Genetic improvement of beta carotene in cassava (*Manihot esculenta* Crantz) landraces

by

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DECLARATION

I Bright Boakye Peprah declare that, the thesis hereby submitted by me, for the degree of Philosophiae Doctor in Plant Breeding at the University of the Free State, is my own independent work and has not previously, been submitted by me for a qualification at another institution of higher education.

I furthermore, cede copyright of the thesis in favour of the University of the Free State.

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DEDICATION

This study is dedicated to my adopted mother, Rev Dr Mrs Elizabeth Yaa Parkes, my wife, Mrs. Felicity Ababio (Adwoa kraa), my father Mr Yiadom Boakye Peprah (late), Lydia Annor (mother) and my wonderful children, Elizabeth Yaa Kyerewaah Peprah, Nana Kwame Boakye Peprah, Nana Afia Agyeiwaah Peprah and Nana Kofi Assim Berko and all my siblings who stood with me throughout the changing scenes of life and to see me arise and shine academically by the grace of God.

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SUMMARY

The aim of this study was to identify farmers' adoption challenges, perceptions and preferences of yellow-flesh cassava. Combining ability and stability of these genotypes were also determined. Total carotenoid content (TCC), proximate values and hydrogen cyanide (HCN) of the yellow-flesh cassava were measured and the retention of carotenoids in boiled biofortified cassava was determined. This information will help breeders to identify genotypes with the best nutritional quality across the tested locations for planting and promotion in Ghana also could provide a basis for implementing a recurrent selection scheme for developing cassava varieties with high levels of carotenoids and dry matter. In all the locations visited, farmers' knowledge on the improved cassava varieties (white flesh) and the yellow-flesh cassava were generally poor among the men and women interviewed, due to their inability to access planting materials, which could be improved by strengthening the cassava seed system for awareness, and increased availability of the varieties to farmers. Very few men and women cultivated improved varieties and yellow-flesh cassava. The young adults, who are the future of the agricultural sector, lacked access to improved varieties and they must be given extra attention to understand the activities of cassava breeding programmes, to empower them to make use of these materials. The general combining ability (GCA) was larger than specific combining ability (SCA) for cassava mosaic disease (CMD), harvest index (HI) and TCC, with predictability ratios (0.98, 0.88 and 0.92 respectively) close to one. Hence, there is a possibility for improvement of the characteristics by selection. Positive significant correlation between pulp colour and TCC (r=0.59) and pulp colour and cortex colour (r=0.58) were observed. Negative significant correlation were seen between CGM and HI (r=-0.50), CMD and RTN (r=-0.45), and HI and RTN (r=-0.51). It implies that these key traits could be effectively combined in a breeding program. In particular, breeders can rapidly screen for high TCC by visually assessing the pulp colour in addition selection for CMD symptoms (in a high disease pressure zone). The selected individuals for pulp colour at early stage screening can then be quantified for carotenoids at later stages, to save cost. Some of the yellow-fleshed genotypes (progenies) displayed comparable dry matter content (DMC) values as their white-flesh elite parents and were selected for multilocational trial testing towards commercial release in Ghana. Findings of this study demonstrated that it is possible to simultaneously select for yield and quality traits, such as DMC, at seedling stage. It was shown that the yellow flesh cassava varieties could be used in a hybridization scheme with local material to combine both TCC and DMC traits with high yield in a CMD resistance background. Carotenoid-rich varieties also

showed variation for important characteristics, which are key drivers of variety adoption in Ghana. In view of this, some cassava varieties, such as IBA090151 and IBA083774, are proposed for release in Ghana. The HCN content of the cultivars varied from location to location and the values observed were below 50 µg g^{-1} and hence can be classified as sweet cultivars (low HCN). The cultivars that were sweet were, however, above the range of the maximum acceptable HCN limit recommended by the World Health Organisation (WHO) and for that reason need to be processed before consumption *(*for example as *fufu*, konkonte, gari). Finally, it is recommended that cassava breeders review their breeding objectives to reflect the preferred traits of end-users, and pay attention to stakeholders' perceptions of the yellow flesh cassava to develop demand driven varieties that will serve the need of end-users. Education to create awareness on the potential advantages and diverse uses of the improved biofortified cassava is also needed.

Keywords: combining ability, cyanide, farmer-preferred traits, nutritional value, provitamin A, yellow flesh cassava

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LIST OF ABBREVIATIONS

AGDP	Agricultural gross domestic product
AMMI	Additive main effects and multiplicative interaction
ANOVA	Analysis of variance
AOAC	Association of official analytical chemists
CBB	Cassava bacterial blight
CGM	Cassava green mite
CIAT	International Center for Tropical Agriculture
CMB	Cassava mealy bug
CMD	Cassava mosaic disease
CNG	Cyanoglucoside
CNP	Cyanogenic potential
CRI	Crops Research Institute
CSIR	Council for Scientific and Industrial Research
CSIR-CRI	Council for Scientific and Industrial Research-Crops Research Institute
CV	Coefficient of variation
DRC	Democratic Republic of Congo
DM	Dry matter
DMC	Dry matter content
EAR	Estimated average requirement
FAO	Food and Agriculture Organization
Fe	Iron
FRW	Fresh root weight
FSW	Fresh shoot weight
GCA	General combining ability
GCV	Genotypic coefficient of variation
GDHS	Ghana Demographic Health Service
GDP	Gross domestic product
GxE	Genotype by environment
GEI	Genotype by environment interaction
GGE	Genotype and genotype by environment interaction
GLSS	Ghana Living Standards Survey
GSS	Ghana Statistical Service

IPAC	Interaction principal component axis
HCl	Hydrogen chloride
HI	Harvest index
HCN	Hydrogen cyanide
IFAD	International Fund for Agricultural Development
IITA	International Institute of Tropical Agriculture
LSD	Least significant difference
MAP	Months after planting
mg	Milligram
MOFA	Ministry of Food and Agriculture
NaOH	Sodium hydroxide
NCD II	North Carolina design II
NIRS	Near infrared spectrophotometer
PCA	Principal component analysis
PCV	Phenotypic coefficient of variation
PPB	Participatory plant breeding
PPD	Post physiological deterioration
PPMED	Policy, Planning, Monitoring and Evaluation Division
PRA	Participatory rural appraisal
pVA	Provitamin A
PVAC	Provitamin A content
RDA	Recommended daily allowance
RAE	Retinol activity equivalents
RCBD	Randomized complete block design
RTN	Storage root number
RTW	Storage root weight
SCA	Specific combing ability
TBC	Total beta carotene
TCC	Total carotenoid content
t ha^{-1}	Ton per hectare
TWT	Total biomass
USAID	United State Agency for International Development
UV	Ultra violet
VA	Vitamin A

VAD	Vitamin A deficiency
WAAPP	West Africa Agricultural Productivity Programme
WHO	World Health Organization
Zn	Zinc

CHAPTER 1

GENERAL INTRODUCTION

Cassava, the fifth most important staple crop in the world, is a widely grown and consumed root crop in Sub-Saharan Africa (Tan, 2015). It is also regarded as the most widely cultivated root crop in the tropical region and a crop that persistently contribute to food security mainly because of its ability to store its matured edible roots in the ground for about three years and unarguably the sixth most important crop (following crops like wheat, rice, maize, potato, and barley) in the world (Saranraj *et al.* 2019). Cassava is consumed in various forms such as boiled roots, fufu, and gari. Gari is very popular with urban dwellers as it is easy to prepare and can be stored for extended periods. In Nigeria, which is a major producer of cassava, it is also used for many industrial applications such as starch, glucose and ethanol production. In other countries of sub-Saharan Africa, including Ghana, the situation is similar, as 30 to 80% of the region's inhabitants consume cassava (Otekunrin and Sawicka 2019). The world cassava production stands at 291 million tonnes in 2017 with leading countries like Nigeria (59 million), Congo DR (31 million), Thailand (30 million), Indonesia (19 million, Brazil (18.9 million), Ghana (18.4 million) ranked 1st, 2nd, 3rd, 4th, 5th and 6th respectively with production in the Africa (177 million in 2017) regarded as the world largest cassava growing region. (FAOSTAT 2019).

The main nutritional component of cassava is carbohydrate, which derives from starch accumulated in the tuberous storage roots. On the other hand, the shoots and leaves of cassva are highly nutritious and are consumed as vegetables in many parts of Africa. It has high levels of protein (7 g per 100 g fresh material) and has high concentrations of lysine, minerals and vitamins (Hahn 1989; IITA 1990; Nweke *et al*. 1994; Fregene *et al*. 2000; Benesi 2005). The woody cassava stem cuttings are used commercially as planting materials (Ekanayake *et al*. 1997; Alves 2002). Cassava is adapted to a wide range of environments. It has good drought and acid soil tolerance, with good performance on degraded soils where other crops often fail (Jones 1959; Kawano *et al*. 1978; Jaramillo *et al.* 2005) and also resilience to climatic shocks (Jarvis *et al*. 2012). Cassava is an excellent alternative for maize for industrial processes in the tropics (Jaramillo *et al*. 2005). Cassava has a high yield potential and is better suited than cereals for production in areas where population pressure and crop failure are a challenge (Nweke 1996; Benesi 2005). A big advantage of cassava is the fact that it can be stored in the ground and harvested when needed, which contributes to food security (DeVries and Toenniessen

2001) and famine alleviation (Nweke *et al*. 2002).

Cassava is a major staple crop, which contributes 22% to agricultural gross domestic product (GDP) (Policy, Planning, Monitoring and Evaluation Division (PPMED) 1991 Ministry of Food and Agriculture (MOFA) 2012) in comparison to the GCP contribution by other crops/products such as maize (5%), rice (2%), sorghum (14%) and millet (14%), cocoa (11%), forestry (7%), fisheries and livestock (5%) (Al-Hassan 1989; Dapaah 1996). It is grown in all 10 regions of Ghana (Okai 2001) and occupies over 90% of the country's farming area (MOFA 2012). A study by Al-Hassan and Diao (2007) showed the potential of cassava to reduce poverty and promote economic growth in northern Ghana, which is among the poorest areas in the country. Cassava has been identified as an importnat commodity that can generate economic growth and fight poverty in a number of reports relating to Ghana's economic growth and development (Dapaah 1991; 1996; Al-Hassan 1993; Nweke 2004).

A limiting characteristic of cassava roots for human or animal consumption is their cyanogenic glucosides content (Kakes 1990). Traditional processing methods of grating, fermenting, boiling and/or drying removes most of the cyanide. Cassava roots are a good source of energy while the leaves provide protein, vitamins, and minerals. However, cassava roots and leaves are deficient in sulfur-containing amino acids (methionine and cysteine) and some nutrients are not optimally distributed within the plant (Montagnac *et al*. 2009a). An additional constraint is the negligible amount of provitamin A content (PVAC) found in the white flesh varieties of cassava cultivated in Ghana. Beta carotene and other carotenoids are a dietary precursor of vitamin A, and are responsible for the yellow to orange flesh colour of storage roots (Degras 2003; Rodriguez-Amaya and Kimura 2004). Vitamin A (VA) is essential for good vision, and contributes to an effective immunity system, and is also involved in cellular differentiation, growth and reproduction. VA deficiency (VAD) is a widespread public health problem in 37 countries worldwide and affects a large percentage of people in areas where cassava is a staple crop, such as in sub-Saharan Africa, northeast Brazil, and Southeast Asia (Njoku *et al*. 2011).

The 2014 Ghana Demographic and Health Survey (GDHS), which was carried out by the Ghana Statistical Service (GSS) and the Ghana Health Service, revealed that Ghana is characterised by rampant malnutrition and high incidence of nutrient deficiency-related diseases (GSS *et al.* 2015). They reported that more than three-quarters of children age 6- 59 months are anaemic. Anaemia rates were found to be higher in rural areas where cassava is the main source of livelihood compared to urban areas (72% vs. 58%). Anaemia was also higher in the northern,

Upper West and Ashanti regions. There is emerging evidence that improving the VA status of people has a synergistic effect on iron (Fe) and zinc (Zn) status. In Ghana, the group most at risk of VAD are pre-school children living in the northern part of the country and women in their childbearing years. In the remote areas of Ghana, where poverty is the most severe, VAD is an endemic problem. Nearly one million children in Ghana do not receive nutritional supplements.

Beta carotene is the most abundant carotenoid in cassava and can be efficiently converted to VA. Beta carotene and vitamin E, ascorbic acid, enzymes and proteins make up the biological antioxidant network, which converts highly reactive radicals (•OH) and free fatty peroxy radicals to less active species. In this way they protect the body against oxidative cell damage (Packer 1992). In human nutritional studies, VA activity is expressed as retinol equivalent and 3.7 mg of cassava beta carotene has the biological (VA) activity of 1 mg retinol. According to Maziya-Dixon (2010) this refutes the previous estimate of about 12 mg of beta carotene in cassava being equivalent to 1 mg of retinol. The average daily requirements of beta carotene equivalent for children is 2.4 mg, for adults it is 3.5 mg while for lactating mothers it is 5.0 mg. (WHO 1995; Ukpabi and Ekeledo 2009). These dietary requirements are not adequately supplied by diets, especially in children, pregnant women, and the poor in several countries, including Ghana.

Dietary diversification, food fortification and/or supplementation are the three strategies that have been used most frequently to prevent VAD. For a variety of reasons these strategies have not been effective to eradicate VAD (West 2003). Harvestplus, which involves a global alliance of research institutions, has initiated the development of micronutrient dense staple crops, also called biofortification, as a fourth strategy to eradicate VAD, with one of the initiatives being specifically the development of biofortified cassava clones with high PVAC in the roots (Dwivedi *et al*. 2012). Conventional breeding techniques can be applied for biofortification, by taking advantage of the genetic variability for micronutrients in different crops (Chavez *et al*. 2005), but genetic transformation is also an option (Welsch *et al*. 2010). The underlying factor to micronutrient problem is the consumption of deficient diet by people, and these techniques can be used to address this problem (Ceballos *et al*. 2013). Fortunately, the conversion of PVAC present in cassava roots into VA in humans has proven to be highly efficient (Thakkar *et al*. 2009). In a VA cassava biofortification breeding programme, the acceptability of its product by farmers and consumers, as well as the bioavailability of the beta carotene in the product should be considered (Njoku 2012).

In the southern part of Ghana, the adoption rates and adoption intensity of the improved cassava varieties in 2007 were 9 and 37% respectively (Dankyi and Adjekum 2007). This is because during the early stages of the breeding process, farmers and consumers were not included in the process (Nweke *et al.* 1994; Benesi 2005; Manu-Aduening *et al*. 2014). In 2017 1176, cassava farmers were interviewed and 80% were aware of the improved cassava varieties. Eighty seven percent (87%), 90%, 82% and 62% of farmers from the forest zone, Transition, coastal savannah and Guinea savannah respectively were aware of improved varieties. Forty one percent (41%) of cassava area was planted to improved cassava variety during the 2014/2015 major season (Acheampong *et al*. 2017)

Plant breeding has shifted towards client-oriented participatory breeding. The principle is that farmers and scientists have equal inputs in the selection process, in a long-term collaborative process that leads to better products. Client-oriented participatory breeding improves breeding efficiency, accelerates adoption, leads to more acceptable varieties, promotes genetic diversity and saves cost through reduction of the breeding cycle (Morris and Bellon 2004; Witcombe *et al*. 2005; Mangione *et al*. 2006; Gyawali *et al*. 2007; Manu- Aduening *et al*. 2014). Therefore, to increase the acceptability and adoption rate for biofortified yellow-flesh cassava cultivars, farmers and consumers would of necessity have to be integrated at the early stages of the research and in the selection of varieties through participatory methods.

The objectives of this study were to:

1. Identify farmers' adoption challenges, perceptions and preferences for yellow flesh cassava through participatory rural appraisal.
2. Determine the combining ability for beta carotene, dry matter content (DMC), cassava mosaic disease (CMD), yield and its related components in some F1 cassava families.
3. Determine genetic variability, stability and heritability for quality and yield characteristics in provitamin A (pVA) cassava varieties.
4. Determine the total carotenoid content (TCC) and HCN in yellow flesh cassava cultivars and also to measure the retention of carotenoids during the processing of biofortified cassava into boiled cassava.

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CHAPTER 2

LITERATURE REVIEW

2.1 Importance of cassava

2.1.1 General importance

Cassava (*Manihot esculenta* Crantz) is regarded as the most widely cultivated root crop in the tropical region (Saranraj *et al*. 2019) and it originated from several centres beginning from the southern edge of the Brazilian Amazon (FAO 2013). It serves as a food for over 900 million people in the tropics and sub-tropics (FAO 1996; Nassar 2005) and also as a source of calories in the human diet with 500 calories per day for more than 500 million people in sub-Saharan Africa, Asia and Latin America (Onwueme 2002). Among all staple crops in sub-Saharan Africa, cassava has been a major staple as it is grown mainly for its storage roots, being the economic part of the crop. As a 'crisis crop', it can be left in the ground for a period of time until shortages arise. Global cassava production in 2012 was 269.1 million ton and 149.4 million tons for Africa (Table 2.1).

Table 2.1 World cassava production (million metric tons) between 2015 and 2018

	2005	**2016**	**2017**	**2018**
World	293.0	288.5	279.3	277.8
Africa	172.7	172.8	168.3	169.7
West Africa	91.4	89.5	91.5	93.0
Nigeria	57.6	59.6	59.4	59.5
Ghana	17.7	17.8	19.0	20.8

FAOSTAT (2019)

Cassava is ranked as the fifth most important staple crop in the world (Tan 2015) and one of the non-native crop in Africa that has achieved staple food status (Tewe 1992). Cassava roots are very rich in carbohydrate, which makes them an important source of dietry energy (FAO 2013). In 2017, the largest producing countries were Nigeria, Congo DR, Thailand, Indonesia, Brazil, Ghana, Vietnam, Cambodia, Angola, Mozambique, Cameroon, Malawi and China, with Africa producing more than half of the world’s total production (FAOSTAT 2017). Low yields have been due to production constraints and abiotic factors (Nweke 1996). Cassava is

tolerant to drought and acidic soils, with reasonable yield on degraded soils where other crops fail (Jaramillo *et al.* 2005) and is hence a very good crop for Africa (Nweke *et al*., 2002). Cassava can serve as an alternative to maize for industrial purposes in the tropics (Jaramillo *et al*. 2005) and is even more suitable than other grain staples in areas where population pressure and crop failure are a challenge (Al-Hassan 1993; Nweke 1996; Benesi 2005). Cassava roots can be stored in the soil and harvested when needed. This makes it a food security (DeVries and Toenniessen 2001) and famine reserve crop (Nweke *et al*. 2002), but also a good industrial crop (Dixon and Ssemakula 2008).

Cassava production stretches through a wide belt from Madagascar in the southeast to Senegal and Cape Verde in the northwest (Raji *et al*. 2001; Benesi 2005). An increase in cassava production in Africa has been reported due to research and better use of agronomic practices, especially in Ghana and Nigeria, and rapid population growth forcing consumers to look for cheaper sources of calories (IFAD and FAO 2005). Cassava leaves and shoots are also used as vegetables in other parts of Africa because of its nutritional value for humans and animals (Ceballos *et al.* 2004) but have no market value in Ghana, since it is not consumed as a vegetable (Angelucci 2013). The seeds are used as medicine and in animal feed formulations (Fregene *et al.* 2000; Benesi 2005). The woody stems serve as cuttings for planting (Ekanayake *et al.* 1997; Alves 2002) and are sometimes sold to generate an income (Alves 2002; Popoola and Yangomodou 2006).

Root crop production, especially cassava, can spur rural industrial development and raise incomes for producers, processors and traders. It will contribute to the food security status of its producing and consuming households (FAO and IFAD 2001).Cassava markets are being expanded in countries like Nigeria and Ghana for its products like; starch and its derivatives, ethanol, glucose syrup, composite flour and gari (Nweke *et al*. 2002; Nweke 2004). This has led to the growing demand for cassava, with cassava increasingly cultivated in large acreages of commercial farms and by farmers' cooperatives (Nweke *et al.* 2002; Nweke 2004; Manu-Aduening *et al.* 2006). There are also excellent opportunities for product and market diversification in several other African countries (Benesi 2005; Al-Hassan and Diao 2007; Dixon and Ssemakula 2008).

2.1.2 Importance of cassava in Ghana

Cassava adoption was very slow in Ghana in early 1980s after its introduction, due to the fact that most of the people in the forest belt preferred plantain, cocoyam whiles in the northern part people preferred sorghum, Maize and millet (Parkes 2011). Its cultivation and utilisation became important following the major crop failure in 1983, with cassava as the key exception to the catastrophe (Manu-Aduening *et al.* 2005). Currently, Ghana is the sixth largest producer of cassava in the world (FAOSTAT 2017), and cassava ranks first among the root and tuber crops in Ghana (IFAD and FAO 2005). This root crop is the main source of carbohydrates to meet the dietary requirements needed by people and is a regular source of income for most rural dwellers.

Cassava is not only a food security crop but also an important industrial crop for the provision of cash and jobs for rural and urban communities (Nweke *et al.* 2002; Dixon and Ssemakula 2008). There is a growing importance of cassava in Ghana (Dapaah 1991; Al-Hassan *et al.* 1993; Manu-Aduening *et al.* 2006) and this has necessitated the development of cassava-based industries in the country. Cassava has tremendous potential in Ghana and Africa's economy for food, feed and industrial uses, and provides cash and jobs for the rural communities (Nweke 2004; Dixon and Ssemakula 2008).

Cassava roots can be consumed in a variety of forms (Amenorpe *et al*. 2006; Baafi and Sarfo-Kantanka 2008). The crop is used as starch and its derivatives, glucose syrup, flour and gari, for ethanol production and as animal feed (Nassar 2006). The cassava industry creates jobs for large numbers of people, mostly women, in sub-Saharan Africa (Haleegoah and Okai 1992; Thro *et al.* 1995). *Fufu* powder is produced from cassava, and in Ghana the true annual potential demand for this product is probably in the order of 1 000 to 17 100 metric ton. Even the lower limit would represent a substantial new opportunity for Ghanaian food manufacturers, albeit one that would not be easy to exploit. The estimated annual demand for fresh cassava roots translates to 2 000 to 34 200 metric ton, all of which could be supplied by Ghanaian farmers (Collinson *et al.* 2001). The agriculture-led economic growth has proven to reduce poverty more than non-agriculture-led growth (Al-Hassan and Diao 2007).

2.1.3 Nutritional value of cassava

Cassava roots are mostly used as starchy product in the world and the fresh foliage is used in several countries as feed for animals and vegetables for human consumption (Cock 1985; Kawano *et al.* 1998). Among the starchy staples, cassava provides approximately 40% more of the carbohydrate consumed than rice and 25% more than maize (Nyerhovwo 2004). The root serves as a significant and cheap source of calories for both human and animal nutrition. Depending on the terrain, type, age of plant and climatic conditions, cassava has most of its nutrients in the roots and leaves, which are the edible parts of the plant. According to El-Sharkawy *et al*. (2012), cassava storage roots are predominantly used as a source of carbohydrate but less for protein, fat, minerals and vitamins. Consequently, cassava is of lower nutritional value than all cereals, legumes, and even some other root and tuber crops, such as yams. There are two types of cassava varieties; sweet varieties (having low HCN), which requires a low amount of processing, whilst bitter varieties require more processing because of its high total cyanide content or cyanogenic potential (CNP). The higher the CNP of a variety, the greater the need to process the root before consumption (Kakes 1990). Two types cyanogenic glycosides (linamarin and lotuaustralin) are synthesised in the leaves of the cassava plant.

Cassava roots have a low level of protein, about 1-2% on fresh weight basis, and also a low level of essential amino acids (Mahungu 1987). The young leaves have a high crude protein content (170 to 400 g kg^{-1} on a dry matter (DM) basis), with almost 0.85% being true protein (Ravindran *et al.* 1983). Some wild relatives of cassava with high levels of protein have been discovered. Genes from these wild relatives have been introduced into *Manihot esculenta*, which has resulted in an increase in protein content of cassava storage roots (CIAT 2002; Ceballos *et al.* 2004; Olalekan A *et al*. 2011). Elsewhere in Africa (Nigeria and Uganda), introgression of genes for higher protein content into local farmers' preferred varieties has started (Njoku 2012).

Latif and Müller (2015) reported that cassava leaves are highly concentrated in vitamins B1, B2 and C, carotenoids, protein and minerals. Cassava leaves, depending on the variety, are also rich in Fe, Zn, manganese, magnesium and calcium and consumed by many as vegetable (Wobeto *et al.* 2006). While the leaves of cassava are nutrient rich, there are issues of bioavailability and crop acceptability in different parts of the world. The mineral content of the roots of cassava is reported to be two to five times lower than that of the plant leaves. A higher amount of VA in the form of pVA carotenoids are contained in the leaves of

cassava compared to its roots (Montagnac *et al*. 2009a). VA is an important micronutrient for the normal functioning of the visual and immune systems, growth and development, maintenance of epithelial cellular integrity and for reproduction (Huang *et al*. 2018). These carotenoids are also useful antioxidants. According to Gan *et al.* (2010), antioxidants are substances that fight free radicals, which cause oxidation of various biomolecules present in organisms. A diet highly concentrated in antioxidants strengthens the human body protection system (Blomhoff *et al.* 2006). Oxidative damage is a cell and tissue damage caused by free radicals (Gan *et al*. 2010). The most efficient way to get rid of free radicals is consumption of antioxidant nutrients such as vitamin C (ascorbic acid), vitamin E and beta carotene, which can be found in large quantities in yellow/orange coloured fruits and vegetables (Rahmat *et al*. 2003). Fiedor and Burda (2014) also suggested that carotenoids have some antioxidant properties. During the chain reaction mechanism of lipid oxidation, carotene reacts with active free radicals to form stable inactive products (Maziya-Dixon *et al*. 2000) thus preventing oxidative reaction from the production of off-flavours in foods and the potential damage of living cells in biological systems. This role played by carotene is independent of its VA activity (Ceballos *et al*. 2002).

In the assessment of nutritional and anti-nutritional composition of cassava leaf protein concentrate from six cassava varieties for use in aqua feed using standard analytical techniques, Oresegun *et al.* (2016) reported the highest crude protein levels, beta carotene levels and lipid levels of 48.85%, 816.92 µg g^{-1} and 13.27%, respectively. The VA content of cassava leaves is comparable to that of carrots and is higher than that reported for legumes and leafy vegetables (Montagnac *et al.* 2009b). However, cassava leaves have some anti-nutritional and toxic substances. These substances interfere with digestibility and uptake of the nutrients, and they might present toxic effects, depending on the amounts consumed.

2.2 Yellow flesh cassava

Yellow flesh cassava genotypes are planted on a small scale in Colombia, Philippines, Jamaica and other African countries like Nigeria, Uganda, Congo DR and Ghana (Oduro 1981). Research has shown that yellow flesh cassava varieties tend to have a low DMC (Akinwale *et al*. 2010), which is associated with poor cooking quality (Vimala *et al*. 2008). Most cassava breeding populations are white with only a few yellow flesh cassava populations found in Amazonia in Brazil (Njoku 2012).

Yellow-fleshed cassava genotypes have high levels of PVAC and their consumption have been suggested as a sustainable approach for addressing VA deficiencies. In cassava, intensity of yellow pigment in roots of some genotypes is strongly associated with beta carotene (Sánchez *et al.* 2006). Wide variation exists in root colour within the global yellow root germplasm, which has a range from pale yellow through orange to pink (Nassar 2007). This variation in root colour is corroborated by wide variation in carotenoid contents within the global cassava germplasm. Yellow flesh cassava has increased the different views on nutritional benefits associated with the crop; and beta carotene (pVArovitamin A) in yellow flesh cassava can sustainably address VAD through the dissemination of pVA cassava varieties in regions where the crop is a major staple (Makokha and Tunje 2005; Nassar and Ortiz 2010). Efforts in breeding yellow flesh cassava genotypes that are high in beta carotene content started in almost 20 years ago, with slow progress in its development and deployment to farmers (Welch and Graham 2005), which might be due to the negative correlation between beta carotene and DMC (Vimala *et al.* 2008; Akinwale *et al.* 2010).

In Nigeria as well as Ghana, most of the cultivated landraces have white fleshed roots with a negligible amount of the pVA pigment. In 2012, the Crops Research Institute (CRI), Kumasi in Ghana, acquired some yellow flesh cassava genotypes with improved agronomic traits from the International Institute of Tropical Agriculture (IITA), in Nigeria. These genotypes are being used as a tool in fighting VAD in areas that lack VA rich food materials.

VAD causes eye damage, mostly in children. About 60% of the dietary VA is produced from pVA or beta carotene and consumption of high beta carotene foods is the most effective way of fighting the deficiency. The amount of beta carotene the human body can absorb from VA cassava is more than twice the value or amount previously reported. (La Frano *et al.* 2012) and this was received with much hope to improve nutrition using food-based interventions. VA status in deficient populations could improve measurably if people switch to these new varieties with high levels of beta carotene (www.harvestplus.org). Since cassava is a major staple crop in Ghana, consumption of yellow flesh cassava varieties containing even moderate amounts of beta carotene can help reduce VAD in the country.

2.3. Cassava biofortification

Biofortification is the process of incorporating micronutrient-dense traits in cassava varieties with good agronomic characters like fresh root yield/weight through conventional breeding or biotechnology. This method provides a more sustainable way of disseminating

micronutrients to rural/remote populations in developing countries. Both conventional and transgenic breeding methods are being employed to develop these varieties. Generally transgenic crops have tended to be a “political hot button” but crops that are a result of conventional breeding have found favour within communities on this account. Recently, there has been a shift in agriculture where the aim is not only to produce more calories to reduce hunger, but the use of more nutrient rich food in reducing hidden hunger (Saltzman *et al.* 2013).

In many developing countries, cassava is regarded as a food security crop but with a number of liabilities. Montagnac *et al.* (2009a) reported that 500 g of cassava meal for an adult can provide an adequate amount of calories, but with an insufficient amount of pVA and protein. Some of the past efforts to improve the micronutrient content of cassava include programmes like Bio-cassava Plus (phase 1, 2005- 2010), dealing with traits like pVA, shelf life, cyanide content and diseases (Saltzman *et al.* 2013).

Harvestplus, an international initiative involving a global alliance of research institutions in both developed and developing countries, seeks to improve nutritional status of vulnerable people in the society using plant breeding in developing staple crops rich in pVA, zinc and iron (Makokha and Tunje 2005). Under this initiative, targets are set such that vulnerable people will receive more than 50% of the estimated average requirement (EAR) using pVA cassava. Countries like Nigeria, Ghana, Uganda and Congo DR are benefitting from this scheme. Three cassava varieties with 25% of the EAR for women and pre-school children were released in Nigeria in 2011, with a possible release in Ghana by 2020. A few lines have been evaluated at the on-farm stage and scientists are currently waiting for their assessment by the national variety release committee. EMBRAPA, a research institution in Brazil, also released three cassava varieties with about 9 ppm pVA, and planting materials have been distributed to farmers in the country (Saltzman *et al.* 2013).

2.3.1 Importance of carotenoids and vitamin A

Carotenoids are described as richly coloured molecules, which are the sources of the yellow, orange and red colours of many plants (Rodriguez-Amaya and Kimura 2004). In the human diet, carotenoids are obtained from fruits and vegetables. According to Clagett- Dame and Knutson (2011), carotenoids are also found in some fungi, bacteria and algae. Green leafy and yellow-orange vegetables and fruits provide significant amounts of beta carotene (pVA carotenoids) (Veda *et al.* 2007). The essential role of carotenoids in humans is pVA

carotenoids serving as precursors of VA (Chávez *et al*. 2005) and is important for optimal growth and cell and tissue differentiation.

In human plasma, the most common carotenoids include beta carotene, alpha carotene, beta cryptoxanthin, lutein and lycopene. They have health-promoting effects together with zeaxanthin. Among the carotenoids , beta carotene has the highest pVA potential and is also the most wide spread (Rodriguez-Amaya and Kimura 2004). Thus, alpha carotene and beta cryptoxanthin exhibit about 50% of the VA activity of beta carotene. Carotenoids have several beneficial effects on human health, including the enhancement of immune response, reduction in the risk of diseases such as cancer, cardiovascular diseases, cataracts, and mascular degeneration. It is also essential for optimal growth and lung development of the newborn during pregnancy. About 40% increase in VA intake for pregnant women, was recommended and a 90% intake for breast feeding women (Njoku 2012).

The president of Ghana in 2005 launched a special initiative aimed at promoting cassava for starch production as well as a potential source of feeds in the livestock industry. The potential of cassava as food and feed can even be increased with enhanced VA, Zn, Fe and protein content (Njoku 2012). This can be a unique opportunity to increase production, and also prove highly nutritious animal feed, flour, food and chips for both the local, and export markets. In Latin American countries, especially Brazil, water extracted from high carotene cassava during starch production is highly nutritious and serves as additional feed for animals (Njoku *et al.* 2011).

VA is a fat-soluble vitamin that exists in three forms; retinol, retinal and retinoid in animal source foods and as pVA carotenoids, (mostly beta carotene), in plant source foods (Wardlaw *et al*. 2004). The retinol is the storage form and is found in the liver until needed by the body. VA is important in the functioning of the immune system and for good vision (FAO/WHO 2002). Latif and Müller (2015) that, VA status, when improved in deficient children, can help improve their resistance to diseases and hence reduce their mortality and illness from infections significantly also reported it. Furthermore, improving VA status in deficient children aged from 6 months to 6 years increases their chances of staying alive longer (UNICEF 2014). It is further reported that possible mortality from measles is reduced by approximately 50%, 40% from diarrheoa and 25-35% overall. VA sources include breast milk, animal milk, liver, eggs, fish, butter, palm oil, mangoes, pawpaw, carrots, orange flesh potatoes and dark green leafy vegetables (Pan American Health Organization, 2005).

The Institute of Medicine Food and Nutrition board (2000) reported that VA activity of beta carotene in foods is one-twelfth of retinol preformed VA. Another advantage of pVA is that it is only converted to VA when the body needs it, to avoid potential toxicity from an overdose of VA (Clagett-Dame and Knutson 2011). Carotenoids have also been shown to be related to the improvement of immune system and lowered risk of degenerative diseases such as cancer, cardiovascular diseases, muscular degeneration and cataracts (Njoku *et al.* 2011).

2.3.2 Dietary recommendations for vitamin A and carotenoids

The recommended dietary allowance (RDA) of VA depends on the amount required to maintain adequate accumulation to support normal functions of the body. The RDA for VA for infants and children is 400-600 µg retinol activity equivalents (RAE; 1333 - 2000 IU). For adolescents (14-18 years) and adults 19 years and above it is 700 µg RAE (2 333 IU). The RDA for females; 900µg RAE (3000 IU) for pregnant women; 750-770 µg RAE (2333 - 2 567 IU) with the upper limit for pregnant women being 3000 µg RAE or 10000 IU for lactating mothers and for women 1200-1300 µg RAE (4000 - 4333 IU) (Institute of Medicine Food and Nutrition board 2000; Institute of Medicine Food 2001). The retinol activity equivalent is used as a measure of VA equivalence in foods. A mixed diet of 12 µg of all trans beta carotene or 24 µg of other pVA carotenoids (alpha carotene, cis-beta-carotene, beta-cryptoxanthin) is equivalent to 1 µg of retinol (Dary and Mora 2002).

2.3.3 Structure and genetics of beta carotene

Structurally, half of VA (retinol) is essentially beta carotene molecules. Alpha carotene and beta cryptoxanthin comprise the remaining half of VA activity. Beta carotene exists as a mixture of trans and cis forms with highly significant levels of the cis isomers compared to the trans form, but with lower VA activity (Rodriguez-Amaya and Kimura 2004).

The quantitative variability of root colour observed in cassava clones suggests that carotenoid transport and accumulation are governed by a number of genes each with a small effect *(*Ferreira *et al.* 2008). Akinwale *et al.* (2010) reported that there are no maternal or cytoplasmic effects in the inheritance of carotene. A segregation ratio of 9:3:3:1 was observed when white root cassava was crossed with yellow flesh cassava, which indicates that beta carotene is controlled by two or more genes. Njenga *et al.* (2014) on the other hand, reported the presence of maternal and cytoplasmic influence on beta carotene inheritance in cassava

Akinwale *et al.* (2010) also reported a negative correlation between DMC and carotenoid while studying African cassava germplasm, but the two traits had a weak positive correlation in Latin American cassava germpalsm (Ortiz *et al*. 2011). The negative correlation reported in Africa germplasm may be due to linkage of the carotenoid genes with that for low DMC in cassava roots (Njoku 2012). It is believed that with time, the linkage will be broken through recombination and selection (Ceballos *et al.* 2013).

2.3.4 Breeding for high beta carotene content

Research was carried out to increase the concentration of bioavailable PVAC in the edible portion of staple crops such as rice, wheat, maize and cassava (Graham and Welch 1996). A broad distribution of concentration less than 0.1 to 2.4 mg carotene/100 g fresh roots has been reported when more than 632 (Iglesias *et al.* 1997) and 2457 (Chávez *et al.* 2005) cassava clones were evaluated.

Molecular marker-assisted selection was employed in the development of quick, inexpensive ways for screening micronutrients in staple crops (Wong *et al.* 2004). This could enhance the introgression of genes into locally adapted cassava varieties. The importance of such a breeding activity will be driven by factors related to bioavailability of type of micronutrient and also willingness on the part of farmers to adopt such varieties. Carotenoid content can be improved simultaneously with yield and its related characteristics. Beta carotene makes the cassava pulp to have yellow to orange colour. It is generally same with other major staple crops (e.g. sweet potato). Yellow flesh cassava roots are thus a good source of carotenoids. Jos *et al.* (1990) reported the potential of increasing carotene content in cassava storage roots through recurrent selection. They could increase the carotenoid concentration of fresh storage roots of cassava in a base population from 4.2 mg kg^{-1} to 14 mg kg^{-1} after two cycles of selection and recombination. A database with more than 3000 samples of cassava genotypes was used to evaluate the potential of near infrared spectrophometry (NIRS) and spectrophotometer devices to predict root quality traits. Maximum TCC and total beta carotene (TBC) were 25.5 µg g^{-1} and 16.6 µg g^{-1} respectively on fresh weight basis (Sánchez *et al.* 2014).

A number of screening factors/parameters are required for better nutritional quality selection and it includes selection of a phenotype with agronomic micronutrient efficiency (Graham and Welch 1996), food processing concerns (Ceballos *et al.* 2012), bioavailability of the nutrients in improved cassava.

Breeding for higher carotene content in cassava could also reduce or delay post physiological deterioration (PPD). Reduced PPD in roots of carotene-rich cassava varieties was attributed to antioxidant property of carotenoids, particularly those of beta carotene, which is the predominant carotenoid in cassava roots (Safo-Kantanka *et al.* 1984). Sánchez *et al.* (2006) and Morante *et al.* (2010) have also reported reduced PPD.

2.4 Growth and development of cassava

2.4.1 Dry matter partitioning and source–sink relationship

During cassava growth, carbohydrates are needed to ensure good development of the leaves (source) to be able to produce DM in the storage roots, stem and growing leaves (sink) (Alves 2002). The amount of DM in cassava roots depends on the genotype as well as environmental factors and DM can vary from 15-45% (Graham *et al.* 1999). On average, about 90% of storage root DM is carbohydrate, and the other components are 4% crude fiber, 3% ash, 2% crude protein and 1% fat (Kawano *et al.* 1978).

High root DMC is important, especially when roots are used as food, feed and industrial raw materials (Tan and Mak 1995). High DMC thus improves the extraction efficiency and economic value of products of home-based and industrial processing. Price differentials for roots are usually paid on the basis of DMC or starch content; hence, improvement of these traits would greatly increase farm income (Kawano *et al.* 1978). Cassava DM is mainly translocated from leaves into the stems and storage roots of the cassava plant. However, it decrease in amounts with time in the leaves during crop growth in the leaves. Between 60 and 75 days after planting, cassava DMC are higher in the leaves compared with stems and storage roots. After that period, the DMC in storage roots increase rapidly, reaching 50 to 60% of the total DM around 120 days after planting (Tavora *et al*. 1995). At harvest (12 months after planting or MAP) DM is highest in the roots, followed by stems and leaves (Alves 2002). The dry matter content in the storage roots are mostly lower during the vegetative and higher during rest period (Edvaldo *et al*. 2006). Excess moisture stress increased dry matter accumulation in rootsock, fibrous and storage roots, but decreased partitioning to stems and leaves (Lahai and Ekanayake 2009). Mtunda (2009) reported that root DMC at 7 MAP was higher than 11 and 14 MAP. In addition, Kawano *et al*. (1987) observed that root DMC tended to be higher at 8 than 12 MAP, and that higher contents were seen at the beginning of the dry season than at the beginning of the wet season. During this period, starch is hydrolysed as a source of energy for the growing leaves.

Maximum levels of DM accumulation depends on genotypes and environmental conditions (Oelslige 1975; Howeler and Cadavid 1983). The importance of growing conditions in determining the maximum levels for DM accumulation suggests that germplasm should be evaluated under different environments to estimate the possible effects of genotype by environment interaction (GEI) during selection. DM accumulation also depends on photo-assimilate availability (source activity) and sink capacity of storage parts. Sink capacity is determined by the number of storage roots and their mean weight. Photosynthetic rate positively correlates with root yield, total biomass and leaf area index, interception of radiation and biomass production. This indicates that photosynthesis increases when photo-assimilates demand is high (Ramanujam 1990).

Cassava has diverse uses and most of the criteria for selecting quality are also diverse, but high starch content and quality (physico-chemical properties) are always required (Benesi 2005). The amount of starch in cassava is usually estimated from DMC, and both are highly correlated (r = 0.810; IITA 1974; CIAT 1975), but the quicker method is to determine the root's specific gravity, which is related to both DMC and starch content (Ellis *et al.* 1982). Specific gravity is obtained by weighing unpeeled cassava roots in air and water using a suitable balance. Estimation of both traits is based on the principle of a linear relationship between specific gravity with DM and starch content (Kawano *et al.* 1987). DMC is known to be relatively highly heritable, although it is influenced by temperature and rainfall patterns. A number of genes with predominantly additive gene effects apparently controls DMC. Simple breeding techniques such as phenotypic mass selection can be used to exploit the additive variations in DMC (Mtunda 2009), and selection for DMC can be highly effective in cassava breeding (Hershey 1987). Cassava varieties with 30% and more DMC are said to have high DMC (Braima *et al.* 2000).

High DMC is associated with post-harvest deterioration (van Oirschot *et al.* 2000; Chavez *et al.* 2005), although the reason for this is unknown. This could have serious consequences for commercial outlets, but not in subsistence agriculture where roots are immediately utilised. Mahungu (1998) reported that there is a shift in the paradigm factor and root yield alone is not sufficient to justify the production of a particular cassava variety. Root DMC is a critical factor, among others.

2.5 Variability in hydrocyanic acid content of cassava

Cassava contains a cyanogenic glucoside, linamarin (2-β-D-glucopyranosyl-oxy-isobutronitrile) in its leaves and tuberous roots which, when acted upon by linamarase (EC3.2.1.21: linamarin β-D-glucohyrolase) in the plant, is hydrolysed into cyanohydrins, which are further hydrolyzed to give HCN (Fukuba *et al.* 1983). Potentially toxic compounds, cyanogenic glucosides can cause acute poisoning and death in humans and animals when consumed in high quantities. Cassava cultivars with less than 50 ppm HCN are harmless (Endris 2007). Cyanogenic potential of some known cassava varieties ranges from less than 10 mg kg^{-1} to more than 500 mg kg^{-1} as HCN on fresh weight basis (O'Brien *et al.* 1994) and it is therefore important to evaluate cassava cultivars for cyanogenic potential.

Various reports have suggested strategies to reduce the cyanide content of processed cassava; improved processing methods during the production of cassava products, such as flour (Cardoso *et al.* 2005) and heap fermentation, can remove twice as much linamarin as does sun drying. In order to produce cassava flour with 10 mg HCN equivalents per kg of flour (ppm) which is the World Health Organization (WHO) safe level, one should use sweet cassava roots having 32 ppm linamarin or less (Bradbury 2004).

The amount of HCN in cassava varies strongly according to genotype, environmental conditions and various parts of the same plant (Food Safety Network 2005; Endris 2007). HCN content of cassava roots have been reported to be lower with potassium fertilisation (El-Sharkawy and Cadavid 2000, Susan *et al.* 2005; Endris 2007). Contrary to that, Attalla *et al.* (2001) reported high HCN level in cassava tuber tissues with increasing rates of potassium fertilizer (K_2SO_4). Production of cyanide in various cultivars is affected by soil, weather and other geographical conditions (Bokanga *et al*. 1994), same cultivars may produce high cyanide in one location and significantly lower value in another (Charles *et al*. 2005). It is therefore necessary to assay cyanide content of cassava root by characterizing for location and genotype before use for human consumption. The recommended WHO maximum acceptable level of cyanide in foods meant for human consumption is below 10 mg kg^{-1} (Bradbury and Egan 1992). The cyanide level of all cassava tubers were found to be above this recommended level (White *et al.* 1998). Cyanide is usually removed from tubers by different processing methods such as fermentation, boiling, steaming, drying and roasting (Cardoso *et al*. 2005).

2.5.1 Measures to control cyanide content in cassava

Regardless of the usefulness of cassava, it has a major drawback as food such as its perishability, its low protein content and its potential toxicity, which are a crucial limitation on its sustainability as a food source in the tropics. Upon crushing the plant tissues, its inherent cyanogenic glucosides are catalytically hydrolyzed to release HCN (Oloya *et al*. 2017). Due to the potential cyanogen toxicity, research efforts have been channeled to mitigate this effect. Three major strategies have been adopted and these are
(i) breeding of acyanogenic cassava varieties; (ii) controlling its metabolism and (iii) removal of cyanogens through processing (Nambisan 2011).

Development of acyanogenic varieties through breeding is probably the ultimate of the three listed approaches; however, it is a long-term solution as research efforts are still underway on this. Both genetic improvement and effective processing methods can be implemented to control cyanogenesis in cassava. There is also the option to manipulate the linamarin metabolism in roots, but this is also a long-term process. Processing remains the most efficient way of controlling cassava cyanogens in the short term (Nambisan 2011). For populations which rely on cassava as a staple, cultivars with low cyanogen (linamarin) content should be grown as far as possible, or alternatively, if high cyanogen or bitter cultivars are used, adequate processing should be done to reduce the cyanogen content to safer levels.

The processing methods used in cassava consuming communities are very diverse. These may include peeling and slicing fresh tubers then boiling, baking, steaming, drying, deep frying, fermentation, grating/pounding followed by drying or roasting. Most of these processing methods are effective in reducing cyanoglucoside (CNG) content to some extent (Bradbury 2006; Nambisan 2011). Depending on the processes used, they usually either lead to hydrolysis of CNG to release acetocyanohydrin and cyanide, which are volatilized and subsequently lost, or the highly soluble CNG and its hydrolytic products are leached out in the water (Nambisan 2011).

When cassava is cut into chunks and boiled, about 80% of the CNG content is removed (Table 2.2). It is therefore important to decant the water after boing, as the CNG in the roots leaches into it. The volume of water should be enough for optimum dissolution of CNG. When bitter tubers are cooked, it is customary to decant the water a few times until the bitterness is reduced to the maximum extent. Sun drying of cassava chips of 10 mm

thickness also removes 80% of CNG. Processes such as baking, steaming and frying result in only around 20% loss of CNG, due to inactivation of linamarase and stability of linamarin at high temperature. As seen in Table 2.2, the process of grating/pounding followed by sun drying is the very effective since it facilitates the enzyme reaction and results in 95–99% removal of CNG (Nambisan 2011). The most efficient processing method by far is fermentation, which removes about 80 to 98% of the cyanogens.

Table 2.2 Effect of different processing methods on the cyanogen content of cassava

Processing method	**Cyanogen retained (%)**	**Mechanism of removal**
Boiling	20-50	Leaching
Blanching and drying	50	Leaching
Baking, frying, steaming	80	Thermal degradation
Sun drying	20-50	Enzyme action
Oven drying	30-50	Enzyme action
Crushing and sun drying	< 5	Disintegration and enzyme action
Grating/fermentation, dewatering, drying	< 2	Disintegration and enzyme action

Nambisan (2011)

In Africa, fermentation is frequently adopted in making products like gari (roasted grated cassava), fufu (boiled and pounded), lufan, casaba and farina. These foods are prepared by a combination of grating/soaking, fermentation, dewatering and drying/roasting. The level of cyanogens in the final processed product is influenced by both the original CNG content and the processing methods applied. This suggests that different methods must be used for processing high and low cyanide varieties.

2.5.2 Effect of processing on the nutritional value of cassava

To increase the shelf stability of products, to facilitate conveyance and sales, reduce cyanogenic content and improve palatability and nutrient bio-availability, cassava must be processed into different products (Nyirenda *et al*. 2011). As with cyanide, simple processing of cassava such as drying cause a reduction in moisture and volume roots (Obilie *et al.* 2004) is drying and it causes a reduction in moisture, volume and cyanide concentration of roots. This prolongs product shelf life (Westby 2002). One of the traditional methods of processing cassava is fermentation (Cardoso *et al.* 2005) and is reported to enhance the nutrient content

of foods through biosynthesis of vitamins, fibre digestibility as well as enhancing micronutrient bioavailability. It also aids in degrading anti-nutritional factors. Bioavailability of micronutrients like vitamin B6, thiamin and carotenoids can also be improved through thermal processing like boiling, as it discharges them from cell walls in the plant matrix (Montagnac *et al* 2009b).

It has been reported that processing cassava affects the nutritional composition of cassava roots through alteration and losses in essential nutrients (Montagnac *et al*. 2009a; 2009b). Analysis of nutrient retention has shown that raw and boiled cassava roots keep the majority of high-value nutrients, except riboflavin and Fe (Onyenwoke and Simonyan 2014). The extent of soaking of cassava significantly affects nutrient retention and content in the processed samples. The longer the soaking, the lower the nutrient retention, especially the minerals.

Lafun and *fufu* processing methods seemed to retain more of the minerals than other processing methods. The gari processing method, which involves grating, predisposes the minerals to easy leaching through draining water. Significant mineral losses in the final products were reported after repeated washings with rehydration and draining in *abacha*. Fermentation and cooking enhanced nutrient content in prepared *eba, fufu* and *amala*. To improve the nutrient contribution of *gari* and *eba* for consumers, two days of fermentation of raw cassava was suggested (Adepoju *et al*. 2010).

With regards to cassava leaves, proximate components showed no difference between the ash, lipid, protein, starch and fiber contents of non-processed, pounded or ground cassava leaves. However, the free sugar component was reduced compaired to non-processed leaves. Processing has been reported to have no effect on the calcium, magnesium, potassium, sodium, phosphorus, copper, manganese and Zn contents of cassava leaves except for Fe (Achidi *et al*. 2008).

2.5.3 Impact of processing on carotenoids

The stability of food nutrients according to Montagnac *et al*. (2009b), is affected by processing and preservation. Processes such as roasting and fermentation have been reported to have severe impacts on carotene (Halvorsen *et al*. 2006). Other processes such as dehydration, blanching and canning may also have effects on the antioxidant property of the carotenoids of some edible plants (van Het Hof *et al*. 2000). However, there is an increased

antioxidant activity upon boiling and steaming of some vegetables such as carrots, lettuce, peppers, potatoes, tomatoes and cabbages (Halvorsen *et al*. 2006).

It has also been reported by Negi and Roy (2000) that beta carotene is sensitive to heat and oxidation during blanching and drying. High temperatures can cause losses of pVA and therefore excessive boiling, roasting and frying at high temperatures affects pVA (Thakkar *et al*. 2009) but low boiling for a few minutes, soaking and chopping can improve bioavailability with little carotenoid loss (La Frano *et al*. 2013).

2.5.4 Bioavailability of carotenoids

The percentage of consumed nutrient that is assimilated and reaches the point of being absorbed during blood circulation is termed as bioavailability (Maiani *et al*. 2009) whilst the proportion of a carotenoid that is transferred from the food matrix to micelles during digestion and made accessible for intestinal absorption, is termed as bioaccessibility (Stahl *et al.* 2000). The fraction of bioavailable pVA that can be changed into retinol is known as bioconversion (Ceballos *et al*. 2013). The efficiency of the process that ingested dietary pVA carotenoids are absorbed and converted to retinol is termed bioefficacy (van Lieshout 2001). In an animal source, vitamin A is readily taken as preformed retinol but from plant sourced foods, it is taken as pVA and therefore needs to be converted into retinol in the human body (Institute of Medicine 2001).

Bioavailability and bioconversion of carotenoids are affected by a number of factors, which include the quantity of fat available in the food (Thurnham *et al*. 2003), carotene types, molecular bonds, amount of carotene contained in a meal, nutritional status of the organism, genetic conditions and organism related conditions (Parada and Aguilera 2007).

2.6. Genotype by environment interaction

GEI occurs when cultivars perform significantly different with marked changes in pattern under different environmental conditions (Dixon *et al.* 1994). It can also be defined as the failure of genotypes to achieve the same relative performance in different environments (Fernandez 1991). Large GEI variation impairs the accuracy of yield estimation and reduces the relationship between genotypic and phenotypic values, thereby reducing progress from selection. It also prevents the extrapolation of results of agronomic evaluations from one location to another, thus requiring expensive trials at multiple locations (Shaffi *et al.* 1992). Hence, it is essential to examine different lines or crosses in several environments to determine their genetic potential and also for releasing genotypes with adequate adaptation

(Baiyeri *et al.* 2008). The relative performance of genotypes across environments defines the importance of GEI for the traits of interest.

Cassava as a crop generally is widely adapted to different ecologies but an individual cultivar can vary in adaptation because cassava cultivars are very sensitive to GEI (Ssemakula *et al.* 2007). Also, the response of an individual genotype to different environments may follow a diverse pattern due to the influence of the climate and soil variations. It is on this pattern that selection for high root yield, pest and disease resistance, and stable root yield is based (Dixon *et al.* 1994). Quantitative traits in cassava associated with root qualities, for example, carotene content, DMC, starch and HCN content may show sizeable interaction with the environment. Therefore, the testing of new lines in different locations (environments) to establish their genetic potential is crucial in cultivar development. In addition to high mean yields, stability of a genotype's performance in different environments is necessary to assist breeders in selecting superior cultivars to meet varying growing conditions. One approach is to reduce the number of replications used in a single field trial, assuming that performance can still be evaluated accurately.

The rate of adoption of biofortified cassava genotypes will largely depend on their agronomic performance, including fresh and dry storage root yield, resistance to major pests and diseases, DMC, HCN, and the stability of these traits over time and space. Although cassava as a crop is widely adapted to a variety of environmental conditions, most of the white fleshed varieties show narrow adaptation with large GEI effects (Dixon *et al.* 1994; Dixon and Nukenine 1997). Ssemakula *et al.* (2007) and Maroya *et al.* (2012) reported a significant GEI for total carotene content and they reported higher impact of genotypic effect than both environment and GEI, indicating fewer environments are necessary to distinguish genotypes with high and stable performance. In contrast, Maroya *et al.* (2010) found a non-significant GEI on beta carotene when evaluating nine yellow flesh cassava genotypes in Ghana.

Kawano *et al.* (1978), Ssemakula and Dixon (2007), Aina *et al.* (2009), Maroya *et al.* (2012) and Thompson (2013) reported significant GEI for DMC. Maroya *et al.* (2012) found higher environmental effects on DMC in contrast to the higher genotypic effects reported by others. Tan and Mak (1995) found that GEI effects were significant for cyanide content when studying the relative influence of genotype, environment and GEI effects on this trait in Malaysia.

In recent years, research has progressively placed attention on increased storage root production. However, agriculture must now not only produce more calories to reduce hunger but also more nutrient-rich food to reduce hidden hunger (Kennedy *et al.* 2003).

2.7 Heritability of characteristics in cassava

Heritability estimates how much variation in phenotypic traits in a population is due to genetic variation among individuals in that population (Klug and Cummings 2005). The two commonly used measures of heritability are broad-sense and narrow-sense. Narrow- sense heritability is more useful than broad-sense since its estimation does not involve dominance and interaction variances. There are many approaches for estimating heritability. Some researchers use the parent-offspring regression approach, or by comparing full-sibs. An analysis of variance (ANOVA) approach can also be used in estimating heritability, correlation and regression.

Heritability estimates are crucial in cultivar development and GEI testing strategies. Studies have shown that PVAC is controlled by a few (~2) major genes and is highly heritable (Menkir and Maziya-Dixon 2004; Grüneberg *et al.* 2005). However, heritability can be overestimated if studies contrast non-pVA and high pVA genotypes (Ewool and Akromah 2017). Both minerals and pVA are mainly driven by additive genes implying good general combining ability for these traits. Important characters such as harvest index (HI) and resistance to diseases such as cassava bacterial blight (*Xanthomonas manihotis*) and *Cercospora* leaf spot (*C. henningsii*) are highly heritable traits (Kawano *et al.*1978) indicating that they will be easily transmitted relatively to progenies.

2.8 Inheritance of nutritional traits

The colour of root parenchyma seems to be simply inherited. A report by Hershey and Ocampo (1989) established that a partially dominant gene determined root colour (white/cream/yellow). Homozygous dominant individuals have yellow roots while homozygous recessive individuals have white roots while the heterozygous individuals have cream roots (Hershey and Ocampo 1989). The inheritance of beta carotene concentration in storage root thus appear to be determined primarily by two genes, one controlling transport of shoot precursors to roots and one responsible for the biochemical processes affecting the accumulation of beta carotene in the root (Akinwale *et al.* 2010).

Although that was true for parents they used, recent studies have shown that the inheritance of root colour, and by implication carotene content may be more complex (Akinwale *et al.*

2010). Although major genes are responsible for the transport and accumulation of carotene in the storage roots, the quantitative variability observed within root colour classes suggested that a number of genes with smaller effects are involved in the accumulation process. The biochemical pathway leading to the synthesis of carotenes has also been long established (Spurgeon and Porter 1980) to support the genetics of this trait. Single breaks in the pathway could culminate in lack or reduced levels of carotene formation.

According to Iglesias *et al.* (1997), there is variation in beta carotene concentration in cassava storage roots in germplasm collection (630 genotypes) and also in the global cassava germplasm collection (about 5 500 genotypes). The authors further reported that sufficient genetic variability exists within the available cassava germplasm, which could help crop breeders to develop cassava varieties that contain enough beta carotene to meet the daily requirements of adults (i.e. 6 mg d^{-1} beta carotene) depending on bioavailability of beta carotene in cassava storage roots. Sánchez *et al.* (2006) reported a range in beta carotene concentrations in fresh roots from 0.1 to 2.4 mg 100 g^{-1}. The relationship between root colour and heritability, as well as the stability of root beta carotene content, to different root-processing techniques, has been studied. Visual screening by using intensity of orange colour to estimate beta carotene content seems feasible. However, it is possible that other forms of carotenoids could also be responsible for the deep yellow colour observed in accessions that have intermediate beta carotene concentrations.

2.9 Mating designs and heterosis

Various mating designs are used by crop breeders and geneticists to develop improved lines through plant breeding. Successful plant breeding schemes involve the correct choice of design and suitable progenitors (Khan *et al.* 2009). Mating design is used to provide genetic information of a trait under investigation and to generate breeding populations to be used as a basis for variety development. It also provides information for evaluating parents, estimation of genetic parameters and gain (Acquaah 2012). Mating design refers to the methods employed by plant breeders to produce progenies in plant breeding. However, the choice of a mating design for estimating genetic variances should be dictated by the objectives of the study, time, cost, space and other biological limitations (Nduwumuremyi *et al.* 2013). Some commonly used mating designs in cassava include polycross, North Carolina (I, II, III), diallel (methods I, II, III, IV)

2.9.1 Combining ability

Combining ability is defined as the performance of hybrid combinations (Kambal and Webster 1965). It helps in the selection of parents for hybrid development and genetic studies (Duvick 2001). Combining ability can be estimated by using the genetic parameters of the parents and the hybrid components (GCA and SCA, respectively) of diallel analysis (Griffing 1956). Sprague and Tatum (1942) first explained the concepts of GCA and specific combining ability (SCA) that underlies the genetic and breeding attributes of a genotype and therefore important in designing an efficient breeding strategy. GCA refers to the average performance of a line in a hybrid combination and is mostly due to additive gene effects. SCA is due to non-additive gene effects and is the case where combinations do relatively better or worse than would be expected based on the average performance of the lines involved (Falconer and Mackay 1996). The relative amount of improvement to be obtained from GCA and SCA is proportional to their variances and it shows the type of gene action controlling a particular trait (Quick 1978). Thus the relative sizes of the predictability ratio [(2GCA/(2GCA:SCA)] have been used to assess the relative importance of GCA and SCA. The closer the ratio is to one, the more important the additive gene effects (Baker 1978). GCA was more important in controlling CMD resistance (Lokko *et al.* 2005; Parkes 2011) and dominance plays an important role in fresh root weight (FRW) but was of little importance in some traits such as DMC (Cach *et al.* 2006). Njenga *et al.* (2014) reported significant GCA for root pulp colour. Selection of crop varieties based on combining ability estimates is useful to identify the most valuable progenitors and families for breeding and cultivar improvement. Both diallel and NCD II mating designs have been used to interpret genetic information on important traits in cassava (Jaramillo *et al.* 2005; Cach *et al.* 2006; Parkes 2011; Njoku 2012). These studies used crosses between heterozygous cassava clones as parents given that inbred lines don't exist in cassava. Rajendran (1989) reported additive gene action and non-additive gene action for storage root yield and yield components (such as HI, storage root number (RTN) and storage root weight) respectively. Root quality traits, namely starch, DMC and HCN content are predominately non-additive (Amma *et al*. 1995). Parkes *et al*. (2013) reported additive gene action for root number and plant height as well as for CMD and cassava bacterial blight in the forest zone of Ghana. Peninah *et al.* (2014) reported additive gene action for root pulp colour in yellow flesh cassava varieties from IITA and root pulp colour correlates directly with beta carotene. They also reported that improving local white cassava varieties for beta carotene does not affect yield. They further suggested that GCA and SCA of selected parents should also be considered due to the additive and non-additive gene effects involved in the inheritance of the trait. Combining

ability of parents and their performance are very important when formulating specific breeding programmes (Rajendran 1989). Diallel crosses are also used to investigate GCA of parents and to identify superior parents for use in cultivar development (Ortiz *et al.* 2001; Yan and Hunt 2002).

The usefulness of both additive and non-additive gene action in the inheritance of yield and yield components has been reported in different studies (Maris 1989; Ruiz de Galarreta *et al.* 2006). However, such information is scant for the yellow flesh cassava breeding population in Ghana. Selecting individuals and families for high DMC and TCC in a population improved for CMD resistance could be an important strategy in breeding for yield stability towards under food and nutrition security.

2.9.2 Diallel and North Carolina design II

The diallel and North Carolina design II (NCD II) mating designs provide genetic information such as combining ability of parents and the inheritance of quantitative traits (Kang 1994). The NCD II mating scheme is a cross-classification design whereby different sets of parents are used as males and females. The design can accommodate more parents than the diallel and provides the same type of genetic information (Hallauer and Miranda 1988). The main effects of males and females correspond to GCA and the female x male interaction to SCA (Jaramillo *et al.* 2005; Cach *et al.* 2006). Both diallel and NCD II mating designs have been used to interpret genetic information on important traits in cassava (Jaramillo *et al.* 2005; Cach *et al.* 2006; Parkes 2011; Njoku 2012).

2.9.3 Heterosis

Crossbreeding or hybridization of two parents often result in positive fitness-related effects in F_1 progeny (Akah 1992; Sprague 1983) and this has been exploited in plant breeding for years. The performance of F_1 progeny that exceed the average parental performance is mostly referred to as hybrid vigour or heterosis (Shull 1908; Parkes 2011) or superiority in performance of hybrids over their parents. It is the interpretation of increased vigour, size, speed of development, resistance to disease and pest, manifested by crossbred lines as compared with their parents. There are two predominant theories that are used to define heterosis; dominance and over- dominance hypothesis (Crow 1952). Dominance hypothesis is produced when deleterious recessive alleles from one individual parent are masked by dominant or partially dominant alleles from in the second individual (second parent) in the hybrid (F1), while over-dominance hypothesis is due to the heterozygous superiority of the

F1 (hybrid) over homozygous individuals. Therefore, increased vigour is proportional to the amount of heterozygosity (Lamkey and Edwards 1999). Heterosis is due to the combined action and interaction of allelic and non-allelic genes and is usually closely and positively correlated with heterozygosity (Burton 1968). There are different types of heterosis, such as mid-parent, better-parent and standard heterosis. Mid- parent heterosis is defined as the difference between the hybrid and the mean of the two parents (Falconer and Mackay 1996).

2.10 Participatory plant breeding

Farmers as well as professional plant breeders have important knowledge and skills that could complement one another. Participatory Plant Breeding (PPB) is based on this concept, and is defined as a range of approaches that involve a mix of participants (including scientists, breeders, farmers and other stakeholders) in different plant breeding stages. Depending on whether researchers or farmers are controlling the breeding process, and the scale on which the work is undertaken (community-centred or research to extrapolate results), two categories can be identified, which may be either farmer-led or formal-led PPB. These concepts are not well understood and are difficult to pinpoint using conventional market research methods. Farmers are often uneducated and unschooled in plant breeding, but they have, in several years, dominated crop production and selected superior cultivars of different crop species (Harlan 1992). They were able to recover and successfully propagate genetic recombinants that exhibited desirable traits (Jauhar 2006) through the collection of volunteer eedlings, which they selected and added to their cultivated genotypes (Manu-Aduening *et al.* 2005; Kizito *et al.* 2007; Peroni *et al.* 2007; Pujol *et al.* 2007). Under formal plant breeding programmes has been low and scientists are yet to fully utilize their knowledge and this has resulted into low adoption of modern varieties bred (Witcombe *et al.* 1999). Farmers have different perceptions and priorities to those of plant breeders (Manu-Aduening *et al.* 2005). Farmers often use specific stable morphological traits like height at first apical branching, petiole colour and culinary attributes such as taste, to differentiate and name varieties (Kizito *et al.* 2007) as well as to indicate preferences. Farmers were reported to consistently base their selections on distinctive traits which they can observe, such as canopy shape, stem colour and root skin colour (Manu-Aduening *et al.* 2014). High CMD incidence and good extension services usually had to increase farmer adoption of improved varieties. Cassava breeding efficiency tend to improve when farmers participate in the early stages of breeding (Dixon *et al.* 2007). Farmer participation in breeding also enhanced farmer ownership of newly developed cultivars, leading to their acknowledgement that they are the ultimate beneficiaries, which often resulted in the rapid adoption of new

improved cultivars (Manu-Aduening *et al.* 2014)

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Chapter 3

Awareness, perception and willingness to adopt yellow flesh cassava through participatory rural appraisal in coastal savannah, forest and forest-transition zones in Ghana

Abstract

Deficiency of micronutrients, such as vitamin A, constitutes a major public health problem in Ghana, which affects mainly children and women. Qualitative and participatory approaches were employed to investigate preferences of producers and processors, their perceptions of, willingness to adopt, and inhibitory factors to adoption of newly developed biofortified cassava cultivars. A total sample of 113 producers and processors participated in 12 focus groups in three cassava growing districts within three agro-ecological zones (forest, transition and coastal) using a semi-structured interview schedule. The results showed that knowledge and use of improved varieties and the new yellow flesh cassava was generally low among men and women farmers in all the three locations visited. Participants preferred other traits such as climate smart varieties, long storability in the soil and mult-purpose varieties in addition to the high yielding trait. Factors that contributed to low technology adoption were lack of awareness, access and availability of new varieties, gender stereotyping, access to extension services and perception of the new varieties. It is recommended that cassava breeders focus more on education of producers and consumers in terms of potential benefits of the biofortified varieties. They should also incorporate the identified trait preferences in their breeding objectives and adopt a multi- stakeholder approach in improving the cassava seed systems in Ghana.

3.1 Introduction

Cassava is grown primarily for its starchy storage roots and is an important staple for more than 800 million people, mostly in sub-Saharan Africa and other parts of the world (Anna *et al.* 2010). In terms of calories consumed, it is the second most important food staple (Tarawali *et al*. 2012) and mostly called Africa's food insurance crop because it gives appreciable yield even in the face of drought and poor soil management (Dixon *et al.* 2003). However, current cassava varieties mainly cultivated in Ghana produce roots which are high in DM but low in VA, protein, fat, minerals and other micronutrients (Ceballos *et al.* 2007; Thakkar *et al.* 2009). VAD constitutes a public health problem and affects mainly children and women. The HarvestPlus, a development programme involving a global alliance of

research institutions, initiated the development of micronutrient-dense staple crops (Bouis *et al.* 2011; Dwivedi *et al.* 2012). Among these initiatives is the development of biofortified cassava clones with high content in the roots (yellow colouration). VA is involved in the normal functioning of the human visual and immune systems and is an essential vitamin. It also affects growth and development, and is involved in the reproduction process and the maintenance of epithelial cellular integrity (ACC/SCN 2000; Combs 1998). Mortality in children can be reduced by 23 to 30% when their VA status is improved (Beaton *et al.* 1993). It is estimated that 75 to 251 million children have subclinical symptoms of VAD (WHO 2009).

Four strategies have been developed and applied to improve the status of VA, especially among the vulnerable groups in Africa. These are dietary diversification, food fortification, supplementation and biofortification. The first three approaches are relatively cost-effective, but have failed to eradicate the problem of VAD, for a variety of reasons (West 2003). Biofortification is an approach that relies on conventional plant breeding and modern biotechnologies to increase the micronutrient density of staple crops, including cassava (Bouis 2003; Nestel *et al.* 2006). It holds promise for improving the nutritional and health status of poor populations in both rural and urban areas of developing countries like Ghana and Nigeria. Cassava is one of the crops targeted for biofortification, as is consumed daily by large numbers of people in sub-Saharan Africa. Significant progress has been made in pVA cassava research in Ghana in the past few years and cultivars are at an advanced stage of release to Ghanaian farmers. The challenge is stimulating wider uptake of the varieties and incorporation into the food systems.

A number of plant breeding programmes in developing countries such as Ghana haven't been impactive due to lack of consideration of farmers' needs and concerns, leading to low adoption rates of released varieties (Kamau *et al.* 2011). Witcombe (2009) reported that the active participation of target clients such as farmers in breeding can help breeders select important traits needed in hybridization, but this aspect is mostly ignored, a phenomenon, which, in part, explains why many farmers at times continue to grow landraces, which have farmer-preferred attributes as opposed to the new officially, released varieties (Witcombe 2009). PPB has been used by various researchers to bring all stakeholders together with the aim of addressing this challenge and narrowing the communication gap between scientists and farmers. These gaps have important implications for productivity and food security. Many scientists have successfully implemented PPB in selecting superior cassava cultivars in various countries. Manu-Aduening *et al.* (2006) implemented a successful PPB

programme for developing superior cassava cultivars in Ghana by involving farmers early in the process (the seedling nursery trial stage). Schofield *et al.* (2009) similarly involved farmers in participatory varietal selection of cassava varieties in the Great Lakes area. Kamau *et al.* (2011) also implemented PPB in Kenya resulting in the successful selection of 30 genotypes (which combined early-bulking and high root quality traits) after a preference tests by farmers in KARI-Kiboko farm (Eastern Kenya),. Several authors, including Morris and Bellon (2004), Mangione *et al.* (2006), Gyawali *et al.* (2007) and Manu-Aduening *et al*. (2014) have confirmed that participatory breeding improves breeding efficiency, accelerate adoption, lead to more acceptable varieties, promotes genetic diversity and saves cost through reduction in breeding cycle.

PPB utilizes many approaches such as participatory varietal selection, surveys, key informant interviews and focus group discussions. In social science, this is termed Participatory Rural Appraisal (PRA) approaches, which is heavily reliant on community participation. This method is designed in a way to involve farmers as partners. Farmers are therefore responsible for gathering and analyzing information, meaning they are not just only serving as sources of information but as analyst. These two aspects of the process provide vital information required by farmers (Kamau *et al.* 2011; Were *et al.* 2012) to guide the uptake of new varieties. The involvement of farmers at some breeding stages could change their conservative behavior and promote the adoption of new genotypes by incorporating their preferred traits (Nduwumuremyi *et al.* 2016). Understanding factors that affect famers' adoption of new cassava varieties, especially yellow flesh cassava, could be useful to plant breeders in designing their research programmes. Thus, breeding objectives targeting the end-user preference could therefore enhance the adoption of new varieties.

3.1.1 Status of yellow flesh cassava in Ghana

Cassava is a perennial crop grown mainly for its storage roots and belongs to the economically important family Euphorbiaceae (Boampong and Sarfo-Kantanka 2014; Amenu 2015). Different varieties (both white and yellow flesh cassava) have been cultivated for many decades across the various suitable cassava growing regions in Africa and within Ghana. However, yellow flesh cassava is known to be cultivated on a limited scale in Colombia, Philippines, Jamaica and some African countries, and Ghana is no exception (Okoro 2015). In the past, local germplasm was collected in parts of Ashanti, Eastern, Volta, Greater Accra, Western and Central regions under the National Agricultural Research Project Root and Tuber Crops Research Programme. These collections were characterised

at CSIR-Crops Research Institute (CRI), CSIR-Plant Genetic Resources Research Institute and the Department of Crop Science, University of Cape Coast (Annor-Frimpong 1991). This germplasm may have contained the common local yellow variety called "*Bankye borodee*" literally translated as "cassava-plantain" due to its pulp colour. In 1998, Boampong and Sarfo-Kantanka (2014) made a total collection of 212 landraces, from which they selected 36 accessions to generate the ethno-botanical information. Among these accessions, was the common local yellow variety "*Bankye borode*e" which was found in farmers' fields. This shows that up to the 20th century, this local yellow variety had been common, though the germplasm kept eroding. With constant genetic improvement and the development of new varieties, farmers adopted new varieties that meet their needs and offer them more satisfaction and livelihood security. As they adopted new varieties, landraces and old varieties were dropped. New developments in the cassava sector led to the loss of most of the landraces, old improved varieties and the popular local yellow flesh variety. Currently, in Ghana, very few farmers, especially the adults, have few stands of the yellow flesh cassava on their farms with very limited planting materials, as identified in this study.

The objectives of this study were i) to assess and determine the importance of cassava select Ghanaian communities in threeecologies; ii) to identify cassava preferred traits by farmers; iii) to investigate farmer/stakeholders' awareness, perception and preference for yellow flesh cassava cultivars and iv) to examine factors that could possibly affect adoption of new cassava varieties in the select communities of Ghana.

3.2 Materials and methods

3.2.1 Brief description of area

The study was conducted in Techiman municipal, Agona East and Ketu North districts in the forest-transition, forest and coastal savannah zones of Ghana, respectively. Geographically, Techiman Municipal lies between longitudes 1°49′ east and 2°30′ west and latitude 8°00′ north and 7°35′ south, Agona East at latitudes 5^{o} 30″ and 5^{o} 50″ north and longitudes 0^{o}35″ and 0^{o}55″ west and Ketu North districts at latitude 6^{o}03'N and 6^{o}20'N and longitude 0^{o}49'E and 1^{o}05'E. (GSS, 2015). These three districts were selected based on the high volumes of cassava produced and the high processing activities that occurs in these locations.

3.2.2 Data collection procedures

Prior to the main field work, there were several engagements with the extension agents and the research team at the various districts, to assist in the mobilization of farmers and processors drawn for the study. All preliminary desk reviews on selecting participants for focus group and key informant interviews and guidelines for conducting focus group to ensure effective field work as described by Elias (2013), were carried out. A mixed method approach to data collection was adopted to collect both qualitative and part of the quantitative data, which focused on demographic and farm/processing level characteristics. The PRA approach was, however, the main method used in the data collection. This approach was adopted to create awareness and enhance the uptake of the yellow flesh cassava varieties in future, when released (Turyahikayo 2014). The qualitative tool used was the focus group discussions, key informant interviews and personal observations. The study instruments were peer-reviewed and enumerators trained on them prior to data collection.

Within each district, four cassava growing villages and 10 participants from each village were sampled for the group discussions. Participants were then conveyed to a central location for the meeting. Four focus group discussions were held in each district, involving producers and processors. A total of 12 focus groups, involving 8-10 persons per group, were held across the three districts. The study combined all three types of questions (engagement, exploration and exit) during the group discussions and the key informant interviews. Conscious efforts were made to have a representation of adult males, adult females and young adults for the discussion.

A total of 113 producers and processors participated, representing a 94.2% rate of response. From the social perspective, adults and young adults were defined based on the community's accepted definition; that is that a young adult was someone between the ages of 18-45 years and adult; above 45 years. Qualitatively, an adult was described as a married person or someone who has given birth and the young adult as the unmarried or an active male or female living under the parents' roof. In Techiman Municipal and Agona East districts, the adults were more than the young adults, so groups were disaggregated based on sex (male and female), however, at Ketu North district, the project team sticked to the planned design (disaggregation based on gender; adults and young adults) and this was the first district visited.

The disaggregation was done to allow full participation of all the targeted direct participants and to elicit gender-based responses. Ten participants made up of five producers and five processors were sampled from 12 communities, with gender considerations in the sampling design. Data collected covered land tenure arrangements, crops grown in the communities, cassava varieties cultivated and processed by men and women, cassava traits preferred by men and women, importance of cassava to study groups, access to productive resources, adoption and dis-adoption of varieties, awareness, preferences and perception of yellow flesh cassava and demographic variables of respondents.

3.2.3 Data analysis

The data collected was analyzed using a content analysis approach as described by Elo and Kyngas (2008), which was also adopted by Esuma *et al*. (2019) in their study on "Men and women's perception of yellow flesh cassava among rural farmers in eastern Uganda" and descriptive statistics. Primarily, the field notes and audio recordings were first transcribed for each of the groups and key informants. The responses were coded to identify emerging themes, categorized and analyzed separately for adult producers, young adults, producers, adult processors and young adults processors. Lastly, comparative analysis across the districts were done to examine consistency in results and draw inferences from the study. The descriptive statistics were used to describe the basic features of the demographic information collected. These included frequencies and means distributions.

3.3 Results

3.3.1 Characteristics of survey sample

Some similarities existed between the two groups; producers and processors, and results are presented in Tables 3.1, 3.2 and 3.3. The study recorded a high percentage of young adults' participation (based on communities' definition); 53.8% of producers and 62.5% of processors, respectively.

Table 3.1 Characteristics of survey sample (qualitative variables)

Characteristics	Producers		Processors	
	Frequency (N=65)	Percentage %	Frequency (N=48)	Percentage %
Sex				
Male	55	84.6	15	31.2
Female	10	15.4	33	68.8
Social groups (as defined by community)				
Adults (>45 years)	30	46.2	18	37.5
Young adults (18-45 yrs)	35	53.8	30	62.5
Head of household				
Yes	53	81.5	20	41.7
No	12	18.5	28	58.3
Marital status				
Married	53	81.5	41	85.4
Single	11	16.9	1	2.1
Divorced	-	-	3	6.2
Widowed	1	1.5	3	6.2
Highest level of education				
No formal education	10	15.4	12	25.0
Basic	46	70.8	34	70.8
Secondary	8	12.3	1	2.1
Tertiary	1	1.5	1	2.1
Residential status				
Native	45	69.2	19	39.6
Permanent settlers	20	30.8	29	60.4
Tenancy arrangements				
Family land	12	18.5		
Ownership	16	24.6		
Purchased	1	1.5		
Rent	25	38.5		
Sharecropping	11	16.9		
Main occupation				
Farmer	65	100	-	-
Farmer-processor	-	-	10	20.8
Processor	-	-	38	79.2
Source of labour				
Hired	33	50.8	11	22.9
Family	8	12.3	12	25.0
Both (hired and family)	24	36.9	25	52.1

Table 3.2 Summary statistics for producers (quantitative variables)

Variables	Minimum	Maximum	Mean	Standard deviation
Age (years)	21	70	43.28	12.01
Farming experience (years)	1	45	17.26	11.31
Cassava farm size (hectares)	0.40	4.80	1.22	0.76
Total cropped land (hectares)	0.40	8.40	2.36	1.54
Years in school	0	15	8.23	4.00
Household size	1	20	7.57	4.04
Net income from cassava (GH¢) ($ 291.32)	150	7000	1524.62	1485.75
Net income from all crops grown (GH¢) ($ 418.17)	150	7500	2598.85	2132.67

Exchange rate: $1=GH¢5.1 (As at 25th APRIL, 2019)

Table 3.3 Summary statistics for processors (quantitative variables)

Variables	Minimum	Maximum	Mean	Standard deviation
Age (years)	30	75	43.83	9.05
Processing experience (years)	2	30	10.44	7.72
Years in school	0	15	5.77	4.09
Household size	1	21	6.69	3.39
Net income from processing ($ 204.40)	480	4500	2105.0	1042.45

Exchange rate: $1=GH¢5.1 (As at 25th APRIL, 2019)

Farming (100%) and processing as already envisaged by the study, provided livelihoods for farmers (100%), and most of processors (79.2%) in the communities. About 21% of processors cultivated cassava to feed their factories. Most producers were defined as small holders with land holdings of less than 2.5 hectares for all cultivated crops. Cassava production alone covered about 52% of the total land area under crop production, which showed the importance of cassava to these communities. Any significant change in the sector could impact a lot of lives. A small percentage (26.1%) of participants had right of

ownership of land compared to 73.9% who either farmed on family lands, who rented or practiced sharecropping.

A number of differences were found between producers and processors. Most of the producers were males (84.6%) while the majority of the processors were females (68.8%). With regards to education, the majority of the producers (70.8%) and processors (70.8%) had attained basic level education, and 12.3% and 2.1% respectively had reached secondary level. Transition from the basic level to the secondary level showed a 58.5% and 68.7% drop for both the producers and processors in the community. Similar results were reported in Uganda, by Esuma *et al*. (2019). Considering the years spent in school, producers had spent more years (8.23) in school than processors (5.77). The majority of the producers (81.5%) were heads of their households compared to 41.7% of the processors. The majority (69.2%) of the producers are indigenes of the study communities compared to 60.4% of processors, who were migrant permanent settlers.

3.3.2 Types of crops cultivated in study area

Farmers in the study area cultivated a variety of crops, both food and cash crops. The crops varied slightly between men and women. Several crops were cultivated in the community by men, women and young adults, which included cassava, plantain, cocoyam, sweetpotato, rice, pepper, maize, yam, tomatoes, cowpea, garden eggs, onion, okro, water melon, oil palm, cocoa, orange, cashew, banana, pawpaw and mango. Cassava ranked first, followed by maize, as food crops in all districts by all gender groups, since cassava was seen to perform well on poor soils and under harsh weather conditions. From the discussions and personal observations, it was realised that it is currently difficult to classify a crop as mainly a “food crop” since all the known food crops are targeted for cash. The tree crops could, however, be classified exclusively as cash crops, which were cultivated mostly by men. A male key participant at Techiman Municipal admitted that the women in the community were very hard working and cultivated all crops, and men also have “muscled” to cultivate everything. Both men and women groups narrated this:

"What makes us grow the cassava and cashew is that, I cannot cultivate the whole of my farm land, so I start with cassava which is 2 years. By the time I harvest, the cashew I added would have started sprouting. I do this because of insufficient capital". Rebecca: cassava farmer in Techiman North.

"It can be used for brewing, binding books and used for the textile industry in particular" Silvanus, male young adult in Ketu North.

"When it comes to farming here, both men and women mostly grow crops equally. Women grow what they want, for instance pepper was previously for women but the men have taken over." Male farmer

"The men have taken over all the crops. They grow every crop here; cassava, vegetable (pepper, okro), cocoyam, plantain" Male participant.

3.3.3 Importance of cassava

The pair-wise ranking was employed to examine the importance of cassava as a food or cash crop. Cassava was confirmed as an important food security and income-generating crop, adapted to different farming systems like intercropping and suitable for multiple food and industrial uses. Cassava was intercropped for several reasons, such as reduced labour cost for weeding and preparing new fields, as mentioned by both men and women, or to provide shade for the tree crops, as mentioned by men. This is also done as a sign of security and to generate extra income, especially among late maturing tree crops.

3.3.4 Access and control of productive resources

The main productive resource considered in this study was land access related to the land acquisition process and size of operation. Investigating the access of men, women and young adults to land and tenure arrangements is critical for any variety uptake. Generally, access to land for farming in the study communities ranged from rights acquired through renting, to the right of permanent use of a piece of land through inheritance, which is termed as "family lands" or by total purchase. There were also some participants who were engaged in sharecropping and this was found common for the majority of the young adults. Participants in the focus group and the other key informants

"We have the mentality that men are stronger than women, so in allocating land, men are definitely given larger areas than women." Male adult farmer.

interviews mentioned this. After a lengthy discussion, it was realized that considering land as a nominal entity, both men and women had right of access, however, the level of access differed by community. While in some communities, women could only access land through their husband or a relative, in other places there were no barriers, as reflected in the supporting quotes below:

Women, however, farmed small sizes of land due to perceived lack of strength and capital resources, as reflected in the quotes in the box below - an issue of stereotyping by the communities.

"If women want land they will get it, as long as they are not lazy they can get land. Women have access and control over inherited lands even in their married homes. Women do not need a man to lead them when renting land." Male adult farmers in Techiman North.

These statements were confirmed by the women and men groups at Techiman Municipal.

"I know that if the land is very big the man will get it. Because they perceive women are not strong enough to cultivate big lands." Female farmer.

3.3.5 Knowledge of improved varieties and source of planting materials

In all the locations visited, most men and women had little knowledge of the improved varieties, since they could not access the planting material. In addition, respondents had the perception that the improved varieties normally referred to as "Agric" were not

poundable and therefore did not serve their fundamental purpose of using it for "fufu". Very few adult males and females cultivated the improved varieties, whose planting materials were received from extension officers or agricultural volunteers in the community. Thereafter, planting materials were mainly sourced from their own farms or from fellow farmers. Very few farmers had contact with agricultural extension officers in the localities.

3.3.6 Cassava varieties cultivated and the preferred traits by participants on gender basis

The varieties cultivated by both producers and processors and their preferred traits, were examined in the districts and it was realised that there was a high level of agreement between men, women and young adults on several varieties, with slight differences among locations. In Techiman, several local and improved varieties, were cultivated. The local varieties included Bensere, *Wenchi bankye*, *Afosa, Nkuruwa, Dakware, Esiabayaa, Bankye kokoo, Nkomti, Bankye borodee* (with yellow root), *Agoro* and *Bankye ahoofe*. The improved varieties were *Afisiafi, Nkabom* and *Bankyehemaa*. The women, especially adults, cultivated more improved varieties than the men. Very few farmers (less than 5%) had stands of yellow flesh improved cassava varieties, and these were mainly cultivated by adults. Only very few young adults were aware of the local yellow flesh cassava, which was known in local language as "*Bankye borodee*", as majority of the young adults had never seen it. The characteristics preferred by women include early maturing (6-12 months), tolerance to abiotic stresses (very harsh weather conditions), quick canopy closure (thus labour saving on weeding), and suitability for multiple food uses (such as *fufu, gari, ampesi* and *kokonte*). Men tend to prefer varieties that yielded higher (with higher income), easy-to-harvest, high DM and having good cooking qualities throughout the season.

In Ketu North District, varieties cultivated by all gender groups includes *Kpenyevi, Hushivi, Dogbovi and Bosomensia*. Adult males and male young adults grew improved varieties such as *Abasafitaa, Ampong* and *Tech bankye*. The females had difficulty accessing these improved planting materials but the men received it through contact with extension agents in this district. This is due to the fact that, women need a man or her husband in some cases to lead them All gender groups preferred varieties that are early maturing (6 months), poundable and high yielding (big roots). Adult male and young male adults preferred varieties that could save labour by closing its canopy early, since males normally did the weeding in that district. Adult females and young female adults needed varieties that could easily be processed domestically into starch while the adult male and young male adults

preferred varieties suitable for industrial uses (for brewing, starch and biscuits) to earn higher income. In general male participants requested for more research on their preferred varieties and landraces to improve the poundability and the storability in preferred materials in the soil.

In Agona East District, the common varieties grown by all gender groups include *Madumaku, Bosomensia, Esiabaayaa, Duafra, Nkonmono, Agage* and *Wodziawonye*. Adult males and females cultivated improved varieties such as *Sika bankye* and *Bankyehemaa*. The study however showed that young adults did not have access to improved planting materials. In this district, the preferred traits relative to adults did not differ significantly by gender. The prime traits of interest were poundability and multiple uses such as *gari, starch, kokonte, ampesi* and other forms of products for industrial uses. Men generally prefer traits like good storability in the soil, ease of harvesting, early canopy formation (for weed suppression), and suitability for industrial uses. Women on the other hand tend to prefer traits such as ease of peeling, high starch content and early canopy formation.

3.3.7 Processors

There were no significant differences in the varieties preferred by producers and processors, as most processors also had farms that fed the processing unit. Men and women processors handled similar varieties. The five important varieties mostly processed were *Bensere, Wenchi bankye, Agric* (improved), *Bankyehemaa* and *Agyiribaa*. The yellow flesh cassava was not common among the processors in the Agona East district.

3.3.8 Traits preferred by processors

Cassava processing was dominated by women. The men who were involved, mostly young adults, depended on hired labour, mostly women, to perform the different stages of processing which they supervised. Some men also processed jointly with their spouses. The main trait of interest for processing by men and women processors was high DM or less moisture content. Not all the varieties had this trait, given that cassava storage roots are sometimes used for “fufu” or “*konkonte*”. Women processors also preferred varieties that could be used for starch and biscuits while the men tend to prefer varieties that have high level industrial value use for starch and ethanol production as well as brewing.

3.3.9 Awareness, perceptions and willingness to adopt yellow-flesh cassava

3.3.9.1 Awareness

The majority (90%) of the adult men and women farmers and processors were aware of yellow flesh cassava, compared to the young adults (2%). Within the young adults, awareness was skewed towards the young male adults who were mostly cultivating family farms. On average, the local yellow variety (*Bankye borodee*) had been known in the community for over 20 years through ancestral lineage. However, at the time of the study, the local yellow flesh cassava variety was described as almost extinct, since only a few men and women were still cultivating it (*bankye borodee*) on their farms. None of the young adult farmers had the material in their field. The absence of the local yellow flesh cassava variety in the communities was mainly attributed to the lack of planting material of the variety. The absence of yellow flesh cassava planting material constrained processors to normally add palm oil to white fleshed root for customers requesting for yellow "*gari*" product.

3.3.9.2 Perceptions

During the discussions, two categories of people were identified based on when it came to their perceptions of yellow flesh cassava. There were some, who had seen and used the varieties before, and there were others whom had never used it. In order to avoid lack of response to study questions (i.e. questionnaire) and inadequacy of information during the elicitation, the team took note of the assumptions and expectations of those who had never had experience with the local yellow flesh cassava variety. This was done because a similar study by Gonzalez *et al*. (2011) recorded high lack of response, which indicated that respondents did not have strong perceptions of the new varieties that were evaluated by the research team and farmers through a participatory approach. Overall, farmers asserted that, if the planting materials were made available, yellow flesh cassava cultivation could increase their income due to the high likelihood and propensity by food vendors/restaurants and processors to use it in their food processing, with higher prospect for increased market share. Some men also said the yellow flesh cassava roots do not store well in the soil. The processors admitted that the processed cassava product *"yellow gari"* command higher premium price than the white or cream type, but having low market demand, and therefore small market share. They have therefore suggested that action be taken to increase the market demand for easy uptake. This was found laudable and could be included in future promotional campaigns to enhance adoption. The other perception information of the yellow flesh cassava are presented in Table

3.4. These perceptions were given based on participants' experience with the local yellow variety or what they have heard from yellow flesh cassava farmers or processors.

Table 3.4a Gender groups' perception on yellow flesh cassava

Perception	Gender group
Substitute for plantain, oil palm and colour additives added	Adult male and adult female
Mealy	Adult male and adult female
Low yielding	Adult male and adult female in Techiman Municipal and Agona East districts
Thinner cassava sticks which may affect yield	Adult female and female young adults
Difficulty in accessing planting material	All gender groups
Less starch	Adult female and female young adults
Small market share	All gender groups
Has more nutrients than the white type	Adult male

The stakeholders who had never cultivated the cassava type could only relate to it by providing their expectations and assumptions

Table 3.4b Gender groups' expectations of the improved yellow flesh cassava

Expectation/ assumptions	Gender group
Market share of the crop type increased through consumer sensitization	All gender groups
Consumers may perceive colour change of cassava product as addition of colour additives or oil palm	Adult female processors
May taste bitter just like some other improved varieties	Female young adult processors
The beta carotene level must be high (i.e. "must be as yellow as plantain or yellow corn")	Male and female young adults
Have qualities of a good cassava ("if possible, improve on local varieties")	All genders
Not serve multiple purposes "yellow cassava can only be used for fufu and gari not products like starch/brewing"	All genders

3.3.9.3 Willingness to adopt yellow flesh cassava

When the willingness of men and women producers and processors to accept the yellow flesh cassava was assessed, the majority of the participants were found to be willing to accept it (92.5%). This is predicated on a number of factors among which are the ready market (for processors and food vendors), for *gari* processing and reduced cost associated with the non-use of palm oil to enhance yellow color in cassava. There wasn't much information or evidence about adoption based on the nutritional and health benefits, so participants were educated briefly to facilitate adoption in future, when introduced. Few had reservations about the local yellow variety because of previous experience, which included its low yield, root colour and slender planting materials. Some of the men were willing to accept the new yellow flesh cassava only if the planting materials could be distributed free of charge, since they hardly pay for cassava stakes which appears not to be commercialized in the districts. All gender groups wanted planting materials to be made easily accessible and available in large quantities and a lot of advocacy to be done to further increase market share.

3.3.10 Factors affecting the adoption of new cassava varieties

Several factors stemming from social, cultural, economic, psychological and institutional considerations have been reported to affect technology uptake (Gonzalez *et al.* 2011). The study found similar factors such as cultural beliefs and norms, land tenure arrangements and allocation, extension contacts, awareness, perception, participants preferred traits and production/processing objective as factors impeding the dissemination and adoption of the yellow flesh cassava. These variables are thus the key drivers of adoption.

3.4 Discussion

The importance of cassava in Ghana cannot be over emphasized, as it is the most important root crop, followed by yams and cocoyams, in terms of quantity produced, but ranks second to maize in terms of area planted. Being a staple for over 800 million people in the world and described as Africa's insurance crop, as it ensures improved welfare and food security for small-holder farmers who cultivate it (Anna *et al.* 2010). The crop has other special attributes such as its ability to adapt to different farming systems, reduce cost of farm operations when intercropped, and its capacity to perform well on marginal soil (Dixon *et al*. 2003). Notwithstanding its importance as a food, the crop is low in essential micronutrients, indicating challenges related to nutritional security for the many people who depend on the crop. A high risk could therefore be perceived, as the majority of resource-

poor who typically are people, usually based in rural areas, rarely consume balanced diets, which are necessary to provide them with the required nutrients for good health (Esuma et *al.* 2019). This is then a developmental issue, which, if not curtailed, could affect agricultural and labour productivity as well as the human capital development (McGovern *et al*. 2017). The biofortification of Ghanaian staple crops is therefore a matter of urgency, as the other nutrition enhancement mechanisms are not readily sustainable (Bouis 2003; West 2003; Nestel *et al.* 2006) So breeding programmes/teams must be readily and rapidly facilitated to expedite action on biofortification of staple crops for the benefit of small holder farmers and consumers.

Beside cassava, farmers intensified crop diversification through the cultivation of other roots crops, cereals, vegetables and tree crops to ensure household security. Diversification is adopted by farmers as a coping strategy to reduce food security risk and stabilize food stocks and welfare. Crop diversification to be more advantageous than sole cropping (Mango *et al.* 2018; Makate *et al.* 2016). Crop diversification is thus explored as a means of developing agricultural resilience, in addition to being the most ecologically feasible, cost- effective and rational ways of reducing uncertainties in agriculture including providing farm households with diet diversity, income and some nutrient levels".

Cassava cultivation is intertwined with several factors such as ethnicity, access to resources (including labour, cash and land), gender and wealth (Adjei-Nsiah and Sakyi- Dawson 2012). The results showed gender differences in access to, and control of land resources. The differences were as a result of economic and cultural factors. The study was conducted in Southern Ghana, but the findings showed that ethnicity and location played a role in the access to and control of resources. While among the Akans, both adult males and females had access to and control of land, and hence could take decisions regarding resource investment and allocation, the "*Ewe*" woman needed a man/her husband in some cases to lead her to the chief before accessing the land. Previous research has demonstrated that there is an association between the decision-making powers women enjoy and the quantity (and quality) of land rights they hold in every society (Chigbu 2019). The study found that women farmers are heterogeneous, have different needs, and are affected differently, especially by their culture, location and economic status in society as realised by this study. Size of land as an economic resource has been found an important determinant of adoption (Lavison 2013). However, in both tribes (Akan and Ewe), women farm sizes were relatively smaller than their male counterparts' farms. This dimension is partly rooted in cultural beliefs and practices though the Intestate Succession (PNDC) Law 111, 1985 and 1998 Children's Act

560 have been enacted. The study areas belonged to the two lineage systems in Ghana; Matrilineal and Patrilineal. In the Akan family system, inheritance is matrilineal; which bonds a child to the mother than the father. Here, women may have right to their lineage lands but in varied proportions compared to that of men (FAO, n.d.; Kutsoati and Morck, 2012). This is because the lineage authority; mainly men allocate more land to males who are heads of households. On the other hand, in the patrilineal system; which the Coastal Savannah belonged, women only access land through marriage and hold it only when the marriage is in force. Whilst in marriage women loose acces to their lineage lands (FAO, n.d.). Land is passed on to the young male adult which gives him the right of access and use.The unmarried young female adult however becomes constrained in accessing land. In Africa and for that matter Ghana, customary laws discriminate against women's rights to land (Fonjong *et al*. 2012). In Ghana, in both lineage systems, the culture styreotype against women; looking upon them as inferior hence the limited access to large proportions of land. The results indicated adult farmers had little knowledge of the improved varieties previously released by researchers and they complained of the difficulty in accessing the planting materials. Most of the young adults, on the other hand, had never heard of the improved varieties. Majority of men and women farmers expressed reservations about the attributes of most improved varieties, as the varieties did not often meet their preferences. These gaps may be a result of poor awareness creation for farmers on the attributes and uses of the released varieties by the appropriate institutions responsible for the development of the varieties and extension services. It is incumbent on research and extension institutions to involve all gender groups in their field trials and demonstrations, and also develop sustainable mechanisms to widely disseminate new varieties widely to end users in order to create more awareness about the good values of new varieties. A new variety or technology can only make a positive contribution to economic growth if it is widely diffused and used by the target group (Uaiene *et al.* 2009). The government of Ghana must make funds available to research institutions and the Ministry of Food and Agriculture to multiply breeder and foundation planting materials for distribution of improved varieties (including yellow cassava) to private seed companies, for further multiplication to generate copious quantities to improve availability to farmers.

Producers and processors, depending on their respective objectives of production, have distinct varietal trait preferences. Farmers are generally assumed to be identical in their trait preferences but differences existed between men, women and young adults (Kamau *et al.* 2011; Traoré *et al.* 2016), since farmers could have multiple objectives. Young adults were

much more interested in cassava with industrial qualities, while women preferred cassava that could be used for domestic processing/multiple food purposes. The men preferred high yielding cassava varieties, which could return more cash and are easy to harvest to reduce drudgery and labour costs. This confirms the heterogeneity of farmers and differentiation in trait preferences (Smale *et al.* 2001; Christinck *et al.* 2017; Teeken *et al.* 2018). Breeders must be concerned about additional preferred traits and include them as additional breeding objectives in order to broaden the genetic base, while, being mindful of the trade-offs that may be expected, too.

Despite the great potential of agricultural varieties and technologies, its adoption by the resource poor smallholder farmer has been slow. Awareness and perception of technology are seen as important determinants that facilitate its uptake (Meijer *et al.* 2015), but unfortunately the knowledge and cultivation of the improved varieties and yellow flesh cassava (pVA cassava) were low, especially among the young adults, who are the future of agriculture. The adults who were aware of yellow flesh cassava did not cultivate it and complained of lack of planting materials in addition to their negative perception of the yellow flesh cassava. Okoro (2015) found that farmers preferred local varieties more to improved varieties, and a similar pattern was observed in this study. Having an understanding of these factors are essential for economists (studying the determinants of growth) and for the developers and disseminators of such technologies (Uaiene *et al.* 2009). Most studies (Uaiene *et al*. 2009; Akudugu *et al.* 2012; Loevinsohn *et al.* 2012; Mwangi and Kariuki 2015; Ponguane and Mucavele 2018) tend to emphasize extrinsic factors such as demographics and farm level characteristics of the adopter as other factors that influence the adoption of innovations. In addition, intrinsic factors such as awareness, perception and attitudes have been found as important drivers of adoption (Meijer *et al.* 2015; Mawusi 2004). A combination of both the intrinsic and extrinsic factors must be studied and considered by economists, breeders and policy makers in making informed decisions.

With the virtual extinction of the landraces with yellow flesh colour, which used to be widely planted about three decades ago (Boampong and Safo- Kantanka 2014), almost all landraces and improved varieties cultivated and processed are white fleshed, with the exception of "*Lamesese*" (yellow flesh) which was released in 2015, unfortunately *Lamesese* though not extensively cultivated, and the carotenoid levels have not been quantified. There is therefore a niche for improved yellow flesh cassava varieties (having high beta carotene) in Ghana. Such varieties must be released and disseminated nationally to improve nutrition security

and health for both farmers and consumers. A high percentage of the farmers and processors willing to accept the yellow flesh cassava, which is heart-warming. However, their expectations and doubts about the yellow cassava varieties need to be addressed through extensive education for wide diffusion. Poor functioning markets hinder resource poor farmers from accessing modern technologies; hence an increased access to credit markets could enable farmers and private sector acquire improved varieties, other inputs and access information on modern technologies. This would thus improve the market performance of Domestic cassava commodity markets. It can also be improved through market surveys so as to help identify specific segments for developed product.The excellence in Breeding and Gender in Breeding are developing gender plus tools that will capture customer profiling and market segmentation to give a clearer picture on traits and prefences by consumers for new cassava varieties developed (Orr *et al.* 2018). The product launching of released varieties will also enhace adoption and utilization of biofortified cassava.

3.5 Conclusions and implications for cassava breeding

White fleshed varieties, cassava, most of which are landraces are cultivated across the different agro-ecologies of Ghana. There is limited farmer knowledge on improved varieties including yellow flesh cassava varieties and there is need to improve this by strengthening the farmers in Ghana to enhance accessibility and availability of these varieties by farmers cassava seed systems. Very few men and women are cultivating improved varieties, and yellow flesh cassava. The young adults, who are the future of the agricultural sector, are severely lacking in access to improved varieties. These cadres of farmers require extra attention to enhance their knowledge and access to new varieties including in addition to improving their capacity to use these materials. Numerous similarities were seen in the preferred traits of men and women; though slight differences were observed between districts. Men preferred varieties that could be stored longer in the soil, are easy to harvest all year round, could compete favorably with weeds and are suitable for industrial processing. On the other hand, women preferred climate smart varieties, and varieties that are labour saving and could easily be processed domestically into starch and *"gari"*. Participants have expressed willingness to cultivate and process the yellow flesh cassava if there is improvement in the availability of planting materials of these varieties, increased awareness and market demand. This study successfully demonstrated the importance of qualitative and participatory approaches to research. It is recommended that cassava breeders review their breeding objectives to reflect the preferred traits of farmers and end users. There is need to pay attention to perception issues of the yellow flesh cassava in order

to develop demand driven varieties that will best meet the need of end users. Education to create awareness on the potential advantages and diverse uses of the improved biofortified cassava is also required. Further studies are proposed to investigate and assign measures/ratings to men and women for the qualitative traits evaluated in this work.

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Chapter 4

Analysis of total carotenoid content, cassava mosaic disease, dry matter content, yield and its related components in F_1 cassava families at two locations in Ghana

Abstract

Cassava is currently ranked as the number one food staple and is the most widely cultivated crop in Ghana. Cassava production in Ghana is around 18.5 million tons with more than 70% of the country's farmers engaged in its production. Since 1993, 26 improved varieties have been released in Ghana and distributed to farmers. In most cases, these varieties were white-fleshed with low or negligible amounts of carotenoids, but were bred and selected for processing into intermediary products (gari, konkonte, starch). Malnutrition is endemic in cassava producing regions of Africa, partly due to the low micronutrient content of this storage root crop, given that cassava is a major component of most household diets. It is for this reason that the development of nutrient dense cassava varieties require much attention to reduce the effect of malnutrition among the poor in a less expensive and more sustainable way. The current study was designed to generate genetic information and also to develop cassava clones that combine good total carotenoid content (TCC) and high dry matter content (DMC) using a 2 x 5 North Carolina II breeding scheme. Ten F_1 families were generated and evaluated across two different locations in Ghana. Results indicate general combining ability (GCA) mean squares were larger than their respective specific combining ability (SCA) mean squares for harvest index, cassava mosaic disease (CMD) and TCC, with over 70% of the total variation explained. These traits are therefore highly driven by additive genetic effects. The positive significant correlations that were observed between pulp colour and TCC, TCC and CMD, pulp colour and CMD, and pulp colour and cortex colour makes screening of large numbers of progenies possible and relatively easier in cassava breeding programme. The female parental used are improved cassava lines selected in IITA with CMD reistance background. This could allow breeders to combine both TCC and CMD at the early stages through visual assessment of pulp colour and CMD symptoms. Through visual screening, large number of genotypes can be screened down to a small number and the few selected can then be quantified for TCC at the later stages of breeding scheme to save costs. One of the parental materials used in this study, P6, showed positive GCA effects for TCC, DMC, CMD and storage root weight (RTW), hence could be crossed into a high DMC background to increase chances of generating clones that combine both TCC and DMC.

4.1 Introduction

The populations of underdeveloped and developing countries often suffer undernourishment and so-called “hidden hunger” as a result of micronutrient deficiencies. Areas in Africa, including Ghana, where cassava is widely consumed, are characterized by rampant malnutrition because its storage roots are low in nutrients such as VA (Ssemakula and Dixon 2007). It is for this reason that the development of nutrient dense cassava varieties needs and deserve much more attention to eliminate the ramifications of malnutrition among the poor in an inexpensive and sustainable way. VAD constitutes a major public health problem and affects mainly children and women. Several programmes in nutrition security have been initiated, for example HarvestPlus, which is a global alliance involving several research institutions, tasked with initiating initiated the development of micronutrient-dense staple crops (Bouis *et al.* 2011; Dwivedi *et al.* 2012) under a joint partnership of the International Center for Tropical Agriculture (CIAT) and IITA. Among its several initiatives is the development of biofortified cassava clones with high PVAC in the roots. On this score, the national cassava breeding programme at CSIR-CRI initiated a breeding programme with the aim of developing high yielding cultivars with high levels of TCC, DMC and disease resistance. Deployment of such cassava varieties could sustainably improve nutrition and reduce prevalence of VAD in communities that heavily depend on cassava, especially the rural populations (Nassar and Ortiz 2010; Esuma *et al.* 2016). Adoption of biofortified cassava genotypes in Ghana will largely depend on their agronomic performance, including storage fresh roots, DMC, resistance to major pests and diseases and the stability of these traits over time and space (Njoku 2012). DMC influences texture after boiling and is also a key parameter in the production of gari (a popular cassava product consumed in Ghana). The strong negative correlation that has been reported between DMC and TCC in African cassava germplasm (Akinwale *et al.* 2010; Njoku *et al.* 2015; Esuma *et al.* 2016) could present a potential challenge for the breeding programmes that are aiming to improve both traits. Chávez *et al.* (2005) and Sánchez *et al.* (2014) found that correlations between TCC and DMC were not statistically significant when analyzing Latin American cassava germplasm.

4.2 Materials and methods

4.2.1 Experimental site

The crossing block for this study was first planted in May, 2015 at the CSIR-CRI in the semi-deciduous forest zone of Ghana located at 1°30’0’’W and 6°42’0’’N, 186 m above sea level, with a bimodal rainfall distribution, with two rainy and one dry seasons. A second

crossing block was established at the same location in 2016 due to a limited number of botanical seeds collected during the first hybridization. The soil of the experimental area (Fumesua) is Asuansi series, a ferric Acrisol with sandy loam top soil over sandy clay.

4.2.2 Progeny development

Seven genetically diverse clones (five yellow flesh cassava at advanced selection stages by IITA and two white-fleshed selected from farmers' fields in Ghana) were used as progenitors (Table 4.1). These progenitors were planted in the crossing block at Fumesua under rain fed conditions. Planting was done using disease-free stakes planted in three row plots of five plants/row with a plot size of 15 m^2. Blocking was used to allow all matings involving a single group of males (two white-fleshed) to a single group of females (five yellow flesh cassava) to be kept intact as a unit (Acquaah 2012). Spacing between and within rows was 1.5 m to ease movement during the pollination process. Weeding was done as deemed necessary. Controlled pollinations were carried out by hand as described by IITA (1990). The seven parents were crossed in a NCD II to produce 10 F_1 families without reciprocals. The seed viability was tested by the floatation method (CIAT 2003). The dry seeds were mixed with water and floated. Non-viable seeds were discarded. At least 110 seeds from each cross were germinated in seed trays in the screen house at the Fumesua station in 2017.

Table 4.1. List of progenitors used in the study

Genotype	Code	Source	RFC	Salient traits
Debor	P1	Farmer	White	High DMC
Wenchi Alata	P2	Farmer	White	High DMC
IBA061635	P3	IITA	Light yellow	CMD resistance, pVAC
IBA070539	P4	IITA	Yellow	CMD resistance, pVAC
IBA070593	P5	IITA	Yellow	CMD resistance, pVAC
IBA090090	P6	IITA	Yellow	CMD resistance, pVAC
IBA070536	P7	IITA	Light yellow	CMD resistance, pVAC

IITA = International Institute of Tropical Agriculture, RFC = root flesh colour, DMC = dry matter content, CMD = cassava mosaic disease, pVAC = provitamin A carotenoids

4.2.3 Seedling nursery evaluation

Seedlings from the 10 F_1 families were transplanted to the seedling nursery at the Fumesua station in the minor rainy season of August 2017 for the purpose of generating planting materials for clonal evaluation. The seedlings were established in single rows at 50 cm by 1 m spacing between and within rows, respectively. Data were collected from each individual stand per family for severity ratings of CMD taken at 1, 3 and 6 MAP and cassava green mite at 6 MAP using a scale of 1 to 5 (1 = no symptoms; 5 = severe symptoms) according to IITA (1990). The seedlings (progenies) were evaluated alongside the progenitors. At harvest (10 MAP), root cortex and pulp colour for individual stands were scored based on a standard colour chart developed by IITA (1990). Individuals were selected based on the ability to produce enough (≥ 12) standard-size cuttings, about 25 cm (4-6 nodes). Clonal evaluation trial

Selected individuals were planted in a randomized complete block design (RCBD) with two replications at two sites: Fumesua and Ejisu. The soils for the trial sites were: Asuasi series, a ferric acrisol with sandy loam topsoil over sandy clay at Fumesua and Amantin series, chronic lixisol with sandy loam topsoil at Ejura. Annual rainfall for the sites during the trial period was 1205 mm at Fumesua and 1311 mm at Ejura. Each individual was represented by a single row plot of three plants per rep. with 2 m alleys between blocks. Planting was done at a spacing of 1 m x 1 m between and within rows. The clonal trials were planted in July 2018 and harvested in June 2019 for root measurements. Progenies were evaluated alongside the progenitors in the clonal trials. Weeding was done as deemed necessary and trials were rain fed.

4.2.3.1 Agronomic and morphological characteristics measured

During the growth period, data were collected on severity of CMD, cassava green mite (CGM) and cassava bacterial blight (CBB). The incidence and severity of CMD, CGM and CBB were scored using a scale of 1-5, where 1 represented no symptoms and 5 severe damage (IITA 1990). The scoring was done at 1, 3 and 6 MAP for CMD; 6 and 9 MAP for CGM; 1 and 3 MAP for CBB and an average score for analysis was determined. Plant vigour was also measured at 3 MAP. At harvest (12 MAP), all plants in a row were uprooted and the biomass bulked to estimate yield components by separately weighing the

fresh roots (kg plot^{-1}) and foliage (kg plant^{-1}) using a Salter Brecknell suspended weighing scale calibrated in kilograms. HI was measured as a ratio of fresh root weight (FRW) to total biomass as:

$$HI = \frac{FRW}{(FRW + FSW)}$$

Fresh root yield was measured as root weight (RTW) assessed on a minimum of two plants per genotype.

DMC was determined by measuring the weight of the storage roots in water and air and the values calculated according to Kawano *et al.* (1998).

The iCheck analytical kit developed by BioAnalyt laboratory was used for measuring the TCC at the laboratory. The extraction was done following the procedure by Esuma *et al.* (2016). Five gram of cassava root sample was taken from each clone, pounded and ground into a smooth and fine paste using a mortar and pestle at the laboratory. Twenty ml distilled water was added to the sample to ease grinding. The resulting solution was transferred into 50 ml calibrated Falcon tubes and shaken thoroughly and 0.4 ml of the solution injected into the iEx^{TM} Carotene vial using the syringe and needle provided with the kit. The vials were placed on a smooth surface and left to stand for 5 min, it was then shaken again and allowed to stand until two solution phases were seen. The absorbance of the upper phase in the vial was measured using the iCheck kit (reading).

TCC ($\mu g\ g^{-1}$) calculated as: (V_S/ W_S) x R

Where Vs= volume of the solution transferred to the falcon tube

Ws= weight of a sample measured

R= final reading by the kit at 450nm wavelength

4.2.4 Statistical design and data analysis

ANOVA was done to determine the significance of genetic differences for the traits/variables measured in the 2 x 5 balanced NCD II experiment; within and across locations. The RCBD model of NCD II

was used for the genetic analysis and it considered the effect of location, both male and female parents, their interaction and each interaction with the location and all of their interactions together.

GCA and SCA effects were also estimated based on the parental effects, and their interaction. All the analyses were done using AGD-R v5.0 (Analysis of Genetic Design with R for Windows) software (Rodriguez *et al.* 2015).

The NCD II Multi-locational RCBD statistical model is:

$y_{ijkd} = \mu + L_d + rep_k(L_d) + g_{ij} + L_d * g_{ij} + e_{ijkd}$

$= \mu + L_d + rep_k(L_d) + m_i + f_j + m_i * f_j + L_d * m_i + L_d * f_j + L_d * m_i * f_j + e_{ijkd}$

y_{ijkd} is the observed value

μ is the mean

L_d locational effect

$rep_k(L_d)$ is the effect of replicate k nested in location d (k=1, 2)

m_i is the male effect (i= 1, 2)

f_j is the female effcet (j= 1, 2,...5)

e_{ijkd} residual

eijkd residualVC	**Henderson (balanced)**
$\boldsymbol{\sigma^2_m}$	$(MS_m - MS_{mf})/srm$
$\boldsymbol{\sigma^2_f}$	$(MS_f - MS_{mf})/srf$
$\boldsymbol{\sigma^2_{mf}}$	$(MS_{mf} - MS_e)/sr$
$\boldsymbol{\sigma^2_m}$	$\frac{(m-1)MS_m + (f-1)MS_f - (m+f-2)MS_{mf}}{Sr(2mf-m-f)}$
$\boldsymbol{\sigma^2_A}$	$4\sigma^2 g$
$\boldsymbol{\sigma^2_D}$	$4\sigma^2_{mf}$
$\boldsymbol{\sigma^2_E}$	$\sigma^2_{gxe} + \sigma^2_e \quad s \quad sr$
$\boldsymbol{h^2_b}$	$\sigma^2_A + \sigma^2_D$
$\boldsymbol{h^2_n}$	$\frac{\sigma^2_A + \sigma^2_D + \sigma^2_E}{\frac{\sigma^2_A}{\sigma^2_A + \sigma^2_D + \sigma^2_E}}$

Variation due to males, females, and males x females were denoted as GCAm, GCAf, and SCA variation, respectively. For CMD, GCA and SCA effects were negative, as the preference is for low values. For all other traits, positive estimates of GCA and SCA effects were used to identify genotypes with high yield and yield components. The relative importance of additive (GCA) and non-additive (SCA) genetic effects in explaining the performance of the progeny for each of the traits was determined by individually expressing the GCAf mean square, GCAm mean square, and the SCA mean square as a percentage of the treatment (crosses) mean square as shown in the formula below (Baker 1978; Hirut *et al.* 2017).

$$GCA/(GCA + SCA)*100 = 2MS\ GCA_{pooled}/(2MS\ GCA_{pooled} + MS\ SCA) \times 100$$

$$MS\ GCA_{pooled=}\ (f-1)MS\ GCA_{f} + (m-1)MS\ GCA_{m}/(m+f-2)$$

Where; MS GCA_{pooled} = mean squares for GCA; MS SCA = mean squares for SCA; f = number of female parents; m = number of male parents; MS GCAf = mean square of GCAf; MS GCAm = mean square of GCAm, respectively.

Average degree of dominance = $(\text{Dominance variance/Additive variance})^{1/2}$

4.3 Results

For all genotypes of the 10 F_1 families, TCC ranged from 1.20 to 9.10 ug/g, with the highest mean (6.14 ug/g) recorded for family P1 x P6 and the lowest (2.98 ug/g) for family P1 x P7 (Table 4.2). Individual DMC values for the evaluated genotypes ranged from 19.70% to 42.4%. DMC with means ranging from 26.86% for family P2 x P5 to 36.28% for family P1 x P3. Family P1 x P6 recorded the highest mean RTW (18.35 kg $plot^{-1}$) while family P2 xP5 recorded the lowest mean RTW (14.34 kg $plot^{-1}$). At the parental level, genotypes P1 and P2 recorded the highest value for DMC and CMD (35.4%; 2.05 and 34.20%; 1.99 respectively) but they had the lowest levels of TCC and RTW compared to the yellow parental genotypes (P4-P7).

The GCA mean squares for female progenitors were highly significant for all traits measured except for RTN (Table 4.3). The SCA mean squares for DMC, RWT and TCC were also highly significant. The GCA mean squares for the CMD and total biomass (TWT) for male progenitors were highly significant as well, while the SCA mean squares were

not. The GCA effects for both male and female and SCA effects were significant for RWT and HI, indicating both additive and non-additive gene effects for this trait. The male and female GCA mean squares, as well as SCA mean squares, showed various levels of significance for CMD, HI, RTN, RTW and TWT, indicating the importance of both additive and non- additive gene effects. Chikoti *et al.* (2016) reported significant GCA and SCA effects for CMD as found in this study for CMD and other traits.

The GCA sum of squares for female progenitors accounted for more of the total variation than the GCA sum of squares for male progenitors for DMC. The SCA sum of squares accounted for 63.03% of DMC cross sum of squares. The GCA: SCA ratio for all the traits were higher than 1 except for DMC (1.00) where there were higher SCA than GCA effects (Table 4.3).

The coefficient of variation (CV) ranged from 4% for HI to 39% for TCC (Table 4.2). Low to high CVs for different traits have been reported in several studies. Pasajee *et al.* (2016) reported high values for TCC (44%). Vasconcelos *et al.* (2017), Tumuhimbise *et al.* (2014) and Njoku (2012) also reported low values of 4%, 5% and 10.8% respectively for DMC.

Table 4.2. Mean performance of progenitors and their F_1 progenies evaluated across two locations in Ghana

Parent/family	No	CMD	DMC %	HI	RTN	RTW kg	TCC µg g^{-1}	TWT kg
P1 (Debor)		2.05	35.40	0.45	28.60	15.77	0.80	20.20
P2 (W.Alata)		1.99	34.20	0.42	27.13	15.36	0.75	22.06
P3 (IBA061635)		1.57	31.66	0.46	26.75	16.77	3.53	19.22
P4 (IBA070539)		1.56	31.84	0.42	29.76	16.42	5.41	22.60
P5 (IBA070593)		1.99	27.40	0.43	26.78	16.09	5.70	20.23
P6 (IBA090090)		2.05	32.27	0.45	26.69	17.15	5.33	21.84
P7 (IBA070536)		1.29	27.69	0.44	29.34	17.45	3.28	22.99
P1 x P3	20	1.39	36.28	0.46	26.39	15.25	3.34	18.47
P1 x P4	17	1.53	27.64	0.43	30.06	15.77	5.68	21.32
P1 x P5	16	1.91	27.94	0.45	29.25	16.38	5.61	20.26
P1 x P6	18	1.90	32.70	0.46	27.18	18.35	6.14	21.93
P1 x P7	16	1.19	27.80	0.46	30.13	18.08	2.98	21.51
P2 x P3	15	1.74	27.04	0.45	27.12	16.29	3.72	19.98
P2 x P4	19	1.59	36.03	0.42	29.46	17.07	5.13	23.88
P2 x P5	14	2.08	26.86	0.40	24.30	14.34	5.79	20.20
P2 x P6	14	2.21	32.24	0.43	26.20	15.94	4.50	21.75
P2 x P7	11	1.39	27.58	0.41	28.59	16.81	3.57	24.49
Grand mean		1.69	30.74	0.44	27.87	16.43	4.19	21.38
S.e.m		0.07	0.83	0.005	0.40	0.25	0.40	0.39
CV%		17.59	11.16	4.39	5.97	6.24	39.57	7.46

CMD = Cassava mosaic disease; DMC = Dry matter content; HI = Harvest index; RTN = Storage root number; RTW= storage root weight; TCC = Total carotenoid content; TWT = Total biomass; CV = coefficient of variation

Table 4.3. Mean squares of crosses and sum of squares for combining ability effects of seven traits evaluated in 10 F_1 families and seven parents across two locations

Source	df	CMD	DMC	HI	RTW	TCC	TWT	RTN
Locations	1	0.779***	9.555ns	0.0103ns	162.409***	272.745***	45.689ns	62.500ns
Genotype	9	6.630***	963.891***	0.033***	97.620***	87.770***	208.877***	233.368**
Male	1	7.829***	10.216ns	0.151***	55.311*	2.259ns	322.891***	248.039**
Female	4	12.478***	798.463***	0.024***	106.284***	173.947***	325.607***	323.104ns
SCA	4	0.494ns	1367.74***	0.013**	99.534***	25.221***	63.642ns	139.965**
Error	618	0.279	8.276	0.005	10.892	1.050	28.986	37.298
GCA_m		13.12	0.12	50.50	6.30	0.28	17.17	15.11
GCA_f		83.57	36.85	31.89	48.38	87.09	69.28	78.24
SCA		3.31	63.03	17.61	45.32	12.63	13.54	6.15
GCA:SCA		4.96	1.00	3.87	1.14	1.02	2.27	3.46
Baker ratio		0.98	0.48	0.88	0.66	0.92	0.91	0.81

*P≤0.05; ** P≤0.01: *** P≤0.001; CMD = Cassava mosaic disease; DMC = Dry matter content; HI = Harvest index; RTN = Storage root number; RTW= storage root weight; TCC = Total carotenoid content; TWT = Total biomass

4.3.1 General combining ability effects

P2 (W.Alata), a white-fleshed progenitor with negligible TCC, had a negative TCC GCA effect of -0.13 (Table 4.4). This indicates the unsuitability of this parent as a good combiner when targeting high carotenoid in the progeny.

In the female parents, P5 showed a significant positive effect for TCC but high negative effect for DMC, although not significant (Table 4.4). In contrast, parents P3 (IBA061635) and P7 (IBA070536) showed negative GCA effects for TCC. Results also showed yellow flesh cassava female parents as having positive positive GCA effects for DMC. However, P7 (IBA070536) was found to have a negative GCA effect for both DMC (but not significant). High positive GCA effects for RTW were identified with genotypes P6 (IBA090090) and P7 (IBA070536).

Table 4.4 General combining ability effects of cassava progenitors for seven traits at two locations in Ghana

Progenitor	CMD	DMC	HI	RTN	RTW	TCC	TWT
P1	-0.10	-0.17	0.01	0.69	0.31	0.08	-0.58
P2	1.68	-0.61	-0.02	-0.78	-0.36**	-0.13*	0.78
P3	-0.11	1.09	0.02*	-1.16	-0.68	-1.14	-2.06
P4	-0.12	1.27	-0.02	1.85	-0.03	0.73	1.32*
P5	0.31*	-3.16	-0.01	-1.14	-1.10	1.02*	-1.05
P6	0.37**	1.71	0.006	-1.22	0.69	0.64**	0.56
P7	-0.39	-2.87	-0.002	1.45	1.00	-1.40	1.72

*P≤0.05; ** P≤0.01: *** P≤0.001; CMD = Cassava mosaic disease; DMC = Dry matter content; HI = Harvest index; RTN = Storage root number; RTW= storage root weight; TCC = Total carotenoid content; TWT = Total biomass

4.3.2 Specific combining ability

Cross P1 x P3 had positive non-significant SCA for DMC but a negative effect for TCC and RWT (Table 4.5). Three crosses, P1 x P6, P2 x P7 and P1 x P3, had significant SCA effects for TCC; the first two (P1 x P6 and P2 x P7) were positive while the last cross (P1 x P3) had a negative significant SCA effect for the trait. Four families (P1 x P4, P1 x P7, P2 x P3 and P2 x P5) showed positive SCA effects for TCC (although not significant). Results showed that Family P1 x P3 and P1 x P6 had significant negative effects for CMD. Baker (1978) explained that when SCA means are not significant, the hypothesis is that performance of single cross progeny can be adequately predicted on the basis of the GCA.

When the SCA mean squares are significant, the relative importance of GCA and SCA should be determined by estimating the components of variance to predict the progeny performance (Fasahat *et al*. 2016). The closer the ratio of $2GCA_{MS}/(2GCA_{MS}+SCA_{MS})$ is to 1, the more important the additive gene effects. The predictability (or Baker ratio) in this study varied from 0.48 for DMC to 0.98 for CMD (Table 4.3). All the studied traits had a ratio closer to one for the combined data except for DMC, indicating the importance of GCA and additive gene effects for most of the traits.

Table 4.5 Specific combining ability effects of parents for seven traits evaluated across two locations in Ghana

Family	CMD	DMC	HI	RTN	RTW	TCC	TWT
P1 x P3	-0.08***	4.79	-0.009	-1.05	-0.83	-0.26***	-0.17
P1 x P4	0.07	-4.03**	-0.009	-0.39	-0.96	0.20	-0.69
P1 x P5	0.01	0.71	0.008**	1.79	0.70**	-0.17	0.61**
P1 x P6	-0.06***	0.20	0.007	-0.20	0.89**	0.74***	0.67
P1 x P7	0.0004	0.28	0.01	0.08*	0.32	0.37	-0.91
P2 x P3	0.06	-4.01*	0.01	1.14*	0.88	0.32	0.02
P2 x P4	-0.09	4.81	0.01	0.48	1.01*	-0.14	0.71
P2 x P5	-0.03	0.07	-0.004*	-1.69	-0.66	0.22	0.50*
P2 x P6	0.04**	0.58*	-0.003	0.29	0.85	-0.69	-0.81
P2 x P7	0.02***	0.50	-0.009	0.01	-0.27	0.43***	-0.87

*P≤0.05; ** P≤0.01: *** P≤0.001; CMD = Cassava mosaic disease; DMC = Dry matter content; HI = Harvest index; RTN = Storage root number; RTW= storage root weight; TCC = Total carotenoid content; TWT = Total biomass

4.3.3 Phenotypic correlation

Some of the traits were significantly correlated (Table 4.6). However, there was no significant correlation between TCC and HI, RTW, RTN, TWT (yield components) but TCC showed positive significant (P<0.01) correlation with pulp colour (colour intensity) and CMD. Positive significant (P<0.001) correlation was recorded for pulp colour and CMD. Negative significant correlation were seen between CGM and HI, CGM and RTN, CMD and RTN, and HI and RTN. Positive and significant (P<0.01) correlation was seen between CMD and DMC, pulp colour and cortex colour, TWT and RTW, RTN and RTW and DMC correlated positively with TCC, though not significantly.

4.3.4 Genetic parameters

Broad sense heritability ranged from 0.68 (DMC) to 0.99 (TCC) and narrow heritability sense across the locations ranged from 0.61 (HI) to 0.91 (CMD, TCC) (Table 4.7). Heritability estimates were classified according to Bhateria *et al.* (2006) as high (>0.50), medium (0.30 - 0.50) and low (< 0.30). All the traits studied recorded high heritability, which indicates that selection could be done using direct recurrent selection to improve the traits.

All the traits had high narrow and broad sense heritability. High broad sense heritability indicated that the traits had high genetic variance, both additive and non-additive. Narrow sense heritability is important for breeding programmes as it estimates the relative importance of the additive portion of the genetic variance that can be transmitted to the next generation.

Table 4.6 Phenotypic correlation of measured cassava characteristics evaluated across two locations

	CGM	CMD	COR	PULP	HI	RTN	RTW	TCC	TWT
CMD	0.17ns								
COR	0.12ns	0.40ns							
PULP	0.23ns	0.77***	0.58**						
HI	-0.50*	0.18ns	0.14ns	0.28ns					
RTN	-0.04ns	-0.45*	-0.28ns	-0.26ns	-0.51*				
RTW	-0.51*	-0.23ns	-0.20ns	-0.03ns	0.38ns	0.49ns			
TCC	-0.02ns	0.55**	0.34ns	0.59**	0.01ns	-0.05ns	0.23ns		
TWT	-0.23ns	-0.26ns	-0.33ns	-0.32ns	0.09ns	0.43ns	0.71***	0.29ns	
DMC	-0.06ns	0.60**	0.10ns	0.34ns	0.00ns	-0.13	0.00ns	0.20ns	0.18ns

*P≤0.05; ** P≤0.01: *** P≤0.001; CGM= Cassava green mite; CMD= Cassava mosaic disease; COR= Colour of the cortex; PULP= Pulp colour; HI-= Harvest index; RTN= Storage root number; RTW= storage root weight; TCC= Total carotenoid content; TWT= Total biomass; DMC= Dry matter content;

Table 4.7 Genetic parameters for various traits studied across two locations in Ghana

Variances/ traits	**CMD**	**DMC**	**HI**	**RTN**	**RWT**	**TCC**	**TWT**
GCA_{male}	0.13	0.40	0.005	89.83	14.86	7.19	18.44
GCA_{female}	0.46	20.26	0	2.27	0.90	27.25	1.32
SCA	0.005	0.33	0.0001	4.26	1.27	1.33	1.25
Genotype	0.21	8.57	0.0006	64.73	11.49	13.89	12.37
Additive	0.83	34.29	0.002	258.92	45.96	55.56	49.49
Dominance	0.02	1.32	0.005	17.02	5.06	5.31	4.99
Environmental	0.07	16.91	0.001	20.28	5.37	0.22	8.21
Broad	0.93	0.68	0.75	0.93	0.90	0.99	0.87
Narrow	0.91	0.65	0.61	0.87	0.81	0.91	0.79
Degree of dominance	0.16	0.20	0.5	0.26	0.33	0.31	0.32

CMD = Cassava mosaic disease; DMC = Dry matter content; HI = Harvest index; RTN = Storage root number; RTW= storage root weight; TCC = Total carotenoid content; TWT = Total biomass

4.4 Discussion

Mean TCC values in this study varied from 2.98 to 6.14 µg g^{-1}, with a grand mean of 4.19 µg g^{-1}, which is comparable to values reported by Esuma *et al.* (2016) in Uganda, and Maroya *et al.* (2012), Njoku (2012) as well as Ssemakula and Dixon (2007) in Nigeria. The mean is, however, lower than those reported by Ortiz *et al.* (2011) and Ceballos *et al.* (2013), both in Colombia. The differences observed could be as a result of many years of breeding for TCC at CIAT (Colombia), the age of the plant as reported by Ortiz *et al.* (2011), and the nature of the parental lines used in generating the breeding populations. In most of the studies reported in Africa, parental lines used were selected solely based on either TCC and DMC (white-fleshed) and used in crosses as either male or female parent for each trait, in a design to combine both DMC and TCC to meet stakeholder demands. In other words, a DMC parent crossed to TCC parent. Mean DMC ranged from 26.86 to 36.28% with a grand mean of 30.19%, comparable to values reported by Esuma *et al.* (2016) and Tumuhimbiase *et al.* (2014), but lower than values reported by Kamau *et al.* (2010). Although the DMC grand mean is lower in this study compared to those reported for landraces grown in Ghana by farmers, some individual genotypes evaluated had higher DMC than that of the commonly grown varieties. These evaluated individuals could be selected and further tested towards release, since the trait is one of the key drivers of cassava variety adoption in Ghana. Earlier PRA work done in this study suggested that farmers would be willing to adopt yellow flesh cassava. The mean RTW ranged from 14.34 to 18.35 kg $plot^{-1}$. These mean values were higher than that of farmer-grown varieties, hence could be adopted by farmers in Ghana, but need to be tested in famers' fields on larger plot sizes. Cassava varieties with high TCC and DMC will be rejected by the National Release Committee if they are susceptible to CMD in Ghana. This confirms the importance of selecting clones that are resistant or tolerant to CMD. In the current study, all families recorded CMD values less than 2 (resistant) as shown in Table 4.2.

Some phenotypic correlations in this study are of special importance for selecting high TCC cassava clones in breeding programmes. Firstly, the positive significant correlation between pulp colour and TCC (Chávez *et al.* 2005; Esuma *et al.* 2016), are good for screening large numbers of progenies of elite breeding lines in the cassava breeding programme. This is because pulp colour is directly impacted by carotenoids thus an indicator for Vitamin A. TCC and CMD, pulp colour and CMD, pulp colour and cortex colour. The correlation between CMD and pulp color (TCC) would need more studies to explain the basis underlying this observation. Edoh et al. (2015) also reported positive correlation between

TCC and CMD when evaluating high beta carotene cassava genotypes at advanced trial in Nigeria. Most elite parent materials in breeding programmes in Africa have been pre-selected for CMD resistance and explains why the parent materials and progenies showed good CMD resistance. It shows both TCC and CMD could be combined and selected for at the early stages by visually assessing the pulp colour and CMD symptoms. The reduced number of selected individuals allow for a comfortable size that could be subjected to more demanding quantitative screening analysis for TCC in the later stages of the programme to save cost. Mbusa *et al.*(2018) reported that beta carotene (TCC for our case) can be measured almost quantitatively through a colour chart (visual assessment) estimates since its field estimates (based on the chart TCC values) significantly correlated with those from the laboratory analysis (quantification) in sweet potatoes. The positive but not significant correlation (0.20) between TCC and DMC is useful for developing cassava varieties that could combine both traits. This could be due to the fact that the female parents used in these studies have been selected over years at IITA-Ibadan. Esuma *et al.* (2016) and Mbusa *et al.* (2018) reported negative correlations between TCC and DMC in both cassava and sweet potatoes.

Ceballos *et al*. (2013) reported an initial negative regression between DMC and TCC in CIAT cassava germplasm, which turned into a positive regression after years of recombination and selection. Hence, several years of recombination and selection could help cassava breeders combine both traits in African breeding programmes.

Negative correlation was observed between CMD, CGM and the yield components (RTN, TWT and RTW). The negative correlation is due to the fact that CMD severity (and other pests and diseases) are scored low index for high resistance and vice versa (i.e. low resistance receives high severity index). So good disease response (low scores) go with high yield response (including yield components). It implies therefore that yield and yiled components respond to selection for resistance to pest and diseases. This is the result of the negative impact of diseases and pests on cassava root yield (Hahn *et al.* 1980; Fokunang *et al.* 2000; Ssemakula and Dixon 2007; Parkes *et al.* 2013). The positive significant correlation between RTN and RTW, and TWT and RWT, has also been reported by several other authors (Akinwale *et al.* 2010; Ntawuruhunga and Dixon 2010; Parkes *et al.* 2013; Chikoti *et al.* 2016). This indicates that the higher the root number, the higher the root weight, and eventually the higher the root yield. The non-significant correlation between RWT and DMC suggested that there was no pleiotropic effect between them, and that they

can be selected for independently. However, there is need for breeding to combine both traits to enhance dry root yield which is critical to the commercialization of the crop. Recombination and selection will therefore need to be fixed through crosses and then selected for at early stages of a breeding programme. Combined yield and DMC selection should therefore be possible at the seedling stage (Tumuhumbiase *et al.* 2014). Lastly, the negative correlation between pulp colour and RTN and RTW has also been reported by Ojulong (2006) who stated that colour is highly correlated with beta carotene and negatively with RWT, which is a key driver for adoption. This means that improving the colour may compromise the root yield. Again, breeding to improve simultaneous selection for both traits into a single genotype should be a breeding objective and should be done for both root color and yield for value addition to the crop.

Genetic information was generated in the current study in order to estimate GCA and SCA values for traits of interest. Proportion sum of squares (SS) of both the GCA and SCA as percentage of total sum of squares were calculated to help determine the relative importance of additive and non-additive effects (Falconer and Mackay 1996). This information is critical in the selection of appropriate progenitors and breeding methods for efficient cassava breeding for CMD, DMC and TCC. GCA SS were higher than their respective SCA SS for CMD, HI and TCC, explaining more than 70% of the total variation. This suggests that additive gene effects are more important for the three traits. Esuma *et al.* (2016) reported that GCA accounted for significantly larger SS than SCA SS for HI and TCC. Baafi *et al.* (2016) reported larger GCA than SCA SS in sweet potatoes for beta carotene. Tumuhimbise *et al.* (2014) also reported larger GCA SS than SCA SS for CMD severity and HI. The relative importance of additive gene action for TCC, CMD and HI was confirmed by the higher Baker's ratios (more than 0.5) for these traits. The results suggest that both TCC and CMD could be enhanced through recurrent mass selection due to the additive nature and high heritability for these traits (Ceballos *et al.* 2013).

Improved varieties released in the 1980s and 1990s by IITA explored additive genetic effects for CMD resistance. The discovery of a dominant CMD2 gene (Akano et 2002) has since facilitated the rapid development of CMD resistance in cassava and breeding programs in Africa have explored this gene in the development of most varieties releases in 2000s and subsequently. The use of both additive genetic variance and dominance gene could enhance the development of more durable and stable CMD resistance genetic background for the introgression of other traits such as TCC and DMC. Further studies need to be done on the

parental lines used in this study to ascertain if any of the parental lines have a pedigree for the CMD2 gene.

However it might be difficult to combine with both DMC and TCC due to the larger SCA SS for the former, an indication of non-additive effects. Kamau *et al.* (2010) and Tumuhumbiase *et al*. (2014) reported that DMC is under the influence of non-genetic effects. Ngailo (2015) also reported larger SCA SS for DMC when breeding sweetpotato for improved yield and related traits in Eastern Tanzania. However, the presence of some yellow-fleshed genotypes having DMC values in the same range as the white-fleshed progenitors is an indication that it is possible to breed for both TCC and DMC in a breeding programme. However, Chikoti *et al*. (2016) reported larger SCA SS (67.9%), indicating the influence of non-additive gene action.

GCA and SCA mean squares were significant for RTN and RTW, which implies that these traits showed significant additive and dominance genetic variances. Chiona (2009) and Balcha (2015) also reported significant GCA and SCA effects for yield and yield parameters in Malawi and Ethiopia respectively, but this contrasted a report by Mbusa *et al.* (2018). GCA SS was larger than their respective SCA SS for RTN and RWT, suggesting the predominance of additive genetic effects, as also reflected in their mean squares. Progenitor P6 (IBA090090) showed positive GCA effects for TCC, DMC, RTW and CMD, hence could be crossed to a genetic background of high DMC to increase chances of generating clones that combine both TCC and DMC.

Narrow sense heritability for the traits were high in general. Lestari *et al.* (2010) reported high broad sense heritability of 87% for number of storage roots. Chiona (2009) also reported high broad sense heritability of 96.9% for the same trait. Heritability in both for narrow and broad sense were high for DMC in this study. Shumbusha *et al*. (2014), Parkes *et al.* (2013) and Chiona (2009), all reported high broad-sense heritability for this trait. Broad sense heritability was generally higher than their respective narrow sense heritability for all traits, indicating the presence of non-additive gene effects for their expression. A high broad sense heritability as found for most traits implies that these traits have a highly heritable portion of variation due to both additive and non-additive gene effects with relatively lower influence from the environment. High heritability of a characteristic can be exploited by plant breeders through selection (Akinwale *et al.* 2010). Narrow sense heritability is more important as it measures the relative importance of the additive portion of the genetic variance that can be transmitted to the next generation of the offspring (Fehr

1991). Hence, the high narrow sense heritability observed for all the traits were good for the breeding programme.

4.5 Conclusions

Data generated from this study can be applied for planning an efficient cassava breeding strategy for breeding of yellow flesh cassava in Ghana. The analysis of variance and the GCA:SCA ratio indicated that the GCA was larger than SCA for CMD, HI and TCC and also with predictability ratios close to 1, indicating the presence of additive gene effects and a possibility for improvement of the characters by selection. Some yellow-fleshed genotypes having DMC values in the same range as the white-fleshed progenitors is an indication that it is possible to breed for both TCC and DMC in a breeding programme in Africa, and this was confirmed by the positive (though not significant) correlation between the two traits. Progenitor P6 (IBA090090) showed positive GCA effects for TCC, DMC, RTW and CMD, hence could be crossed to a genetic background of high DMC to increase chances of generating clones that combine both TCC and DMC. Findings of this study showed that yield and quality characteristics can be selected simultaneously, such as DMC early stages of the breeding cycle.

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Chapter 5

Genetic variability, stability and heritability for quality and yield characteristics in provitamin A cassava varieties

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Abstract

Cassava is widely consumed in many areas of Africa, including Ghana, and is a major part of most household diets. These areas are characterised by rampant malnutrition, because the tuberous roots are low in nutritional value. Provitamin A (pVA) biofortified cassava varieties have been developed by the International Institute for Tropical Agriculture, but adoption of these varieties in Ghana will largely depend on their agronomic performance, including fresh root weight, dry mater content (DMC), resistance to major pests and diseases, mealiness, starch content and the stability of these traits. Eight pVA varieties with two white checks were planted in three environments for two seasons to determine stability and variability among the varieties for important traits. There were significant variations in performance between varieties and between environments for cassava mosaic disease, storage root number, fresh root weight and starch content. High broad-sense heritability and genetic advance were observed for all traits, except DMC, and could be exploited through improvement programmes. This study identified the best performing enhanced pVA varieties for traits, which are key drivers of variety adoption in Ghana. In view of this, some varieties can be recommended for varietal release after on-farm testing. The study also showed the possibility of tapping heterosis after careful selection of parents.

5.1 Introduction

The populations of underdeveloped and developing countries often suffer undernourishment and "hidden hunger" as a result of micronutrient deficiencies (Maroya *et al.* 2010). Areas in Africa, including Ghana, where cassava is widely consumed, are characterised by rampant malnutrition because the tuberous roots are low in nutrients such as VA (Ssemakula and Dixon 2007). It is for this reason that the development of nutrient

dense cassava cultivars needs more attention to eliminate the ramifications of malnutrition among the poor in an inexpensive and sustainable way. VAD constitutes a public health problem and affects mainly children and women. Recently, different programmes such as HarvestPlus, involving a global alliance of research institutions, initiated the development of micronutrient-dense staple crops (Bouis *et al.* 2011; Dwivedi *et al.* 2012). Among these initiatives is the development of biofortified cassava clones with high PVAC in the roots.

Adoption of biofortified cassava varieties in Ghana will largely depend on their agronomic performance, including FRW, DMC, resistance to major pests and diseases, starch content and the stability of these traits over time and space. DMC influences texture after boiling, and is also a key parameter in the production of gari (a popular cassava food in Ghana). According to Ceballos *et al.* (2017), there is no negative relationship between carotenoids and DMC, thus, making it possible to identify varieties with high PVAC and acceptable levels of DMC.

GEI is the result of inconsistent performance of varieties across environments. The expression of genes that control key agronomic traits in cassava is influenced by both abiotic and biotic stresses, which can lead to GEI (Kang 2002). Breeders face the GEI challenge by evaluating genotypes in several environments to ensure that they have good and stable performance (Acquaah 2012). Several statistical models have been developed to interpret GEI data to understand stability. Scientists have highlighted weaknesses and strengths of these models, which includes commonly used ones like additive main effects and multiplicative interaction (AMMI) as well as genotype and genotype by environment interaction (GGE) biplots. Several studies on cassava have used AMMI for assessment of GEI effects for traits and storage root yield (Kvitschal *et al.* 2006; Aina *et al.* 2007), carotenoid and DMC (Maroya *et al.* 2010; Esuma *et al.* 2016) and early bulking of storage roots (Agyeman *et al.* 2015). The AMMI model was reported to capture a large portion of the GGE sum of squares and uniquely separates main and interaction effects as required for most agricultural research purposes (Gauch 2006). Yet, the AMMI biplot does not have the most important feature of a true biplot, namely the inner-product property and this biplot does not display the discriminating ability and representativeness view of a biplot, which is effective in evaluating test environments. Hence, the GGE biplot has been proposed to effectively identify the best-performing genotypes across environments, identify the best genotypes for mega-environment delineation, whereby specific genotypes

can be recommended for specific mega-environments and evaluate the yield and stability of genotypes (Yan and Kang, 2003; Yan and Tinker, 2006).

This study was designed to evaluate yellow flesh cassava clones across locations for DMC, CMD, CGM, starch content, yield and its related characteristics; to determine the magnitude of genotype, environment, and GEI effects on these traits, and to identify stable and high performing clones for DMC and FRW using GGE biplots.

5.2 Materials and methods

5.2.1 Varieties, experimental sites and design

Ten varieties were evaluated, of which eight were selected from sets of yellow flesh cassava clones previously acquired from IITA and the other two varieties were white- fleshed landraces obtained from farmer fields in Ghana (Table 5.1). Trials were conducted over two seasons, May 2015 - May 2016 and June 2016 - June 2017 at three locations situated in different agroecological zones. Fumesua (forest), Ejura (forest transition) and Kokroko (transition). Each planting season was considered an environment, giving a total of six environments. Temperature and rainfall data were recorded during the experimentation period as well as soil nutrient profile of the fields prior to planting the trials (Table 5.2). Trials were laid out in a RCBD with three replications, each consisting of four rows of seven plants, giving a plot size of 28 plants. Planting was done at a spacing of 1 × 1 m. To increase chances of sprouting and uniform plant establishment, all stakes used for planting were generated from the middle portions of mature stems. Replications were separated by 2 m alleys. Weeding was done when necessary and experiments were entirely rain fed.

Table 5.1 Provitamin A and white flesh cassava genotypes used for the study

Genotype	Code	Status	Source	Pulp colour
IBA090090	G1	Improved	IITA	Yellow
IBA090151	G2	Improved	IITA	Yellow
IBA070557	G3	Improved	IITA	Yellow
IBA085392	G4	Improved	IITA	Yellow
Debor	G5	Landrace	Farmer	White
IBA083774	G6	Improved	IITA	Yellow
IBA070593	G7	Improved	IITA	Yellow
IBA070539	G8	Improved	IITA	Yellow
UCC	G9	Released	CSIR-CRI	White
IBA083724	G10	Improved	IITA	Yellow

5.3 Data collection

The varieties were evaluated at monthly intervals, starting at 1 MAP to 9 MAP, for their reaction to CMD and CGM. Damage symptoms were scored on a scale of 1 - 5, where 1 = no symptoms and 5 = very severe symptoms (IITA, 1990). Only the score of the most severely affected plants were recorded in a plot. For each trial, TCC, DMC, FRW and HI were measured at 12 MAP. The inner two rows of each experimental plot constituted a net plot of 10 plants for measurement of the traits. Biomass from harvested plants was bulked to estimate yield components by separately weighing the FRW and foliage (FSW). HI was computed from the measure of FRW and FSW as: HI = FRW/(FRW + FSW).

Root samples from each plot (5 kg) were weighed in air (Wa) using a balance after cleaning the soil and other debris from the roots. The root samples were again weighed in water (Ww). The same container was used to weigh the sample in both air and water.

Specific gravity was calculated as: X = Wa/(Wa – Ww)

DMC and starch content were calculated using the following formulas:

DMC = 158.3 x specific gravity - 142 (Kawano *et al.*, 1987)
Starch content = (210.8 x specific gravity) - 213.4 (Howeler, 2014).

The mealiness was measured by taking a small portion of the boiled sample and pressing it between the thumb and the index finger. When it is soft and can form a sticky paste, it is considered mealy and suitable for 'ampesi' (that is boiled and eaten) or for 'fufu'. On the other hand, the hard and difficult to press root will not form a sticky paste and is considered non-mealy. However, non-mealy genotypes can be used for cassava dough 'agbelima', or dried for 'konkonte', cassava chips or processed into gari. Mealiness is measured on a scale of 1 - 4 (1 = non-mealy 2 = mealy, 3 = very mealy and 4 = excellent) (Parkes, 2011). The plant vigour was measured 3 MAP in terms of how the plants germinated. The scale for measurement was 1 - 4, (1 = very poor, 2 = poor, 3 = good, 4 = very good).

Table 5.2 Characteristics of the six trial environments

	Season 1 (May 2015 – May 2016)			Season 2 (June 2016 – June 2017)		
Parameter	Edubiase (Env1)	Kokroko (Env2)	Pokuase (Env3)	Edubiase (Env4)	Kokroko (Env5)	Pokuase (Env6)
pH	5.3	6.2	7.1	5.6	5.7	6.9
OM (%)	2.0	0.9	2.2	2.4	0.4	2.0
N (%)	0.3	0.03	0.1	0.1	0.1	0.2
P (ppm)	3.4	3.1	5.8	3.9	4.6	4.7
Ca (ppm)	3.0	1.8	5.1	3.1	2.3	5.8
Mg (ppm)	2.3	1.2	1.7	1.1	2.1	1.4
K (ppm)	0.8	0.6	0.1	0.1	3.2	0.1
Zn (ppm)	13.9	1.5	34.1	13.1	1.8	44.0
B (ppm)	0.4	0.6	0.2	0.3	0.8	0.2
Cu (ppm)	25.9	0.9	44.0	24.0	0.7	54.0
Fe (ppm)	17365.6	3775.6	5939.38	18365.6	3752.6	6139.28
Mn (ppm)	1618.63	470.98	1367.72	1508.63	533.72	1478.71
Rainfall (mm)	2100.0	892.9	1072.4	2350	1160.9	1420.0
Min T (^{o}C)	21.7	32.0	30.2	21.3	32	31.0
Max T (^{o}C)	29.1	35.0	35.8	31.1	34	34.8
Latitude	4°40'0''N	7°39'1.57''N				5°42'0''N
Longitude	1°38'0''W	1°56'56.48''W				0°16'36''W
Altitude	136.1	482.1				45.7

OM = Organic matter content; Min T = Minimum temperature; Max T = Maximum temperature

TCC was measured following the method of Rodriquez-Amaya and Kimura (2004). Fresh cassava roots of three different sizes; small, medium and large, were washed with tap water to remove dirt and debris, allowed to dry and then peeled. The peeled roots were washed with deionized water to avoid contamination and dried with tissue under a subdued light to protect carotenes. Root samples and extracts were protected from the light as much as possible. Roots were cut longitudinally in half and then the two halves were cut again longitudinally into quarters. Each quarter would therefor include tissue from the periphery, mid-parenchyma and core of the root, as well as proximal, central and distal sections (Chávez *et al.*, 2008). The two quarters of each root were then ground and mixed for a uniform sample. The sample was then packaged into aluminum foil, placed into a whirl pack and labeled. Ten gram of the test sample was transferred into a clean dried mortar, and about 3 g of Celite was added to the test sample to ease maceration of the cassava tissues as well as filtration. Cold acetone (50 ml) was first added in the mortar. The mixture was crushed with a pestle until fine and then filtered.

Extraction was repeated three times with cold acetone to ensure complete extraction. The extract was filtered using a Buchner funnel with 90 mm filter paper and rinsed with cold acetone.The combined extract was transferred into a separation funnel with 5 ml distilled water and 20 ml petroleum ether. Deionized water (500 ml) was dispensed through the walls of the separation funnel to wash the acetone down. Brine solution was added to break any emulsion formed in the ether extract. The petroleum ether extract containing the carotenoids was partitioned in the upper layer in the separating funnel, and the aqueous layer was gradually discarded. The extract was then transferred gradually into a 25 ml volumetric flask using a small funnel with sodium sulfate on top of cotton wool to dry any excess water. Petroleum ether was added to the extract in the volumetric flask and transferred into a 30 ml glass bottle. Aliquots of the extracts were transferred into a cuvette and was read using an UV-Vis spectrophotometer at wavelength of 450 nm from which absorbance readings was obtained and TCC (µg g^{-1}) calculated as:

$$TC = [A \times \text{volume (ml)} \times 10^4]/[A^{1\%}_{1cm} \times \text{sample weight (g)}]$$

where A = absorbance; volume = total volume of extract 25 ml, $A^{1\%}_{1cm}$ = absorption coefficient of beta carotene in PE (2592).

All procedures for carotenoid extraction and measurement were performed in subdued light and samples were analyzed within 24 hours of harvesting. TCC was measured only in one year without replication to confirm status of genotypes as pVA enriched.

5.4 Data analysis

Data were subjected to ANOVA and AMMI analysis for FRW, DMC, RTN and starch of 10 cassava varieties obtained per plot across environments, using Genstat software Release 17.0 (2011). Genetic effects were considered fixed, and location and season effects random. The GGE biplot method outlined by Yan (2002) was used to display the G and GEI patterns in the data in a biplot. The which-won-where pattern, which is an intrinsic property of the GGE biplot rendered by the inner-product property of the cassava genotype environment data set, was also visually presented. In addition, the GGE biplot was used to identify high yielding and adapted cassava varieties as well as suitable test environments.

Stable varieties for each environment were selected from AMMI analysis and principal component (PCA) axes were extracted and statistically tested by Gollob's (1968) F-test procedure (Vargas and Crossa 2000). Phenotypic correlation coefficients and PCA and its biplots were analysed using Genstat software Release 17.0. Trait components and magnitude of variation responsiveness to selection were calculated according to Okwuagwu *et al.* (2008). Expected genetic advance of the mean for each trait was calculated according to Allard (1960). Genotypic and phenotypic variances were calculated according to Obilana and Fakorede (1981).

5.5 Results

5.5.1 Analysis of variance

In the combined ANOVA (Table 5.3) the main effects (genotype, location and year) were highly significant ($P < 0.001$) for all traits evaluated except CMD and significant ($P < 0.05$) for location and year.

Values for separate seasons were only presented for the five main yield components, as the focus was on the data of the seasons combined. Values of DMC, FRW and HI were significantly higher in the second season than the first (Table 5.4) due to more favourable growing conditions in the second season.

Combined over two seasons FRW ranged from 18.99 to 32.67 t ha^{-1} with a mean of 23.43 t ha^{-1} (Table 5.5). Genotype IBA083774 had the highest yield of 32.67 t ha^{-1}, while the lowest

value of 18.99 t ha^{-1} was recorded by IBA085392. DMC ranged from 23.19% to 30.26% with a mean of 27.38%. The local cultivar recorded the highest (30.25%) DMC, followed by IBA083774 (29.39%) and IBA085392 recorded the lowest value (23.19%). CMD scores ranged from 1.0 to 2.17 with a mean of 1.15. All the yellow flesh cassava varieties had a severity score of 1.0 with the exception of IBA070593 (1.17). The local cultivar recorded the highest severity score (2.17) to CMD. All the elite cassava genotypes from IITA recorded higher TCC values than the local check used. Genotype IBA083774 with the highest FRW recorded the lowest TCC values among the IITA materials and the local check with the highest DMC recorded the lowest TCC value.

Table 5.3 Analysis of variance and contribution of main effects to variation for measured characteristics across three environments in two growing seasons

	Source	Df	SS	MS	% of total SS
CMD	Genotype	9	21.45	2.38***	65.10
	Location	2	0.23	0.12ns	0.70
	Year	1	0.27	0.27*	0.82
	Gen.loc	18	1.43	0.08ns	4.34
	Gen.year	9	1.67	0.19***	5.07
	Loc.year	2	0.21	0.11ns	0.64
	Gen.loc.year	18	1.68	0.09*	5.10
	Error	118	6.00	0.05	
DMC	Genotype	9	883.66	98.19***	39.96
	Location	2	245.34	122.67***	11.10
	Year	1	189.11	189.11***	8.55
	Gen.loc	18	191.27	85.75***	8.65
	Gen.year	9	57.47	6.39*	2.60
	Loc.year	2	171.50	85.75***	7.76
	Gen.loc.year	18	86.49	4.80ns	3.91
	Error	118	380.27	3.22	
Starch	Genotype	9	443.13	49.24***	39.96
	Location	2	123.03	61.52***	11.10
	Year	1	94.83	94.83***	8.55
	Gen.loc	18	95.91	5.33***	8.65
	Gen.year	9	28.82	3.20*	2.60
	Loc.year	2	86.01	43.00**	7.76
	Gen.loc.year	18	43.38	2.41ns	3.91
	Error	118	190.70	1.62	
FRW	Genotype	9	2758.90	306.40***	12.02
	Location	2	4413.48	2206.74***	19.22
	Year	1	3029.08	3029.08***	13.19
	Gen.loc	18	1499.40	83.30ns	6.53
	Gen.year	9	671.50	74.61ns	2.92
	Loc.year	2	2189.76	1094.88***	9.54
	Gen.loc.year	18	1648.67	91.59*	7.18
	Error	118	6685.96	56.66	
RTN	Genotype	9	9060.10	1006.70***	16.39
	Location	2	3390.30	1695.20***	6.13
	Year	1	1566.50	1566.50***	2.83
	Gen.loc	18	10419.90	578.90***	18.85
	Gen.year	9	3031.20	336.80ns	5.48
	Loc.year	2	1073.10	536.50ns	1.94
	Gen.loc.year	18	5854.50	325.20*	0.11
	Error	118	20725.90	175.60	

*P≤0.05, ** P≤0.01 ***P≤0.001; CMD = cassava mosaic disease; DMC = dry matter content; FRW = fresh root weight; RTN = storage root number

Table 5.4 Means of five traits measured in two growing seasons (2015/2016 and 2016/2017) in 10 genotypes across six environments in Ghana

	Traits	DMC	FRW	CMD	HI	RTN
Genotype	Year	%	t ha^{-1}			
IBA90090	1	26.00a	18.71a	1.0a	0.36a	39.22a
	2	27.91b	20.67a	1.0a	0.36a	43.11a
IBA090151	1	26.14a	21.00a	1.00a	0.38a	55.24a
	2	29.10b	29.06b	1.00a	0.41a	56.89a
IBA070557	1	28.93a	19.11a	1.00a	0.34a	44.78a
	2	28.60a	26.06b	1.00a	0.43b	42.89a
IBA085392	1	22.61a	12.78a	1.00a	0.28a	29.11a
	2	23.77b	25.06b	1.00a	0.33b	56.00b
IBA083724	1	27.95a	21.63a	1.00a	0.28a	53.56a
	2	30.37b	27.61a	1.00a	0.43b	60.22b
IBA083774	1	28.82a	28.28a	1.00a	0.45a	53.56a
	2	29.96a	37.06b	1.00a	0.47a	59.44a
IBA070593	1	24.59a	16.71a	1.11a	0.37a	34.89a
	2	28.15b	21.67a	1.22a	0.34a	36.00a
IBA070539	1	23.03a	15.79a	1.00a	0.42a	34.22a
	2	24.75b	29.17b	1.00a	0.52b	48.00b
UCC	1	26.97a	23.89a	1.33a	0.55a	44.22a
	2	29.48b	29.06b	1.00b	0.46b	43.89a
Local check	1	28.54a	15.43a	2.44a	0.42a	45.22a
	2	31.98b	29.94b	1.89b	0.46b	46.11a
Mean	1	26.36a	19.33a	1.19a	0.38a	43.36a
	2	28.41b	27.53b	1.11a	0.42b	49.26a

DMC = dry matter content (%); FRW = fresh root weight (t ha^{-1}); CMD = cassava mosaic disease; HI = harvest index; RTN = storage root number (t ha^{-1}). Means followed by the same letter are not statistically different

Table 5.5 Mean values of nine traits measured in 10 genotypes across six environments in Ghana

Genotype	FRW	RTN	TWT	DMC	Starch	HI	CMD	CGM	Mealy	TCC
IBA090090	19.69	41.17	35.79	26.95	13.24	0.45	1.00	1.27	1.94	10.37
IBA090151	25.03	56.06	39.04	27.62	13.72	0.34	1.00	1.05	1.67	12.73
IBA070557	22.58	43.83	34.42	28.76	14.52	0.39	1.00	1.11	1.89	7.78
IBA085392	18.99	42.56	40.62	23.19	10.59	0.31	1.00	1.16	1.50	11.74
IBA083724	24.62	56.67	30.23	29.16	14.81	0.36	1.00	1.12	1.89	6.58
IBA083774	32.67	56.50	39.97	29.39	14.97	0.46	1.00	1.50	1.06	3.12
IBA070593	19.19	35.44	33.72	26.37	12.83	0.35	1.17	1.61	1.94	16.00
IBA070539	22.48	41.11	23.27	23.89	11.08	0.47	1.00	1.38	1.66	13.79
UCC	26.47	44.06	24.84	28.23	14.15	0.51	1.17	1.33	2.50	3.13
Local	22.69	45.67	38.64	30.26	15.68	0.44	2.17	1.67	3.33	0.78
Grand mean	23.43	46.31	34.04	27.38	13.54	0.41	1.15	1.33	1.93	8.60
S.e.d	6.15	10.82	7.89	1.47	2.06	0.05	0.18	0.47	0.76	1.56
CV %	32.10	28.60	28.40	6.60	9.40	15.50	19.60	43.20	48.20	59.70

FRW = fresh root weight (t ha^{-1}); RTN = storage root number (t ha^{-1}); TWT = total biomass (t ha^{-1}); DMC = dry matter content (%); HI = harvest index; CMD = cassava mosaic disease; CGM = cassava green mite; TCC = total carotenoid content (µg g^{-1})

5.5.2 Additive main effects and multiplicative interaction analysis

Combined AMMI ANOVA (Table 5.6) showed that genotype, environment and GEI effects were highly significant (P<0.001) for CMD, DMC, FRW, RTN and starch. IPCA1 mean squares were highly significant (P<0.001) for all traits except FRW, which was significant at P<0.01. IPCA1 and IPCA2 accounted for more than 70% of the total variation observed in GEI, which was confirmed by the significant (P<0.001) GEI effects for all traits

Table 5.6 AMMI analysis of variance for measured characteristics

Source	df	CMD	DMC	RTN	FRW	Starch
Genotype	9	2.38***	98.18***	1006.7***	306.5***	49.24***
Environment	5	0.14***	121.19***	1206.0***	926.5***	60.77***
GEI	45	0.11***	7.45***	429.0***	84.9***	3.74***
IPCA1	13	0.28***	15.83***	770.3***	127.5**	7.94***
IPCA2	11	0.07ns	3.72ns	396.8***	89.4*	1.87ns
Residual	21	0.02	4.210	234.60	56.10	2.11
% GEI due to IPCA1		76.29	61.40	51.87	43.40	61.39
% GEI due to IPCA2		15.89	12.23	22.61	25.76	12.20

*P≤0.05, ** P≤0.01 ***P≤0.001, CMD = cassava mosaic disease; DMC = dry matter content; RTN = storage root number; FRW = fresh root weight; GEI = genotype by environment interaction; IPCA = interaction principal component axis

5.5.3 Correlations, genetic components and principle component analysis

RTN and TWT, FRW and TWT, TWT and vigour, HI and RTN, HI and FRW, CMD and mealiness, RTN and FRW and CGM and HI were highly significantly positively correlated. Significant negative correlations were observed between TWT and HI as well as between vigour and HI showed (Table 5.7).

The magnitude of the phenotypic coefficient of variation (PCV) was higher than their corresponding genotypic coefficient of variation (GCV) for all the traits studied. The PCV ranged between 8.55% and 26.09%, with CMD showing the highest value, followed by TWT and with DMC recording the lowest value. Heritability was generally high for all characters and varied from 41.34% for RTN to 88.89% for CMD (Table 5.8).

Table 5.7 Phenotypic correlations coefficients for 10 traits measured on 10 cassava genotypes across six environments in Ghana

Traits	CGM	CMD	DMC[c]	HI	Mealy	RTN	FRW	TWT
CMD	0.13							
DMC	0.03	0.18*						
HI	0.33	0.05	0.08					
Mealy	-0.01	0.29***	-0.001	-0.01				
RTN	0.20**	0.03	0.16*	0.45***	-0.07			
FRW	0.19*	-0.05	0.07	0.62***	-0.18*	0.69***		
TWT	-0.18*	-0.13*	-0.02	-0.43***	-0.13	0.30***	0.36***	
Vigour	-0.12	-0.04*	0.14	-0.22**	-0.03	0.07	0.05	0.33**

*P≤0.05, ** P≤0.01 ***P≤0.001, CGM = cassava green mite; CMD = cassava mosaic disease; DMC = dry matter content; HI = harvest index; RTN = storage root number; FRW = fresh root weight; TWT = total biomass

Table 5.8 Coefficients of variation, heritability and genetic advance for five traits of 10 cassava genotypes planted in six environments

Traits	Genetic parameters				
	Mean	GCV	PCV (%)	H^2_b	Gas
FRW	25.33	15.58	17.63	78.39	26.27
RTN	46.31	10.39	16.15	41.34	13.76
TWT	34.04	14.04	18.10	60.10	22.40
Starch	13.55	11.44	13.14	75.95	20.55
DMC	27.38	8.00	8.55	87.55	15.41
CMD	1.15	24.35	26.09	88.89	47.77

GCV = genotypic coefficient of variation (%); PCV = phenotypic coefficient of variation (%); H^2_b = broad-sense heritability (%), Gas = expected genetic advance of the mean; FRW = fresh root weight; RTN = storage root number; TWT = total biomass; DMC = dry matter content, CMD = cassava mosaic disease

From the PCA (Table 5.9) the first three principal components (PC) had eigenvalues higher than one and accounted for 83.93% of the total variation. PC1 accounted for 40.50% variation and RTN, DMC and starch were the principal contributors. PC2 accounted for 27.51% of the variation with TWT, vigour and CGM contributing most to

the variation. PC3 accounted for 15.92% of variation with FRW, mealiness, CMD and HI being the main contributing factors.

Table 5.9 Principal component analysis of 10 quantitative traits in 10 cassava genotypes showing eigenvectors, eigenvalues, individual and cumulative percentage of variation explained by the first three principal components axis

Characters	Eigenvectors		
	PC1	PC2	PC3
RTN	0.34	0.27	0.06
FRW	0.35	0.23	0.47
TWT	-0.09	0.52	-0.32
Mealy	0.28	-0.33	-0.41
DMC	0.43	0.16	-0.14
Starch	0.43	0.16	-0.14
Vigour	0.12	0.49	-0.13
CMD	0.34	-0.27	-0.37
CGM	0.24	-0.31	-0.06
HI	0.32	-0.19	-0.56
Eigenvalue	4.05	2.75	1.50
Individual	40.50	27.51	15.92
Cumulative	40.50	68.01	83.93

RTN = storage root number; FRW = fresh root weight; TWT = total biomass; DMC = dry matter content; CMD = cassava mosaic disease; CGM = cassava green mite; HI = harvest index

5.5.4 GGE biplot for average dry matter content, fresh root weight, starch and stability of varieties

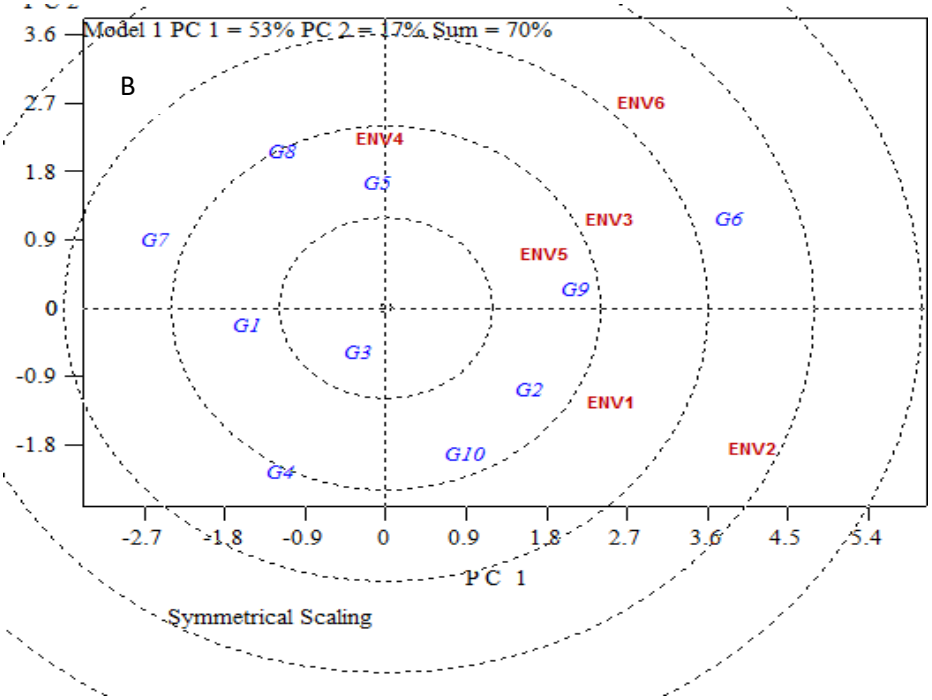
The biplot (Figure 5.1) showed that PCA1 and PCA2 explained 91% of variation for DMC. DMC was highest in genotype IBA083724 (G10), followed by IBA083774 (G6) and local (G5). IBA085392 (G4) had the lowest DMC value. Varieties IBA090151 (G2) and IBA070539 (G8) were more stable with genotype IBA070593 (G7) being the most unstable. Genotype IBA083774 (G6) had the highest mean for FRW, followed by UCC (G9), IBA090151 (G2), while IBA070593 (G7) ranked the lowest (Figure 5.1). In terms of stability, varieties IBA090090 (G1) and UCC (G9) were most stable.

5.5.5 The best performing genotype in each environment and mega-environments for dry matter content, fresh root weight and starch content

PC1 explained 77% and PC2 14% of variation, both reflecting 91% of the DMC variation (Figure 5.2). PC1 explained 53% and PC2 17% of variation in FRW, reflecting a total 70% of variation.

A convex-hull drawn on the varieties from the origin of the biplot gave five sections with IBA070593 (G7), IBA085392 (G4), IBA090090 (G1), IBA083774 (G6) and IBA083724 (G10) as vertex varieties. G10 was the best variety in three environments (Edubiase, Env 4; Pokuase, Env 3; and Kokroko, Env 2) and G6 was best in three other environments (Edubiase, Env 1, Kokroko, Env 5 and Pokuase, Env 6) for DMC (Figure 5.2A). The biplot grouped all the environments into two groups, suggesting two mega-environments. The first mega-environment had environments Env 4, Env 3, Env 2 and Env 6 with varieties IBA083724 (G10), UCC (G9), Local (G5), IBA090151 (G2) and IBA083774 (G6) as the best performers and the second mega-environment had environments Env 5 and Env 1, with genotype IBA070557 (G3) performing best.

For FRW, IBA083774 (G6), IBA070539 (G8), IBA070593 (G7), IBA085392 (G4) and IBA083724 (G10) were the vertex varieties for the five sections of the biplot (Figure 5.2B). The biplot grouped all the environments into two groups, suggesting two mega-environments. The first mega-environment had environments Env 1, Env 2, Env 3, Env 5 and Env 6 with varieties UCC (G9), IBA090151 (G2) and IBA083774 (G6) as the best performers and the second mega-environment had environment Env 4, with genotype Local (G5) performing the best.



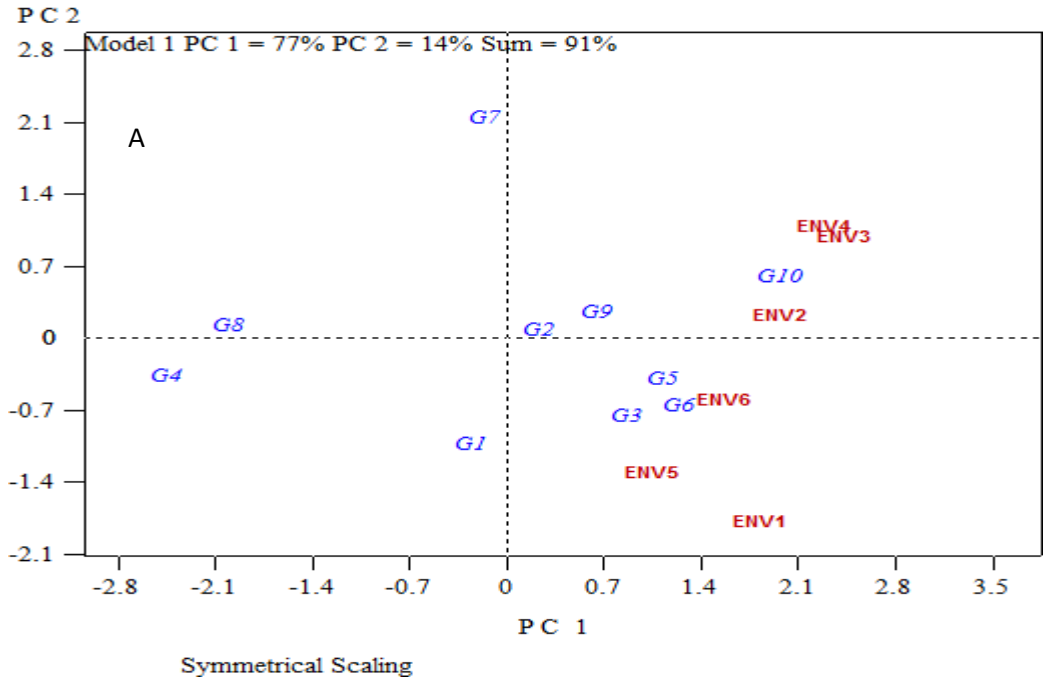
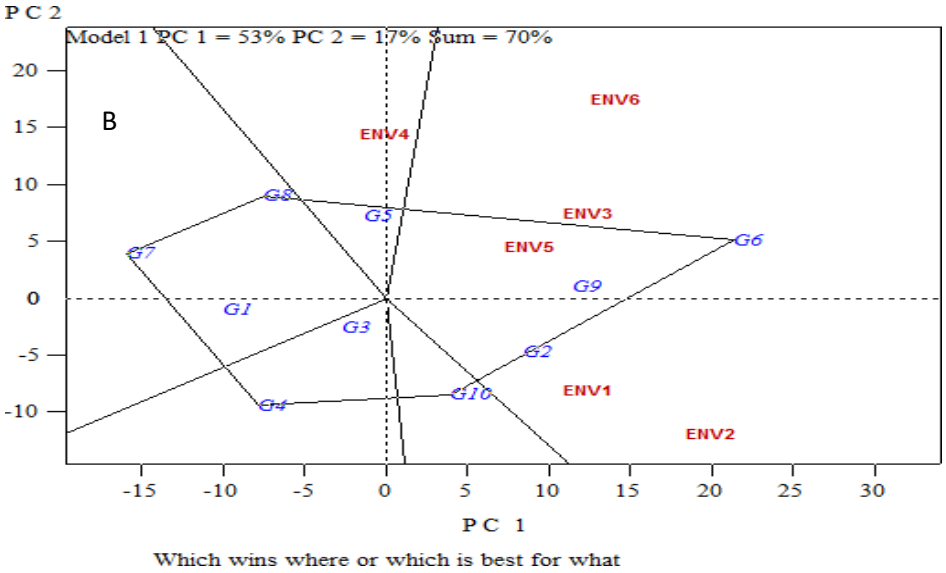


Figure 5.1 GGE biplot showing (A) dry matter content and (B) fresh root weight mean performance and stability of 10 cassava genotypes



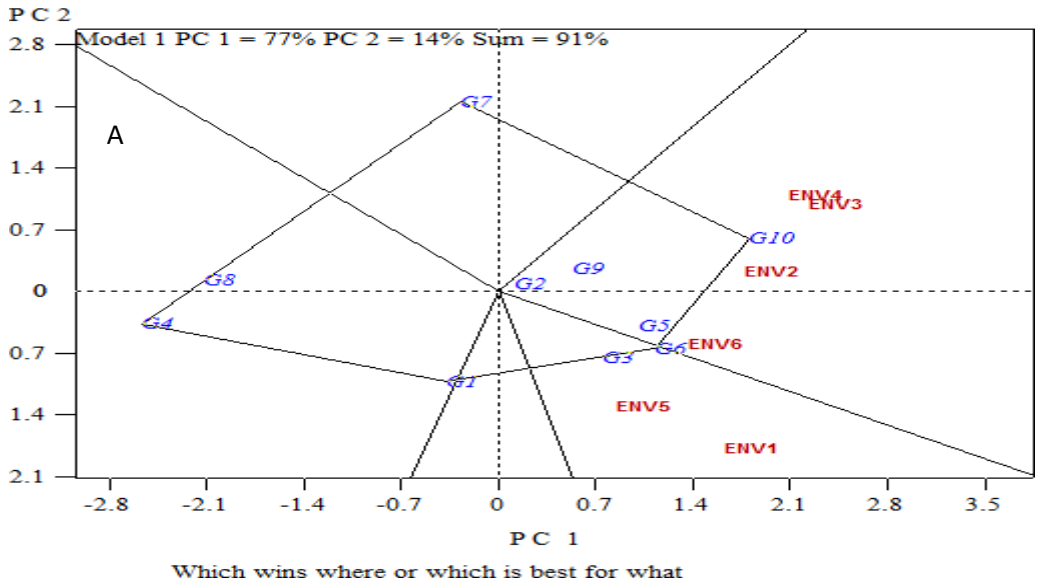


Figure 5.2 Which wins where GGE biplot for best cultivars for (A) dry matter content (B) fresh root weight in different environments

5.6 Discussion

DMC, FRW, starch, CMD, mealiness and RTN are key drivers for cassava variety adoption (Abdoulaye *et al.* 2014; Esuma *et al.* 2016) in Ghana. All the yellow flesh cassava varieties in this study had higher TCC values than the local and improved check. Three of the yellow flesh cassava varieties (IBA090I51, IBA083774 and IBA083724) recorded higher FRW than the checks. In terms of DMC, the local variety was not statistically different from varieties IBA083774 and IBA083724, which recorded the highest FRW and CMD score.

There were significant variations in mean performance of varieties for CMD, RTN, FRW and starch, which are some of the most important traits for consumer acceptance (Owusu and Donkor 2012), in different environments. TCC-rich cassava cultivars could be selected using on-station trials in one location and selected cultivars can be subjected to multi-location evaluation where the focus is on other important traits of cassava for variety adoption (Esuma *et al.* 2016)

The significant genotype effects observed for the traits studied indicated that varieties were significantly different; hence, genetic improvement could be achieved through hybridization. The significant GEI (from AMMI analysis) for CMD, DMC, RTN, FRW and starch, indicated variation in genotypic responses to different environments and this underlined the importance of the multi-environment testing of newly developed varieties.

The combined ANOVA for CMD, DMC and starch indicated that genotype main effect accounted for 65.10%, 39.96% and 39.96% of variation, respectively. This was confirmed by the small difference between their PCV and GCV values. Selection for such traits could be fairly easy due to the close association between the genotype and the phenotype

Cassava breeding aimed at selecting desired genotypes is linked with GCV, heritability estimates, genetic advance as percentage of the population mean and other genetic parameters for important traits (Idahosa *et al.* 2010). The magnitude of the heritability of the selected traits studied were generally high. Pradeepkumar *et al.* (2001) reported that heritability estimates together with genetic advance contribute to improved selection response. The low PCV values for DMC, in this study have also been reported by other authors (Kundy *et al.* 2015; Ewa *et al.* 2017). The generally higher values of PCV than

their corresponding GCV values for traits indicated the considerable role of the environment in the expression of these traits; hence, the variation in the varieties are due to both genotype and the environment. The high heritability values for the measured traits indicate the presence of a larger portion of heritable variation, which would aid selection. RTN with quite high heritability and low genetic advance could pose a challenge if selection is based only on this trait. Esuma *et al.* (2016) confirmed a strong negative correlation between DMC and TCC. Ceballos *et al.* (2013) reported simultaneous gains for both TCC and DMC through rapid selection. There is need in Ghana to combine these two important traits in the breeding programme. The best yellow flesh cassava varieties identified in this study could be the starting material for this improvement.

Correlations among traits play an important role in plant breeding as it can improve selection efficiency. The positive significant correlation between FRW and RTN, TWT, HI, DMC and starch, suggests that an increase in mean value of any one of these character pairs would significantly increase the mean of the other (Akinwale *et al.* 2010). The negative significant correlation of HI and TWT is very important in cassava breeding, where the ultimate focus is on yield/weight (storage roots) which correlates positively with HI. However, varieties must also produce prolific stems from planting material which is also related to TWT. The negative correlations between CMD and FRW, TWT and vigour confirms the potential storage root yield losses that can be caused by the disease, which was confirmed by Parkes *et al.* (2013). There was also a significant positive correlation between CMD and mealiness. Landraces are more susceptible to CMD and most landraces in Ghana are mealy.

5.7 Conclusions

This study showed the best performing TCC-rich varieties also have variation for important traits of cassava, which are key drivers of variety adoption in Ghana. In view of this, varieties IBA090151, IBA083774 and IBA083724 can be considered for varietal release after on-farm testing. The study also revealed that the yellow flesh cassava varieties could be used in a hybridization scheme with the local material to combine both TCC and DMC traits with high yield in a CMD resistance background.

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Chapter 6

Proximate composition and cyanide content, and total carotenoid retention after boiling of yellow-flesh cassava cultivars

Abstract

Biofortified yellow-flesh cassava is important in countries with high cassava consumption, to improve the vitamin A status of its population. In this study, 10 cultivars were evaluated over three locations for proximate composition and cyanide content as well as retention of carotenoids after boiling. There were significant differences ($p<0.001$) in the crude fiber, fat, protein and ash content of all the cassava genotypes investigated. All yellow flesh cassava genotypes recorded higher protein values than the local cultivars across all locations, except for cultivar IBA085392. The cyanide content of the cultivars varied between locations, but was within the range of sweet cassava cultivars, but in excess of the maximum acceptable limit recommended by the WHO. However, the cyanide can be reduced in cassava cultivars with several processing methods such as roasting, frying, boiling, and fermentation. Micronutrient retention is an important aspect of research on biofortified crops, because a loss of micronutrients during processing and cooking reduces the nutritional value of biofortified foods. TCC of fresh and boiled cassava was measured by spectrophotometer. TCC of fresh peeled cassava was 1.18 - 18.81 µg g^{-1} on a fresh weight basis, whereas in boiled cassava TCC was 1.01 - 13.36 µg g^{-1}. All the yellow flesh cassava genotypes recorded higher TCC values in both the fresh and boiled state than the white-fleshed varieties used as checks.

6.1 Introduction

Cassava roots are a staple food that provides carbohydrates and energy for more than 2 billion people in the world, while representing the main source of carbohydrate and energy for the approximately 700 million people living in the tropical and sub-tropical areas (Ferraro *et al*. 2015).

VAD is a widespread nutritional disorder in low-income countries and is still a public health concern globally. The insufficient intake of VA over a long period of time causes xerophthalmia that may lead to irreversible blindness. Subclinical deficiency of VAD can aggravate diseases such as diarrhea and other infectious diseases (FAOSTAT 2003).

Yellow flesh cassava cultivars rich in pVA are part of the outputs of an international biofortification effort by HarvestPlus, the IITA, CIAT and other National Agricultural Research institutions, to reduce VA and other micronutrient deficiencies through the development of staple food crops with enhanced micronutrient content. Replacing the white-fleshed cassava varieties grown by most farmers with new high pVA (yellow) cassava varieties to address micronutrient and health needs of people, could benefit an estimated 20 million children under 6 years of age, who are currently at risk from diseases associated with VAD. Biofortified staple crops with higher micronutrient density, including yellow flesh cassava varieties biofortified with pVA carotenoids, have been developed to improving food and nutrition security reducing micronutrient deficiencies across the world (Saltzman *et al*. 2016). These biofortifies crops directly contributes to the attainment of Sustainable Development Goal 2 in eradicating all forms of hunger, including hidden hunger. Biofortified cassava varieties developed by conventional breeding techniques have been released in the main cassava- producing countries of Africa, such as the DRC, Ghana and Nigeria.

In 2013, 15 yellow-fleshed cassava cultivars with TCC levels between 4 - 18 μg g^{-1} were obtained from IITA-Nigeria. These cultivars have been tested across the various agro-ecologies in Ghana for their agronomic performance by the CRI towards possible commercial release in 2020. Good cooking quality of cassava storage roots is an important parameter in selecting cassava for human consumption. Other factors important for selection are hydrocyanic acid content, starch, fiber, cooking time, flavour, consistency and cooked pulp texture (Wheatley 1987).

Cassava roots are the main source of calories in communities where it grown and consumed and their nutritional composition is therefore important. The roots consist mostly of starchy flesh (about 80% to 90% of the total weight of the root) with water making up a large proportion of this flesh (Wheatley and Chuzel 1993; Harris and Koomson 2011). The water content for cassava is in the range of 60.3% to 87.1% (Padonou *et al*. 2005). In cassava flour the moisture is much lower and was reported to vary from 9.2% to 12.3% (Charles *et al*. 2005) and 11% to 16.5% (Shittu *et al*. 2007). Moisture content is very important in shelf life of cassava flour since levels higher than 12% allows for microbial growth, which significantly reduce its shelf life (Padonou *et al*. 2010; Harris and Koomson 2011). Cassava contains very low levels of protein of about or 1 - 3% on a DM basis (Buitrago 1990; Charles *et al*. 2005) and between 0.4 and 1.5 g 100^{-1} g fresh weight (Bradbury and Holloway 1988).

So cassava is therefore much more starchy than cereals such as maize and sorghum that have about 10 g protein 100 g^{-1} fresh weight (Montagnac *et al.* 2009). Cassava plants are very valuable, as they produce more weight of carbohydrate per unit area than other staple food crops under comparable agro-climatic conditions. Unfortunately, the roots have very low nutritional value due to low protein content and high starch content. About 50% of the crude protein in the roots consists of whole protein and the other 50% of free amino acids (Zvinavashe *et al*. 2011). Raw cassava root has more carbohydrate than potatoes and less carbohydrate than wheat, rice, yellow maize, and sorghum on a 100 g basis (Montagnac *et al.* 2009).

The aim of the HarvestPlus program is the improvement of micronutrient content of crops to such an extent that it will impact on human nutritional and health status in a way that can be measured. Equally important is not to negatively affect agronomic characteristics of the crop, such as yield and disease resistance. The process of developing biofortified crops include factors such as nutrient retention after harvesting, how much of the crop is consumed, and whether the biofortified crop is acceptable to the consumer. The bioconversion to provitamin A to retinol in the case of pVA rich foods (called bioavailability), is also an important factor. The mechnisms must also be in place for large-scale dissemination of the biofortified crop, which may differ in specific target countries (Boy and Miloff 2009). Carotenoids are very sensitive to light, heat and physical handling, which leads to losses during the processing of yellow flesh cassava roots into commonly consumed products (Maziya-Dixon *et al.* 2015). Total carotenoid retention is therefore largely dependent on specific cultivars and processing methods used to prepare products (Jaramillo *et al*. 2018).

The pVACs target level for cassava, set to reach 50% of the EAR for children and pregnant women in the DRC and Nigeria, assumes that up to 50% of pVACs in peeled roots is lost during processing, storage, and cooking (Saltzman *et al*. 2013; Anderson *et al*. 2017). Carotenoid retention higher than 50% in boiled cassava has been reported in different studies (Chavez *et al*. 2007; De Moura *et al*. 2015). A study in Kenya demonstrated that feeding 2 – 4 year old children with boiled yellow fleshed cassava improved their VA status (Talsma *et al*. 2016). Cassava is mainly traded in Ghana either as dry pieces of fermented cassava roots, *konkonte*, that are milled into cassava flour to prepare *banku*, or as fermented cassava paste, *bankye mole*, used to prepare *koko*. Cassava is also boiled and pounded with plantain to prepare *fufu*.

Generally, *fufu* in Ghana is prepared by cooking peeled cassava in boiling water, whereas *chikwangue* is prepared by precooking and steaming fermented cassava paste (Avouampo *et al*. 1995; Humpal *et al*. 2012). In Nigeria, a study found that apparent carotenoid retention in *fufu* prepared with fermented cassava flour was 17 – 32%, but no information on true retention was presented (Omodamiro *et al*. 2012). The same study also found that apparent retention of carotenoids was 86 – 90% when *fufu* was prepared with a wet paste without a drying step. Another study in Nigeria reported true carotenoid retention between 12 and 36% when processing biofortified cassava roots into *fufu*, using fermented cassava paste without a drying step (Maziya-Dixon *et al*. 2015). Although there is some level of information on carotenoid retention in cassava in other parts of the world, it is limited for a country like Ghana, making it difficult to estimate its potential impact on VA status of children and women in the country.

Despite its nutritional and commercial benefits, cassava contains toxic substances that limit its utility, the most important being cyanogen, which is responsible for the bitter taste of some cassava cultivars (FAO 2000). Cassava cultivars are therefore classified into two major types: bitter and sweet (Ubwa *et al*. 2015) on the basis of the cyanogenic content. "Sweet" cassava variety roots contain less than 50 mg kg^{-1} HCN on fresh weight basis, whereas those classified as "bitter" varieties may contain up to 400 mg kg^{-1} HCN (FAO 2008). However, the level of cyanide in the cassava roots can be effectively reduced with different processing and fermentation methods (Emurotu *et al*. 2012).

Cyanide is the result of the enzymatic hydrolysis of molecules such as linamarin, lotaustralin, and acetone cyanohydrin (Marcus and Adesina 2001; Asegbeloyin and Onyimonyi 2007). Cyanide is stored in vacuoles of cassava cells, and is known to be more concentrated in leaves and the root cortex compared to root parenchyma (Cardoso *et al*. 2005). Linamarin and linamarase react when cassava cells are mechanically damaged during harvesting. They then release acetone cyanohydrin, and this then decomposes to release cyanide (Omotioma and Mbah 2013), either by hydroxyl nitrile lyase or spontaneously when the pH is higher than 5 (Orjiekwe *et al*. 2013). Several neurological diseases, including ataxic neuropathy, cretinism, and xerophthalmia are seen in areas where cassava is the staple food, and this has been attributed to cyanide poisoning (Emmanuel *et al*. 2012; Abraham *et al*. 2016). Cyanide can also cause thyroid disorders, goiter and stunting in children (Mburu *et al*. 2013). Cassava toxicity levels vary depending on altitude, geographic location, period of harvesting, crop variety and seasonal conditions (Ndam *et al*. 2019). Several cases of

cassava poisoning has been recorded in Nigeria, all resulting from improper fermentation and processing of cassava. Cyanide exposure of more than 50 mg kg^{-1} caused symptoms such as headache, weakness, changes in taste and smell, irritation of the throat, vomiting, lacrimation, abdominal colic, pericardial pain and nervous instability (Ifeabunike *et al*. 2017).

Cyanide content of cassava is higher during drought periods due to water stress in the plant (Ojo *et al*. 2013). In Mozambique, more than 55% of fresh sweet roots became extremely toxic during drought periods, a trend which was also observed in the Democratic Republic of Congo (Gitebo *et al*. 2009) and other countries in Africa (Cardoso *et al*. 2005). Cassava must therefore be processed to make it safe for consumption. Numerous processing techniques are used in cassava eating countries. These techniques often improve palatability, extend shelf life but also decrease cyanogenic potential of cassava (Bradbury 2006). The aim of the present study was to determine the TCC, proximate values and HCN in yellow flesh cassava cultivars and to measure the retention of carotenoids during the processing of biofortified cassava into boiled cassava. This will help breeders to identify genotypes with the best nutritional quality across the tested locations for planting and promotion.

6.2 Materials and methods

6.2.1 Varieties, field trials and sample preparation

The same varieties used in Chapter 5 were used for this study, with the only difference being that Cape Vars, a commercially released white fleshed variety, was used as one of the controls, together with a white fleshed local landrace (Husivi). Trials were conducted from May 2015 - May 2016 at three locations situated in different agroecological zones. Fumesua (forest), Cape Coast (Rain forest) and Ohawu (Coastal savannah). Trials were laid out in a RCBD with three replications, each consisting of four rows of five plants, giving a plot size of 20 plants. Planting was done at a spacing of 1 × 1 m. Replications were separated by 2 m alleys. Weeding was done when necessary and experiments were entirely rain fed. The soils for the trial sites at Fumesua is Asuasi series, a ferric acrisol with sandy loam top soil over sandy clay. At Cape Coast, it is Benya series, Acrisol with deep yellowish red to yellowish brown, well to moderately well drained alluvial clays and Ohawu have Toje- Alajo series, a loamy top soil over sandy loam soil. Annual rainfall for the sites during the trial period was Fumesua (1205 mm), Cape Coast (1295 mm) and Ohawu (1024 mm).

The fresh roots were sampled randomly for each variety before they were washed and peeled. The samples were transported immediately to the laboratory from the fields. Samples from the apical, middle and distal portions of the roots were cut into small cubes, packed and heat-sealed in laminated bags of 1 kg each, and stored in a cool place until used. A total of 60 samples from each location were analyzed for various characteristics (30 fresh samples and 30 boiled samples obtained from ten genotypes with three replications) (Table 6.1).

Table 6.1 Provitamin A and white cassava genotypes used for the study

Genotype	Code	Status	Source	Pulp colour
IBA090090	G1	Improved	IITA	Yellow
IBA090151	G2	Improved	IITA	Yellow
IBA070557	G3	Improved	IITA	Yellow
IBA085392	G4	Improved	IITA	Yellow
Husivi	G5	Landrace	Farmer	White
IBA083774	G6	Improved	IITA	Yellow
IBA070593	G7	Improved	IITA	Yellow
IBA070539	G8	Improved	IITA	Yellow
Cape Vars	G9	Released	CSIR-CRI	White
IBA083724	G10	Improved	IITA	Yellow

6.2.2 Proximate analysis

Determination of moisture and dry matter content

The moisture and DM of the fresh cassava genotypes were determined using the AOAC (1990) method. Two gram of each sample was weighed (W_1) into a dry evaporating dish of known weight and the sample spread evenly within the dish with a spatula. The dish (partially open) was placed in the air oven and dried for three hours at 105^{o}C. After drying, the dish was closed and transferred to the desiccator, and then reweighed (W_2) after cooling to determine the weight of the sample. Measurements were taken in duplicate and were calculated as follows:

Moisture (%) = $(W_1 - W_2)/W_1$

Where W_1= Weight (g) of sample before drying

W_2= Weight (g) of sample after drying

DMC was obtained by subtracting the percentage moisture from the total percentage:
DMC = 100% - Percentage moisture.

Determination of crude protein

The solution turned green and clear. The sample solution was then transferred into a 100 ml volumetric flask and made up to the mark with distilled water. Twenty-five ml of 2% boric acid was pipetted into a 250 ml conical flask and two drops of a mixed indicator (20 ml of bromocresol green and 4 ml of methyl orange) was added; and placed into the decomposition chamber of the distillation apparatus, 15 ml of a 40% NaOH solution was added. Ten ml of the digested sample solution was then introduced into a Kjeldahl flask. The condenser tip of the distillation flask was then dipped into the boric acid in the conical flask. The ammonia in the sample solution was then distilled into the boric acid until it changed completely too bluish green. The distillate was then titrated with 0.1 N HCl solution until it became colourless. The percentage total nitrogen and the crude protein were determined. Measurements were done in triplicate.

$$\text{Percent total nitrogen (\% N)} = \frac{(\text{Titre value} - \text{Blank value}) \times 0.01 \times 14 \times 100}{1000 \times 5 \times \text{weight of sample}} \times 100\%$$

Percentage protein (% Protein) = Percent total nitrogen (% N) x 6.25 Where: atomic weight of nitrogen = 14, volume of titrant = 5 ml, blank value = 0.45, Molarity of acid (HCl) = 0.01, Volume of digest = 100 ml

Determination of crude fat

Crude fat was determined based on the Sohxlet extraction method of the AOAC (1990). Two g of the sample was weighed into a muslin thimble and inserted into the extraction column with the condenser connected. Petroleum ether as extracting solvent (200 ml) was poured into a 250 ml round bottom flask of known weight and fitted into the extraction unit. The flask was then heated at 60^{o}C for 2 hours on a hot plate. Losses of the solvent due to heating were prevented by the condenser, which cooled and refluxed the evaporating solvent. After extraction, the thimble was removed, and the solvent salvaged by distillation. The round bottom flask containing the fat and the residual solvent was placed on a water bath to evaporate the solvent, followed by further drying in an oven at 103^{o}C for 30 min to completely evaporate the solvent. The flask was then cooled in a desiccator and weighed. Measurements were done in triplicate.

Percentage fat = $(W_1-W_2) / W_1$

Where W_1 = weight of the sample before heating

W_2 = weight of sample after heating

Determination of crude fiber

Crude fibre was determined by the AOAC (1990) method. The defatted sample (from crude fat determination) was transferred into a 750 ml Erlenmeyer flask and 0.5 g of asbestos was added. Boiling 1.25% H_2SO_4 (200 ml) was added and the flask was immediately set on a hot plate and a condenser connected to it. Boiling occurred within 1 min and the sample was digested for 30 min. The content of the flask was passed through a filter paper into a funnel and subsequently washed with boiling water until the washings were no longer acidic. The sample was washed back into the flask with 200 ml boiling 1.25% NaOH solution. The condenser was again connected to the flask and the content of the flask was boiled for 30 min. It was then filtered through the filter paper and thoroughly washed with boiling water until washing was no longer alkaline. The residue was transferred to a clean crucible with a spatula and the remaining particles washed off with 15 ml ethanol into the crucible. The crucible with its content was then dried in an oven overnight and cooled in a desiccator and weighed. The crucible with its content was then ignited in a furnace at 600^oC for 30 min, cooled and reweighed. Measurements were done in triplicate.

Percentage fibre = $(W_1-W_2) / W_1$

Where W_1 = weight of the sample before heating

W_2 = weight of sample after heating

Determination of ash content

Ash content was determined by using the AOAC (1990) method. Each sample of 2g was weighed into a weighed porcelain crucible. The crucible with its content was placed in a furnace and preheated to 600^oC for 2 hours. The sample was then allowed to cool in the furnace to 250^oC. The crucible and the ash were then transferred into an oven at 100^oC for 30 min cooling. The crucible with its contents were then cooled in a desiccator. The weight of the crucible and its content was recorded. Measurements were done in triplicate.

Percentage ash = $(W_1-W_2) / W_1$

Where W_1 = weight of the sample before heating

W_2 = weight of sample after heating

Determination of carbohydrate content

Total percentage carbohydrate was determined by adding the total values of crude protein, crude fat, crude fibre, moisture and ash constituents of the sample and subtracting it from 100% (Onyeike and Oguike 2003).

Percentage carbohydrate =100 – (% moisture + % ash + % crude protein + % crude fat + % crude fibre)

Determination of TCC using the spectrophotometer

One gram of each fresh sample of yellow flesh cassava cubes was weighed and ground using a mortar and pestle. Pyrogalol was added to facilitate the grinding and to prevent oxidation. Methanol (25 ml) was then added and the mixture was transferred into a conical flask corked with filter paper by vacuum filtration. Acetone (25 ml) was added to the residue to ensure maximum extraction of carotenoids. The volume of the filtrate was recorded as the volume after first extract. The filtrate was then poured into a separating funnel (where the tap was closed) fixed to a retort stand. Petroleum ether (20 ml) was placed into the separating funnel before the extract was added. Distilled water was finally added. A separation of yellowish coloured organic solvent and colourless inorganic solvent was observed. The tap of the separating funnel was opened for the inorganic solvent to run out into a beaker placed under the funnel, and finally discarded. Distilled water was again added to the sample for washing. The inorganic solvent was collected and discarded. Washing was repeated several times until the carotenoid solution was clear. All excess distilled water was discarded. A funnel was then placed in a conical flask under the separating funnel and the organic solvent containing the carotenoids was collected. The volume of the organic solvent was recorded as the volume of the second extract. A glass cuvette was filled with the organic solvent extract and the absorbance was read at 450 nm. The extraction was read in triplicate using a T80 UV/VIS spectrophotometer.

TCC was then calculated using the formula:

$$TCC\ (\mu g\ g^{-1}) = \frac{A \times V\ (ml) \times 10^4}{A^{1\%}1cm \times P\ (g)}$$

Where A = is the absorbance

V = Total extract volume after second extraction

P = Sample weight

$A^{1\%}1cm = 2592$ (beta carotene extinction coefficient in petroleum ether)

The TCC of the boiled root samples of the yellow flesh cassava was also determined using the spectrophotometry method. First the cubes were placed in laminated polythene bags and boiled in water at 100^{o}C for 20 min. One gram of each boiled sample was ground using a mortar and pestle. Pyrogalol was added to facilitate the grinding and to prevent oxidation. Methanol (25 ml) was then added and the mixture transferred into a conical flask corked with filter paper/vacuum filtration. Acetone (25 ml) was also added to the residue to ensure maximum extraction of carotenoids. The volume of the filtrate was recorded as the volume after first extract. The filtrate was then poured into a separating funnel and 20 ml petroleum ether was already added to the separating funnel. Distilled water was finally added. The same procedure was then followed as for the raw samples

6.3 Data analysis

Data was subjected to ANOVA using SPSS, version 21. Results were presented as means ± standard deviations. Differences between means were considered significant at $p<0.05$ using the Duncan multiple range test (Eleazu and Eleazu 2012).

6.4 Results

Moisture content

The moisture content (Table 6.2) ranged from 50.48% to 83.84% at Cape Coast, 62.20% to 80.07% at Fumesua and 56.31% to 90.43% at Ohawu. Genotype Husivi (local) recorded the overall highest moisture content per fresh weight at the Ohawu locations. At Cape- Coast, genotype IBA085392 had the highest moisture content and Cape Vars the lowest content. At Fumesua and Ohawu, genotypes IBA070539 and Husivi (local) had the highest moisture content, respectively.

Carbohydrate content

Carbohydrate content of samples from Cape-Coast, Fumesua and Ohawu ranged from 12.85% to 45.79%, 14.90% to 40.41% and 6.85% to 38.82% respectively (Table 6.2). The highest value was recorded for genotype Cape Vars (white-fleshed) across the three locations. The local (white fleshed) and improved variety (Cape Vars) recorded higher carbohydrate content than most of the yellow flesh cassava genotypes.

Table 6.2 Percentage moisture and carbohydrate content of fresh cassava varieties from three different locations

	Moisture content (%)			Carbohydrate content (%)		
Variety	Cape-Coast	Fumesua	Ohawu	Cape Coast	Fumesua	Ohawu
I090090	70.4±13.1^{abcd}	76.6±0.2^{c}	70.3±1.0^{bc}	26.9±13.1^{abc}	20.1±0.1^{ab}	25.8±0.8^{de}
I090151	79.5±10.3^{cd}	66.9±0.3^{ab}	64.8±2.0^{b}	17.9±9.8^{ab}	29.0±0.1^{cd}	6.9±1.9^{ef}
I070557	66.9±0.78^{abcd}	62.2±3.7^{a}	66.9±0.2^{b}	30.7±0.4^{bcd}	34.2±0.2^{d}	27.3±1.7^{de}
I085392	83.8±4.45^{d}	64.7±7.0^{a}	69.7±0.9^{bc}	12.9±4.4^{a}	30.2±9.5^{cd}	27.2±1.0^{de}
I083724	58.2±11.7^{ab}	67.3±0.3^{ab}	76.6±6.3^{cd}	38.9±11.8^{cd}	28.5±0.6^{bcd}	19.9±6.2^{bcd}
I083774	66.2±0.26^{ab}	62.2±3.7^{a}	67.5±5.0^{b}	34.4±0.1^{bcd}	34.9±1.3^{d}	24.5±10.9^{cde}
I070593	66.8±3.1^{abcd}	73.3±5.1^{bc}	82.8±3.5^{de}	29.0±5.7^{abcd}	24.5±5.1^{bc}	15.0±3.4^{abc}
I070539	50.7±2.0^{a}	80.1±0.5^{c}	83.7±4.3^{cde}	45.6±2.1^{d}	14.9±1.9^{a}	13.0±4.4^{ab}
Cape Vars	50.4±11.8^{a}	54.8±1.1^{ab}	56.3±5.7^{ab}	45.8±11.6^{d}	40.4±0.8^{bcd}	38.8±5.4^{f}
Husivi	66.2±2.6^{abc}	63.1±0.2^{a}	90.4±2.0^{a}	30.7±2.6^{bcd}	34.3±0.2^{d}	33.0±2.6^{a}
p-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Values are presented in means and ± standard deviations. Means along the same column with different superscripts are statistically different (P<0.05)

Protein and fat contents

Protein content ranged from 0.01 to 1.45% with genotype IBA070557 recording the highest value among the samples from Cape Coast, followed by genotype I090151 which recorded 1.32% and 1.26% at Fumesua and Ohawu respectively. (Table 6.3). The protein content of the white fleshed variety *(husivi)* was generally lower than most of the yellow flesh cassava genotypes across all locations. Fat content ranged from 0.05% to 1.24% with genotype I070539 recording the highest value at Cape Coast and Fumesua followed by genotype I070557 at Ohawu (1.16%). Generally, samples from Ohawu recorded the highest protein content followed by those from Fumesua and Cape Coast. For Fat content, genotypes from Cape Coast had the highest, followed by Fumesua and Ohawa.

Crude fiber and ash content

Crude fiber is the part of food made up of cellulose and lignin. Genotype IBA083724 (2.62 %) had the highest fiber followed by genotypes IBA085392 and I083774 (2.57%) all at the same location, Fumesua (Table 6.4).Genotypes from Fumesua recorded the highest crude fibre, followed by those from Ohawu and Cape Coast.

Ash content is indicative of inorganic constituents (such as minerals) and for cassava, it generally ranges from 1% to 2%. Genotype IBA083724 (Ohawu) had the lowest ash content

of 0.02% and is therefore likely to contribute less minerals in the diet when consumed. Almost all the yellow flesh cassava genotypes had higher ash content relative to the white fleshed variety (local). Generally, genotypes from Cape Coast had the highest ash content, followed by those from Fumesua and Ohawa

Table 6.3 Protein and fat content of cassava varieties from three different locations

	Protein content (%)			**Fat content (%)**		
Variety	Cape-Coast	Fumesua	Ohawu	Cape Coast	Fumesua	Ohawu
I090090	0.24 ± 0.01^{d}	0.28 ± 0.01^{d}	0.45 ± 0.01^{e}	0.92 ± 0.1^{cd}	0.07 ± 0.04^{a}	0.74 ± 0.1^{cd}
I090151	0.32 ± 0.01^{f}	1.32 ± 0.01^{a}	1.26 ± 0.01^{c}	0.72 ± 0.1^{ab}	0.87 ± 0.3^{cd}	0.05 ± 0.001^{a}
I070557	1.45 ± 0.01^{a}	0.58 ± 0.01^{f}	0.85 ± 0.01^{g}	0.94 ± 0.2^{cd}	1.16 ± 0.04^{d}	1.14 ± 0.5^{d}
I085392	0.01 ± 0.01^{a}	0.37 ± 0.01^{a}	1.12 ± 0.01^{h}	0.96 ± 0.1^{cd}	0.27 ± 0.3^{ab}	0.12 ± 0.03^{ab}
I083724	0.31 ± 0.01^{f}	0.25 ± 0.01^{c}	0.67 ± 0.01^{f}	1.05 ± 0.04^{de}	0.12 ± 0.1^{a}	0.47 ± 0.24^{bc}
I083774	0.19 ± 0.01^{c}	0.17 ± 0.01^{a}	0.84 ± 0.01^{g}	0.96 ± 0.03^{cd}	0.74 ± 0.1^{bcd}	0.84 ± 0.1^{cd}
I070593	0.08 ± 0.01^{b}	0.18 ± 0.01^{b}	0.27 ± 0.01^{c}	0.67 ± 0.03^{ab}	0.40 ± 0.4^{abc}	0.07 ± 0.03^{a}
I070539	0.27 ± 0.01^{f}	0.26 ± 0.01^{c}	0.37 ± 0.01^{d}	1.24 ± 0.1^{e}	1.22 ± 0.02^{d}	0.30 ± 0.10^{ab}
Cape Vars	0.02 ± 0.01^{a}	0.10 ± 0.01^{a}	0.23 ± 0.01^{b}	0.54 ± 0.001^{a}	0.45 ± 0.3^{c}	0.27 ± 0.10^{ab}
Husivi	0.03 ± 0.01^{a}	0.18 ± 0.01^{b}	0.13 ± 0.01^{a}	0.82 ± 0.03^{bc}	0.17 ± 0.2^{a}	0.05 ± 0.0001^{a}
p-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Values are presented in means and standard deviations. Means along the same column with different superscripts are statistically different ($P<0.05$)

Table 6.4 Crude fiber and ash content of fresh cassava varieties across three different locations

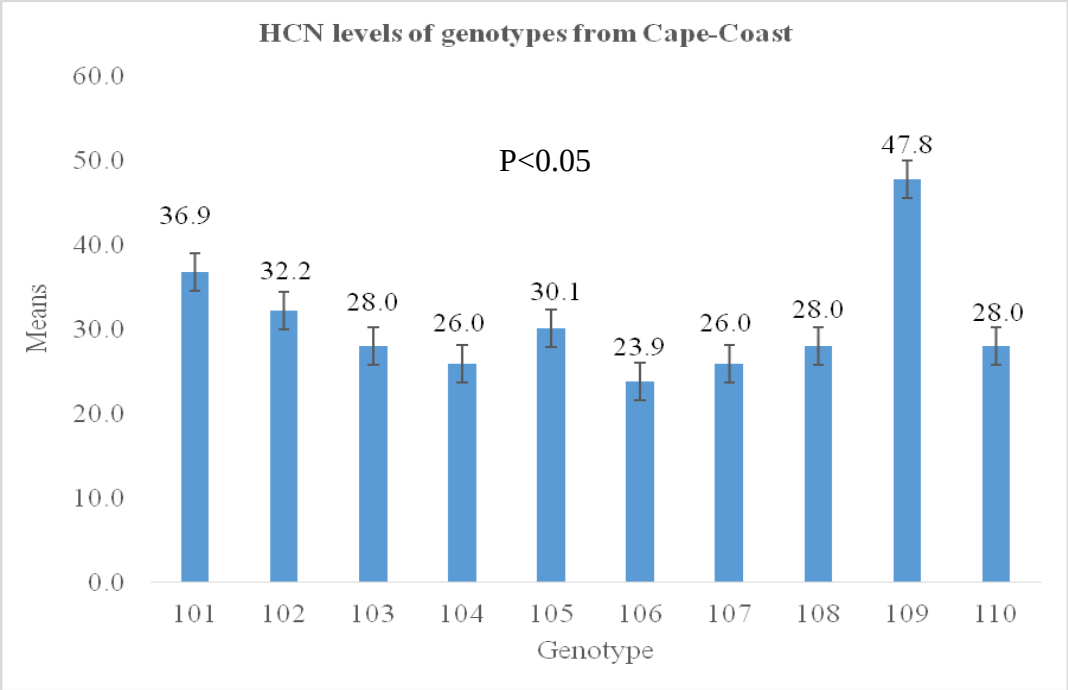
	Crude fibre			Ash content (%)		
Variety	Cape-Coast	Fumesua	Ohawu	Cape Coast	Fumesua	Ohawu
I090090	0.75±0.1^{ab}	2.53±0.2^{c}	2.07±0.03^{d}	0.72±0.1^{ab}	0.42±0.1^{a}	0.62±0.3^{ab}
I090151	1.04±0.2^{abc}	2.48±0.1^{c}	1.33±0.2^{ab}	1.02±0.3^{ab}	0.60±0.4^{a}	0.65±0.4^{ab}
I070557	0.47±0.3^{a}	1.24±0.1^{ab}	1.72±0.04^{c}	1.020.7^{ab}	0.59±0.08^{a}	2.11±2.0^{ab}
I085392	0.94±0.21^{abc}	2.57±0.2^{c}	1.49±0.01^{bc}	1.39±0.1^{ab}	1.94±2.0^{a}	0.37±0.04^{ab}
I083724	1.04±0.5^{abc}	2.62±0.04^{c}	2.23±0.1^{de}	0.82±0.3^{ab}	1.17±0.3^{a}	0.02±0.003^{a}
I083774	1.02±0.1^{abc}	2.57±0.2^{c}	1.47±0.04^{bc}	1.22±0.3^{ab}	0.47±0.04^{a}	2.34±2.30^{b}
I070593	1.34±0.6^{bc}	1.16±0.4^{a}	1.03±0.04^{a}	2.09±1.9^{b}	0.42±0.1^{a}	0.77±0.04^{ab}
I070539	1.42±0.03^{c}	2.53±0.2^{c}	1.72±0.04^{c}	0.72±0.1^{ab}	2.07±2.4^{a}	0.90±0.001^{ab}
Cape Vars	1.02±004^{abc}	1.74±0.3^{b}	2.54±0.4^{a}	0.72±0.04^{ab}	0.69±0.3^{a}	0.79±0.003^{ab}
Husivi	1.04±0.5^{abc}	1.65±0.3^{b}	2.1±0.01^{d}	0.52±0.4^{ab}	0.57±0.04^{a}	0.45±0.10^{ab}
p-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Values are presented in means and standard deviations. Means along the same column with different superscripts are statistically different (P<0.05)

Hydrogen cyanide content

The highest and lowest HCN content of the fresh cassava from Cape Coast were 47.76 µg g^{-1} and 23.88 µg g^{-1} in Cape Vars and IBA083774, respectively (Figure 6.1). The HCN of samples significantly differed (p>0.05). For Fumesua, the highest HCN content (43.1 µg g^{-1}) was recorded in genotype IBA070557 and IBA070593 with genotype IBA085392 having the lowest value (9.9 µg g^{-1}) (Figure 6.2). The highest HCN content was found genotype

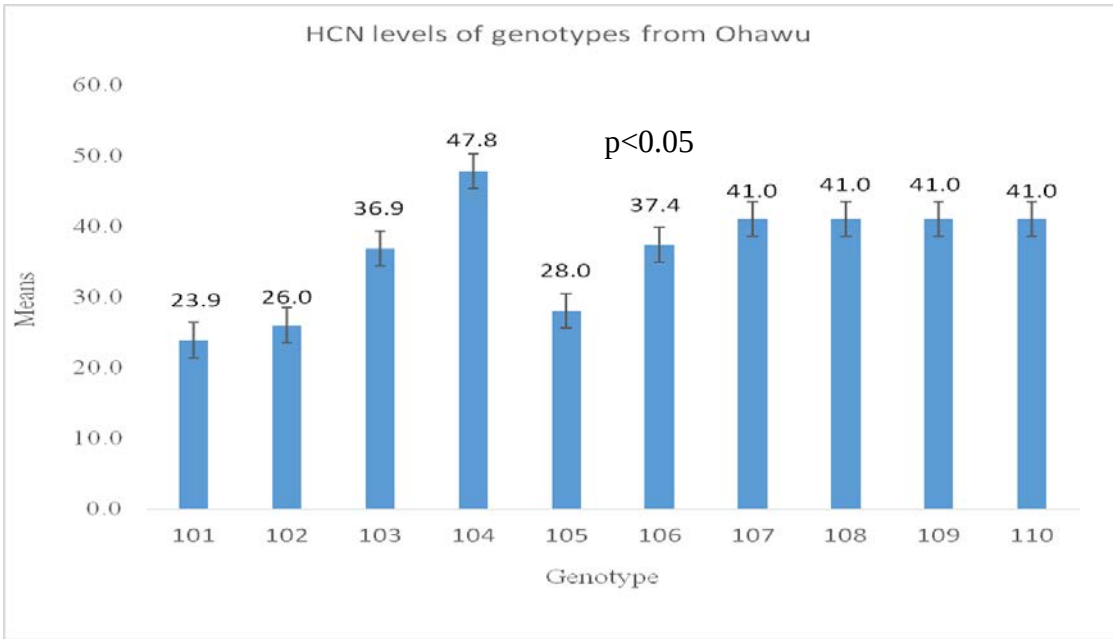
Figure 6.1 Hydrogen cyanide content of yellow flesh cassava from Cape-Coast



Key: 101 to 110 represents varieties I090090, 1090151, I070557, I085392, I083724, I083774, I070593, I070539, Cape Vars and Husivi

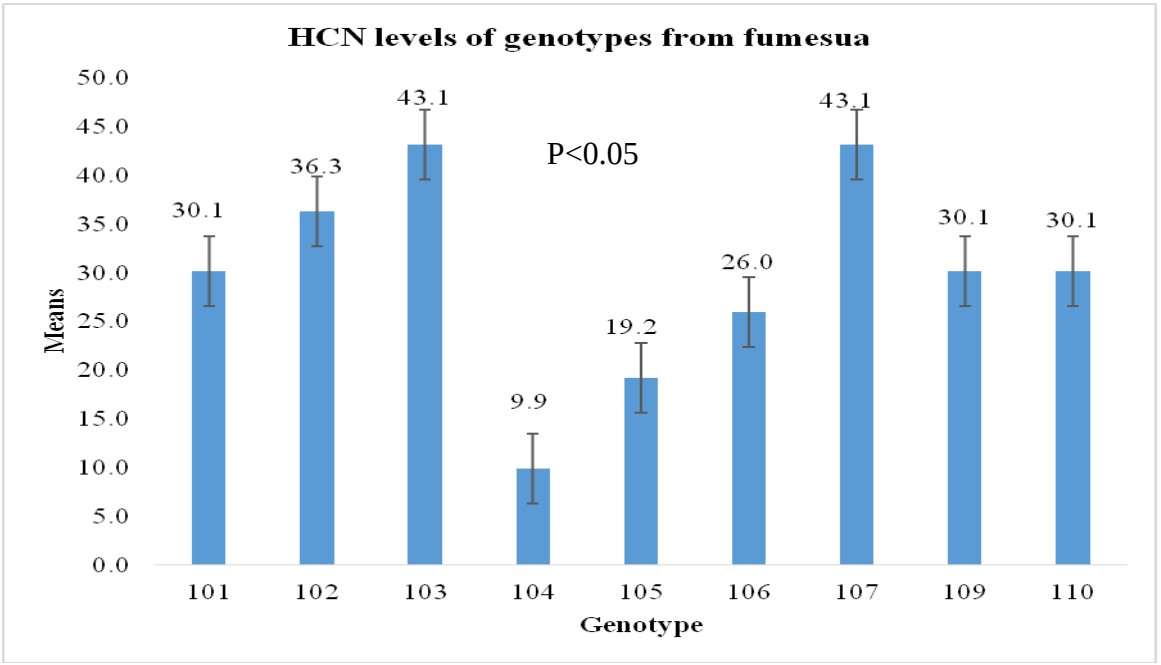
IBA085392 (47.8 µg g^{-1}) and the lowest in genotype IBA090090 (23.9 µg g^{-1}) in Ohawu. Across the three sites, genotypes Cape Vars from Cape Coast, IBA070593 and IBA070557 both from Fumesua, and IBA085392 from Ohawu had the highest HCN levels of 47.8 µg g^{-1}, 43.1 µg g^{-1} and 47.8 µg g^{-1} respectively. Whilst samples IBA083774, IBA085392 and IBA090090 from Cape Coast, Fumesua and Ohawu respectively had the lowest HCN values of 23.9 µg g^{-1}, 9.9 µg g^{-1} and 23.9 µg g^{-1}. Ohawu recorded the highest mean value for HCN (36.40 µg g^{-1}), followed by Cape Coast (30.69 µg g^{-1}) with the lowest from Fumesua (29.73 µg g^{-1}). There was no statistically significant differences in HCN levels across the three locations (30.00±7.04, 37.78±7.02, 29.73±10.02 at P=0.10). Genotype Cape vars recorded the highest mean value (39.63 µg g^{-1}) for HCN, followed by genotypes IBA070557 (36.00 µg g^{-1}) and IBA070593 (36.00 µg g^{-1}) across all the three locations. Genotype IBA083724 recorded the lowest HCN mean (25.77 µg g^{-1}).

Figure 6.2 Hydrogen cyanide content of yellow flesh cassava from Ohawu



Key: 101 to 110 represents varieties I090090, 1090151, I070557, I085392, I083724, I083774, I070593, I070539, Cape Vars and Husivi

Figure 6.3 Hydrogen cyanide content of yellow flesh cassava from Fumesua



Key: 101 to 110 represents varieties I090090, 1090151, I070557, I085392, I083724, I083774, I070593, I070539, Cape Vars and Husivi

Table 6.5 Comparison of hydrogen cyanide content of cassava genotypes from different locations

Variety	Cape-Coast	Ohawu	Fumesua	Mean±SD	P-value
I090090	36.90	23.90	30.10	30.30±6.50	0.75
I090151	32.20	26.00	36.30	31.50±5.19	
I070557	28.00	36.90	43.10	36.00±7.59	
I085392	26.00	47.80	9.90	27.90±19.02	
I083724	30.10	28.00	19.20	25.77±5.78	
I083774	23.90	37.40	26.00	29.10±7.26	
I070593	26.00	41.00	43.10	36.70±9.33	
I070539	28.00	41.00		34.50±9.19	
Cape Vars	47.80	41.00	30.10	39.63±8.93	
Local	28.00	41.00	30.10	33.03±6.98	
Mean±SD	30.69±7.04	36.40±7.82	29.77±10.72		0.20

Total carotenoid content

The colour of the cut crosss section of fresh roots show colour ranges that depicts the level of TCC, this is visually assessed by colour chat. Actual levels are determined in the labouratory. For this study, the concentration of TCC in the fresh roots ranged from 1.18 µg g^{-1} (Cape Vars) for samples from Cape Coast to 18.81 µg g^{-1} (I070539) for samples from Ohawu. For the boiled analysis, TCC ranged from 1.01 µg g^{-1} (Cape Vars) for samples from Cape Coast to 13.86 µg g^{-1} (I083724) for samples from Fumesua. Boiling was found to decrease the total carotenoid content of the different cultivars across all three locations. For both boiled and fresh samples, there were differences in TCC content across the three locations. Fresh samples in Fumesu recorded the highest, followed by Ohawu and then Cape Coast (10.71±4.27 µg g^{-1}, 10.61±4.27, 5.87±3.16; p= 0.02 respectively). The same trend was observed after boiling.

Table 6.6 Total carotenoid content (µg g^{-1}) of fresh and boiled cassava genotypes across three different locations

	Fresh				Boiled			
Variety	Cape-Coast	Fumesua	Ohawu	P-value	Cape Coast	Fumesua	Ohawu	P-value
I090090	6.11±004	14.56±0.04	10.00±0.04		5.09±0.04	11.29±0.04	8.43±0.04	
I090151	5.98±0.04	11.44±0.04	8.52±0.04		5.98±0.04	10.64±0.04	7.08±0.04	
I070557	11.99±0.2	11.78±0.07	9.87±0.05		5.22±0.09	11.49±0.06	6.44±0.04	
I085392	4.80±0.04	14.05±0.04	11.51±0.04		4.64±0.04	10.51±0.04	8.70±0.04	
I083724	4.63±0.08	14.52±0.04	11.70±0.07		3.42±0.04	13.86±0.07	11.50±0.04	
I083774	6.81±0.04	13.84±0.04	9.89±0.04		5.22±0.04	11.81±0.04	7.63±0.04	
I070593	8.15±0.04	10.11±0.04	18.81±0.08		7.20±0.04	9.21±0.04	16.91±0.06	
I070539	7.81±0.04	14.06±0.04	15.74±0.04		4.71±0.2	12.38±0.04	12.08±0.04	
Cape Vars	1.18±0.04	1.34±0.04	5.14±0.08		1.01±0.04	1.00±0.04	4.74±0.04	
Local	1.49±0.04	1.36±0.04	4.90±0.04		1.04±0.04	1.02±0.04	4.56±0.08	
M±SD	5.87±3.16	10.71+5.15	10.61±4.27	0.02	4.27±2.0	9.10±8.85	8.85±3.78	0.02
p-value	**<0.0001**	**<0.0001**	**<0.0001**		**<0.0001**	**<0.0001**	**<0.0001**	

6.5 Discussion

The results of the proximate analysis of the different cassava genotypes samples from three different locations revealed good variation for all traits with ranges of 50.48 - 90.4% for moisture content, 6.85 - 45.79% for carbohydrate, 0.01 - 1.26% for protein, 0.07 - 1.24% for fat, 0.47 - 2.62% for fibre and 0.37 - 2.34% for ash. Significant differences ($p<0.001$) were found amongst the genotypes for each of the proximate analysis parameters. In general, the observed ranges were below values reported by Otache *et al.* (2017) and Rajapaksha *et al.* (2017). The maximum limit for the crude fibre and fat content observed agreed with values reported by Eleazu and Eleazu (2012). However, the values observed for fat content was higher than the values reported by Adegbola *et al.* (1992) and Ifeabunike *et al*. (2017).

The carbohydrate values obtained in this study were lower than values reported by other studies such as Okpako *et al.* (2008) which had a range of 62.0-72.4%, Christopher *et al.* (2016) (87-89%) and Otache *et al*. (2017) with 85-89%. The results in this study generally indicated that yellow flesh cassava tend to have less carbohydrate than white-fleshed

varieties.

Crude ash content is usually indicative of inorganic constituents (minerals such as K, Zn and Ca) and for cassava, it generally ranges from 1% to 2%. Ash contents represents the total mineral content in food after it has been burned at a very high temperature. The ash and protein contents were lower than values reported by other studies (Idugboe *et al*. 2015; Otache *et al.* 2017), but were similar to those reported by Emmanuel *et al.* (2012) and Eleazu and Eleazu (2012) from six yellow and white cassava varieties cultivated in Umudike, Nigeria.

Cyanide concentrations vary in different cassava genotypes according to the altitude, geographical location and seasonal and production conditions (Oluwole *et al*. 2007). Reports have shown that age, variety and environmental conditions influence the occurence and concentration of cyanide in various parts of the cassava plant and at different stages of development (Charles *et al*. 2005), hence the genotypes need to be tested at different ages of maturity for further inferences.

Cassava is classified as sweet or bitter based on a total cyanide content of less or more than 50 mg kg^{-1}, respectively. In drought conditions, there is an increased total cyanide content due to water stress (Cardoso *et al*. 2005). Thus, a variety is considered to be "sweet" under one set of conditions may be "bitter" in a different geographical location or climatic conditions (Hadayat *et al*. 2016). Values from 15 - 400 mg kg^{-1} fresh weight of total cyanide in cassava roots have been reported in different studies (FSANZ 2008), and there were reports of even higher levels, depending on where the crop was grown (Oluwole *et al*. 2007; Cardoso *et al*. 2005). However, the rates can be reduced in cassava with different processing and fermentation methods. The observed levels of cyanide obtained in the present study showed that all the genotypes sampled could be classified as sweet varieties. The values were lower than those reported by Ubwa *et al*. (2015) but it is not advisable to eat it raw since the range is above the acceptable limit (10 mg kg^{-1}).

A loss of micronutrients during processing and cooking is undesirable, as it reduces the nutritional value of biofortified foods. Hence, micronutrient loss must be considered, and breeding targets set appropriately so that biofortified foods will add sufficient micronutrients to the diet to have a positive impact. It is therefore very important that biofortified crops should be able to retain sufficient levels of micronutrients after typical processing, storage

and cooking practices for biofortification to be successful.

All eight tested genotypes had TCC higher than the farmer preferred varieties (Husivi) and the improved check (Cape Vars) in both fresh and boiled states. TCC of fresh peeled cassava of the evaluated genotypes measured by spectrophotometer was 1.18 µg g^{-1} to 18.81 µg g^{-1} on fresh weight basis whereas in boiled cassava it was lower between 1.01 µg g^{-1} and 13.36 µg g^{-1} across the three locations. In general, there was a decrease in TCC level during boiling. This is in contrast to findings by van Jaarsveld *et al*. (2006) reported that carotenoid retention was better when sweet potatoes were boiled for the shortest possible time compared to methods like drying, frying and roasting that caused reduced retention. Boy and Miloff (2009) also reported that boiling has higher TCC retention compared to other processing techniques in sweet potatoes.

Different factors separately or combined, such as heat, light, oxygen and enzymes, can lead to major or minor losses of carotenoids in yellow cassava during processing into consumable products (Chavez *et al*. 2007; Eyinla *et al*. 2019). The losses observed in the study for boiled roots could also be due to carotenoid isomerization and oxidation, which is the breakdown of trans-carotenoid to their cis-isomers due to increased content with moisture and heat treatment during boiling (Bendich 1993). Gari is also one of the most popular products of cassava processing in Ghana and sub-Saharan Africa and it has been reported that extended roasting during its processing results in higher carotene content (De Moura *et al*. 2015). Gari may therefore be a useful way of bioefficiently utilizing biofortified cassava in VA deficient population. Further studies on more varieties commonly used for cassava dough, fufu, konkonte and gari may be needed to ascertain how yellow flesh cassava varieties may may respond for TCC during processing into Ghanaian food forms.

Findings by other authors like Eyinla *et al*. (2019), Bechoff *et al*. (2018) and Taleon *et al*. (2019) confirm TCC loss patterns in cassava products consumed in sub-Saharan Africa. The result further suggests that the current yellow flesh cassava genotypes being evaluated could provide more VA in diets and contribute to reduction of health challenges associated with VAD, which is widespread in Ghana and sub-Saharan Africa. Following the agricultural transformation agenda in Ghana (Modernization of Agriculture in Ghana), which has resulted in the availability of improved varieties (including biofortified cassava) there is a great need to scale up micronutrients in staple foods produced in the country (Edoh *et al*. 2016).

Even though the impact of consuming yellow flesh cassava products on VA serum concentration is not yet fully established in VAD populations in Ghana, the results give an indication that yellow flesh cassava varieties are better than white-flesh in terms of carotenoid and protein contents and have the chance of reducing VAD in Ghanaian populations where it is still endemic. Beta carotene in cassava storage roots does not play a role in photosynthesis, as it is located in the cell chloroplasts (Czygan 1980) where it is found in lipid droplets or bound to a protein that is released during cooking, thereby enhancing its bioavailability.

6.6 Conclusions

In view of the importance of cassava to the economy of Ghana, and given its role as a major crop in alleviating hunger in Africa, genetic improvement of the crop to address food and nutritional needs of Ghana and the continent has been recognizied. The crop has to be improved for productivity, proximate composition and safe cyanide content. The yellow flesh cassava varieties evaluated have better TCC levels than varieties grown by Ghanaian farmers. With increasing awareness on the toxicity of cyanide in cassava and given that cassava is mainly consumed as processed food, yellow-fleshed cassava are effectively safe for consumption as food to enhance vitamin A. Biofortification of cassava cultivars presents a viable and promising intervention for tackling VAD in disease-burdened populations of sub-Saharan Africa. The WHO is advocating food and nutrition security and yellow flesh cassava could be a key driver for this purpose. Considering that cassava is consumed principally as processed foods, further studies are needed to ascertain the TCC losses in processing of the various food forms the crop is consumed. There is also the need to develop new farmer-preferred cassava varieties that combine high TCC with high DMC to increase their rate of adoption, since most of these varieties/landraces already possess some important traits like high DMC and stability, which are key drivers of cassava adoption. Further breeding of cassava varieties with higher TCC is ongoing in Ghana and in many other countries. It is therefore expected that more varieties will be available in the nearest future with increased adoption rates and increased pVA intake.

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Chapter 7

General conclusions and recommendations

Cassava is currently ranked as the number one food staple and the most widely cultivated crop in Ghana. Cassava production in Ghana is around 18.4 million ton per year and more than 70% of farmers are engaged in its production, contributing about 22% to agriculture GDP. Several improved varieties (26 in total) have been released and disseminated to farmers in Ghana since 1993. In most cases, the released varieties were white-fleshed with low or negligible amounts of carotenoids but were bred and selected for processing into intermediary products (gari, flour, konkonte). Malnutrition is endemic in cassava producing regions of Africa, partly due to the low micronutrient content in this root crop, which is a major component of most household diets. It is for this reason that the development of nutrient dense cassava cultivars should be prioritized and given more attention to eliminate or minimize malnutrition among the poor, in a sustainable inexpensive way.

Several white fleshed varieties, most of which are traditional landraces, are still cultivated across the different agro-ecologies of Ghana. Knowledge of these improved varieties especially for the yellow flesh cassava are highly limited. This could be easily addressed by strengthening and improving the cassava seed systems. Very few men and women farmers cultivate improved varieties and yellow-flesh cassava. Information indicates that young adult farmers, who typical represent the future of Ghanaian agriculture, are lacking access to improved varieties and must be given extra attention in all cassava programmes to enhance their knowledge and capacity to efficiently optimize cultivation of improved varieties. In general, several similarities were found among men and women for preferred traits that makes it easy to breed for the same traits for both sexes although slight differences were found for preferred traits across locations. Men typically tend more to prefer varieties that could be stored longer in the soil, easily harvested all year round, favorably competitive with weeds and highly suitable for industrial processing. On the other hand, women tend to more prefer varieties which are climate smart (well adapted), labour saving (canopy dense for weed suppression) and easily suited for processing into starch and “gari”. With increased availability of planting material and improved awareness and market demand for the new improved, farmers have high interest and willingness to adopt, cultivate and process yellow flesh cassava.

The mean TCC values in this study varied from 2.98 to 6.14 µg g^{-1}, with a grand mean of 4.19 µg g^{-1}, which is comparable to values reported in Uganda and Nigeria. The mean is however, lower than those reported in Colombia. Although the DMC grand mean is lower in this study as compared to those reported for landraces grown in Ghana by farmers, some individual genotypes evaluated in this study had higher DMC than that of the commonly grown varieties. These genotypes could be selected and further tested towards release, since the trait is one of the key drivers of cassava variety adoption in Ghana. Earlier PRA work done in this study suggested that farmers would be willing to adopt yellow flesh cassava varieties if FRW and DMC are high.

The study also generated relevant information to understand the genetics of key traits of interest to farmers to aid the implementation of efficient cassava breeding programme for Ghana to meet the needs of the cassava community. The ANOVA and the GCA:SCA ratio indicated that the GCA was larger than SCA for CMD, HI and TCC with predictability ratios close to 1, indicating the presence of additive gene effects and a possibility for improvement of the characteristics by selection. The phenotypic correlations among the studied traits revealed a positive significant correlation between pulp colour and TCC, TCC and CMD, pulp colour and CMD, and pulp colour and cortex colour. This study identified materials that were good for TCC and CMD resistance implying both traits could be jointly selected for and improved at the early breeding stages including the ability to visually assess the pulp colour (for TCC) and CMD symptoms. Visual assessment of pulp color could rapidly reduce the number of materials at the seedling nursery or clonal evaluation trials rapidly to just few numbers which then be quantitatively assessed to reduce cost at the later stages of breeding. Some yellow flesh cassava progenies were found to showed favorable good response for increased DMC values. This was evident in the positive correlation found between TCC and DMC (though not significant). This is an important finding, as it confirms the report by Ceballos *et al.* (2013) that DMC, which is a key driver for cassava adoption, can be improved alongside TCC. One of the parents utilized in this study, P6 (I090090) showed positive GCA effects for TCC, DMC, RTW and CMD, and would be very suitable for use in crosses (with another parent with good complementary genetic background) to develop improved cassava genotypes combining both TCC and DMC efficiently.

Findings of this study also demonstrated that it is possible to simultaneously select for yield and quality traits, such as DMC at early stages. The study also showed that some of the TCC- rich varieties evaluated showed very good performance and meets other key traits of

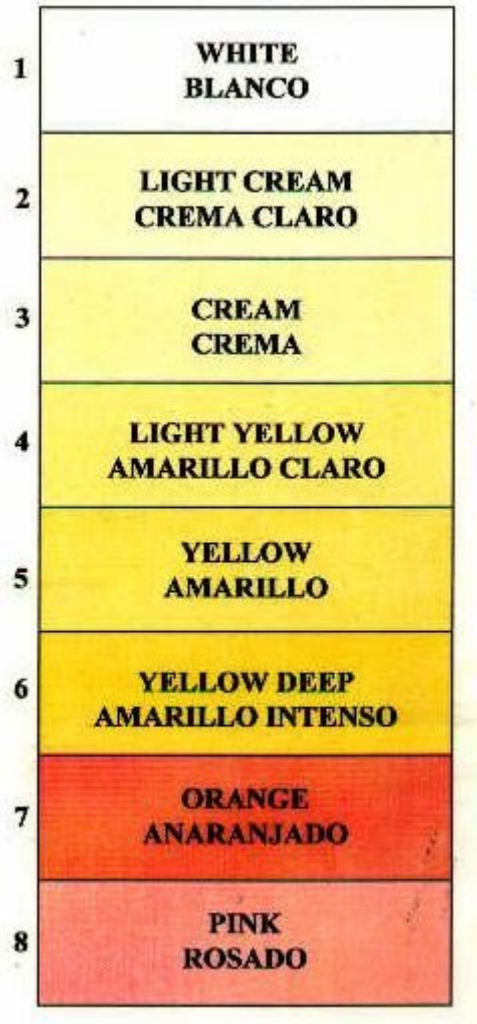
farmer interest critical for their adoption in Ghana. In view of this, varieties IBA090151, IBA083774 and IBA083724 can be considered for varietal release after on-farm testing. The study further revealed that these yellow flesh cassava varieties can be used as parents in crosses with the local material to improve landraces grown by farmers for TCC, DMC, high yield and CMD resistance.

The WHO is advocating for improvement of food and nutrition security worldwide. Yellow flesh cassava could be a key driver for this purpose. This reiterates the need for research to reduce micronutrient losses related to processing of biofortified crop products and to further increase the level of TCC concentration in farmer preferred landraces/varieties to increase rate of adoption, given that a number of varieties or landraces grown by farmers already possess other key important traits like DMC and stability. Further breeding initiatives to develop higher TCC in cassava varieties is ongoing in Ghana as with other parts of the world. It is expected that these efforts will provide superior varieties highly elevated TCC. The results indicate that with increased consumption of boiled biofortified cassava there could be improvement in pVA intake in areas where VAD exists. People would also have to be educated on best processing methods that retain more TCC so that they can maximize benefits from the consumption of biofortified foods. The HCN level in the yellow flesh cassava tested in this study were well within the permissible levels recommended by WHO for human consumption for the various food products in Ghana.

This study demonstrated the importance of exploring participatory approaches to research for impactive results. It is recommended that cassava breeders review their breeding objectives to reflect the preferred traits of end users, and pay attention to stakeholders' perceptions of yellow flesh cassava to develop demand driven varieties that will serve the need of end users. Education to create awareness on the potential advantages and diverse uses of the improved biofortified cassava is also needed. Further studies are proposed to investigate and assign measures and ratings to men and women for the qualitative traits recorded. It is also recommended that, in developing high TCC and DMC varieties, breeders should carefully select good parental lines with high breeding values for both traits and other farmer prime traits of interest.

Appendices

Appendix 1: Colour chart for visual assessment of carotenoid content based onpigmentation of root parenchyma



Source: Dr Elizabeth Yaa Parkes, harvestPlus cassava breeder, IITA- Ibadan, Nigeria

Appendix 2: Baseline study on trait preferences and perception of yellow flesh cassava by men and women chain actors

A checklist through participatory approach

Date of interview:..... Time of interview: .....

Name of interviewer: .....

Name of recorder: .....

Location of interview: .....

Tool 1: Farmers' checklist

A. Socio-demographics characteristics of cassava farmers

Name	
Sex	
Age	
Marital status	
Level of education	
Farming experience	
Farm size for cassava	
Land tenure system	
Main occupation	
Labour source	
Residential status (Native or settler)	
Head of household	

Note: For the land tenure system, **probe**: who are entitled to ownership of land in the community (men and women, settlers and native)

B. Farm level characteristics

Farm production

1. What was the average total food crops farmed last year?
2. List the main food and cash crops grown by men and women **(Use pair-wise ranking)**
3. Importance of cassava in the community
4. Percentage of the farm grown to cassava
5. Do men and women have equal access to and control over cassava farms? If no, what actions can be taken to increase women access and ownership of land
6. Challenges in expanding the cassava field

Varietal information in the community

1. Please list the cassava varieties grown as given by each participant
2. Kindly give the meanings of the variety names given
3. Rank varieties per participant and as a group
4. Is cassava cultivated as a sole or intercrop and why?
5. Types of cassava roots colour grown and why?
6. Which type of cassava roots are mostly liked by customers (white or yellow) and why?
7. For each of the variety grown, what do you like about it?
8. For each of the variety grown, which do you want improved.
9. What traits do you prefer in cassava varieties (eg. what do you like to see in new varieties)?
10. Tell us about some traits that are distinct for men and women (probe for the reasons).
11. Tell us about the kinds of activities men and women engage in during the cassava cropping calendar (Start from land search to marketing).
12. Which aspects of cassava cultivation are believed to be difficult for men and women

Information on planting material and credit access

1. Source of planting material
2. In the last **five years,** has anyone received any new varieties? (Please tell us how you got these varieties)

3. In the last **five years,** has anyone bought any new varieties? (Please which varieties and why)
4. Source of information on improved varieties? (probe for opportunities and challenges in accessing those varieties)
5. Do you produce your own cassava stems, buy some, or get it from somewhere, why?
6. If you buy or get it from somewhere, why
7. What are the challenges in accessing new varieties
8. Are there opportunities of credit for cassava production? If yes, what are the sources?
9. How easy is the process of accessing credit by men and women? (probe for who gets credit easily and why)

Adoption and dis-adoption

1. How long do you use a particular variety before discarding (local and improved), provide examples.
2. In this group how many have ever used improved cassava varieties?
3. If you have never used it, why not?
4. How many are still using it?
5. For those who have stopped, why?
6. Which varieties have you abandoned?

Preferences, perception and willingness to accept yellow flesh cassava

1. How many of you are aware of yellow flesh cassava?
2. Since when did you become aware (take per participant) of it?
3. For those who are aware, how many have ever cultivated yellow flesh cassava?
4. How many are still cultivating it?
5. What did you like / still like about the yellow flesh cassava?
6. What do you want improved?
7. Where do you get planting materials?
8. What are the challenges in accessing planting materials?
9. How do you perceive the yellow flesh cassava compared to white in terms of:
    - Taste

- Texture (when cooked)
- Appearance: Colour
- Nutrients
- Marketability (which one sells faster)
- Price (which one has higher price)
- Uses

10. How many are willing to cultivate yellow flesh cassava and why?
11. If not willing to cultivate, why?

Appendix 3 Tool 2: Processors' checklist

C. Socio-demographics characteristics of cassava processors

Question/Aspect	Responses
Name (optional)	
Sex	
Age	
Marital status	
Level of education	
Processing experience	
Amount of roots/ bags processed every week	
Main occupation	
Labour source	
Residential status (Native or settler)	
Head of household Yes [1] No [0]	
Nett income (estimate): (After expenses)	

Note: For the land tenure system, **probe**: who are entitled to ownership of land in the community (men and women, settlers and native)

D. Background

1. Which kind of jobs/activities do women and men do in the processing business (Activity profiling**)**

2. Describe the process for accessing cassava roots (probe for the differential opportunities or challenges for accessing the sources of these roots)
3. Who are the key stakeholders in the business of cassava roots (probe for the women and men in the trade and why)
4. Identify gender based constraints in the cassava processing
5. What challenges are in expanding the cassava processing business

Varietal information in the community

1. Please list the cassava varieties processed as given by each participant
2. Kindly give the meanings of the variety names given
3. Rank varieties per participant and as a group
4. Do you process each cassava variety separately or together and why
5. Types of cassava roots colour processed and why
6. Which type of cassava roots are mostly liked by customers (white or yellow) and why
7. For each of the variety grown, what do you like about it
8. For each of the variety grown, which do you want improved
9. What traits do you prefer in cassava varieties (Eg. what do you like to see in new varieties)
10. Tell us about some traits that are distinct for men and women (probe for the reasons)
11. Which aspects of cassava processing are believed to be difficult for men and women

Information on planting material and credit access

1. Do you have your own farm
2. If yes, source of planting material
3. In the last **five years,** has anyone received any new varieties? (Please tell us how you got these varieties)
4. Source of information on improved varieties? (probe for opportunities and challenges in accessing those varieties)
5. Do you produce your own cassava stems or buy some or get it from some why?
6. If you buy or get it from somewhere, why
7. What are the challenges in accessing new varieties

8. Are there opportunities of credit for cassava processing? If yes, what are the sources?
9. How easy is the process of accessing credit by men and women? (probe for who gets credit easily and why)

Adoption and dis-adoption

1. How long have you process a particular variety before discarding (local and improved), provide examples
2. In this group how many have ever processed improved cassava varieties?
3. If you have never processed, why not?
4. How many are still processing?
5. For those who have stopped, why?
6. Which varieties have you abandoned

Preferences, perception and willingness to accept yellow flesh cassava

1. How many of you are aware of yellow root cassava?
2. Since when did become aware (take per participant)
3. For those who are aware, how many have ever processed yellow root cassava?
4. How many are still processing?
5. What did you like / still like about the yellow flesh cassava
6. What do you want improved
7. Where do you get yellow flesh roots to process?
8. If from your own farm, where did you get planting materials?
9. What are the challenges in accessing yellow flesh roots/ its planting material?s
10. How do you perceive the yellow flesh cassava compared to white in terms of:
    - Taste
    - Texture (when cooked)
    - Appearance: Colour
    - Nutrients
    - Marketability (which one sells faster)
    - Price (which one has higher price)
    - Uses
11. How many are willing to process yellow flesh cassava and why?

12. If not willing to process, why?

Appendix 4: Scoring the pulp colour using colour chat

Appendix 5A and 5B: Eliciting information from stakeholders through a PRA

Appendix 6: Pearson phenotypic correlation for some cassava traits

Pearson Correlation Coefficients, N = 20										
Prob > |r| under H0: Rho=0										
	CGM	CMD	CORT_COL	PULP_COL	HI	RTN	RTW	TCC	TWT	DMC
CGM	1	0.16688	0.12345	0.2271	-0.49521	-0.03972	-0.51294	-0.02271	-0.23443	-0.057
CGM		0.4819	0.6041	0.3356	0.0264	0.868	0.0207	0.9243	0.3198	0.8113
CMD	0.16688	1	0.3957	0.77168	0.17597	-0.44956	-0.23005	0.54842	-0.25133	0.59704
CMD	0.4819		0.0842	<.0001	0.458	0.0467	0.3292	0.0123	0.2851	0.0054
CORT_COL	0.12345	0.3957	1	0.57959	0.14467	-0.28015	-0.19977	0.33654	-0.33167	0.09944
CORT_COL	0.6041	0.0842		0.0074	0.5428	0.2316	0.3984	0.1468	0.1531	0.6766
PULP_COL	0.2271	0.77168	0.57959	1	0.27598	-0.25987	-0.03062	0.58975	-0.32291	0.33529
PULP_COL	0.3356	<.0001	0.0074		0.2389	0.2685	0.898	0.0062	0.1649	0.1484
HI	-0.49521	0.17597	0.14467	0.27598	1	-0.50741	0.3841	0.01111	-0.09499	0.00351
HI	0.0264	0.458	0.5428	0.2389		0.0224	0.0945	0.9629	0.6904	0.9883
RTN	-0.03972	-0.44956	-0.28015	-0.25987	-0.50741	1	0.49008	-0.05152	0.42546	-0.12906
RTN	0.868	0.0467	0.2316	0.2685	0.0224		0.0283	0.8292	0.0615	0.5876
RTW	-0.51294	-0.23005	-0.19977	-0.03062	0.3841	0.49008	1	0.22903	0.70884	0.00344
RTW	0.0207	0.3292	0.3984	0.898	0.0945	0.0283		0.3314	0.0005	0.9885
TCC	-0.02271	0.54842	0.33654	0.58975	0.01111	-0.05152	0.22903	1	0.28791	0.20481
TCC	0.9243	0.0123	0.1468	0.0062	0.9629	0.8292	0.3314		0.2184	0.3864
TWT	-0.23443	-0.25133	-0.33167	-0.32291	-0.09499	0.42546	0.70884	0.28791	1	0.16712
TWT	0.3198	0.2851	0.1531	0.1649	0.6904	0.0615	0.0005	0.2184		0.4813
DMC	-0.057	0.59704	0.09944	0.33529	0.00351	-0.12906	0.00344	0.20481	0.16712	1
DMC	0.8113	0.0054	0.6766	0.1484	0.9883	0.5876	0.9885	0.3864	0.4813	

Appendix 7: Scoring for mealiness