8.6	2	4
TR 0713	8.2	2	4