GENETIC DIVERSITY, STARCH PHYSICOCHEMICAL PROPERTIES AND CYANIDE LEVELS OF FARMER PREFERRED CASSAVA LANDRACES IN THE EASTERN ZONE OF TANZANIA

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EXTENDED ABSTRACT

Cassava is an important staple crop and its starch is an important source of biomaterial for food and non-food industries. Processing of cassava is very important due to its rapid postharvest deterioration and cyanide content, but it can also add value and provides employment opportunities. There is a correlation between cyanogenic poison and poverty in communities where cassava is a staple food. Therefore development of starches occupies a central position in the quest for cassava commercialization, thus stimulating increased cassava production. Moreover,the farmer will realize profit from cassava production and hence improved food security at farmer's level. To meet such a high demand of cassava in Tanzania, cultivar selection, production and processing need to be improved. A study was therefore conducted to understand farmer preference for cassava landraces, cassava genetic diversity present in the farmer's field and to assess suitability of commonly grown cultivars for commercialized starch production for different applications and cyanogenic potential.

The specific objectives were:

i) To gather farmer's indigenous knowledge on cassava variety selection and conservation in the Eastern zone of Tanzania.

ii) To characterize farmers preferred cassava landraces in Eastern zone of Tanzania using morphological and molecular techniques.

iii) To assess variation in physicochemical characteristics and functional properties of selected cassava starch.

iv) To determine the effect of genotype, location and harvesting time on fresh root yield, starch yield and root cyanide content of selected cassava landraces.

Participatory rural appraisal approaches were used to gather farmers' knowledge on conservation of cassava genetic resources. During the survey 52 cassava landraces were

collected and characterized. Farmer's decision on landrace to be grown was the main factor which was found to influence cassava diversity in the fields. Farmer's decision was based on of diverse factors from food security, market forces and culinary attributes. Seed flow occurs as farmers exchange or buy from local market also contributed to the diversity found in the farmer's fields. Fresh root yield followed by early maturity were the most preferred attributes. The genetic relationship and diversity of 52 farmers preferred cassava landraces were successfully characterised using morphological and Single Nucleotide Polymorphisms (SNPs) data. The results of analysis showed a substantial diversity in cassava germplasm found in farmer's field. Both morphological (genetic distance of 1.18 to 0.15) and SNPs (genetic distance of 0.076 to 0.002) analysis revealed considerable variability among cassava landraces and cluster analysis did not segregate landraces according to geographic location. In general, the internal branches of the dendogram from SNPs analysis were short while external branches were long, indicating that within group variability was higher than between groups. The most divergent cultivars revealed by morphological analysis were *Kichooko*, *Mbega*, *Shibatumbo* and *Pusuu*, and SNPs analysis revealed *Mbega* and *Mzungu Mweupe* to be highly diverse.

Of the 52 collected cassava landraces, six commonly grown were identified and further analysis was done to assess variation in physicochemical characteristics, starch yield and cyanogenic potentials. Moreover,analysis has shown that there is a difference in physicochemical characteristics between landraces ($p \leq 0.05$), and can be targeted to different industrial applications. The study also illustrated genotypic difference in starch yield and cyanogenic potential as previously described by other authors. Among landraces, *Kiroba* showed potential for maximum starch yield (12.8 t ha^{-1}) followed by *Msenene* (12.3 t ha^{-1}) and third was Kilusungu (10.2 t ha^{-1}). The optimal harvest time for

maximum starch yield was found to be 12 months after planting for most cultivars. The cyanide content of cassava landraces was between 15 and 800 ppm across all trial sites. Moreover *Kilusungu* displayed high cyanide (400 ppm) levels compared with cultivars across all trial site. This cultivar exhibited a high potential for starch production as displayed by near average starch yield compared with other cultivars.

Partial least square discriminant analysis (PLS-DA) was done to distinguish among cassava starches based on the physicochemical and functional properties. When only starch functionality properties were considered, landrace *Nyamkagile* was the most divergent among landrace, followed by *Kalolo* and *Msenene*, with setback viscosity, solubility at 90 °C and syneresis at -20 °C underpinning this differentiation. *Msenene* and *Kilusungu* had high swelling power, which makes them potentially suitable for use as thickeners and binding agents for food and non-food uses. *Msenene* also had a relatively low setback viscosity after cooling, and low syneresis, (p>0.05), desirable properties in starches for gelling agents and thickeners in refrigerated and frozen food products. *Kibandameno* starch had the highest enzyme digestibility and lowest particle size distribution (p<0.05) compared with starches. This makes the cultivar suitable for making glucose syrup, adjuncts in breweries (fermentation stock), low fibre feed and sweeteners.*Nyamkagile*(p<0.05) had the lowest digestibility and may find application in food for individuals wishing to manage their glycemic index such as diabetic and overweight patients. Based on this study, farmer's knowledge was documented and diversity found in farmer's field was confirmed by morphological descriptors and SNP analysis. SNP markers were able to discriminate morphologically similar landraces (*Kasunga* and *Nyamkagile*) and morphologically different landraces, *Pusuu* and *Pushuli* were found by SNPs analysis to be genetically near identical. The advantage of SNP to discriminate closely related individuals has been shown by this study. This collection

revealed a wide range of genetic diversity and represents a valuable resource for trait improvement enabling capture of farmer preferred traits in future cassava breeding programmes. Other desirable traits can be exploited and incorporated during breeding. Data generated from this study will help the breeders to devise more appropriate and cost effective breeding strategies and will aid in deciding which germplasm to conserve.It is recommended thatappropriate policies need to be put in place in favour of development of starch industries. It is is also argued to devise germplasm conservation strategy to prevent loss of germplasm and ensure conservation of desirable traits.

LIST OF PUBLICATIONS

i.Mtunguja, M.K., Laswai, H.S., Muzanila, Y.C. and Ndunguru, J. (2014). Farmer's Knowledge on Selection and Conservation of Cassava (*Manihot esculenta*) Genetic Resources in Tanzania**.***Journal of Biology, Agriculture and Healthcare* 4 (10): 122- 129.

ii. Mtunguja, M.K., Ranjan, A., Laswai, H.S., Muzanila, Y.C., Ndunguru, J. and Sinha, N.R. (2015).Genetic diversity of farmer preferred cassava landraces in Tanzania based on morphological descriptors and Single Nucleotide Polymorphisms. *Journal of Plant Genetic Resources (first view).*

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DECLARATION

I, Mariam Kom Mtunguja, do hereby declare to the Senate of the Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

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Mariam Kom Mtunguja (PhD Candidate)	Date

The above declaration confirmed by

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Prof. Henry S. Laswai (Supervisor)	Date
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Prof. Yasinta C. Muzanila (Supervisor)	Date
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Dr. Joseph Ndunguru (Supervisor)	Date

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DEDICATION

I dedicate this work to my father, Omary Mandia; my mother, Grace Mamsap and my late grandfather Vincent Gao.

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LIST OF ABBREVIATIONS

AEA	Average Environment Axis
AEC	Average Environment Coordinates
AFLP	Amplified Fragment Length Polymorphism
AFM	Atomic Force Microscope
AMMI	Additive Main Effect and Multiplicative Interaction
ANOVA	Analysis of Variance
CIF	Cost of Insuarance and Freight
CLD	Chain Length Distribution
C-NMR	Carbon 13- Nuclear Magnetic Resonance
DAP	Days After Planting
DigRaw	Digestibility of Raw starch after 24 h incubation
DM	Dry Matter
DNA	Deoxyribonucleic Acid
FAO	Food and Agriculture Organization
FRY	Fresh Root Yield
GBS	Genotype By Sequencing
GBSS	Granular Bound Starch Synthasase
GEI	Genotype by Environment Interactions
GGE	Genotype and Genotype by Environment interaction (G+ GE)
GPS	Global Positioning System
HPAEC	High-Performance Anion-Exchange Chromatography
HQCF	High Quality Cassava Flour
IITA	Institute of Tropical Agriculture
MAP	Months After Planting
NGS	Next Generation Sequencing
PC	Principal Component
PKT	Peak temperature

PLS-DA	Partial least squares discriminant analysis
PST	Pasting temperature
PV	Pasting viscosity
RAPD	Random Amplified Polymorphic DNA
RC	Relative crystallinity
RS	Reducing sugars
RVA	Rapid Viscosity Analyser
SEM	Scan Electron Microscope
SGAP	Starch Granule Associated Protein
SNPs	Single Nucleotide Polymorphisms
SOL70	Solubility at 70 °C
SOL90	Solubility at 90 °C
SPSS	Statistical Package for Social Sciences
SRN	Storage Root Number
SS1	Starch Synthase
SSR	Simple Sequence Repeat
SSSA	Soil Science Society of America
SV	Setback viscosity
SWPW 70	Swelling power at 70 °C
SWPW 90	Swelling power at 90 °C
SY	Starch yield
Syn re	Syneresis at 4°C
SynRf	Syneresis at -20°C
TAN	Tropical Ataxic Neuropathy
TRA	Tanzania Revenue Authority
UPGMA	Unweighted Pair Group Method using Arithmetic averages

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Cassava (*Manihot esculenta* Crantz) is a perennial woody shrub with an edible root and grows in tropical and subtropical countries. The starchy tuberous roots of cassava provide more than half of the calories consumed by more than 800 million people in Sub-Saharan Africa (SSA), Latin America and Asia (Lebot, 2009; Burns*et al*., 2010).Cassava is also the world's fourth most important staple crop after rice, wheat and maize, and plays an essential role in food security, especially in those regions prone to drought and poor soils. Its growth characteristics make it an important staple crop preferred by resource-poor farmers in many tropical countries (Taylor *et al.,* 2012). Cassava has ability to grow and produce on low nutrient soils, where cereals and other crops do not grow well and is suitable for incorporation in various cropping systems (Lekule, 2006; Montagnac *et al*., 2009). These attributes make cassava a mainstay of smallholder farmers in the tropics,where agricultural inputs are limited (Fregene *et al*., 2000).

In Tanzania, cassava is mainly grown as a subsistence crop and it is the third important source of calories after maize and rice (Jeremiah and Kulembeka, 2007). Tanzania is one of the largest producers of cassava in Africa and ranks fourth after Nigeria, Democratic Republic of Congo and Ghana (Van der Land and Uliwa, 2007) and produced 7 million tonnes of cassava in 2008 (FAOSTAT, 2010). Cassava is cultivated and produced in all regions of Tanzania but is mainly produced in Mwanza, Mara, Lindi, Tanga, Ruvuma and Coast regions (Jeremiah and Kulembeka, 2007). Commercialization of cassava

starch has not yet been exploited in Tanzania.Starch which is used for many industrial applications in the country is obtained from corn, and mainly imported from USA and China. FAO urges developing countries to strengthen their rural economy and boost cassava farmers' incomes by converting it into high value starch (FAO, 2006). There are a lot of benefits of using cassava as a source of starch for industries in Tanzania, considering that, cassava is produced within the region and is widely available thus will provide a cheaper source of starch compared with imported corn starch. This will promote cassava as cash crop and help in poverty alleviation in cassava growing regions while the export of excess crop will contribute to the nation's foreign exchange earnings.

Substantial diversification of cassava germplasm has taken place since its introduction in Africa. Farmers grow relatively large numbers of crops and varieties in trying to reduce risks of food insecurity. Farmers distinguish genotypes by using morphology and several studies have revealed the importance of farmer's indigenous knowledge in germplasm collection, conservation and genetic improvement (Gullberg *et al.,* 2007; Mkumbira *et al*.,2003). Molecular approaches have become more efficient in measuring genetic diversity, as they directly quantify genetic variability at DNA level (Kawuki *et al*., 2009). Molecular markers have also provided a powerful tool for researchers in crop breeding. Polymerase Chain Reaction (PCR) based molecular markers are widely used based on their advantage in speed and sensitivity. This study is therefore aimed at characterizing cassava landraces in Eastern zone of Tanzania for genetic diversity. The physicochemical properties of starch in relation to function were also assessed. Furthermore, appropriate genotype, location and harvest time for optimal starch yield and cyanide levels were studied.

1.2 Cassava Biology and Physiology

1.2.1 Biology

Cassava is a perennial shrub of the dicotyledonous family Euphorbiaceae, and important for its starchy root which can be harvested between 6-24 months after planting (Alves, 2001). Cassava can be propagated using stem or seeds, but farmers prefer using stem cuttings. Cassava plant also produces seedlings. These seedlings are genetically segregated due to reproduction by cross pollination.

1.2.2 Plant developmental and growth

Cassava sprouting occurs 5-12 days after planting (DAP) whereby adventitious roots emerge from the bottom surface of the stem cut, followed by emergence of small leaves at the top. After 90-180 DAP maximum root and leaf growth is attained and branches are formed.Partitioning of dry matter from leaves to root occurs between 180 and 300 DAP, during which rapid root bulking is observed (El-Sharkawy, 2003).

Carbohydrates produced during photosynthesis have to be distributed for growing leaves and storage roots. During the early days of crop cycle (60-75 DAP) carbohydrates accumulate more in leaves. After that deposition in the storage roots increases rapidly reaching up to 50- 60% of the total dry matter at around 120 DAP (Alves, 2001). The period of maximum rates of dry matter accumulation depends on genotype and growing conditions. El-Sharkawy (2003) reported that at high altitude more time is required compared to tropical conditions where the growth is fast.

1.2.3 Agronomy and environmental effect on cassava physiology

Water plays an important role in transpiration and photosynthesis as it regulatesstomata opening (Ulukan, 2008).Cassava requires annual rainfall between 1000 and 3000mm, but

can tolerate low rainfall if well distributed (Lebot, 2009). Substantial water supply is required during the period of root and shoot initiation3-5 MAP (Month After Planting, MAP).Water deficit during this period can reduce yield (Vandegeer *et al*., 2012; Santisopasri *et al*., 2001). Studies have also shown that there is no significant yield reduction after 5 MAP if cassava experiences water deficit(Alves, 2001; Vandegeer *et al*., 2012; El-Sharkawy, 2012).One advantage of cassava compared to other crops, is that it can resume growth after period of prolonged drought (Vandegeer *et al*., 2012). Furthermore importance of rainfall distribution and ability of the soil to retain water plays a significant role in cassava production. Areas with rain showers equally distributed throughout the year are suitable for cassava production. Also, sand clay loam soils are favorable because of their good water holding capacity (Benesi*et al*., 2008; El-Sharkawy, 2007). When grown on very poor soils under prolong drought, cassava crop reduces both its leafy canopy and transpiration water loss(El-Sharkawy, 2007; Vandegeer *et al*., 2012). Remaining leaves continue to be photosynthetically active but at reduced rate (El-Sharkawy, 2007).Temperature affects sprouting, leaf formation and therefore plant growth in general. Temperature ranging from 25 to 29°C isfavorableto cassava growth but it can tolerate temperature as low as 12°C and as high as 40°C(Lebot, 2009).

Cassava is highly efficient in nutrient absorption thus adapted to poor degraded soils with low pH, high levels of exchangeable Aluminium and low concentrations of phosphorus. Nutrients availability in the soil is important to cassava production and differences in cassava yield have been reported with changes in micronutrient supply. Phosphorus is essential for development of root systems (Ulukan, 2008) and cassava plant is well adapted to P absorption than cereals because of its association with

mycorrhizal hyphae (Howeler,2002). Potassium stimulates net photosynthesis and translocation of food reserve in the tuberous root. Increased starch content with increased potassium have been reported (Benesi *et al.,* 2008).Nitrogen is a building block for chlorophyll and protein. N deficiency cause declinein yield due to decrease in photosynthesis rate and total leaf area (Cruz *et al*., 2003). The ability of cassava plants to take up water and nutrients depends on the soil pH (Ulukan, 2008),lime application may cause yield increase but high rates may induce micronutrient deficiency. Cassava can tolerate low pH, but not pH above 8 (Howeler, 2002).

1.2.4Cassava cyanogenesis

Cyanide is toxic to most living organisms due to its ability to bind to metals (Fe, Zn and Cu) functional groupsor ligands of many enzymes. Plants produce cyanide as a by-product of ethylene synthesis or a defensive chemical against herbivores. Furthermore, cyanide may regulate development or seed germination in some plants and affect the alternate respiratory pathway (McMahon *et al.,* 1995).The plant produces two cyanogenic glucosides, linamarin (95%) and a small amount of lotaustralin (5%). If the plant is attacked by predators, an enzyme, linamarase, catalyzes breakdown of the glucosides to give hydrogen cyanide (Fig. 1)(Cumbana *et al.,*2007). Cassava is one of the cyanogenic plants and farmers using cassava as staple food prefer bitter varieties.Linamarin has bitter taste hence high cyanide cassava roots are normally bitter and contain >100ppm cyanide, and therefore termed bitter cassava (Nhassico *et al*., 2008).

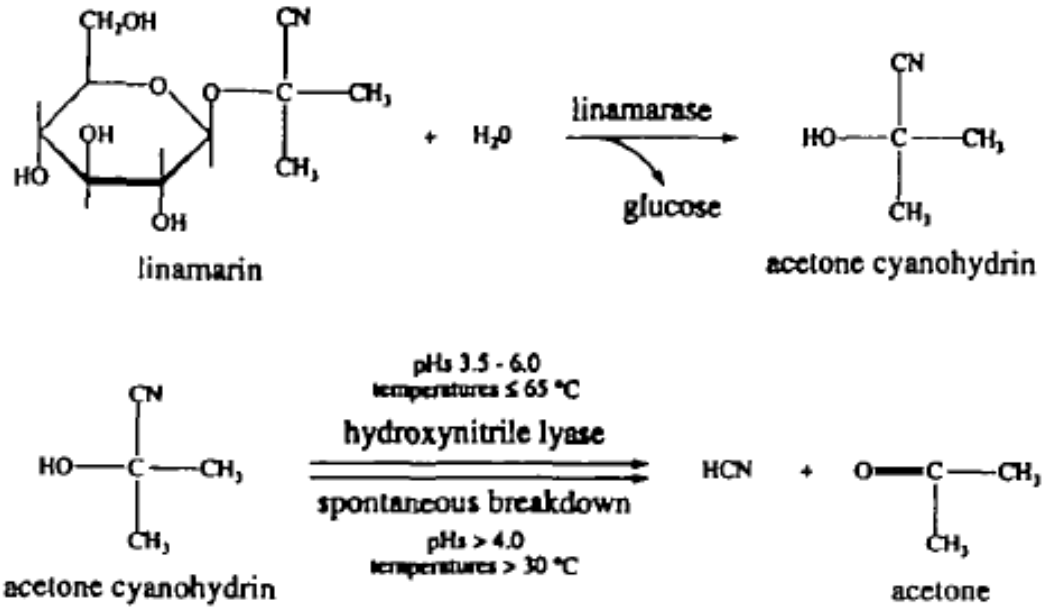

Figure 1: Cyanogenesis from linamarin (adapted from McMahon *et al.,* 1995)

Acute cyanogen poisoning resulting from eating poorly processed bitter varieties (above 10mg HCN equivalent/kg DW)can result in *konzo*, a paralytic disorder or in some cases death (Adamolekun, 2011).*Konzo* outbreaks in Tanzania (Mlingi *et al.,* 1992), Mozambique (Cumbana *et al*.,2007; Cliff *et al*., 2011) and Democratic Republic of Congo (Chabwine *et al*., 2011) has been reported. Farmers are knowledgeable in distinguishing bitter and sweet cultivars (Mkumbira *et al.,* 2003)and also processing technologies forreducingroot cyanogen to safe limits (Chiwona-Karltun *et al*., 2000).Shortcuts in processing usually are the cause of cyanogen poisoning which occurs mainly during shortage of food especially during drought and wars. Cyanogenic potential of cassava has been reported to be cultivar and environment dependent (Burns *et al*., 2012; Bradbury *et al*., 2013). Also, drought in early development stages of cassava plant has significant effect on cyanide concentration of both leaves and roots (Vandegeer *et al*., 2012).

1.3Cassava Genetic Diversity and Farmer's Knowledge

Farmers have been maintaining diversity of crops in their farms. This diversity ismainly due to crop preference and food security. Cassava famers have maintained more than six cultivars grown in large quantities and other cultivars grown in few stands around their farm (Oluwole *et al.,* 2007). Farmers keep even the low yielding varieties and will never discard them from genepool, a practice used as risk management strategy (Elias *et al.,* 2000). Preference of cassava varies according to intended use. In areas where cassava is staple crop, bitter cultivars are preferred. Sweet cultivars are grown for fresh cassava market as breakfast snack, mostly in urban areas (Benesi *et al*., 2010).Knowledge of genetic diversity produces a better understanding of germplasm. It provides higher efficiency tool during genotype sampling, which result in the biologically oriented choice of crosses and gene introgression from exotic germplasm. It also provides a base used to recommend cultivars when the goal is to increase the genetic basis of commercial cultivars (Vieira *et al.,* 2007). To obtain a more complete understanding of the degree of genetic divergence it is necessary to consider the molecular and morphological data separately.

1.3.1Morphological descriptors

Morphological characterization has been used in quantifying genetic distance. Famers can accurately distinguish cultivars using their morphological descriptors (Mkumbira*et al.,* 2003). Even though,cultivars with similar features are sometimes confused by farmers(Elias *et al*., 2000). The most common descriptors used by farmers to differentiate cultivars include color traits, shape of central lobes and branching habit (Benesi *et al*., 2010;Elias*et al*., 2001). However, phenotype expression is influenced by genotype and environment interactions, leading to a low accuracy of quantitative genetic parameter estimates (Vieira *et al*., 2007).

1.3.2Molecular markers

Molecular markers are not subject to environmental influence and thus considered superior to morphological markers (Máric *et al.*, 2004). DNA based genetic markers allow for direct assessment of variation within genotypes and have been used to analyze genetic diversity, construction of genetic maps and trait mapping. Different approaches have been used to study DNA sequence differences of different crop population; Random Amplified Polymorphic DNA (RAPD) (Herzberg *et al.,* 2004), Amplified Fragment Length Polymorphism (AFLP) (Benesi *et al*., 2010; Elias *et al*., 2000), Simple Sequence Repeat (SSR) (Kawuki *et al.,*2009; Masumba, 2007) and Single Nucleotide Polymorphisms (SNPs) (Kawuki *et al*., 2009).These markers have been used extensively to identify genes controlling traits of economic importance and develop crop varieties to suit different crop preferences. These traditional marker systems have to be adjusted in terms of number and type of marker to suit specific study purpose.

Next Generation Sequence (NGS) technology provides sequencing of the whole genome thus allowing development of thousands of markers in a single experiment (Rafalski, 2002). NGS also allow sequencing of many individuals at a time thus provide increasing affordable tool. This is possible by the use of barcoding, where by individual genotypes are given specific labels and are easily identified during analysis (Kilian and Graner, 2012). NGS create thousands of SNPs discoveries in a single experiment as it allows direct analysis of sequence difference between individual at a large number of loci. Genomics data derived from NGS will also enable researchers to identify which chromosome fragment is derived from which parent in the progeny line, thereby identifying clear crossover events occurring in every progeny line and placing markers on genetic and physical maps without ambiguity. Eventually, this will help in

introducing specific chromosome regions from one cultivar to another. Therefore, it can be anticipated that NGS technologies will be particularly useful for developing and confirming introgression lines for a trait of interest. It also facilitates genomic assisted breeding and hasten the development of transformation technologies for crops because it will become easier to modify genes with the increasing availability of genomic data (Varshney, 2009).

Single Nucleotide Polymorphisms(SNPs) are the ultimate form of molecular genetic marker, as a nucleotide base is the smallestunit of inheritance. SNPs represent a single nucleotide difference between two individuals at a defined location (Edwards *et al.,* 2007). SNPs are biallelic in nature and the information provided by SNPs is most useful when several (usually two to four) closely spaced SNPs completely define haplotypes in the region being examined (Rafalski, 2002). SNPs are perfect markers because the sequence information provides the exact nature of the allelic variation (Berkman *et al.,* 2012).

Kawuki *et al*. (2009)compared genetic analysis using 26 SNPs identified based on direct sequencing of 9 genesand 12 SRR markers. Both analyses revealed the same genetic diversity according to region of origin. Though SNPs analysis did not have sufficient information on the genetic relationshipbutthis SNPs analysis involved only a small portion of the genome(9 genes).Since SNPs occur at high frequency in a genome therefore can be targeted as useful genetic markers (Berkman *et al.,* 2012).

1.4Starch

Starch granule is a form of storing energy for plants and its synthesis takes place in the amyloplast. It is well organized, densely packed and insoluble in water make it

appropriate for storing plant energy. Starch granule are mostly found in seed, tubers, stem and root (Pérez and Bertoft, 2010).Starch yield amount to 80% of dry cassava root (Zhu, 2015) though great variations exist in starch yield between cassava genotypes as reported by several studies (Bennesi *et al.,* 2008; Tumuhimbise *et al.,* 2014). This is true because starch biosynthesis is achieved throughcomplex coordination and interaction of different groups of enzymes (Tetlow, 2011). These enzymes include; starch synthases, starch branching and starch debranching enzymes which are involved in amylopectin synthesis. Granule Bound Starch Synthase(GBSS) is involved in amylose synthesis (Tetlow, 2011). Amylose and amylopectin constitute major component of starch and their proportionvaries between species.

1.4.1 Starch production and utilization

Starch interaction with different components makes it suitable for different food and non-food applications (Waterschoot *et al*.,2015). Starch has been utilized in different industries as stabilizer, binder, sweetener, thickening agent and fining agent inpaper printing. For some of the industrial applications starch has to go through modification to suit processing condition.Starch extraction from cassava is easier compared to cereals, due to tissue structure and relatively low amount of lipids and protein content (Moorthy, 2002). Starch from cassava has unique properties when compared to other tuber and cereal crops. It is differentiated by its low fat, protein and ash content. It has also low amylose content compared with amylose containing starch. Furthermore, it has small to negligible amount of phosphorus (Zhu, 2015). This attribute make cassava starch suitable for application in different industriessuch as textile, processed food, plywood, paper and pharmaceutical (Breuninger*et al*., 2009; IITA,2011).

According to IITA report (2011), Tanzanian industries can consume up to 47000 tonnes of cassava processed into industrial raw materials such as High Quality Cassava Flour (HQCF), starch and cassava chips, which can substitute expensive imports in breweries, textile, food, adhesive, bakeries and animal feed industries. The average starch import is 7 667 tonnes per year (Table 1), which is equivalent to CIF value of 3.2 million US dollars (TRA, 2011). Therefore, cassava farmers and starch producers can benefit if the available demand for starch in Tanzanian' industries is exploited.

Table 1:Aggregate starch imports in Tanzania

Year	Quantity(tonnes)	CIF value(million USD)
2004	7,897	2.9
2005	6,476	2.7
2006	7,030	2.95
2007	9,313	3.98
2008	9,506	3.6
2009	5,781	3.2
Average	**7,667**	**3.2**

Source: Tanzania Revenue Authority (2011)

1.4.2 Starch granule chemical composition

Starch is α-glucan polymer containing two major components amylose and amylopectin (Copeland *et al.,* 2009). Amylose is predominantly linear (1,4) linked α-glucan and with very few branches (<1%). Amylopectin is highly branched, with (1,4) linked α-glucan and (1,6) branch points(99%) (Tester *et al*., 2004). Amylose and amylopectin differ in functional properties. Amylose has high tendency to retrograde and produces tough gels and strong films. Amylopectin is more stable in water dispersion and produces soft gels and weak films. Amylose was considered linear but it was later discovered that cannot be

completely hydrolyzed by ß-amylase. The incomplete hydrolysis of amylose was due to branching of some molecules. Branching characteristic of amylopectin is reported to be difficult to assess since the branching points are thought to be located in amorphous region and crystalline lamella consist of amylopectin double helices (Pérez and Bertoft, 2010). Branching pattern and chain length distribution plays a significant role in influencing functional properties of starch and subsequent application in different industries (Perez *et al*., 2009).

Starch granule chemical composition plays a significant role in influencing starch properties and starch products derived from it (Perez *et al*., 2009). General understanding of starch composition and structure is crucial for optimized functionalities for starch. Apart from amylose (14-29%) and amylopectin, starch is constituted of other minor components. These minor components include phytoglycogen (intermediate material between amylose and amylopectin), starch lipids, Starch Granule Associated Protein (SGAP) and phosphate mono esters, and are thought to be incorporated into the granule during synthesis (Baldwin 2001). SGAP are protein retained in the starch granule after following extraction and washing techniques to obtain starch (Tetlow 2006).They are different from storage proteins and are tightly bound to surface as integrated part of starch structure (Baldwin, 2001).SGAPs are mainly enzyme left over after starch biosynthesis, the enzyme include Starch Synthase (SS1) (57kDa), SSII (77kDa), SSII (110-140 kDa) and Granular Bound Starch Synthase (GBSS) (60KDa) (Baguma, 2004). Starch from different botanical origins have different contents of SGAP.SGAP are alleged to be involved in influencing viscoelasticity of starch matrix during gelatinization.Starch lipids are mainly found on cereal starch granule, and are mainly found as free fatty acids or phospholipids. Maize starch is comprised of small amounts of free fatty acids and trace phospholipids, whereas rice starch contains substantial

amountsof phospholipids and little free fatty acids. Tuber and root starch contain insignificant (negligible) amount of starch lipid. Lipids and phospholipids form stable complex with starch granule and thus result in restricted swelling (Chung *et al*., 2011). Apart from phospholipids content, starch also comprises of substantial amounts of phosphate monoesters. These phosphate monoestersare covalently bound to amylopectin, thus reported to influence crystallinity and consequently gelatinization enthalpy (Hoover, 2001). It also influences clarity of starch paste and viscosity, while presence of phospholipids results in opaque and lower viscosity pastes (Copeland*et al*., 2009).

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1.4.3Starch granule morphology,size and its crystalline nature

Starch granule is made up of alternating amorphous and crystalline shells which are known as growth rings (Fig.2). Amorphous zones are thought to contain amylopectin in less ordered state, interspaced with amylose and crystalline zone containingbundles of amylopectin double helices (Biliaderis, 2009).Starch from different botanical originsexhibits different morphological structure. Different starch shapes have been observed from spherical, oval polygonal and disc shape. Large granules (A-type) of wheat starch exhibit disc shape whereas small granules (B-type) are spherical in shape(Jane, 2006)Cassava starch granulesare mostly round with flat surface while sweetpotato starch granules are mostly polygonal (Moorthy, 2002). Starch granule diameter varies from <1µm (amaranths and pigweed) to 100µm (canna starch) (Hoover, 2001).

Different techniques have been used to study granule surface structure and molecular organization. Scan Electron Microscope (SEM)(Pérez and Bertoft, 2010)and Atomic Force Microscope (AFM) (Waduge,2012) are some of imaging techniques in enhancing understanding of starch granule surface. The former has advantage of allowing the sample to be imaged under ambient conditions. Both techniques reveal the presence of

‘blocklets’, resistance unit material in a chemically degraded starch. Alternating ring crystalline and semi-amorphous amylopectin lamella exists in the blocklet (Peréz and Bertoft, 2010). Blocklet size has been hypothesized to influence susceptibility of starch granule to enzyme catalyzed hydrolysis.

Carbon 13- Nuclear Magnetic Resonance (C-NMR)investigation revealed starch granule contains more double helices that are not part of extended crystalline lamella and that amylopectin is more of semicrystalline shells. The branches of amylopectin molecule form double helices that are arranged in crystalline domain (Peréz and Bertfort).Crystallinity is a measure of ordered structure (packing) of amylopectin side chain and double helices in the starch granule (Delcour *et al*., 2010).Starches crystalline structure is described as either A,B or C type crystalform (pattern), C type is a mixture of A and B (Singh *et al*., 2003). A type starches possess tightly packed double bonds while B type have more open packing of helices. A type has side branches scattered in both amorphous and crystalline regions but has shorter branching points compared to B type. B type starch has most branch point clustering in amorphous region. Hydration rate is lower in compacted A-type while B type has more interhelical water (Singh *et al*., 2003). Normally, cerealstarches have A type, while tubers have a B type with exception of cassava which has A type (Tester *et al*., 2004) or mixture of both (Zhu 2014)and legume that have C-type(Tester*et al*., 2004). Most of starch granules have degree of crystallinity between 15 and 45%,depending on botanical source (Peréz and Bertoft, 2010). Crystallinity is considered to influence overall granule functional properties like gelatinization temperature and enzyme digestibility. Differences in susceptibility to enzyme hydrolysis between A and B type raw starch have been reported (Lehmann and Robin 2007; Tester *et al*., 2004).

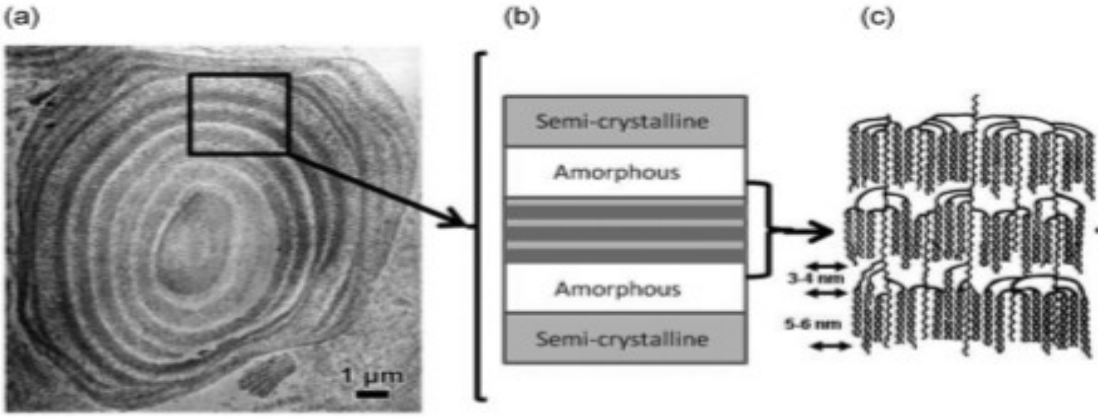

Figure 2:Starch granule cross section showing growth rings of amylopectin. (a)ultrathin section of waxy maize hydrolyzed by H_2SO_4 and stained with uranyl acetate and lead citrate; (b) alternation of semi-crystalline and amorphous rings; (c) cluster model of amylopectin(adapted from Peréz and Bertoft, 2010).

1.5.4Structural transition related to physical properties

The basic underlying factor that influences the functional properties of starch is its crystalline order. When starch is heated in excess water above certain temperature, disruption of molecular order occurs and crystalline structure melts. Gelatinization of starch during this collapse of the crystalline structureand starch granule undergoes irreversible swelling and amylose leaching (Biliaderis, 2009). This process is accompanied by irreversible changes in granule swelling, uncoiling and dissociation of double helices and starch solubility (Singh *et al*., 2003). During cooling this suspension will form an elastic gel as a result of formation hydrogen bonding between starch chains. These molecular interactions occurring during cooling is what is known as retrogradation (Hoover, 2001). Initial gel firmness during retrogradation is attributed to the formation of amylose matrix gel and crystallization of amylopectin.Amylose has high tendency to retrograde and produce tough gels and strong films while amylopectin is more stable and produces soft gels and weak films. During storage of starch gel, helical association of amylopectin and crystallization of amylopectin outermost short branches occurs.This phenomenon is termed ‘syneresis’ (Charoenrein and Preecha, 2010). High level syneresis

is undesirable for starch used for making frozen or refrigerated food products (Doue *et al.,* 2014).

Starch can absorb water 30% of their weight, at room temperature without any visible changes. When heat is applied starch granule absorbs more water and swells. At a certain temperature, polysaccharides will start to leach out of the granule mainly amylose (Biliaderis 2009).Swelling capacity is the ability of the starch to hydrate under specific condition and greater swelling capacity is an indication of weaker binding forces in starch granule (Copeland *et al.,* 2009).Swelling capacity and solubility increases with increase in temperature. Increased temperature in the presence of water,weakens the binding forces holding the starch molecules thereby developing starch molecule mobility(Lawal *et al.,* 2011). Lipid and phosphate monoester influences the swelling power of starch granule. Phosphate monoesters increase swelling due to its negative charge, while lipid starch complexes inhibit swelling. This is evident for cassava (no lipids) and potato (contains phosphate monoesters) starchesthat have high swelling power compared to others types of starches (Waterschoot *et al.,* 2015). Starch with high swelling power is preferred for use as food thickener and binding agent as glue and pastes in non-food industries (Doue *et al.,* 2014).

Viscosity development during pastingis accompanied by changes in rheologicalbehavior of heated starch suspension as a result of granular swelling and solubilization of macromolecules upon gelatinizationand further increase in viscosity and formation of gel network. It encompasses changes in granule organization proceedings after onset of gelatinization (Waterschoot *et al.,* 2015). The change in viscositycan be measured by using Rapid Visco Analyser (RVA). Pasting temperature is that at which rise in viscosity

is detected. Peak viscosity is the highest viscosity reached and cold paste is that measured at the end of heating and cooling cycle. During cooling, starch suspension increase in viscosity as leached out amylose forms gel network. Starches with high granule swelling power are prone to granule breakdown at higher temperature than their counterparts (Singh *et al*., 2003).

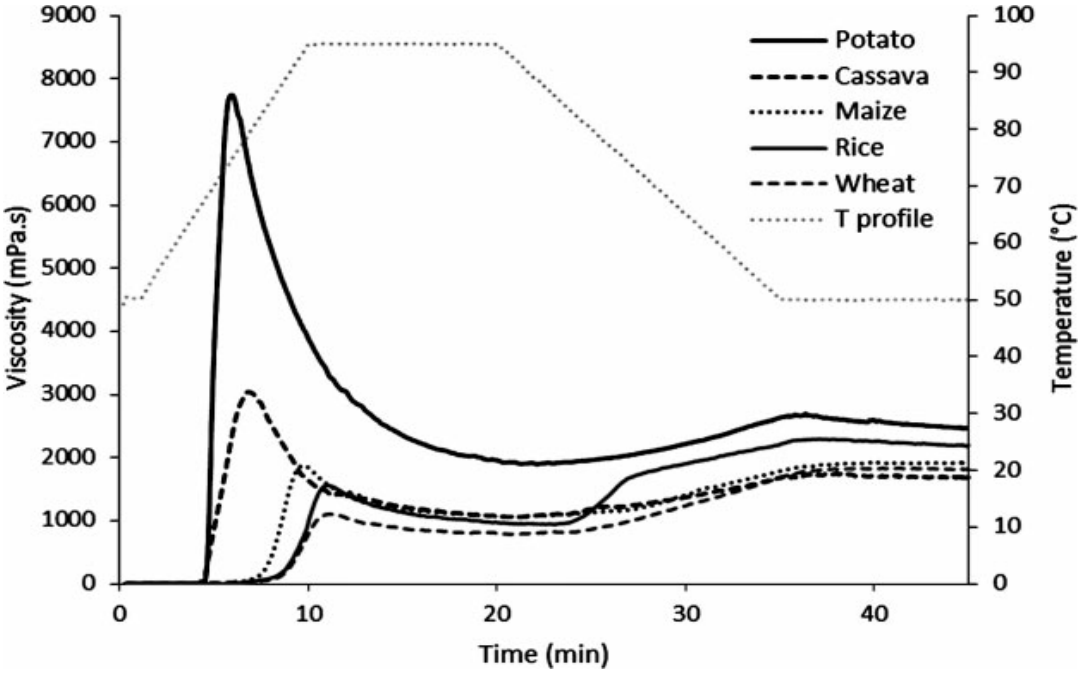

Figure 3:Pasting profiles of 8.0 potato, cassava, maize, ice and wheat starches in water measured with RVA (adapted form Waterschoot *et al.*, 2015)

1.4.4 Starch digestibility

Digestibility of starch by enzymes into glucose, is of nutritional importance for application of starch in different food industries (Copeland *et al.,* 2009). There is a wide variation in digestibility among starch from different botanical sources. These differences in digestibility of native starch areattributedto several granule characteristics, such as nature(source), granule size, amylose-amylopectin ratio and amylose chain length (Noda *et al.,*2008). The susceptibility of starch granule to enzyme hydrolysis is

known to be affected by crystalline structure. Chung*et al*. (2011) observed a difference in digestibility of starch granule, A-typestarches were more susceptible than B-type and C-type was intermediate. Starch that is not rapidly degraded (slow digestible starch) by upper gut has been shown to have healthy benefits. The slow digestible starch offers slow release of glucose in blood stream resulting in reduced postprandial glycemic and insulin response after consumption of food. This has advantage of maintaining a slow increase in blood glucose levels(Lehman and Robin, 2007;Copeland *et al.,* 2009). Starch processing results in increased digestibility at values higher than 90% at the end of incubation.Starch processing results in destruction of starch crystallinity and molecular organization and renders them susceptible to enzyme attack (Singh *et al.,* 2010).

1.5 Problem Statement and Justification

Processing of cassava is very important due to its rapid postharvest deterioration and high cyanide content, but it can also add value and provide employment opportunities. Postharvest handling, processing and marketing of cassava has to be carefully considered if their contribution to the livelihood of poor people is to be increased. Cassava starch is amenable to use in various applications both dietary and industries including gelling, bakery, textile pharmaceutical and paper industries. Development of starches also occupies a central position in the quest for their commercialization hence increasing production at farmer level. To meet such high demands of cassava in Tanzania,cassava cultivar selection, production and processing need to be improved.

Better understanding of physicochemical properties of cassava starch will help to identify different industrial applications of cassava starch to drive cassava varieties towards commercialization. Genotypic variation has been reported in cyanide content;

pasting properties and starch yield. The observed differencesare indications that cassava landraces can be targeted for use in different food products and industries. Farmers and starch producers can benefit if starch extraction is optimized in terms of choice of appropriate cultivars, locations and correct timing of harvesting and proper method of starch extraction. Cultivars with high cyanide content have been reported to have high dry matter and therefore can be targeted for starch production. This will reduce the risk for cyanogenesis outbreaks and also provide income for farmers and hence improve their food security. To target starch production for maximum profit, farmers need information on the right cultivars and optimal harvesting period.

Genetic identity of asexually reproduced crops is of fundamental importance. There is lack of one to one relationship between farmers' variety names and a single clone (Gullberg *et al.*, 2007).Farmers give names to landraces according to their local languages or phenotypic appearances or names of a personswho introduced the cultivars in a particular area. Across geographical location landraces can bear different names but can be genetically the same. Diverse of cassava cultivars exist in farmers' fields, hence an insight into genetic divergence of the available germplasm is crucial for breeding and conservation strategies. This will help in avoiding/ not to introducelandraces, which have the same genetic value or originating from same gene pool as local germplasm. The work done by Masumba (2007) for cassava landraces in Southern, Lake and Eastern zones of Tanzania, comparing the genetic similarities between local and improved cassava landraces using 12 SSR markers revealed that genetic similarities do exist between local and improved cassava grown in a region.This implies that there exists a rich diversity (gene pool) in farmer field yet to be exploited, for cassava improvement programs. A study of genetic diversity of farmers preferred cassava landraces in Eastern zone, will therefore establish a knowledge base of these varieties so that they can be targeted for

increased productivity and hence increased starch yield for income generation.Although significant work on cassava has been done on breeding varieties with resistance to diseases and pests (Kanju *et al*., 2007), there exists a gap in knowledge on genetic diversity, starch physicochemical properties and cyanide levels in farmers preferred cassava landraces in Tanzania. Therefore, this study aims to increase efficient use of local germplasm as well as cassava products in diverse industries. Being a multipurpose crop that feeds people in Tanzania, information on cassava genetic diversity, starch characteristics, cyanide levels and potential for industries will help to target and improve cassava production, processing and commercialization and in turn improve livelihood of rural people.

1.6Objectives

1.7.1 Overall objective

To study genetic diversity, starch physicochemical properties and cyanide level in cassava landraces in eastern zone

1.6.2 Specific objectives

i) To collect farmer's indigenous knowledge on cassava variety selection and conservation in Eastern zone of Tanzania.

ii) To characterize farmers preferred cassava landraces in Eastern zone of Tanzania using morphological and molecular techniques.

iii) To assess variation in physicochemical characteristics and functional properties of cassava starch.

iv) To determine the effect of genotype, location and harvesting time on fresh root yield, starch yield and root cyanide content of cassava landraces.

1.7 Organization of the Thesis

The thesis is organized in three chapters. The first chapter comprises of introduction and key highlights on cassava biology, agronomy, cyanogenesis, diversity and starch characteristics. The chapter ends with a brief description of methodology and data analysis. Chapter two consists of four sections (papers), each addressingone of the four specific objectives of the study. The third chapter contains the overall conclusion and recommendation based on the study findings.

1.8Materials and Methods

1.8.1Location of study

This study was done in the Eastern zone of Tanzania andpurposive sampling was employed to identify districts and villages withhigh cassava production. Major cassava growing districts were selected including Mkuranga (Coast), Muheza (Tanga) and Morogoro rural (Morogoro).

1.8.2Survey and collection of landraces

Participatory methods were used in data collection and structured questionnaire(Appendix 1) wasadministeredto cassava farmers. Before administered, questionnaire were pretested at Chambezi and after that necessary corrections were made. During the survey landraces with their passport data (Appendix 2) were collected (Fig.4).

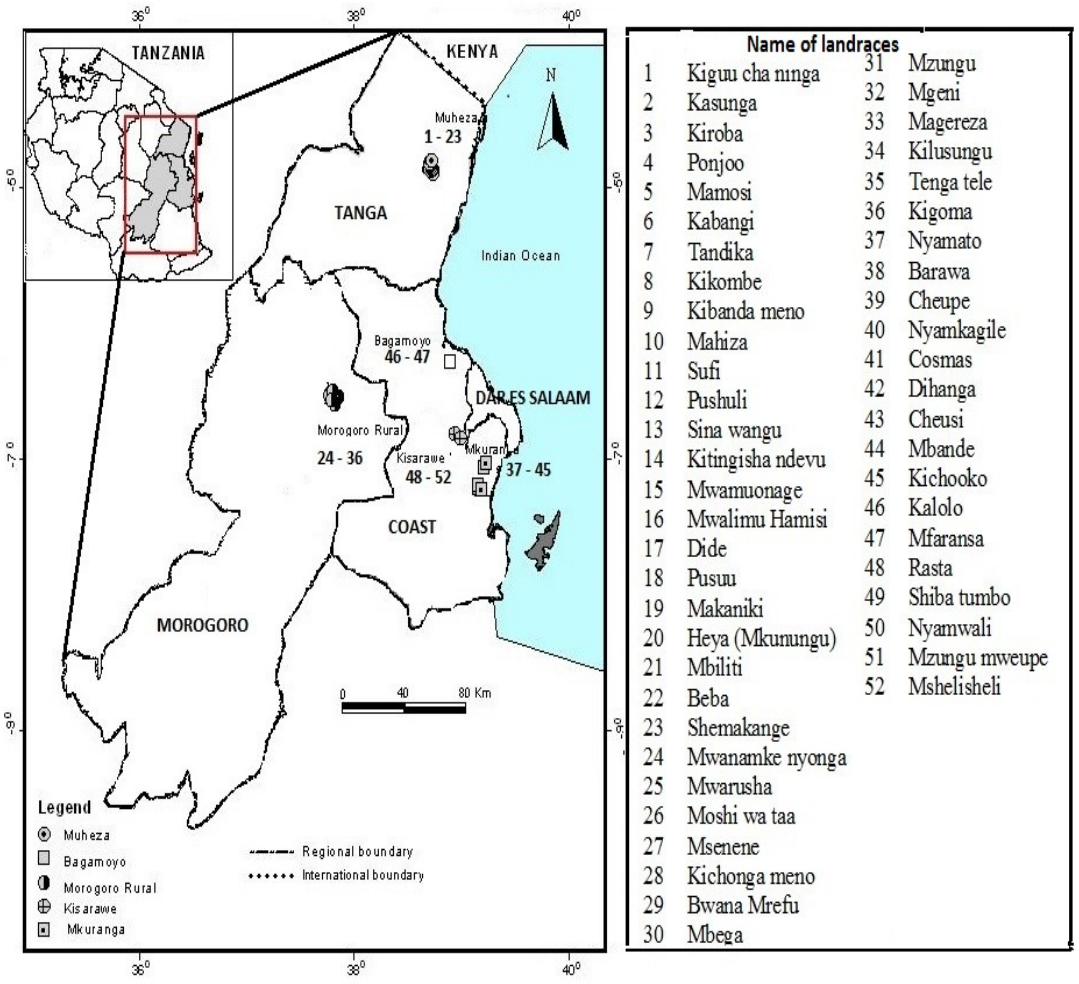

Figure 4: Map of Tanzania showing areas where 52 cassava landraces were collected

1.8.3Morphological and Molecular characterization of cassava landraces

Fifty two cassava landraces were collected and planted in Chambezi, Bagamoyo in three replicates and morphological characterization (Appendix 3 and 4) was done according to Fukuda *et al.*(2010).DNA extraction for molecular characterisation was done according to Dellaporta *et al.* (1983).Library preparation and sequencing of DNA was done using Illumina genome sequencing (Monson-Miller *et al*., 2012) (Appendix 5).

1.8.4Starch characterization and study on the effect of genotype, location and harvesting time on root yield, starch yield and root cyanide content of cassava landraces

A total of six commonly grown cassava landraces were selected and planted in a split plot design replicated three times using plot size of 5m x 5m with spacing of 1 m x 1m. Cassava starch was extracted according to Forsyth *et al.* (2002) and starch characterization parameters were collected. For GxE study, three locations namely, Bagamoyo, Amani and Magadu were used for the study. Starch yields, dry matter, starch content and cyanide content were collected at three harvesting periods (9, 12, 15 months after planting (MAP)).

1.8.5 Data analysis

All collected data were summarized into Excel Window program (2010). STATA 12 was used to analyze descriptive statistics and regression analysis for survey data. Starch characterization data were subjected to Analysis of Variance (ANOVA) to determine statistical differences. When statistical differences were found, mean separation was done using Tukey's multiple comparison test ($p \leq 0.05$). Pearson correlation (r) was done to elucidate the degree to which variables analyzed were related (SPSS 16). Multivariate analysis PLS-DA was performed using Metaboanalyst online tool (TMIC, Canada).Clustering was performed by Unweighted Pair Group Arithmetic Method (UPGMA) using NTSYS 2.1(Rohlf, 2009) for morphological and molecular characterization data. ANOVA was performed for yield, dry matter, starch content and cyanide content for individual GxE trails using GENSTAT 11. Genotype by Environment interactions (GEI) for starch yield and cyanide were further analysed using GGE biplot (Genotype and Genotype Environment interaction, GGE) methodology to explicate superior and stable landraces.

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CHAPTER TWO

Paper One: Farmer's Knowledge on Selection and Conservation of Cassava (Manihot esculenta) Genetic Resources in Tanzania (published).

Paper two: Genetic diversity of farmer preferred cassava landraces in Tanzania based on morphological descriptors and Single Nucleotide Polymorphisms(published).

PAPER THREE

Assessing variation in physicochemical, structural and functional properties of root starches from novel Tanzanian cassava (Manihot esculenta Crantz.) landraces

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Abstract

Cassava is an ideal 'climate change' crop valued for the efficient production of the starch stored in its roots. Here, the physicochemical properties and functionality of starches isolated from six cassava landraces were explored to determine (i) how they varied compared to those described in published studies of cultivated cassava, (ii) the extent of variation in starches among the landraces, and (iii) their potential utility as value-added food and biomaterials. For most parameters assayed, the landraces exhibited a narrower range of values compared to published data, perhaps indicating a local preference for a certain cassava-type. Among landraces, dry matter (30-39%), amylose (11-19%), starch (74-80%) and reducing sugars content (1-3%) varied when expressed on a dry weight basis ($p \leq 0.05$); however, only one of the six genotypes differed in crystallinity and mean starch granule particle size, while glucan chain distribution and granule morphology were the same. In contrast, the starch functionality features measured: swelling power, solubility, syneresis, and digestibility differed ($p \leq 0.05$). This was supported by Partial least square discriminant analysis, which highlighted the divergence among the cassavas based on starch functionality, permitting suggestions for the targeted uses of these starches in diverse industries.

Key words:*Manihot esculenta*, cassava starch, starch functionality, starch structure, starch digestibility

1.0 INTRODUCTION

The cassava (*Manihot esculenta* Crantz) has recently been the focus of concentrated research efforts to fully exploit its potential as a sustainable multipurpose crop [1, 2]. Cassava is valued primarily for its starch, which accumulates to 80% of the root dry weight and is an important source of calories for over 800 million people in Sub-Saharan Africa, Asia and South America, especially among food-insecure communities [1, 3, 4]. Cassava is also cultivated as a cash crop as there is a growing demand for the starch to be used as a feed, biofuel, and raw material for different industries [4]. Cassava starch is particularly suited to make paper, textiles, confectioneries, sweeteners, fructose, alcohol and monosodium glutamate, due to its high purity, resistance to acid, and unique pasting properties [5-7]. There has been a recent surge in the popularity of cassava, reflected in a 60% increase in global production between 2000 and 2012, with further projected increases, fueled by several favorable characteristics of cassava compared to cereals [1]. Cassava show higher productivity and adaptability to environmental stresses, require fewer agricultural inputs [8], and have a flexible production and harvesting window [9, 10]. This ability to produce roots with high starch yields useful as food, fuel and polymer even under adverse conditions, has led to the designation of cassava as an ideal 21st century 'climate-change' crop [1].

Starch accumulates as round and irregularly-shaped granules of approximately 13 μm in cassava roots [11]. Starch consists of two large molecular weight glucan polymers called amylose and amylopectin, which occur in an approximate 20:80 ratio in non-mutant cassava [11]. Both glucans are made up of chains of α-1,4 linked glucoses which are intermittently branched by α-1,6 glucosidic linkages [12]. There are branch points placed

approximately every 20 glucoses in amylopectin but only every 4-100 glucoses in amylose [13]. The branching pattern in amylopectin is precise, so that clusters of glucan chains of 3-4 distinct lengths are produced in an orderly array [14], which is essential for the formation of a semi-crystalline starch macromolecule [12, 15]. Collectively, the precise packing of glucoses within the starch molecule, the amylose-to-amylopectin ratio, and glucan chain distribution, all influence granule morphology and size [12, 15, 16].

The functionality of starch depends on its molecular structure [14, 17]. In many cases, starch is used after gelatinization i.e. after it is heated in excess water [18]. This leads to the collapse of starch molecular order and to irreversible changes in water uptake, granule swelling, solubilization, and viscosity and pasting development [15]. These processes and the properties of the resulting gels and pastes formed during subsequent cycles of freezing and thawing, or after enzymatic hydrolysis, can indicate the suitability of starch for various applications [14, 17]. To monitor these changes, granule solubility, swelling, gelatinization, pasting, retrogradation, syneresis and digestibility are often assessed [15]. These starch functional properties sometimes correlate with specific granule characteristics. For example, the *in vitro* digestibility of many native starches correlates with crystalline organization [12], particle size [19] and the ratio of amylose and amylopectin [20], but because of the complex interplay of multiple starch features on functionality, it can be difficult to identify these relationships.

Because of the growing importance of cassava as a subsistence and cash crop, and its great versatility of uses, our aim was to characterize the physicochemical and functional

properties of starches from six understudied famers-preferred landraces commonly grown in Eastern Tanzania (Fig. 1). Propagation of landraces aids *ex-situ* conservation of locally-adapted genetic resources [21], and documenting their organoleptic properties is important both for scientific cataloguing of existing natural diversity, and to help farmers, industrial end-users and breeders select the most appropriate genotypes for their immediate need and for future breeding purposes. There is genetic evidence using Simple Sequence Repeat markers for distinct genetic subpopulations among African cassava with a "pivotal position for Tanzanian landraces" [22]. However, the starches from cassavacultivars adapted to grow in this region have yet to be studied. These genotypes may be distinct from those grown in Asia, South America and other parts of Africa [11, 22-24], and in addition, were not subjected to intense plant breeding and may synthesize starches with distinct profiles [25]. Such data could broaden our knowledge of starch-structure function and provide a wider selection of starches for commercial application. In current study, we comprehensively investigated the compositional, structural and functional properties of starch extracted from the Tanzanian cassava landraces, and use both correlation and multivariate analyses to understand the diversities of these starches and their foreseeable end-uses (Figure 1).

2.0 MATERIALS AND METHODS

2.1 Materials

This study used starch isolated from six farmer-preferred cassava landraces collected in February 2012 from the Eastern region (36.98°E 6.83°S) of Tanzania. These cassava landraces were: *Nyamkagile, Kiroba, Kalolo, Kibandameno, Kilusungu* and *Msenene*(Supplemental data, Figure 1).A randomized complete block design replicated

three times using plot size of 5 x 5 m^2 with spacing of 1 x 1 m^2 was deployed. Roots were harvested 9 months after planting. Three healthy plants were selected from each plot and harvested (Supplemental data, Figure 1). Marketable roots were selected and immediately brought to the laboratory for analysis to avoid deterioration. Starch was obtained from the entire root.

2.2 Methods

Dry matter content. Cassava root dry matter (DM) was determined according to Benesi *et al*. [26]. Fresh cassava roots were grated. Exactly 200 g of grated cassava root (w_1) were put in pre-weighed petri dishes (w_0). The samples were oven dried at 60 ^oC for 72 h, and weighed (w_2) after removal from the oven. DM was calculated as follow;

$$\text{DM } (\%) = \frac{w2-w0}{w1-w0} \times 100$$

Starch extraction and purification. Cassava starch extraction was done according to Forsyth *et al*. [27] with slight modification. Ground cassava samples were homogenized with 0.3% (w/v) Sodium metabisulphite. The homogenate was filtered through 4 layers cheesecloth. The process was repeated 3-4 times until a clear solution was obtained. The filtrate was centrifuged for 5 min at 3200 *g* (Eppendorf Centrifuge 5810R) and the supernatant was discarded. The residue was washed with sodium dodecyl sulfate (SDS), centrifuged and then washed five times with nanopure water to get rid of the SDS and sodium metabisulphite. The starch was then washed twice in 100% toluene and the supernatant removed. The starch was left to dry overnight in a laminar hood at 37 ^oC.

Starch content and reducing sugars. Starch content was quantified by an enzymatic method according to AOAC method 996-11 [28] with a slight modification. The starch

sample (0.1 g) in a glass test tube was mixed with 0.2 mL of 80% (v/v) ethanol. Then, 110 U thermostable alpha amylase (diluted with 0.08 M phosphate buffer, pH 6) was added and the mixture was incubated in a boiling water bath for 6 min. A 4 mL of 200 mM acetate buffer (pH 4.5) and 3U of amyloglucosidase were then added and the sample was then incubated in a shaker water bath at 60 ^{o}C for 3 h. The reaction was terminated by boiling for 30 min. The samples were filtered through Whatman No. 42 filter paper and the amount of glucose wasdetermined by glucose oxidase peroxidase (GOPOD) method. Starch samples of 0.1 mg were boiled in 80% (v/v) ethanol and sugars were extracted. Sugars were quantified by adding dinitrosalicyclic (DNS) acid to ethanol-soluble extracts, boiling, and recording absorbance at 540 nm in a spectrophotometer [29].

Amylose/amylopectin content. Amylose was determined using an Amylose/amylopectin assay kit (K-AMYL, Megazyme International Ireland Ltd., Wicklow, Ireland). For each measurement 20 mg of purified starch was used. Cassava starch samples were solubilized in DMSO, and the amylose was separated from amylopectin using Concanavalin A as previously described by Tanadul *et al.*[29]

Phosphate Content. This was determined spectrophotometrically as described by Prokopy [30]. Starch was mixed with ammonium molybdate and antinomy potassium tartrate under acidic conditions to form an antimony-phospho-molybdate complex. The absorbance of the complex was measured at 880 nm and corresponded to the concentration of phosphorous.

Starch granule particle size distribution and morphology. At least 10 mg of purified starch was injected in the Microtec Analysette 22, laser scattering particle size distribution analyzer (Quebec, Canada). The frequency of granule size distribution was recorded [29]. The morphology and diameter of the cassava starch granule were estimated using Scanning Electron Micrographs (SEM*)* on the basis of the scale bar provided on SEM micrographs [31].

Relative crystallinity (%). X-ray Diffractograms of purified starch sampleswere obtained using a Scintag XDS 2000-ray diffractometer with XGEN4000 Scan Generator model (Scintag, Sunnyvale, CA), operating at current of 40 mA, filament of 3.23 Å, and power of 1.8 kV. The scanning range was 2-40° 2θ and the scan speed was 0.02° per second [31]. The degree of crystallinity was quantitatively estimated by calculating the relative peak intensity following method previous described by Wang *et al*. [32] with slight modification. A smooth curve connected with the peak baseline was computer-plotted on diffraction. The area above the smooth curve was regarded as the crystalline portion (A_c), while the lower areas between the smooth curve and a linear baseline that connected the two points of intensity at 2Θ of 5-30° was taken as amorphous section (A_a). The upper diffraction area and the total area were measured using the image tool software (UTHSCSA, San Antonio, Texas). The ratio of upper area to total diffraction area was taken as the relative crystallinity (RC).

$$RC\ (\%) = A_c / (A_c + A_a)\ X\ 100.$$

Branch Chain Length Distribution of Amylopectin by High-Performance Anion-Exchange Chromatography with pulsed amperometric detection (HPAEC-PAD)

The branch chain length distribution of amylopectin was determined following the method of Bertoft [33]. The starch samples were debranched using isoamylase (4 U, isoamylase from *Pseudomonas sp*., 1000 U/ml, Megazyme, Ireland)and pullulanase (2.88 U pullulanase M1 from *Klebsiella planticola*, 720 U/ml, Megazyme, Ireland) and analyzed by high-performance anion-exchange chromatography (Dionex ICS-5000 system, Sunnyvale, CA), equipped with a pulsed amperometric detector (HPAEC-PAD). The anion-exchange column, i.e. CarboPac PA-100 column with a guard column was used for analysis. A gradient eluent was composed of eluent A (150 mM NaOH) and eluent B (150 mM NaOH, 500 mM NaOAc) with the gradient elution (130 min) described as follows: 0-9 min, 15-36% B; 9-18 min, 36-45% B; 18-110 min, 45-100% B; 100-112 min, 100-15% B; 112-130 min 15% B. The flow rate of eluent was at 0.25 mL/min flow rate throughout the analysis.

Assessment of starch functionality

Swelling power and solubility. Swelling power andsolubility of cassava starch suspended in deionized water and heated at 70 ^{o}C and 90 ^{o}C were determined by the method previously described by Wang *et al*. [34].

Syneresis. Starch samples were suspended in deionized water (6% w/v) and were heated in a boiling water bath for 30 min with constant stirring. After cooling to room temperature, the samples were stored at -20 ^{o}C and 4 ^{o}C for 22 h, and later cooled in water bath (30 ^{o}C) for 1.5 h. The samples were then centrifuged at 1050 *g* for 20 min.

Syneresis at -20^{o}C and 4^{o}C was determined as the amount of water released after centrifugation.

Rapid Visco Analysis (*RVA*). Rapid Visco-Analysis (RVA) of 8% (w/v) starch slurry was done on an AR1000-N Rheometry (TA Instrument, New Castle, DE), following method described by Thitisaksakul *et al*. [35]. The temperature profile was as follows: hold at 50 °C for 1 min, heat to 95 °C over 4.5 min, hold at 95 °C for 3 min, cool to 50 °C over 4.5 min, and finally hold at 50 °C for 1 min. All RVA readings were done in triplicate.

*Starch digestibility***.** The digestibility of starch samples was estimated following the method by Salman *et al*. (2009) [36] with slight modifications. The starch (20 mg) was incubated in a shaking water bath at 37 ^{o}C in 5 mL sodium acetate buffer (100 mM, pH 4.5) containing a mixture of porcine pancreatic amylase (Megazyme™: 14 U) and amylogucosidase (Megazyme™; 0.33 U). An aliquot of 0.1 mL of incubation mixture was collected at 30 min, 1, 2, 6 and 24 h and heated in boiling water bath for 10 min. The amount of glucose produced was measured after incubating the aliquot with Megazyme™ glucose oxidase peroxidase reagent (GOPOD) for 20 min at 50 ^{o}C. Absorbance was read at 510 nm and converted to the amount of glucose against a D-Glucose standard. To examine the enzyme digestibility of the gelatinized starch samples, 2 mL of water was added to the starch before the assay. The content was mixed by vortexing for 1 min and heated in boiling water bath with gentle stirring for 20 min. After heating, the samples were placed in water bath at 37 ^{o}C to equilibrate and the above procedure for digestibility was followed.

Statistical analysis

The data reported are an average of three independent biological replicates derived from roots originating from separate plants. The data were then subjected to Analysis of Variance (ANOVA) to determine statistical difference. Statistical analyses were determined to be different at a significance level of $p \leq 0.05$ or $p \leq 0.01$. When statistical difference was found, mean separation was done using Tukey's multiple comparison test ($p \leq 0.05$). Pearson correlation (r) was done to elucidate the degree to which variable analyzed were related (SPSS 16, IBM, CA). Multivariate analysis PLS-DA was performed using Metaboanalyst online tool (TMIC, Canada).

3.0 RESULTS AND DISCUSSION

3.1 Starch physicochemical properties and dry matter content

Table 1 contains various chemical and compositional properties of the cassava starches studied. Attempts were made to compare the data generated here with published studies, with the caveat that differences in tuber developmental age, growth conditions and analytical techniques used between studies may introduce undesirable variables that mask genotypic effects [14, 37]. Among the genotypes, dry matter and amylose content varied most, then starch content, and reducing sugars, while mean particle size and relative crystallinity showed the least variation (Table 1).

3.1.1 Cassava tuber carbohydrate composition

Dry matter content. Cassava landraces tend to have a lower dry matter content compared to improved clones[11]. In an extensive screen of cassava germplasm, the 3272 landraces examined had an average dry matter of 32.8% while the values for the 772 improved clones was higher i.e. 36.7% [11]. Among genotypes values ranged from 30.6% for *Kilusungu* to 39.5% for *Kibandameno* (Table 1)and average dry matter was 34.1%, which is intermediate between the landraces and improved clones studied by Sanchez *et al.*[11]. Although it is difficult to draw accurate conclusions, it is possible that some of the Tanzanian genotypes, especially *Kibandameno*,may have been subjected to a greater degree of selection than others.

Starch content and reducing sugars. Starch varied among the Tanzanian landraces from 74.3% and 80.3% (Table 1), which is a much narrower range, compared to 70.4% to 89.9% reported by Nuwamanya *et al.*[24] for Ugandan cassava cultivars. The average

starch content of genotypes usedcin this study was 78.3%, lower than the 84.5% reported for all genotypes by Sanchez *et al.*[11]. The reducing sugar in our analysis was also higher (average 1.89%) than the average values obtained from the landraces (1.25%) and improved clones (1.56%), studied by Sanchez *et al.*[11]. Roots with a high reducing sugars content are associated with low dry matter and starch content, and is evident for *Kilusungu* and *Kalolo* (Table 1). High dry matter accumulation was selection criteria during domestication of cassava. It is therefore believed to be a trade-off between diverting resources for abiotic and biotic stress responsive mechanisms needed to deal with varying and harsh conditions, versus the greater investment in dry matter afforded by culturing improved varieties on farms [25]. Taken together, our data on dry matter, starch and sugars suggest that the Tanzanian landraces may have been selected for higher yield attributed to the accumulation of non-starch dry matter, in addition to adaptability to biotic and abiotic stress compared to other cultivated cassava.

Amylose content. The proportion of starch accumulated as amylose has a profound effect on starch functionality [38], however Sanchez *et al.*[11]showed that there was no significant difference in average amylose content among landraces and improved germplasm. Amylose content among the landraces in this study ranged from 11.9% (*Kibandameno)* to 19.4% (*Nyamkagile andKilusungu*) (Table 1). These values are lower and are less varied than the 16.96% to 28.8 % amylose reported by Mweta *et al*. [39] for Malawian cassavas and the 15.2% to 26.5% reported by Sanchez *et al.*[11] however, differences in analytical methods can lead to such discrepancies [37]. It should be noted that SDS-PAGE analysis of starch granule intrinsic proteins of these starches indicated that there was a higher level of a 63 kD protein presumed to be granule bound starch synthase I (GBSSI; which makes amylose), extracted from *Nyamkagile*, which has an

amylose content of 19 %, while the level was lower in *Kibandameno* of amylose content 11.9% (Supplemental data, Fig. 2). The amount of GBSSI protein extracted from starch varies proportionally to the amylose content of that starch [29] and is supportive of the amylose values reported here.

Phosphorouscontent. This element is present in root and tuber starches as a phosphate monoester [40], however phosphorous content in our starches was too low to be quantified spectrophotometrically. A similar result was also reported by Peroni *et al*. [38] and may be explained by the fact that the phosphorous in cassava starch is not bound to amylopectin, and is thus easily washed out during purification [5].

3.1.2 Starch granule size distribution and morphology

Differences in granule size distribution may influence starch functionality [41], and were examined by Scanning Electron Microscope (SEM; Supplemental Figure 3) and laser diffractometry (Table 1, Supplemental Figure 4). There was no apparent difference in granule size and morphology among the cassava landraces studied. Most of the granules were round, but a few polygonal granules were observed by SEM, similar to those seen in other studies [38, 39]. A distinct small and large granule size class was observed by SEM, which was also previously reported by Gumul *et al.*[42].

The laser diffractometer provided a better estimate of starch granule size distribution (Table 1; Supplemental Fig. 3). Mean granule size ranged from 12.5 and 13.8 μm, which represents more narrow variability than the 11.3 to 15.7 μm range found in four Sri Lankan cultivars [23, 43]. *Kibandameno* was the only cultivar that varied and had the smallest mean particle size (12.5 μm).

Table 1: Physicochemical composition of cassava landraces and their purified starches*

Cultivars	Dry Matter (%)	Starch content (%) dry weight	Total Reducing Sugars (%)	Amylose Content (%)	Mean Particle size (µm)	Granule size volume percent distribution (%)			RC (%)
						Small (<12 µm)	Medium (12-25 µm)	Large (25-48 µm)	
Nyamkagile	33.6±0.4^{b}	80.3±0.4^{b}	1.03±0.2^{a}	19.4 ±0.4^{c}	13.33^{b}	46.19^{a}	52.04^{a}	1.76^{a}	37.9 ± 1.1^{a}
Kibandameno	39.5±0.6^{d}	80.0±0.5^{b}	1.43±0.1^{ab}	11.9±0.5^{a}	12.50^{a}	49.87^{a}	49.14^{a}	0.96^{a}	41.4 ± 0.8^{b}
Kilusungu	30.6±0.5^{a}	77.1±1.5^{ab}	2.12±0.7^{ab}	19.2±0.3^{c}	13.21^{b}	42.58^{a}	57.37^{a}	0.00^{a}	37.0 ± 0.4^{a}
Msenene	33.4±0.4^{b}	78.4±1.5^{ab}	1.75±0.4^{ab}	17.1±0.3^{b}	13.78^{b}	42.02^{a}	55.36^{a}	2.62^{a}	37.4 ± 0.4^{a}
Kalolo	30.8±0.8^{a}	74.3±1.5^{a}	3.10±1.03^{b}	16.9±0.3^{b}	13.09^{b}	44.05^{a}	55.96^{a}	0.02^{a}	36.0 ± 0.3^{a}
Kiroba	36.7±0.5^{c}	80.2±0.8^{b}	1.96±0.2^{ab}	17.2±0.4^{b}	13.43^{b}	42.33^{a}	57.32^{a}	0.37^{a}	36.1 ± 0.5^{a}

RC= Relative crystallinity.

Values with different letters in the column differ significantly (p value<0.05);

*Mean ± SEM for three independent biological replicates.

3.1.3 Starch crystallinity

X-ray diffractometry was used to estimate the crystallinity of cassava starches (Table 1). Cassava starch may be of the A- or C_a-type crystallinity [37]. The diffraction pattern (Fig.1) showed prominent peaks at 2Θ=15.2, 23.4 and a doublet at 17.2 and 18.2, which is typical of the type A crystallinity [24, 36, 44, 45]. The relative crystallinity (RC) ranged from 36.1 to 41.4%, which is similar, but slightly higher than the values reported for Thai cassava, which averaged 35.8% [44]. *Kibandameno* was the only genotype that differed significantly having the highest value of 41.4% (Table 1). Interestingly this genotype had the lowest amylose (11.9%)consistent with the negative correlation between starch crystallinity and amylose content observed in many starches [46].

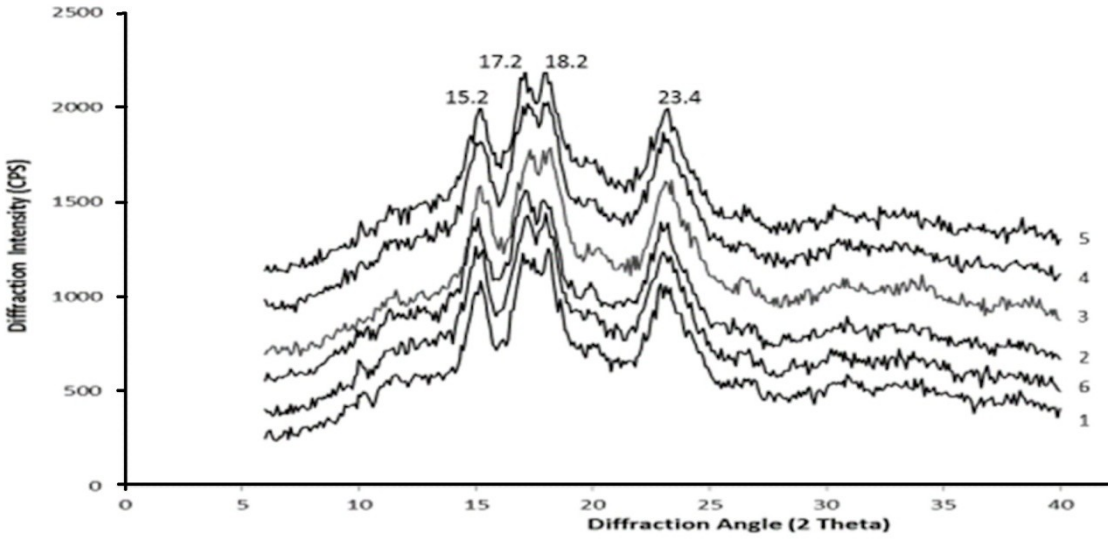


Figure 1: Wide angle X-ray diffraction patterns of cassava starch. The graph was offset for clarity and the scan signal shown after 6°2θ. There was no peak at 5.6°2θ which would indicate a C_A-type crystallinity 1=*Nyamkagile*; 2=*Kibandameno*; 3=*Kilusungu*; 4=*Msenene*; 5=*Kalolo* and 6=*Kiroba*

3.2 Branch Chain Length Distribution (CLD) of Amylopectin

Differences in glucan chain length distribution (CLD) can influence starch properties and there are relatively few reports of amylopectin branch CLD in cassava starch. For example, starches with a high proportion of very short glucan chains (degree of polymerization; DP = 6-9) of amylopectin may interfere with the normal crystalline order of starch [47], while longer chains (DP>18) may provide greater stability [23, 48].

The amylopectin chain lengths were classified into four classes and the frequency of occurrence of each determined as done by Franco *et al.*[49] for comparability (Table 2; Figure 2). There was no significant difference in amylopectin CLDs among the landraces [34, 50]. Chains of DP 13-24 (medium chains) occurred with greatest frequency (38.6-38.9%) while long chain lengths with DP 25-36 were of the lowest frequency (18.1-18.6%) for all sample analyzed ($p \leq 0.05$).

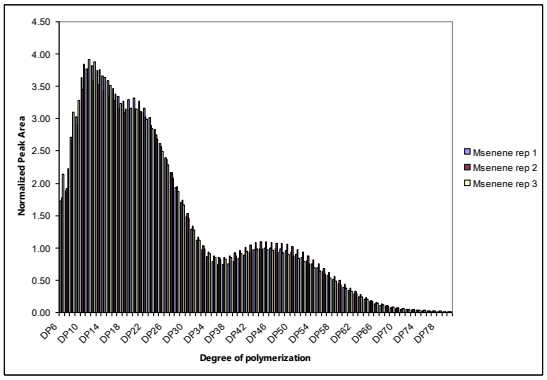
As noted, there is no extensive published data on the CLDs of different cassava starches. The cumulative studies cited in Charoenkul *et al.*[50], suggest that cassava CLD is bimodal with two peaks that occur from DP 10-15 (Peak I) and DP 36-44 (Peak II) respectively, and a shoulder at approximately 16-22 DP. These may represent the B_2+B_3, A, and B_1 chains of amylopectin respectively [50]. In our work, Peak I occurred at 12 DP, Peak II at 44 DP and the shoulder at 20 DP respectively (Fig.2). Our data for the short chains were similar to the DP 12 for Brazilian cassava starch [49] and DP 11-12 for Thai cassava starch [50]. The DP of the longer chains were more varied among the published studies; it ranged from DP 43.3-44.7 for our landraces, which is lower than the DP 47 reported by Franco *et al.*[49] and the values for our samples were narrower

compared to the DP 40-44 published by Charoenkul *et al*. [50]. No significant differences in CLD were observed among starches of cassava genotypes analyzed in those other studies.

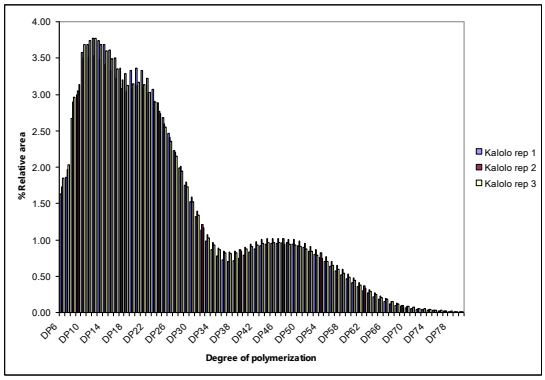
Table 2: Frequency of chain length distribution (CLD) ofamylopectin isolated from different cassava starch cultivars [a].

	Chain length distribution (CLD; %)			
Cultivars	**DP 6-12**	**DP13-24**	**DP 25-36**	**DP>37**
Nyamkagile	20.5	38.6	18.5	22.4
Kibandameno	21.4	38.8	18.3	21.5
Kilusungu	20.6	38.9	18.5	22
Msenene	21	38.9	18.1	22
Kalolo	20.5	38.8	18.6	22
Kiroba	20.7	38.8	18.4	22.2

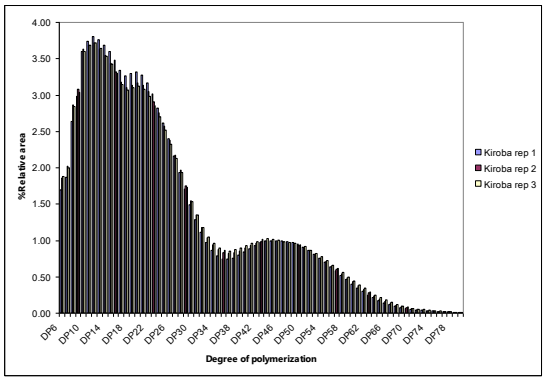
[a] Sum of peak-area ratios (%) of a group with defined degree ofpolymerization (DP)



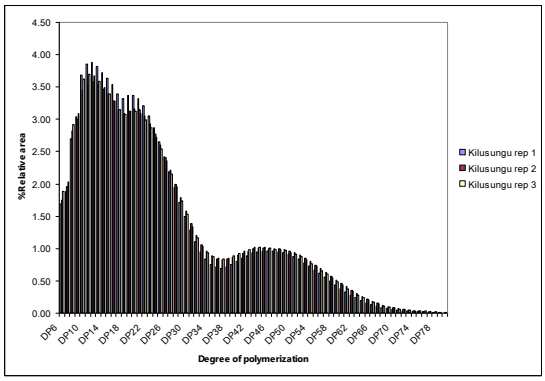
(a) *Msenene*



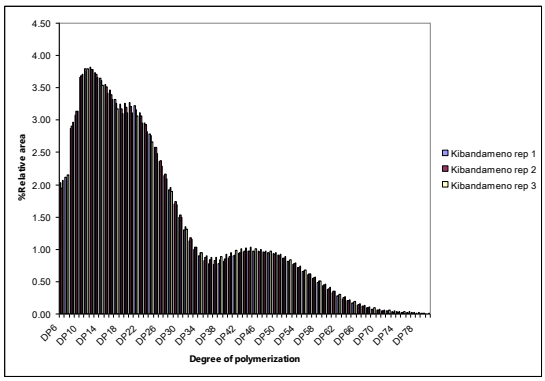
(b) *Kalolo*



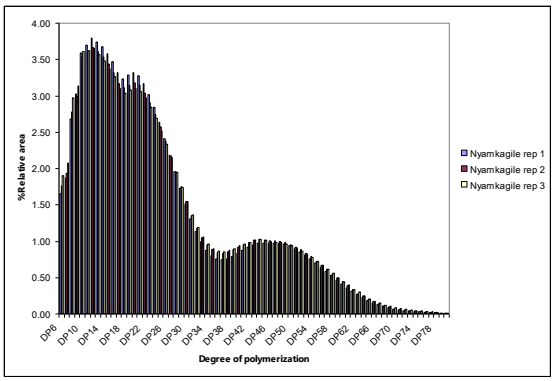
(c) *Kiroba*



(d) *Kilusungu*



(e) *Kibandameno*



(f) *Nyamkagile*

Figure 5: Branch-chain length distributions of debranched amylopectin of isolated starches from cassava landraces analyzed by HPAEC- PAD. Values were derived from three biological replicates, where each replicate constituted one root

3.3 Swelling Power, Solubility and Syneresis

Starch swelling power (SP) and solubility depend on the capacity of the starch molecule to hold water through hydrogen bonding after gelatinization. They both measure the strength of interaction between water molecules and glucan chains [51]. As thermal energy increases, the bonds between glucan chains relax and the granules absorb water and swell [52]. There were differences in swelling power (SP) among the landraces in this study, attributed to disparities in bonding forces within the starch granule [15]. *Nyamkagile* had the lowest SP at both temperatures (Table 3), which indicates strong forces holding the granules, thereby enabling them to resist swelling. Low SP in this cultivar may be due to its higher amylose content (19.4%; Table 1) which impedes granule expansion [23]. *Kilusungu* and *Kiroba* had the lowest solubility at 90 °C (Table 3), indicative of strong intra-granular organization, as more energy is required to losen these molecular forces [34].

Syneresis is the tendency of a starch gel to release water when subjected to repeated cycles of freezing and thawing during storage, and is an undesirable property for most applications. Assaying this component is important if a starch is to be used in refrigerated (4 ^{o}C) or frozen (-20 ^{o}C) food products [53]. There was a significant difference (Table 3) ($p \leq 0.005$) in syneresis among the landraces. *Msenene* had the lowest syneresis at both storage temperatures, indicating its suitability for low temperature applications. The higher levels of amylose accumulated in *Nyamkagile* may influence the higher syneresis observed at -20 ^{o}C (Table 1 and 3) while in *Msenene,* low syneresis may be due to the slightly lower amylose content (17.1%) observed although other factors are likely to influence.

Table 3: Swelling power, water solubility, syneresis and pasting properties of starches from cassava landraces

Cultivars	Swelling power (g/g)		Water Solubility (%)		Syneresis (%)		Pasting properties				
	70°C	90°C	70°C	90°C	4°C	-20°C	PST (°C)	PV (m.PaS)	PT (°C)	BV (Pas)	SV (Pas)
Nyamkagile	8.9±0.8^{a}	13.5±0.2^{a}	3.0±0.3^{a}	6.0±0.3^{ab}	23.0±1.6^{ab}	57.7±2.3^{c}	69.6±0.6^{a}	0.64±0.10^{a}	89.9±0.7^{a}	0.30±0.05^{a}	0.21±0.04^{a}
Kibandameno	10.3±0.6^{abc}	14.2±0.3^{a}	3.0±0.5^{a}	7.4±0.1^{bc}	26.8±1.6^{b}	38.3±3.3^{ab}	67.5±0.9^{a}	0.72±0.10^{a}	80.5±4.6^{a}	0.46±0.09^{a}	0.29±0.04^{a}
Kilusungu	12.3±0.4^{c}	16.3±0.8^{b}	2.8±0.5^{a}	5.0±0.3^{a}	21.5±1.8^{ab}	38.3±1.7^{ab}	66.4±0.3^{a}	0.77±0.20^{a}	76.6±2.6^{ab}	0.57±0.09^{a}	0.28±0.08^{a}
Msenene	11.7±0.3^{bc}	16.0±0.9^{b}	2.1±0.4^{a}	7.9±0.7^{c}	16.7±1.7^{a}	31.7±1.7^{a}	66.7±0.9^{a}	0.54±0.10^{a}	76.8±3.4^{ab}	0.44±0.06^{a}	0.11±0.01^{ab}
Kalolo	9.5±0.3^{ab}	15.7±0.3^{b}	3.9±0.6^{a}	8.5±0.3^{c}	38.3±1.7^{c}	48.3±2.8^{bc}	67.7±0.4^{a}	0.64±0.10^{a}	88.1±0.5^{a}	0.36±0.02^{a}	0.28±0.05^{a}
Kiroba	11±0.3^{abc}	14.5±0.5^{ab}	3.3±0.03^{a}	5.3±0.2^{a}	28.7±0.7^{b}	36.7±2.0^{a}	67.5±0.6^{a}	0.68±0.04^{a}	85.1±1.3^{a}	0.31±0.06^{a}	0.39±0.03^{a}

PST= pasting temperature, PV= peak viscocity, PT= peak temperature, BV= breakdown viscocity, SV= set back viscocity

Values with different letters in the column differ significantly (p value<0.05)

*Mean ± SEM for three independent biological replicates.

3.4 Starch Pasting Properties

Pasting properties were examined using rapid visco-analysis (Table 3). Starch pasted at temperatures between 66.4 and 69.6 ^{o}C, similar to those found in Brazilian (67.4 ± 0.2 °C) [38] and Thai (67-70 °C) [45] cassavas. In the 3272 cassava landraces surveyed by Sanchez *et al.* (2009), pasting values varied from 58 to 71 °C, but among the cassava cultivars it was 60 - 69.7 °C [11]. Our values aligned closely with those of improved clones and may have been an attribute that faced stronger selection pressure due to human preference [54]. We found no significant difference in pasting temperature among cultivars, which is in accordance with data reported by Charoenkul *et al*.[45] for 12 Thai cassava, among which 11 were highly bred varieties with different cooked root texture (i.e. mealiness and firmness). This may mean that differences in the DP of the long chain fraction among different regional cassavas (see section 3.2) were not sufficient to affect pasting.

3.5 Starch Digestibility

The rate of digestibility of raw and gelatinized starches *in vitro* by amylase and amyloglucosidase are shown in Figure 4. The digestibility of raw starches varied amongcultivars($p \leq 0.001$) with *Kibandameno* having the highest digestibility rates at all time points assayed except 0.5 h. It is of note that *Kibandameno* also had the highest proportion of small granules, highest crystallinity and the lowest amylose content, this could explain the high rate of digestibility observed for raw starch [29] (Fig.3).

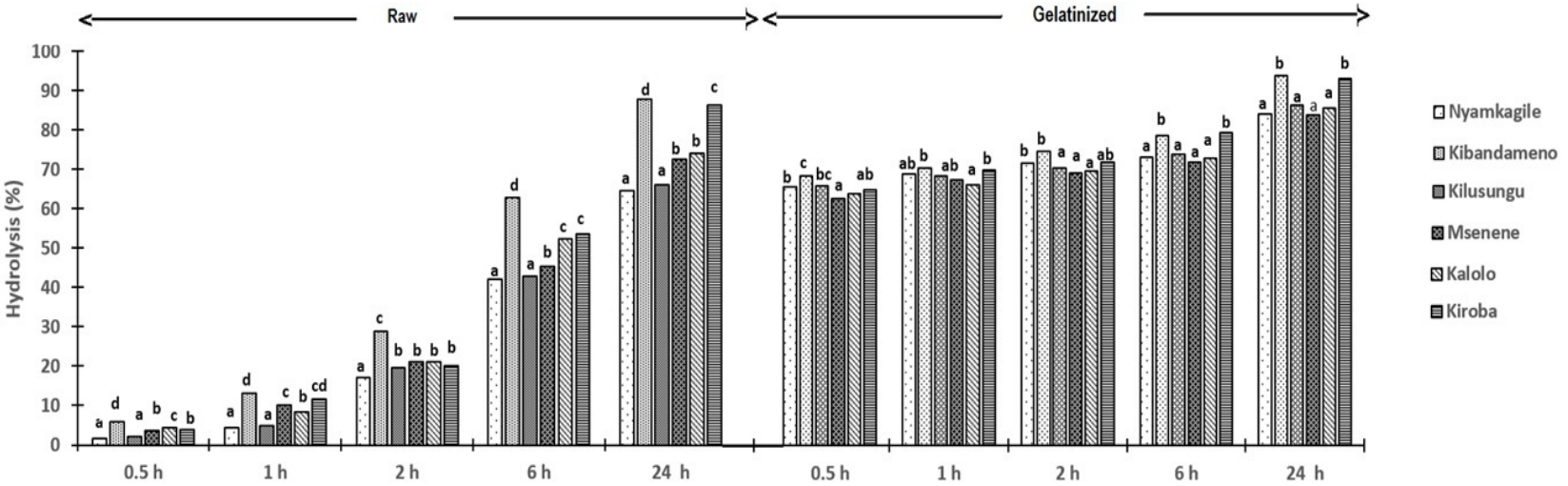


Figure 3: Enzymatic digestibility of raw and gelatinized cassava starch at different time intervals: 30 minutes, 1, 2, 6 and 24 hours. Bars with different letters within the same time point, implies are significantly difference (p<0.05) in digestibility among landraces

Gelatinization increases starch susceptibility to enzyme attack, as a result of destruction of starch's crystalline structure [12, 20]. Gelatinized starches from cassava may be more susceptible to enzyme hydrolysis than that of other roots, due to its A-type crystalline structure [6, 43, 44]. Hung and Morita [55]reported digestibility rate after 24 h of approximately 91 % for cassava which is within the range of our samples (85 - 95%; Fig.3). Gelatinized samples were rapidly degraded (approximately 3-fold faster), especially in the initial stages (30 minute, 1 and 2 h) compared with the raw starches (Fig.3). There were fewer differences among the starches analyzed, however after 6 and 24 h *Kibandameno* and *Kiroba* were digested to a greater extent than the other landraces (Fig. 3). In support of this, Moorthy and Padmaja [56] observed that the digestibility of cooked starch did not correlate with amylose content as it did with raw starch The conditions used for gelatinization may have made the starches more uniform in terms of accessibility to the starch by degradation enzymes used here.

3.6 Correlation between the Physicochemical and Functional Properties of Cassava Starches

Pearson's correlation coefficients were calculated to determine the relationship among the physicochemical and functional properties of starch (Table 4). Although there were few or no significant differences among cultivars for some starch features, small variations may nonetheless affect some aspects of starch functionality. This could be reflected in significant correlations among data, which in some instances may be causal. Such relationships ($p \leq 0.01$) were found among starch physicochemical properties and functional attributes (Table 4). Amylose content was inversely proportional to starch solubility, digestibility and RC. Wickramasinghe *et al.*[43]also reported significant negative correlation between amylose content and digestibility.

However, amylose did not correlate with either swelling or pasting properties as have been reported by other authors [24, 34, 39, 44]. This might have been due to the narrow range of amylose content data obtained from cassava cultivars used in this study, which were mainly locally grown landraces. Furthermore our analysis did not find significant correlation between starch granule particle size with digestibility and amylose content as has been previously shown by Colonna *et al.* and Lehman and Robin [19, 20] for cassava starch However, Luengwilai *et al*. [57]working with tomato fruit starches and Srichuwong *et al*. [44] with starches from different botanical sources, found no correlation between digestibility and starch particle size, which is consistent with our finding. Starch content was negatively correlated with RC, but it is difficult to draw significant biological meaning from this relationship.

Correlations were also found between the starch functional properties assessed. Swelling power negatively correlated with pasting temperature and peak temperature, but positively correlated with breakdown viscosity (Table 4). Peak temperature was also positively associated with the syneresis at -4 ^{o}C and -20 ^{o}C. *Msenene* and *Kilusungu* had the highest swelling power and lowest peak temperature compared to other cultivars (Table 3). Low peak temperature is an indication that the starch granules swell best at the lowest temperature before their physical breakdown. Granule swelling reduces amount of free water and thus increases peak viscosity, which is followed by rapid breakdown. Furthermore, starch granules from these landraces have shown the ability to absorb more water compared to other cultivars [16]. Significant interactions among pasting properties has also been observed by others [58].

Table 4: Pearson's correlation coefficient (*r*) for physicochemical and functional properties of cassava starch

	AC	SC	RS	DM	SWP	SOL	SN4	SN20	DIG	PST	PV	PKT	BV	SV
SC	0.36													
RS	0.06	0.01												
DM	-0.30	0.2	0.28											
SWP	0.19	0.01	0.52*	0.36										
SOL	-0.57**	-0.6	0.40	0.41	0.41									
SY4	-0.25	0.23	0.34	0.23	0.01	0.40								
SY20	0.07	-0.35	-0.17	-0.49*	-0.39	-0.31	0.24							
DIG	**-0.73****	0.14	0.04	0.31	-0.16	0.36	0.26	-0.21						
PST	0.12	-0.02	-0.41	-0.52*	**-0.72****	-0.45*	0.15	**0.63****	-0.12					
PV	0.01	-0.38	0.52*	0.26	0.31	-0.15	0.08	-0.01	0.07	-0.28				
PKT	0.11	0.15	-0.13	-0.37	-0.55*	-0.23	0.47*	**0.712****	-0.04	**0.81****	-0.33			
BV	-0.03	-0.27	0.31	0.41	**0.66****	0.16	-0.20	-0.39	-0.12	**-0.7****	**0.68****	**-0.82****		
SV	-0.10	-0.28	0.46*	0.19	-0.13	-0.10	0.36	0.13	0.43	0.08	**0.61****	0.19	-0.03	
RC	**-0.62****	**-0.72****	-0.29	-0.20	-0.39	0.10	-0.25	0.16	0.24	0.23	0.04	-0.12	0.06	0

AC=amylose content, SC=starch content dry base, RS= reducing sugars, DM= dry matter, SWP= swelling power, SOL=solubility, SN4=syneresis at 4 °C, SN20=syneresis at -20 °C, DIG= digestibility, PST=pasting temp, PV=peak viscosity, PKT=peak temp, BV=breakdown viscosity, SV=setback viscosity, RC= relative crystallinity.

** $p \leq 0.01$ and * $p \leq 0.05$

3.7 Multivariate Analysis

In order to gain an insight on the extent of variance among cassava landrace based on the physicochemical and functional starch properties, the data in this study were subjected to PLS-DA analysis. The PLS-DA algorithm is a supervised multivariate technique that seeks to maximize differences among groups by minimizing within group variation [59]. The PLS-DA score plot (Fig.4A) of all of the data showed clear separation among some landraces, indicative of differences in starch properties. Based on their coordinates on the plot *Kalolo* and *Nyamkagile* were most disparate among the genotypes, *Kibandameno* could be distinguished from the remaining genotypes (although there was some overlap), while *Kilusungu*, *Msenene and Kiroba* clustered together(Fig. 4C). PLS-DA loading scores (Figure 4B) explicated the reason for the separation seen on the PLS-DA scores plot. Values close to the origin on the graph make little contribution to the separation, whereas those further away make a bigger contribution [60]. Reducing sugars, set back viscosity and to a lesser extent solubility at 90 °C were the primary determinants of the PLS Score plot.

When only starch functionality data was considered, there was better separation among the landraces. *Nyamkagile* was the most disparate and *Kalolo* and *Msenene* were also distinct, only overlapping with one other genotype on the plot (Fig.4C). In contrast, *Kilusungu* shared features with three other genotypes suggesting core commonality in starch functional features among them. Setback viscosity, solubility at 90 °C and syneresis at -20 °C were most important in defining the coordinates of each genotype relative to each other (Fig.4D). It is of interest that although *Kibandameno* was most different in starch structure and composition compared with other cultivars (Table 1) these features did not lead to a corresponding disparity in starch functional attributes assayed (Fig.4C), highlighting the lack of congruency in starch structure-function relations.

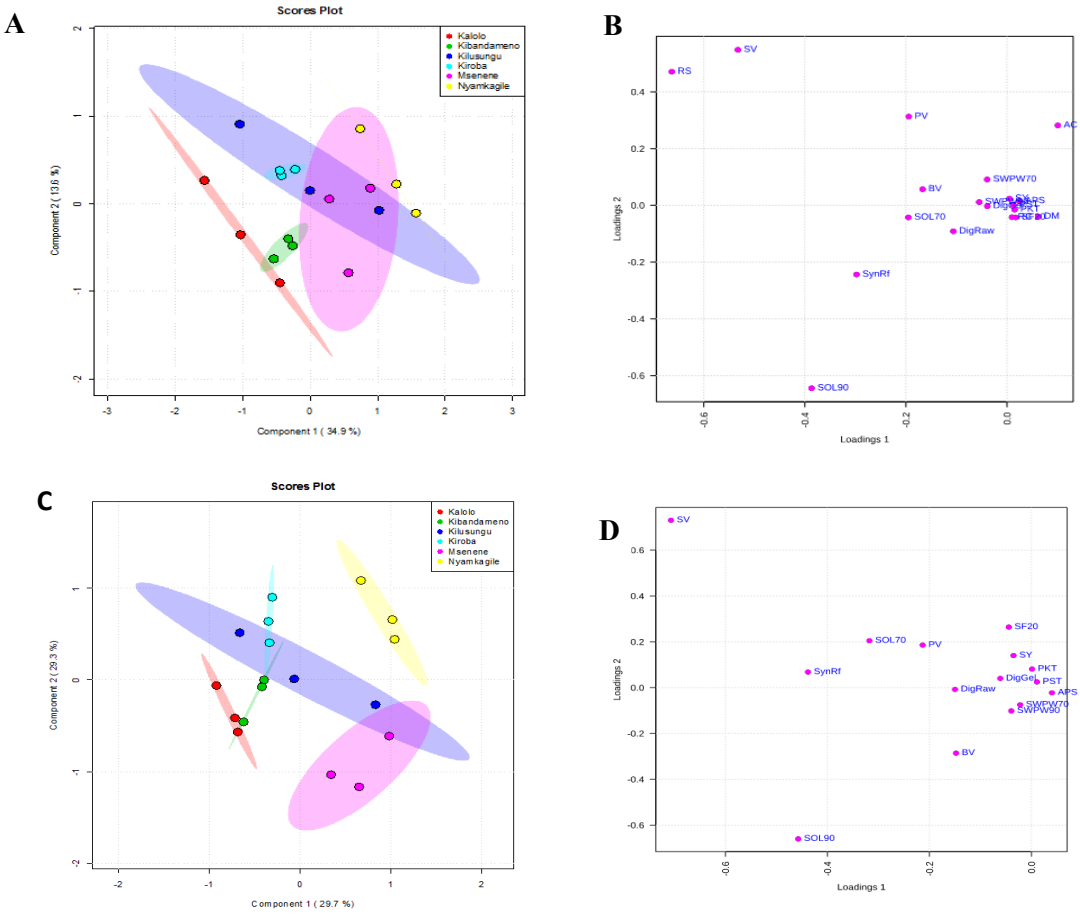


Figure 4: Partial Least Square Discriminant Analysis (PLS DA) of cassava starch (A) PLS-DA score plot showing the separation of cassava landraces using all data collected in this study. (B) PLS DA loading scores showing the parameters that contributed to the separation of cassava landrace seen in the score plot. (C) Starch functionality PLS-DA score plot showing the separation of cassava landraces using functionality data only. (D) Loading scores for the score plot in 4C.

Key is as follows: AC= amylose content, APS= average particle size, BV= breakdown viscosity, DigGel = digestibility of gelatinized starch after 24h incubation, DM= dry matter, PKT= peak temperature, PST= pasting temperature, PV= pasting viscosity, RC= relative crystallinity, RS= reducing sugars, SOL70= solubility at 70 °C and SOL90= solubility at 90 °C, SV= set back viscosity, SWPW 70= swelling power at 70 °C; SWPW 90= swelling power at 90 °C, SY = Starch yieldDigRaw= digestibility of raw starch after 24 h incubation; SynRf= syneresis at -20°C, Syn re= syneresis at 4°C.

3.8 Conclusions

The physicochemical and functional properties of starch from cassava cultivars grown in Tanzania were investigated to gain insight into their potential industrial application. The landraces explored here showed a narrower range of values for most starch parameters compared with cassavas in other studies. Dry matter, starch and sugar content data suggest that these cassavas were selected for yield and perhaps also for resistance to stress [11, 22, 25]. Although there was great similarity among starch properties measured, the genotypes could be distinguished from each other by PLS-DA. Differences in starch swelling power, solubility, syneresis and digestibility were observed during this analysis, indicating that these genotypes could be targeted for use in different food and non-food industries even though they were not always statistically significant ($p>0.05$). *Msenene* and *Kilusungu* landraces had high swelling power, which makes them potentially suitable for use as thickeners and binding agents for food and non-food uses. *Msenene* also had a relatively low setback viscosity after cooling, and low syneresis, ($p>0.05$), desirable properties in starches for gelling agents and thickeners in refrigerated and frozen food products. Similarly, *Kilusungu* had the highest peak viscosity, and a low pasting and peak temperature ($p>0.05$). This may indicate a potential difficulty that may occur when mixing this starch paste, as there may be a resultant high viscous load. *Kibandameno* starch had the highest enzyme digestibility and lowest particle size distribution ($p\leq0.05$) compared to other starches, this makes the cultivar suitable for making glucose syrup, adjuncts in breweries (fermentation stock), low fiber feed and sweeteners.*Nyamkagile*($p\leq0.05$) had the lowest digestibility and may find application in food for individuals wishing to manage their glycemic index such as diabetic and overweight patients or as efficient feedstock for biofuels.

Acknowledgements

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PAPER FOUR

Effect of genotype and genotype by environment interaction for total cyanide content, fresh root and starch yield in farmer preferred cassava landraces in Tanzania.

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Abstract

Starchy tuberous roots of cassava provide food for people but also find application in various industries.High starch yield is the important trait for commercial cassava production for the starch industries. Furthermore, cyanide present in cassava root pose a health challenge in the use of cassava for food. Cassava genotypes have varying maturity periodsthat are also environmental dependent. This study aimed at identifying suitable cassava cultivars and optimum time of harvest to maximize starch production across three environments. The study found significant difference between genotypes ($p \leq 0.001$), genotype by environment interaction ($p \leq 0.001$)and also harvest period ($p \leq 0.001$) in starch yield and cyanide content. Optimal harvesting period for maximum starch yield was 9 months after planting for most landraces. Kiroba recorded high starch yieldsof 17.4, 12.7 and 8.2 t ha^{-1} at Chambezi, Amani and Magadu respectively.Kilusungu recorded highest cyanide content of 300-400 ppm across all locationsbut Kiroba recorded highest cyanide values of 800 ppm, 15months after planting at Chambezi site. Kilusungu and Nyamkagile hadconstant cyanide contentduring the growing period at Magadu. Moreover, Kalolo and Kilusungu showed the same trend in cyanogenic potential at Amani site during the growing period.GGE biplot analysis further confirmed that Kiroba was a superior cultivar in terms of starch yield. Kilusungu had the highest cyanide contentand average starch yield, therefore it can be suitable for use in starch production. Nyamkagile was stable with low cyanide content andbelow average starch yield, therefore not recommended for starch production but safe for food.

Keywords: *Manihotesculenta*; cyanogen; crop management; GGE biplots; maturity period: starch yield.

1.0 INTRODUCTION

Starchy tuberous root of cassava provide food for people in sub-Saharan Africa and Latin America (Nassar and Ortiz, 2007). Apart from culinary requirements, cassava starch can also find application in various industries such as pharmaceuticals, cosmetics, biopolymers, textile and biofuels (Nassar and Ortiz, 2007). Cassava productivity (8.5 t/ha fresh weight) in Tanzania is low(Mkamilo and Jeremiah, 2005) compared with average world potential of 35 t/ha (Lebot, 2009), due to various production constrains (El-Sharkawy, 2004; Siritunga and Sayre, 2004).Consequently, farmers have struggled to increase area of production rather than productivity.

The increasing demand of starch for Tanzania industries, which in 2011 was estimated at5,781 tonnes per year,was equivalent to 3.2 million US dollar (TRA report, 2011). This demand can be met by cassava starch and the potential to fulfill that demand is available. Cassava farmers can benefit from marketing cassava to starch producers thereby improving their income and food security (Tonukari, 2004). To achieve this, adequate information is required on the right cassava cultivars and appropriate harvesting period so as to maximize cassava production and increase income. Maximum yield of cassava is attained after full development of the canopy, beyond which the root growth decreases (Sagrilo *et al.,* 2006). Cassava has varying maturity period depending on genotype (Ceballos *et al*., 2004). Moreover studies have reported that definite optimal harvest time for cassava is genotype and environment dependent (Benesi *et al*., 2008). There is a need to maximize cassava farmer's income by selection of genotypes with high productivity of both dry root and starch yield per unit time.

Santisopasri *et al*. (2001) working on six Thailand cassava cultivars revealed that environmental conditions during both initial and late growth stage are necessary for

satisfactory root growth and starch quality. Water stress in early development stages of cassava affects its productivity (El-Sharkawy, 2006; Santisopasri *et al*., 2001).Insufficient maturity impairsstarch quantity and quality (Santisopasri *et al*., 2001). Furthermore, high productivity of cassava will not be achieved until the right environmental conditions have been met (Benesi *et al*.,2008). Therefore, appropriate location for growing cultivars for potential high dry matter production should be sought off precisely.

Cyanide is produced by plants as by product of ethylene metabolism, or as reduced form of nitrogen storage and defense against attack by herbivoes (Chiwona-Karltun *et al*., 2004; Møller, 2010). Cassava plant produce high quantities of cyanide compared to other crop and it is mainly concentrated in leaves and roots. The cyanogenic potential varies with genotype (Burns *et al*., 2012) and within the same genotype; cyanide is also affected by planting season and soil type (Bokanga *et al.,* 1994). The same genotype can taste sweet in one locality and bitter in another (Bokanga *et al*., 1994; Bradbury *et al*., 2013). Konzo is the irreversible upper motor neuron damage caused by consumption of high dietary cyanogen, especially from insufficiently processed cassava roots. It occurs to children and women of child bearing age. Also high dietary cyanogen has been reported to cause tropical ataxic neuropathy (TAN) in elderly and stunted growth in children (Nhassico *et al*., 2008). During food shortage short cuts in processing cause Konzo outbreak (Mlingi *et al.,* 2011; Siritunga and Sayre, 2004).

Farming communities where cassava is a staple food, prefer bitter (high cyanogenic) varieties as protection from predators and thieves (Mkumbira *et al.,* 2003; Benesi *et al*., 2010). Bitter varieties are preferred fortheir high yield (Bradbury *et al.,* 2013) and

production of flour with characteristic taste preferred by cassava farmers (Chiwona-Karltun*et al.,*2004). Most bitter varieties have long durability (Mkumbira *et al.,* 2003; Chiwona-Karltun *et al.,* 2004), are not susceptible to theft (Sirutunga and Sayre, 2004) thus can be stored underground and harvested piecemeal. As a result,food insecurefarmers prefer growing bitter cassava. Commercial starch production will increase demand of cassava in the market and reduce dependency on bitter varieties as food reserve thus decreasing chances of cyanide exposure and associated risks.

Only few local cassava cultivars have been evaluated for dry matter (Mtunda, 2009) and in less diverse environmental conditions. In this study, six farmer preferred landraces were evaluated in diverse environment in terms of temperature range, rainfall distribution and soil conditions. These landraces have survived biotic and abiotic stresses for decades and are preferred by farmers. This characteristic makesthem ideal candidates for recommendation for commercial production of both food and starch. Therefore, the present study aimed to determine the effect of genotype and environmental conditions on starch yield and cyanogenic potential across sites and harvesting periodsand finally to evaluate stability and cultivar superiority.

2.0 MATERIALS AND METHODS

2.1 Cassava Landraces and Trial Design

Six farmer preferred cassava landraces collected from Eastern zone of Tanzania in January 2012 were used for this study. The landraces included; Nyamkagile, Kibandameno, Kilusungu, Msenene, Kalolo and Kiroba. A split plot design replicated three times using plot size of 5m x 5m with spacing of 1 m x 1m was deployed, whereby

the landraces were main plots and harvest date as sub plots. Trials were planted during the rainy season (March, 2012), and harvesting took place in three rounds namely; 9, 12 and 15 months after planting (MAP).

2.2 Location of Trial Sites and Edaphic Conditions

Trials were set at Chambezi (Bagamoyo), Amani (Muheza) and Magadu (Morogoro municipality) in April 2012 up to July 2013 (Table 1). Each planting set continued for 15 months, and harvesting was done in three rounds, 9, 12 and 15 months after planting (MAP). Three healthy plants were selected for each plot and marketable roots were harvested, counted (RTN) (Supplemental data Table 1) and weighed (SRM). For starch and drymatter analysis, roots were brought immediately toDepartment of Food Science and Technology laboratory, Sokoine University of Agriculture (SUA). Weather data for minimum and maximum temperature and monthly total rainfall of trial sites were recorded. Soil samples were collected from the top 30 cm at different three positions for each location (Gullberg, 2007). Soil samples were kept in bags, labelled and transported to Soil Science laboratory of Sokoine University of Agriculture for determination of soil pH, phosphorus, calcium and potassium contents according to methods by SSSA (1996).

Table 1: Description of location for the study

Location of the trial (ward/district)	GPS coordinates	Agro- ecological zone	Altitude (m)	Temperature Range (°C)		Annual rainfall (mm)
				Min	Max	
Chambezi-Bagamoyo	S 06.55318 E 38.9148	Coastal plains	46	19-23	29-31	800-1000
Amani- Muheza	S 5.1088 E 38.67373	Eastern plateux and mountain block	542	15-18	27-30	800 -1500
Magadu-Morogoro	S 6.84706 E37.65448	Eastern plateux and mountain block	1100	19-23	29-31	600 - 1000

2.3 Total Fresh Root Yield (FSRY)

FSRY was calculated from the formula:

$FSRY = (SRM \times 10{,}000)/(STRN \times 1000)$

SRM =storage root mass

STRN= storage root number

2.4 Root Dry Matter Content

Cassava root dry matter (DM) was determined according to method described by Benesi *et al*. (2008). Fresh cassava root were grated and exactly 200 g of the grated cassava root (W_1) were put in pre-weighed petri dishes (W_0). The samples were oven dried at 60 ^oC for 72 h, and weighed (W_2) after removal from the oven. DM was calculated as follows;

$$\text{DM (g/kg)} = \frac{W2-W0}{W1-W0} \times 1000$$

2.5 Starch Content (SC) Expressed As Percentage of Total Fresh Weight of Cassava

Starch extraction was conducted following method by Benesi *et al*. (2010) with slight modification**.**Exactly 500 g of fresh peeled cassava from the three root samples were chopped using water as extraction solvent in a laboratory blender. The tissues were broken to liberate the starch granules and fiber was separated by sieving through double layer of cheese cloth. After washing several times, starch was oven dried at 50-55°C for 72 hour and then weighed (Wo).

Starch content (SC g/kg) was calculated as = Wo/500 *1000

2.6 Total Starch Yield

Starch yield was obtained as the product of fresh cassava yield and starch content expressed as t ha^{-1}

Starch yield = SC* FSRY.

2.7 Cyanide Content

Cyanide content was determined following a picrate paper method as illustrated by Bradbury (2009). A sharp pointed knife was used to take a 100 mg sample adjacent to the center of the transverse section of the tuber. Tuber sampling was standardized to account for known tuber variation in cyanogenic glycoside concentration and analysis was done within an hour after the harvesting. Readings were obtained from colour charts and cyanide content of fresh root was recorded as ppm. For each set of analysis, standardization was done with standard paper and a blank provided with the kit.

ppm =mg HCN equivalents/kg fresh root

2.8 Data Analysis

Data analysis was done using GENSTAT 11 program. Analysis of variance (ANOVA) for all the traits was performed for each site separately. Then, combined ANOVA was performed with replication as blocking factor while genotype, location and round of extraction as fixed effect. Contributions of all the source of variation to sum squares were calculated for each site. Effect of genotype by environment interaction were significant and further analysis using GGE biplots was done on starch yield and cyanide content. GGE biplots analysis was performed to elucidate landrace's superiority, stability and also which won where in relation to starch yield and cyanide content (Yan and Tinkers, 2006).

3.0 RESULTS

3.1 Weather and soil condition of the trial sites

Total annual rainfall was 827, 1605.3, and 694.4 mm in 2012 and 1024.3, 1599.3 and 570.6 mm in 2013 for Chambezi, Amani and Magadu respectively (Figure 1). Amani had

a well spread rainfall throughout the year, followed by Magadu. Mean monthly maximum temperature range for the growing period were 29.9 - 33.8°C for Chambezi, 24.5 - 32.5°C for Amani and 28 - 36.2°C at Magadu. Minimum monthly temperature range was 18.5- 23.7°C for Chambezi, 11.5 - 17.5°C for Amani and 15.4 - 22.4°C for Magadu (Fig. 1). Both sites had slight acidic soils, but Magadu soil was the most acidic (pH 5.5) compared with sites. Chambezi had sand soils, Amani had sandy, clay/ loamy soils while Magadu had sand/ clay. Cation exchange capacity (CEC) measures the ability of soil to hold essential nutrients and its availability to plants (soil fertility). Amani soil had high CEC (16.2 cmol/kg) and Chambezi had the lowest (8.2 cmol/kg). Chambezi soil had high Fe (41.6mg/ kg) content but low in organic matter (0.3%), while Magadu had low Fe (1.556mg/kg) and high organic matter (1.6%) compared with sites (Table 2).

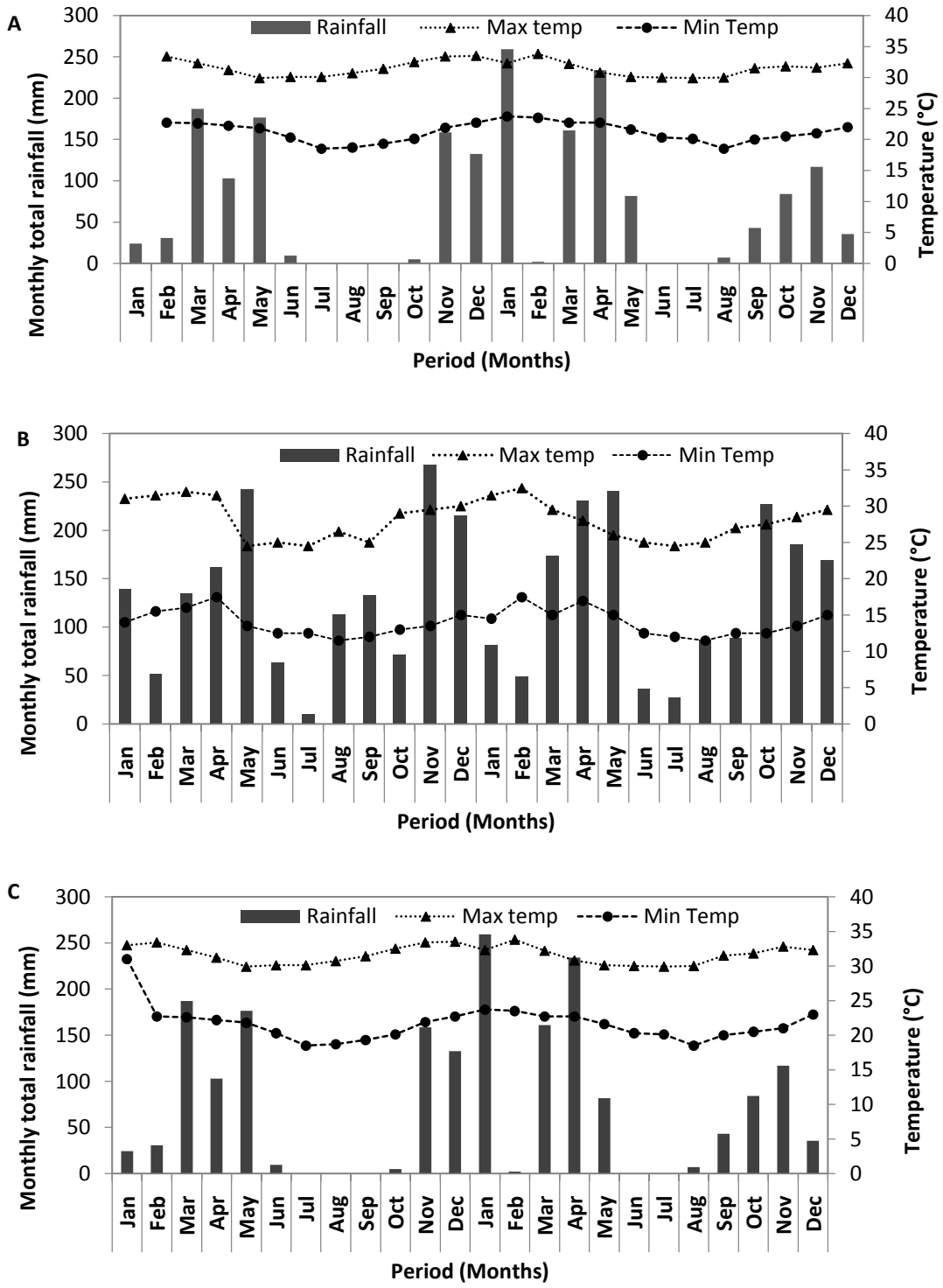

Figure 1: Temperature and rainfall data for the three trial sites A) Chambezi B) Amani and C) Magadu taken from January 2012 to December 2013

Table 2: Soil data from the three trial sites

Parameters		**Trial sites**		
		Chambezi-Bagamoyo	**Amani-Muheza**	**Magadu-Morogoro**
Soil pH (in H_2O)		5.8	6	5.5
P.S.O	Clay	6	20	46
	Silt	3	7	8
	Sand	91	73	4.6
Texture class		**Sand**	**Sand/ clay/ loam**	**Sand/ clay**
Fe(mg/kg)		41.6	17	1.55
N%		0.05	0.11	0.15
% OC		0.3	1.2	1.6
Ext.P /Pbry (mg/ kg)		15.6	8.49	6.84
CEC (cmol/kg)		8.2	16.2	13.8
Exchange bases (cmol/kg)	Mg^{2+}	0.03	0.22	0.23
	Na^{+}	0.27	0.35	0.42
	Ca^{2+}	1	4.5	4.1
	K^{+}	0.3	0.4	0.3

P.S.O= Particle Swarm Optimization; ExtP=; OC=organic matter; CEC= cation exchange capacity

3.2 Effect of harvesting time (Months After Planting, MAP) on cassava yield, dry matter, starch content and cyanide content

There was high significant difference ($p \leq 0.001$) in trial sites (Tables 3, 4, 5 and 6) for main effect (landraces and harvest rounds) and interaction. Fresh root yield varied significantly with harvest round for both locations. It increased with harvest rounds where 15MAP had the highest yield and 9MAP had lowest except for Kalolo (at Chambezi site) and Kibandameno (at Chambezi and Amani sites) whereby at 12 MAP produced lowest yield. Chambezi site had the highest average yield at all harvest rounds (MAP) while Magadu site had the lowest. Kiroba and Msenene had the highest average

yields for all rounds, while Kalolo recorded highest yields of 10.5 t/ha at 9MAP at Bagamoyo site. Optimal harvest time of 12 MAPfor Kiroba was observed for all locations and Nyamkagile was 15MAP (Table 3).

Significant differences ($p \leq 0.001$) in dry matter and starch content were observed among landraces. Significant difference ($p \leq 0.001$) in starch content among landraces and harvest roundswere also observed, and an increase in dry matter and starch was evident from 9MAP to 12MAP but decrease at 15MAP. This trend was observed for all locations and is different from what has been observed from yield data. Amani site recorded highest overall average dry matter at 12MAP. Harvest rounds had no profound effect on dry matter for landrace Nyamkagile. Lowest starch content was observed at 15MAP for all landraces at all sites, with exception of Kilusungu, which had the highest at Bagamoyo site.

Variations in cyanide content were highly due to genotype (G) and genotype by environment interaction (GE) ($p \leq 0.001$). Nyamkagile at Magadu site was the only landrace which had cyanide content below 30 ppm. Kibandameno also had low cyanide content at Bagamoyo site at 9 and 12 MAP. The trend showed that each landrace gave different response at each harvesting time and it varied with location. Nyamkagile was the only landrace which showed decrease in cyanide content with age. However, the trend and cyanide concentration differed for each environment as demonstrated by significant genotype by environment interaction ($p \leq 0.001$).

Table 3: Fresh root yield (t ha^{-1}) of six cassava landraces evaluated in three sites at three different harvesting rounds (MAP)

	CHAMBEZI				AMANI				MAGADU			
Landraces (G)	**9MAP**	**12MAP**	**15MAP**	**Mean¥**	**9MAP**	**12MAP**	**15MAP**	**Mean¥**	**9MAP**	**12MAP**	**15MAP**	**Mean¥**
Kalolo	10.5	8.3	19.4	12.7	2.5	3.4	9.8	5.2	1.6	3.7	8.7	4.7
Kibandameno	7.5	5.0	11.0	7.8	5.6	2.9	24.6	11.0	1.9	7.9	10.0	6.6
Kilusungu	7.3	7.3	17.6	10.7	5.5	7.8	18.3	10.5	3.7	12.4	11.9	9.3
Kiroba	4.5	23.0	24.8	17.4	6.5	13.0	18.7	12.7	2.1	9.9	12.6	8.2
Msenene	6.4	12.4	39.4	19.4	9.0	14.4	13.8	12.4	1.2	7.9	6.4	5.2
Nyamkagile	7.4	8.7	19.4	11.8	5.1	7.1	11.6	7.9	1.6	5.2	9.3	5.3
Mean+	7.3	10.8	21.9	13.3	5.7	8.1	16.1	10.0	2.0	7.8	9.8	6.6
CV (%)			7.2				7.8				10.0	
LSD for G			1.75***				0.88***				0.45***	
LSD for MAP			0.91***				0.51***				0.50***	
LSD G*MAP			2.42***				1.29***				1.07***	
SE for G			0.78				0.28				0.2	
SE for MAP			0.45				0.18				0.24	
SE G*MAP			1.19				0.45				0.52	

*****p≤0.001; **p≤0.01; CV=Coefficient of variation; SE=standard error; LSD=Least significant difference; G=landraces (genotypes); MAP= Months After Planting (harvest rounds); Mean¥=mean over three harvest rounds, Mean+= overall landrace site mean**

Table 4: Root dry matter (DM) content (g/kg) six cassava landraces evaluated in three sites at three different harvesting rounds (MAP)

	CHAMBEZI				AMANI				MAGADU			
Landraces (G)	**9MAP**	**12MAP**	**15MAP**	**Mean¥**	**9MAP**	**12MAP**	**15MAP**	**Mean¥**	**9MAP**	**12MAP**	**15MAP**	**Mean¥**
Kalolo	308.0	350.3	285.1	314.5	371.1	388.9	346.6	368.9	344.3	390.9	335.8	356.8
Kibandameno	395.3	411.3	378.1	394.9	397.4	416.8	373.8	396.0	404.0	446.4	373.3	407.9
Kilusungu	306.4	328.6	311.0	315.3	404.2	438.4	382.3	408.3	362.3	383.6	347.2	364.4
Kiroba	367.4	377.7	335.7	360.3	397.5	405.2	375.8	392.9	356.2	405.4	344.7	368.8
Msenene	334.4	365.3	310.4	336.7	349.9	369.3	298.3	339.1	370.1	382.1	328.5	360.2
Nyamkagile	335.6	354.3	332.8	340.9	414.4	425.6	380.1	406.7	402.7	417.2	388.9	402.9
Mean+	341.2	364.6	325.5	343.7	389.1	407.4	359.5	385.3	373.2	404.3	353.0	376.8
CV(%)			1.3			2.5					2.5	
LSD for G			5.7***			8.1***					8.6***	
LSD for MAP			3.0***			3.6***					6.8***	
LSD for G*MAP			7.9***			10.4***					15.6**	
SE for G			2.6			2.6					3.9	
SE for MAP			1.5			1.2					3.3	
SE for G*MAP			3.9			3.5					7.7	

*****p≤0.001; **p≤0.01; CV=Coefficient of variation; SE=standard error; LSD=Least significant difference; G=landraces (genotypes);**

MAP= Months After Planting (harvest rounds); Mean¥=mean over three harvest rounds, Mean+= overall landrace site mean

Table 5: Root starch content (g/kg) of six cassava landraces evaluated in three sites at three different harvesting rounds (MAP)

	CHAMBEZI				AMANI				MAGADU			
Landraces (G)	**9MAP**	**12MAP**	**15MAP**	**Mean[¥]**	**9MAP**	**12MAP**	**15MAP**	**Mean[¥]**	**9MAP**	**12MAP**	**15MAP**	**Mean[¥]**
Kalolo	229.8	261.4	211.9	234.4	279.3	292.7	260.9	277.6	256.6	291.9	249.0	265.8
Kibandameno	316.3	329.0	300.0	315.1	319.4	334.9	300.4	318.2	315.2	348.2	291.1	318.2
Kilusungu	236.5	253.7	240.9	243.7	319.5	346.6	302.3	322.8	283.2	299.9	269.8	284.3
Kiroba	295.1	303.5	268.8	289.1	321.1	327.0	303.3	317.1	284.2	323.5	274.1	294.0
Msenene	262.1	286.3	243.4	263.9	278.7	294.2	237.6	270.2	288.0	297.4	255.7	280.4
Nyamkagile	276.2	291.6	275.3	281.4	337.4	346.6	302.8	328.9	322.9	334.6	311.8	323.1
Mean[+]	269.3	287.6	256.7	271.2	309.2	323.7	284.5	305.8	291.7	315.9	275.2	294.3
CV (%)		1.3				1.4				2.4		
LSD for G		0.41***				5.2***				6.6***		
LSD for MAP		0.24***				2.8***				5.1***		
LSD G*MAP		0.60***				7.2***				11.7**		
SE G		1.9				2.3				2.9		
SE MAP		1.2				1.4				2.5		
SED G*MAP		3.0				3.6				5.6		

*****p≤0.001; **p≤0.01; CV=Coefficient of variation; SE=standard error; LSD=Least significant difference; G=landraces (genotypes);**

MAP= Months After Planting (harvest rounds); Mean[¥]=mean over three harvest rounds, Mean[+]= overall landrace site mean

Table 6: Starch yield (t ha^{-1}) of six cassava landraces evaluated in three sites at three different harvesting rounds (MAP)

	CHAMBEZI				AMANI				MAGADU			
Landraces	**9MAP**	**12MAP**	**15MAP**	**Mean$^{¥}$**	**9MAP**	**12MAP**	**15MAP**	**Mean$^{¥}$**	**9MAP**	**12MAP**	**15MAP**	**Mean$^{¥}$**
Kalolo	2.4	2.2	4.1	2.9	0.7	1.0	2.6	1.4	0.4	1.1	2.2	1.2
Kibandameno	2.4	1.7	3.3	2.4	1.8	1.0	7.4	3.4	0.6	2.8	2.9	2.1
Kilusungu	1.7	1.9	4.2	2.6	1.8	2.7	5.5	3.3	1.1	3.7	3.2	2.7
Kiroba	1.3	7.0	6.7	5.0	2.1	4.3	5.7	4.0	0.6	3.2	3.4	2.4
Msenene	1.7	3.6	9.6	5.0	2.5	4.3	3.3	3.3	0.4	2.4	1.7	1.5
Nyamkagile	2.1	2.5	5.3	3.3	1.7	2.5	3.5	2.3	1.6	1.7	2.9	5.3
Mean^{+}	1.9	3.1	5.5	3.5	1.8	2.6	4.7	3.0	0.6	2.5	2.7	1.9
CV (%)			7.2				7.8				10.0	
LSD for G			0.4***				0.23***				1.9***	
LSD for MAP			0.3***				0.16***				0.13***	
LSD G*MAP			0.7***				0.4***				1.02***	
SE for G			1.3				0.1				0.09	
SE for MAP			0.91				0.1				0.06	
SE G*MAP			0.27				0.2				0.16	

*****p≤0.001; **p≤0.01; CV=Coefficient of variation; SE=standard error; LSD=Least significant difference; G=landraces (genotypes);**

MAP= Months After Planting (harvest rounds); Mean$^{¥}$=mean over three harvest rounds, Mean^{+}= overall landrace site mean

Table 7: Cyanide content (ppm) of six cassava landraces evaluated in three sites at three different harvesting rounds (MAP)

	CHAMBEZI				AMANI				MAGADU			
Landraces	**9MAP**	**12MAP**	**15MAP**	**Mean¥**	**9MAP**	**12MAP**	**15MAP**	**Mean¥**	**9MAP**	**12MAP**	**15MAP**	**Mean¥**
Kalolo	100	100	200	133.3	400	400	150	316.7	200	100	100	133.3
Kibandameno	30	100	50	60.0	100	100	100	100.0	50	100	75	75.0
Kilusungu	40	600	400	346.7	400	400	150	316.7	400	400	400	400.0
Kiroba	50	100	800	316.7	200	100	200	166.7	100	100	40	80.0
Msenene	50	75	100	75.0	100	50	40	63.3	100	100	100	100.0
Nyamkagile	125	100	100	108.3	200	100	75	125.0	30	15	25	23.3
Mean+	65.8	179.2	275	173.3	233.3	191.7	119.2	181.4	146.7	135.8	123.3	135.3
CV (%)			7.1				13.9				7	
LSD for G			19.8***				30.6***				14.21***	
LSD for MAP			10.9***				21.6***				8.39***	
LSD G*MAP			26.7***				53.0***				20.01***	
SE for G			5.4				11.24				5.5	
SE for MAP			3.5				7.28				3.8	
SE for G*MAP			8.9				18.4				9.5	

*****p≤0.001; **p≤0.01; CV=Coefficient of variation; SE=standard error; LSD=Least significant difference; G=landraces (genotype);**

MAP= Months After Planting (harvest rounds); Mean¥=mean over three harvest rounds, Mean+= overall landrace site mean

3.3 Variation in Traits in Response to Effect of Genotype and Environment

Harvest round (MAP) and interaction between landraces (G), location (L) and rounds (G x L x MAP)were most important factors that contributed to variability in fresh root yield and starch yield (Table 8). Furthermore, landraces (genotypes) have contributed the most to the variation of starch (40.6%) and cyanide (38.8%) content than other sources of variation. Kiroba performed well in terms of fresh root yield at Amani site and Chambeziwhereas Msenene had the highest fresh root yield at Chambezi and the lowest fresh root yield at Magadu. Nyamkagile yielded high at Chambezi site and low yield were observed at Magadu. Furthermore, Nyamkagile had the highest starch content at Amani and Magadu sites, while Kibandameno had the highest starch content at Chambezi. Nevertheless, Kalolo had the lowest starch content across all locations.

In terms of starch content the trend per site was Amani> Magadu> Chambezi was last. For fresh root yield, Chambezi> Amani> Magadu (Table 8 and supplemental Figure 1). Chambezi had sandy soils, which is suitable for cassava production because of easy rootpenetration and expansion of the growing root during carbohydrates partitioning. Amani had sand clay loamy soils, which are appropriate for water retention and thus provided a good distribution of soil water for long period after the start of dry season. Magadu had lowest yields, which might have been attributed to the clay soils and relatively lowest amount of rainfall received during the growing period when compared to the other sites.Kilusungu recorded high mean cyanide content across the environment and Msenene had the lowest at Chambezi and Amani.Bagamoyo site exhibited increase in cyanide content, Amani site displayed a decrease trend and Magadu displayed almost constant trend over the growth period (Table 8, supplemental Fig. 1).

Table 8: ANOVA table for fresh root yield root starch content, starch yield and cyanide content for six cassava landracesfrom Tanzania

		Fresh root yield (t ha^{-1})				Root starch content (g/kg)			
Source of variation	**df**	**Sum of Squares (SS)**		**Contribution to SS (%)**	**Mean square**	**Sum of Squares (SS)**		**Contribution to SS (%)**	**Mean square**
Replicates	2	3.9			1.9	8.9			4.5
Location (L)	2	1261.2	***	14.43	630.6	33491.8	***	19.26	16745.9
Landrace (G)	5	652.8	***	7.47	130.6	70546.4	***	40.56	14109.3
Harvest (MAP)	2	3345.6	***	38.29	1672.8	36338	***	20.89	18169
G x MAP	10	438.6	***	5.02	43.9	2069.4	ns	1.19	206.9
G x L	10	698.3	***	7.99	69.8	19924.9	***	11.46	19.92
G x L x MAP	34	2218.8	***	25.39	65.3	6663.6	***	3.83	196
Residual	106	119.3		1.36	1.1	4892			46.2
Total	171	8738.5				173935			
		Starch yield (t ha^{-1})				**Root cyanide content (ppm)**			
Source of variation	**df**	**Sum of Squares (SS)**		**Contribution to SS (%)**	**Mean square**	**Sum of Squares (SS)**		**Contribution to SS (%)**	**Mean square**
Replicates	2	0.61			0.30	3.7			3.7
Location (L)	2	214.31	***	31.90	107.16	43672.2	***	1.61	21836
Landraces (G)	5	59.95	***	8.92	11.99	1051211.1	***	38.80	210242
Harvest (MAP)	2	77.46	***	11.53	38.73	11938.9	***	0.44	5969.4
G x MAP	10	39.19	**	5.83	3.92	328983.3	***	12.14	32898
G x L	10	47.43	***	7.06	4.74	369450.0	***	13.64	36945
G x L x MAP	44	220.81	***	32.87	5.02	888444.4	***	32.79	37019
Total	**181**	**671.73**				2709400			

*****p≤0.001; **p≤0.01; n=not significant; G=Landraces (Genotypes); L=location; MAP=harvest rounds**

3.4 Superiority and stability for starch yield and cyanide content for cassava landraces

The potential of cassava as a biomaterial for different industries depends on its yield and starch content and consequently starch yield. Also cassava use for food is constrained by its cyanide content. However, from this study these two important traits have been found to be significantly influenced by genotype by environment interaction. Further analysis was done using GGE biplot to elucidate the stability of the landraces under different environments. The cultivar superiority measure (CSM) encompasses calculations (across environments) of the mean square difference between the performance of a variety and the best variety within a given environment, measuring mean performance and stability simultaneously. GGE biplots enables visual comparison of the location and genotype studied and their interaction. The GGE biplots for starch yield and cyanide were generated to allow evaluation of performance of each cassava landrace.

The GGE biplot analysis showed that PC1 and PC2 accounted for 91.4 and 8.12% (total = 99.49) variation respectively for starch yield (tones/ha) (Fig. 2). The average environment coordinates (AEC) of the GGE biplot (Fig. 2A) is based on genotype scaling. It shows mean starch yield and stability of the six landraces in three environments (sites). The average environment coordinates (AEC) measures the stability of the genotypes over specific environment. A stable genotype willbe very close to the AEC and will have a short perpendicular line. Therefore,Kiroba had high starch yieldat Chambezi and Amani.Likewise, Kilusungu and Nyamkagile were stable though they had below average starch yield (Fig 2A). Msenene was the most stable across the environment but yielded lower atChambezi. Kiroba was superior in terms of starch yield

and performed well at Chambezi site. In terms of test environment, Magadu had shortest vector from AEA, implying that it has little discriminating information on genotype and was the least representative. Chambezi site had the longest vector indicating the highest discriminative and representative (Supplemental data Fig.2).

Kibandameno was theleast stable and Kalolo had the least starch yielder among all landraces evaluated. Nyamkagile was also stable but produced just below the average starch yield across all locations. The GGE biplot depict landrace which had the best performance at each location (which- won- where biplot). A convex hull with three vectors was formed with Kiroba, Nyamkagile and Kalolo as the vertex cultivars (Fig.1B). Kiroba was winning landrace in mega environment (Chambezi and Amani), while Kibandameno was winner at Magadu. No environment fell into sector with Kalolo as the vertex, indicating that it was not the best in any environment. Fig1C illustrated ideal genotype (the center of concentric circles), which should have both high mean performance and high stability across environment. An ideal genotype should be a point on the AEA in a positive direction and having the longest vector length. From the plot it can be seen that Kiroba is closer to the ideal genotype followed by Msenene, indicative of high yielding and adapted genotypes.

The GGE biplot analysis for cyanide content (ppm) showed that PC1 and PC2 accounted for 77.1% and 13.5% respectively (Fig. 3 A, B and C). The analysis showed that Kilusungu was stable and had the highest cyanide content across all environments. Kiroba hadhighest cyanide content landrace at Chambezi. Kibandameno, Msenene and Nyamkagile had the lowest cyanide content across environment. Nyamkagile wasalso

stable. Chambezi was the most discriminative environment and Amani was least discriminative (Fig. 3 A).

Fig. 3B illustrate that both trial sites formed a mega environment, this implies that they both had comparable discriminative ability for cyanide content. This can also be explained by length of their vectors as shown in Fig. 3A. Consequently, in terms of cyanogenic potential, the best to grow in all environments and which will have safe cyanide limits is Nyamkagile.

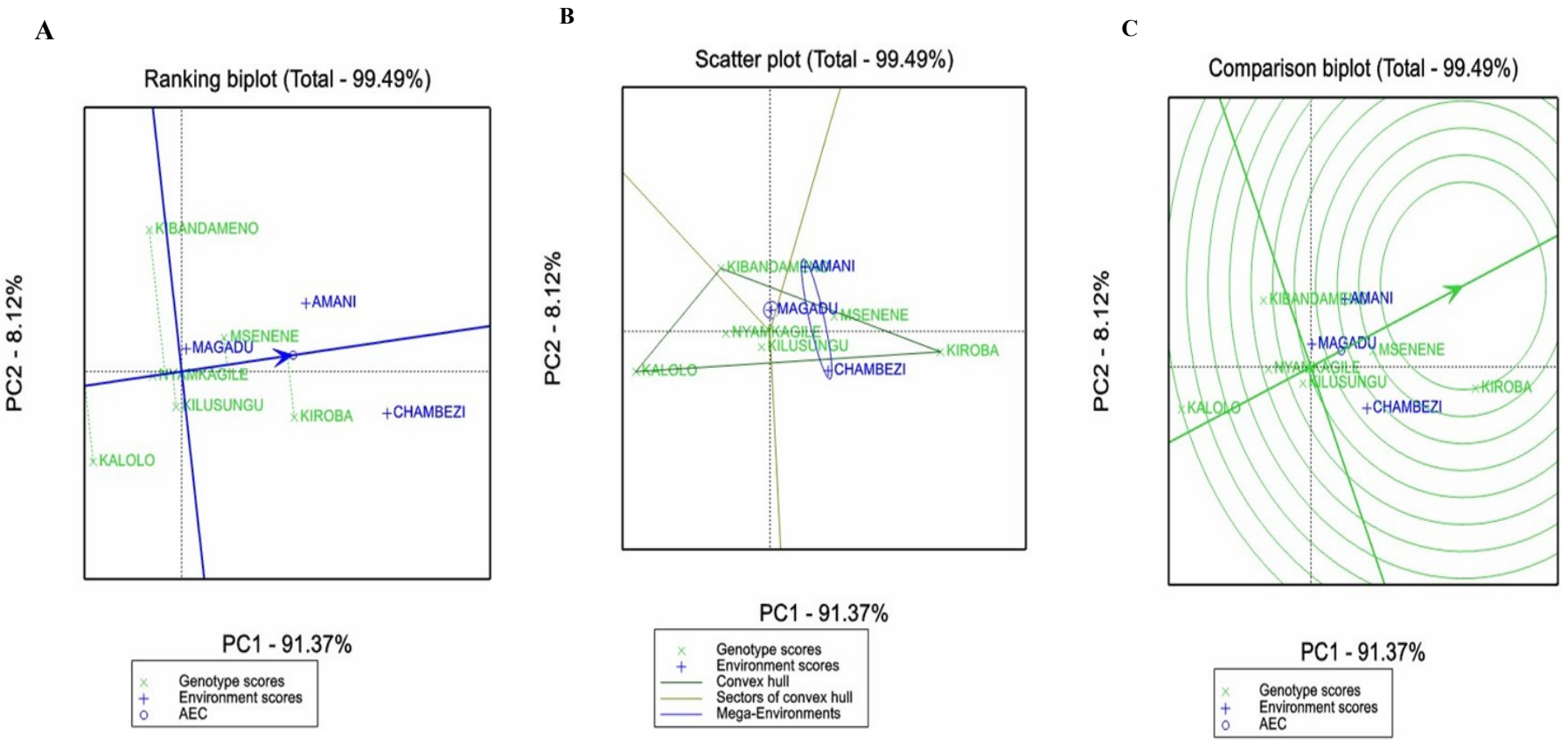

Figure 2: GGE biplots A) showing ranking of cassava landraces based on mean cassava starch yield and stability performance across three environments B) scatter plot for which- won- where (superiority) showing the best landrace for each environment C) the average environment coordination (AEC) view to rank landraces relative to an ideal genotype (center of the concentric circle).

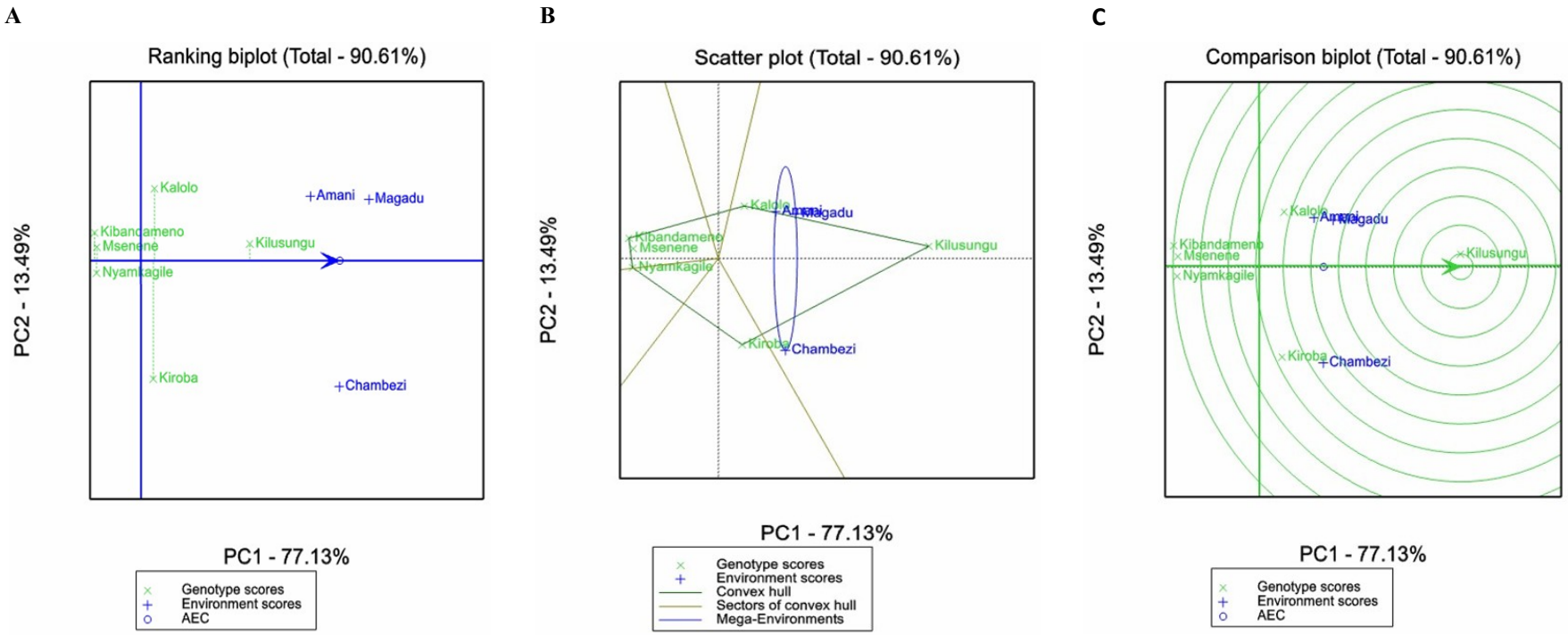

Figure 3: GGE biplots A) showing ranking of cassava landraces based on mean cassava cyanide content and stability performance across three environments B) scatter plot for which- won- where (superiority) showing the winning landrace for each environment C) the average environment coordination (AEC) view to rank landraces relative to an ideal genotype

4.0 DISCUSSION

Yieldincrease was observed from 9 to 15 MAP, and a huge increase was noticed between 12 and 15 MAP except for Magadu site where increase was not very pronounced. This could be explained by dry period that existed between January and March and thus low carbohydrate partitioning in the storage root (Santisapori *et al*., 2001; El-Sharkawy, 2006). Chambezi site recorded average high yields compared to other sites across all harvesting periods. After commence of rainfall period (March – May) huge increase in yield was observed at 15MAP. In contrary dry matter and starch content increased up to 12MAP and decreased at 15MAP, a trend also observed by Sagrilo *et al.* (2006) except for Magadu site. Accumulation of drymatter increased up to 12MAP, until physiological rest was reached. From that period onwards reserve carbohydrates are mobilized for synthesis of new vegetative growth (Sagrilo *et al*., 2006; Benesi *et al*., 2008). Therefore, during this period a decrease in dry matter and starch is usually observed. Furthermore, this trend can partly be explained by dry spell experienced between January and March. During this period the plant mobilizes stored energy for use (El-Sharkawy, 2006). In contrary, starch content for Kibandameno at Bagamoyo, remained the same for 9 and 12 but decreased at 15 MAP**.** At both harvest rounds cultivars recorded average high starch content at Amani site. This can be partly explained by soils at Amani having high Potassium content compared to the other sites and the fact that it received rain showers throughout the year.This observation has been also reported by Benesi *et al.* (2008).

Previous studies showed that cyanide content varied significantly with genotypes and across environment (Burns *et al*., 2012). Bokanga *et al.* (1994) reported that even though genotypes have varied cyanogenic potential, soil composition and water supply during

growth also affect roots and leaves HCN content. This study found significant difference ($p \leq 0.001$) between landraces and also significant GEI, therefore confirming previous studies.

McMahon *et al.* (1995), reported that cyanogenic potential of cassava is also age dependent, implying that cyanide production varies with plant growth**.** Bokanga *et al* (1994) also reported that cyanogenic potential concur with vegetative growth, that when the tuberous root growth becomes the sink, cyanogenic potential starts to decrease. The trend was not the same for most landraces used in this study and varied across the environments. In this study, only Nyamkagile exhibited decrease in cyanide content at both trial sites. This further demonstrated the genotype dependency of cyanide production (Bokanga *et al*., 1994). Furthermore, the mechanism involving HCN metabolism is very complicated and involves several genes (Møller, 2010). At Amani site there was no cyanide content difference with MAP except Nyamkagile which showed decrease with MAP. Also Kibandameno increased from 50 to 100 ppm and then decrease to 75 ppm. At Amani site, the landraces did not exhibit considerable water stress as shown by rainfall data. However, at Chambezi site, cassava experienced 5 months of drought through the 15 months growing period. This can explain the observed trend at Amani (Cardoso *et al*., 2005**)**. Furthermore cyanide content at Chambezi, increased at 12 MAP, which might be due to dry spell experienced by plants in February 2013(after 9MAP harvest). It has been also reported by El-Sharkawy (2006) and Burns *et al*. (2012) that during water stress cassava plant exhibits high cyanide content compared to the well-watered cassava. This could be explained by Kiroba, which recorded high cyanide content (800 ppm) during 15 MAP at Bagamoyo site. Cyanide value of 1090 and

1550 ppm have been reported by Mlingi and Bainbridge (1994) for Tanzania bitter cassava cultivars.

Analysis by GGE bilpot further elucidated the performance of landraces across trial sites and which was due to GEI (Akinwale *et al*., 2011; Yan and Tinkers, 2006) for cyanide content and starch yield. In terms of starch yield, Kiroba was winning (superior) at Chambezi and Amani, and Msenene was a winning landrace at Magadu site. Kilusungu had the highest cyanide across all environments but had average starch yield and which was stable across environments.

5.0 CONCLUSION

Environment (trial sites) and genotypes (landraces) had a profound effect on all the traits analyzed. These variations indicatesa significant genetic diversity present in farmer fields and that can be trapped to increase yield potential for cassava at different locations. The study has confirmed effect of genotype and genotype environment interaction on cyanide content of cassava roots. Kilusungu and Kalolo, were bitter landraces used during this study. Kalolo displayed low yield and starch content compared to the other landraces thus cannot be suitable for starch production. Kilusungu displayed high cyanide and average starch yield (at 12 or 15MAP) and was stable across environments. Therefore, it can be suitable for starch production.The study has also showed the potential toxicity of Kilusungu. Consequently, as a safety measure, farmers should be emphasized on the need for its proper processing prior to consumption. For maximum starch yield, Kiroba should be harvested at 12MAP and highest starch content for Kiroba was recorded at Amani, although in terms of starch yield it was higher at Chambezi (because of higher

yields). Therefore,Kiroba can be recommended for starch production at Chambezi and Amani and Msenene should be targeted forMagadu. Amani and Bagamoyo formed a mega environment, thus shared same superior landraces for maximum starch yield.

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CHAPTER THREE

3.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

3.1 Conclusions

Cassava continues to be an important staple crop due to its starchy root and ability to grow where other crops have failed. The surge in the popularity of cassava is reflected in a 60% increase in global production between 2000 and 2012 as reported by Food and Agricultural Organization. Increased population and climate change are among factors making cassava popular as a future crop. Tanzania cassava production has to be coupled with this increased demand and cassava starch production as a support towards economic development of rural people. This study has established a base towards development of cassava starch industries for use by different industries. During the study, farmers' knowledge was documented and the importance of incorporating farmer's knowledge during research intervention was confirmed. Farmer's decision to grow or abandon certain crop varieties comprise of several interrelated factors. These factors have led to diversity of germplasm found in farmer's field. Also, the diversity found in farmer's field was confirmed by morphological descriptors and SNPs analysis. The power of SNPs to discriminate closely related individuals has been shown by this study. This collection revealed a wide range of genetic diversity and represents a valuable resource for trait improvement enabling capture of farmer preferred traits in future cassava breeding programmes.

The germplasm comprised of diverse set of desirable traits, which are important to cassava farmers. These traits can be further exploited and incorporated in breeding

programmes to improve our local cassava germplasm. Data generated from this study will help the breeders to devise more appropriate and cost effective breeding strategies and will aid in deciding which germplasm to conserve. Potential toxicity of cassava landraces in Tanzania has been shown in this study. So as cassava continues to be an important staple crop, production expansion should be coupled with effective detoxification method. This will help in lessening cyanide exposure risks to cassava consuming communities. The study has also showed that farming communities where cassava is staple preffer bitter varieties to reduce risk of food insecurity. Commercialiation of will help fill the gap of food insecurity through promotion and development of cassava starch industries. Farmers will benefit for selling their cassava to cassava producing industries and thus improve their income and livelihood.

In this study, landrace like Kiroba, Msenene, Nyamkagile, and Kilusungu, can be targeted for starch production and its starch characteristics are suitable for application in different industries. These landraces can further be exploited for starch production for different industries in the country, especially Kilusungu, which is a bitter variety.

3.2 Recommendations

Genetic diversity that exist in the farmer filed could be exploited for improved cassava productivity. The information will help breeder to choose which cassava genotype to use for improvement. It will also help to exploit desirable traits found in our local germplasm and incorporated them during breeding so to improve our cassava landraces.

From the study it has been found that there is a trendamous loss of germplasm, Root and Tubers Research Centres are argued to devise germplasm conservation strategy to prevent loss of germplasm and ensure conservation of desirable traits, which can be used in the future breeding program. The data generated from this study will help the breeders to devise more appropriate and cost effective breeding and conservation strategies and will assist in deciding which germplasm to conserve.

Awareness creation of potential hazards of cyanide exposure, especially low doses (due to consumption of insufficiently processed cassava) and promotion of appropriate processing technologies for cyanide removal in areas where cassava is grown and consumed is essential. Farmers should therefore be trained in proper processing technologies to remove cyanide. Also, Extension officers in cassava growing areas need training and be provided with simple picrate kits so as to assess levels of cyanide in local varieties and devise appropriate control/ processing measure. Mass education on effect of low doses cyanide exposure to cassava eating community need be conducted to rescue those who would otherwise fall victims of their ignorance.

This study has shown potential that exists for Tanzania cassava that could be exploited for starch production for different industrial uses, thus to contribute to economic development in Tanzania. The government should provide suitable environment for medium scale industries to start up this processing and put in place appropriate policies and regulations so that our industries can start using the locally made starch. Appropriate policies and regulations should be put in place to strengthen starch producing industries so as to operate in a conducive environment in favour of development of these industries.

The following are the major areas that need to be addressed so as to favour the development of cassava and cassava starch industries and starch use in Tanzania:

i. Government should put in place appropriate polices in favour of development of starch industries. Local industries should be given incentives and favourable environment so as to develop. Also, strict guidelines on the use of cassava starch in our industries especially paper and textile need to be developed to widen the use of this starch as a way of sustainably expanding cassava production in the country.

ii. This study should be extended in other cassava growing areas in Tanzania, which are also outstanding cassava growing area especial in Lake zone (Mwanza and Mara), Central (Dodoma), Southern (Mtwara and Lindi) and Southern highlands (Songea) to evaluate the suitability of available germplasm for statch production.

Future Perspective and Recomnedations for futher studies:

i. Further studies on effect of temperature and drought in relation to cassava yield and cyanide content on the commonly grown landraces need to be conducted. Also further studies on assessing storage durability for sweet versus bitter cassava cultivars is needed.

ii. This study did not evaluate starch physicochemical changes occurring as subjected to harvest period and environmental condition. Further studies therefore needto be conducted to explore this area.

iii. Research should be done to examinecorrelation between dry matter and cyanide content of cassava.

iv. Differences in genotypic cyanogenic potential of cassava landraces have been shown therefore appropriate technologies for cyanide removal which are cultivar specific should be sought of. Also further studies on residual levels of cyanogens in the final products so that to make sure produst are within cyanide safe limits.

APPENDICES

Appendix 1: Semi Structured Questionnaire for the Survey on Exploring/ Collecting Farmer's Knowledge on Cassava Cultivar Selection, Conservation and Postharvest Issues in Eastern Zone of Tanzania (also used as checklist during focus group discussion)

A: Household characteristics

1. Name of village: ……..
2. Ward :……. District: ……
3. Farmer's name: …… Age: ……
4. Name of interviewer …….
5. Is the household male or female headed? a) Male b) Female
6. Level of Education
   a) Primary education b) Secondary education c) College d) None
7. How many people are in your household? (Number)……..

B: Origin of variety

1) Do you grow different cassava varieties?
a) yes b) no
2) If yes in 1 above, list the names of varieties, source and purpose of growing

S/N	Variety /landrace name	Is it local (L) or new (N) variety?	Source of the landraces	When did u start growing	Purpose of growing	Rank of preference/

C: Selection criteria for variety

1. Do you make selection of variety before planting: a) yes b) no
2. In the household who is making variety selection decision
   a) father b) mother c) children d) both

3. What criteria do you use to select cassava cultivar before planting?
   ……
   ……
   ……

4. How do you categorize/ identify cultivars?
   ……
   ……

a) leaf morphology b) stem color d) taste e) texture

5. How do you differentiate landrace if they both look alike?
   ……
   ……
   ……

6. Mention the criteria that you prefer mostly in variety selection

S/N	Criteria	Rank

7. Why do you grow different varieties of cassava?
   ……

   ……

   a) Taste b) early maturity c) pest and disease resistance

   d) Marketing qualities e) food security f) starch content

8. Which variety(s) do you preferred to grow?
   ……

   ……

9. Why do you prefer the variety mentioned in Qn 8 above:
   a) taste b) early maturity c) pest and disease resistance

   d) Processing characteristics e) shelf stability f) others

10. Can you distinguish between bitter and sweet varieties? a)yes b) no;
11. How do you distinguish between bitter and sweet varieties?
    ……
    ……
    ……
    ……

12. If yes in Qn 10, mention the names of sweet and bitter varieties you grow

S/N	Variety name	Category Sweet(S)/bitter(B)

D: Production and management practices (agronomic practices)

1. Give information on your household agricultural land

Plots	**Size(acres)**	**Farming type (intercrop?)**	**Is cassava grown**	**Location of farm**

2. Total area of cassava production

S/N	**Variety name**	**Area of production (acress)**	**Planting period**	**Maturity period**

3. How do you prepare the cultivars before planting in the farm?
   ……
   ……

4. Which cropping system are you practicing?

a) intercropping b) crop rotation c) relay cropping d) others (specify)……

5. Which other crop(s) is planted with cassava
   ……
   ……
   ……What is the planting systemused?.....
6. Do you maintain soil fertility in cassava? a)yes b) no
7. If yes at Qn 7, how do you maintain this soil fertility?
   a) by crop rotation b) using fertilizer c) mulching d)other (specify)…….
8. What type of soil is used for cassava production?
   ……
9. Do you weed? a) yes b) no
10. If yes at Qn 10, How many times do you perform the weeding per year?
    ……
    ……
11. What are other traditional practices on management of cultivars?
    ……
    ……
12. What is the fresh cassava yield per acre?

S/N	Variety name	Local (L) or improved (I)	Yield per acre/hectrate

E: CONSERVATION

1. How do you make sure the good varieties are conserved?
   ……

   …….....

   ……

   ……
2. What is the conservation status of each known variety
   a) Threatened b) extinction c) not threatened

S/N	Variety/landraces	Conservation status

3. Why do you think some of the cassava varieties have been lost over time (extinction)?

   ……

   ……
4. From whom did you acquire cassava variety selection and conservation knowledge?
   ……

   ……

F: UTILIZATION OPTIONS

1. What is the primary purpose of growing cassava:
   a) food b) marketing c) famine reserve d) both e) none f) others(specify)…….

2. What is your secondary purpose of growing cassava: a) food b) marketing c) famine reserve d)none f) others

3. Which are the other uses of the crop?
   ……

   ……
4. Which post harvest method is used to preserve cassava?
5. And why?
   ……

   …..……
6. How do you prepare the cassava for home consumption?
   a) steaming b) frying c) drying and milling d) others e) both a,b and c

7. How do you prepare the cassava for marketing?
   a) steaming b) frying c) drying and milling d) sold fresh c)both
8. How is it done?
   ……

   ……
9. Do you process cassava into starch? a) yes b) no
10. If yes to Qn 9 which method do you use?
    ……

    ……
11. If yes to Qn 9 which variety do you prefer for starch extraction?
12. Where do you sell starch?.....
13. Which variety do you prefer for home consumption?.....
    Why?.....
14. Which variety is preferred for marketing purposes?.....
    Why?.....

Appendix 2: Passport data for 52 collected cassava landraces

Name of cultivar	Name of farmer	Village	Hamlet	Ward	District	Latitude (°)	Longitude (°)
Kiguu cha ninga	Mariam Athuman	Mamboleo	Kiwanjani	Nkumba kibanda	Muheza	5.16481	38.7277
Kasunga	Mariam Athuman	Mamboleo	Kiwanjani	Nkumba kibanda	Muheza	5.16481	38.7277
Kiroba	Mariam Athuman	Mamboleo	Kiwanjani	Nkumba kibanda	Muheza	5.16481	38.7277
Ponjoo	Omary Issa	Kibanda	Semhina	Nkumba kibanda	Muheza	5.17369	38.73623
Mamosi	Omary Issa	Kibanda	Semhina	Nkumba kibanda	Muheza	5.17369	38.73623
Kabangi	William Kiama	Kwemhosi	Kwemhosi	Nkumba kibanda	Muheza	5.14745	38.70201
Tandika	William Kiama	Kwemhosi	Kwemhosi	Nkumba kibanda	Muheza	5.15106	38.7046
Kikombe	Kombo Lugendo	Kwemhosi	Kwemhosi	Nkumba kibanda	Muheza	5.14297	38.71474
Kibanda meno	Asha Omary	Kisiwa	Kwemshuza	Nkumba kibanda	Muheza	5.15577	38.72145
Mahiza	Asha Omary	Kisiwa	Kwemshuza	Nkumba kibanda	Muheza	5.15675	38.72069
Sufi	Asha Omary	Kisiwa	Kwemshuza	Nkumba kibanda	Muheza	5.15564	38.7214
Pushuli	Anna Raphael	Ubembe	Majengo	Nkumba kibanda	Muheza	5.15093	38.73624
Sina wangu	Mariam Akilimali	Ubembe	Majengo	Nkumba kibanda	Muheza	5.14841	38.73374
Kitingisha ndevu	Mary Kipande	Bagamoyo	Bagamoyo	Tongwe	Muheza	5.13125	38.71547
Mwamuonage	Said Kaskazi	Bagamoyo	Bagamoyo	Tongwe	Muheza	5.12986	38.71306
Mwalimu Hamisi	Dismas Patric	Masiwa	Hoima	Tongwe	Muheza	5.11168	38.71076
Dide	Charles Msheangi	Masiwa	Hoima	Tongwe	Muheza	5.09742	38.71954
Pusuu	Frank Msami	Masiwa	Hoima	Tongwe	Muheza	5.10419	38.71243
Makaniki	Alex Mbogo	Masiwa	Hoima	Tongwe	Muheza	5.08303	38.72296
Heya (Mkunungu)	Alex Mbogo	Masiwa	Hoima	Tongwe	Muheza	5.08303	38.72296
Mbiliti	Dismas Magembe	Masiwa	Masiwa	Tongwe	Muheza	5.71183	38.71183
Beba	George Mnyonjo	Masiwa	Masiwa	Tongwe	Muheza	5.08382	38.72954
Shemakange	Dismas Magembe	Masiwa	Masiwa	Tongwe	Muheza	5.71183	38.71183
Mwanamke nyonga	Miko Mwenyigoha	Pangawe	Pangawe kati	Mkambarani	Morogoro rural	6.80268	37.79243
Mwarusha	Miko Mwenyigoha	Pangawe	Pangawe kati	Mkambarani	Morogoro rural	6.8019	37.79224
Moshi wa taa	Juma Swanga	Pangawe	Pangawe kati	Mkambarani	Morogoro rural	6.80164	37.78845

..ctn Appendix 2: Passport data for 52 collected cassava landraces

Name of cultivar	Name of farmer	Village	Hamlet	Ward	District	Latitude (°)	Longitude (°)
Msenene	Asha Nondo	Pangawe	Pangawe kati	Mkambarani	Morogoro rural	6.80164	37.7885
Kichongameno	Asha Nondo	Pangawe	Pangawe kati	Mkambarani	Morogoro rural	6.80164	37.7885
Bwana Mrefu	Rajab S. Mpingo	Mkambarani	Mkambarani	Mkambarani	Morogoro rural	6.77141	37.81506
Mbega	Rajab S. Mpingo	Mkambarani	Mkambarani	Mkambarani	Morogoro rural	6.77154	37.81493
Mzungu	Rajab S. Mpingo	Mkambarani	Mkambarani	Mkambarani	Morogoro rural	6.77141	37.81506
Mgeni	Mashaka Abdalah	Kisiwa	Kiroka	Kiroka	Morogoro rural	6.86468	37.83802
Magereza	Rajab Ali	Kisiwa	Kiroka	Kiroka	Morogoro rural	6.86456	37.83807
Kilusungu	Hamis Mkembe	Kisiwa	Kiroka	Kiroka	Morogoro rural	6.86448	37.83801
Tenga tele	Hatibu Kauli	Kisiwa	Kisiwa	Kiroka	Morogoro rural	6.86412	37.83806
Kigoma	Juma Nassor	Kiroka	Msavu	Kiroka	Morogoro rural	6.8354	37.8096
Nyamato	Abdul Majid	Sotele B	Sotele B	Kisiju	Mkuranga	7.32316	39.2179
Barawa	Abdul Majid	Sotele B	Sotele B	Kisiju	Mkuranga	7.32325	39.21798
Cheupe	Abdul Majid	Sotele B	Sotele B	Kisiju	Mkuranga	7.32325	39.21798
Nyamkagile	Maulid Jongo	Sotele A	Sotele A	Kisiju	Mkuranga	7.29873	39.23723
Cosmas	Maulid Salehe	Lukanga	Lukanga	Lukanga	Mkuranga	7.47808	39.16002
Dihanga	Maulid Salehe	Lukanga	Lukanga	Lukanga	Mkuranga	7.47752	39.16017
Cheusi	Yusuph Ndelele	Kilembea	Lukanga	Lukanga	Mkuranga	7.45631	39.15883
Mbande	Yusuph Ndelele	Kilembea	Lukanga	Lukanga	Mkuranga	7.45631	39.15883
Kichooko	Yusuph Ndelele	Misasa	Lukanga	Lukanga	Mkuranga	7.48468	39.19238
Kalolo	Mzee Doto	Buma	Chambezi	Kiromo	Bagamoyo	6.55414	38.90112
Mfaransa	Rashid Kongoromoko	Buma	Chambezi	Kiromo	Bagamoyo	5.544532	38.91947
Rasta	Hamis Chamlungu	Masaki	Kihesa	Masaki	Kisarawe	7.08511	38.94057
Shiba tumbo	Hamis Chamlungu	Masaki	Kihesa	Masaki	Kisarawe	7.08511	38.94057
Nyamwali	Hamis Chamlungu	Masaki	Kihesa	Masaki	Kisarawe	7.08511	38.94057
Mzungu mweupe	Tishi Mohamed	Gumba	Kihesa	Masaki	Kisarawe	7.11231	38.99633
Mshelisheli	Tishi Mohamed	Gumba	Kihesa	Masaki	Kisarawe	7.11245	38.99623

Appendix 3: Morphological characterization of farmers preferred cassava germplasm in Eastern zone of Tanzania data collection sheet at 6Month After Planting (6MAP)

Site: Chambezi- Bagamoyo. Date of planting:7/03/2012 Scheduled: 6 MAP

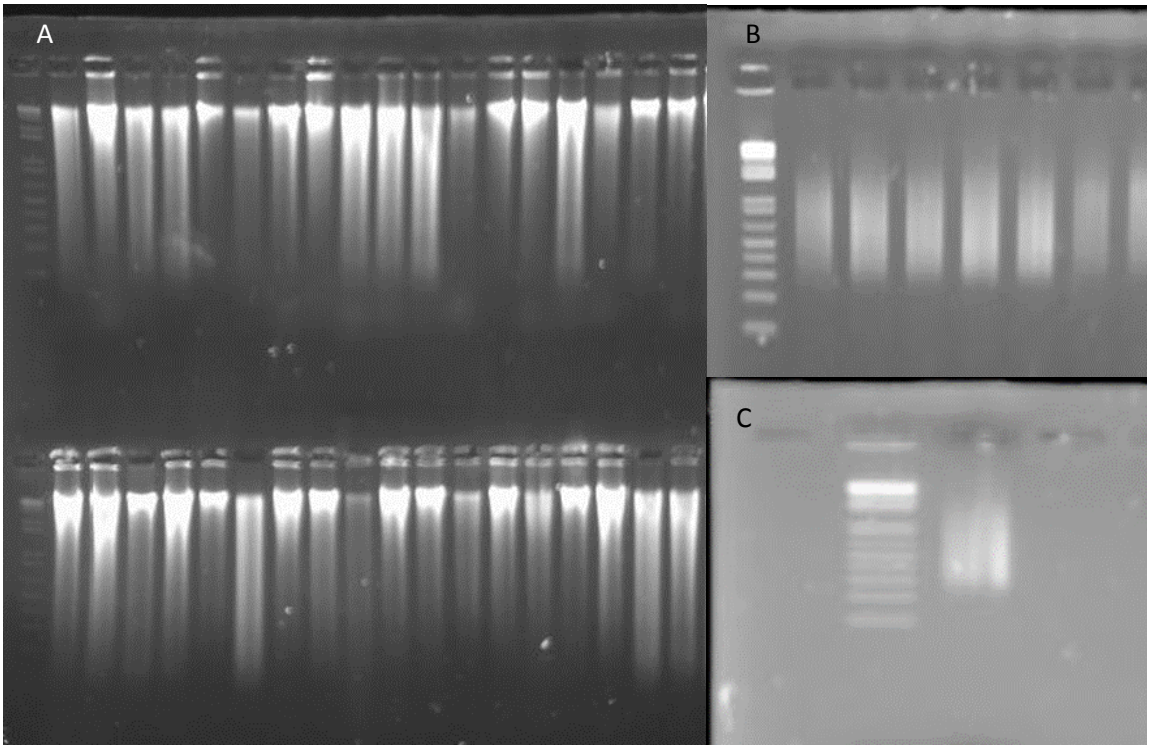
SN	Trait	Accession code								
1	Color of apical leaves									
2	Pubescence on apical leaves									
3	Leaf retention									
4	Shape of central leaflet									
5	Lobes margins									
6	Color of leaf									
7	Number of leaf lobes									
8	Length of leaf lobes (1st)									
	2nd									
9	Width of leaf lobes (1st)									
	2nd									
11	Color of petiole									
12	Petiole length (1st)									
	2nd									
13	Color of leaf vein (health plant)									
14	Orientation of petiole									
15	Flowering									
16	Pollen									

Appendix 4: Morphological characterization of farmers preferred cassava germplasm in Eastern zone of Tanzania data collection sheet at 9 Month After Planting (9MAP)

Site: Chambezi, Bagamoyo. Date of planting: 7/03/2012 Scheduled: 9 MAP Date….

SN	Trait/Score	Accession code								
17	Prominence of foliar scar									
18	Color of stem exterior									
19	Color of stem cortex									
20	Color of stem epidermis									
21	Distance between scars									
22	Growth Habit of stem									
23	Color of branches									
24	Length of stipules									
25	Stipules margin									
26	Fruit									
27	Seed									
28	Plant height									
29	Height of first branch									
30	Levels of branching									
31	Branching habit									
32	Angle of branching									
33	Shape of plant									
34	No of storage root/plant									
35	No of commercial root/pant									
36	Extent root peduncle									
37	Root constrictions									
38	Root shape									
39	External color of storage root									
40	Color of root pulp									
41	Color of root cortex									
42	Cortex; ease peeling									
43	Texture of root epidermis									
44	Root taste									
45	Cortex thickness									
46	Dry matter									
47	Starch content									
48	Harvest index									

Appendix 5: DNA Gel electrophoresis showing A) DNA extracted from 52 cassava landraces B) Cassava DNA samples after size reduction and ligation during DNA libraries preparation C) 52 cassava DNA libraries pooled together ready for sequencing



Appendix 6: A table showing number of SNP and their distribution per chromosome and position on cassava genome V5.0. (Supplemental material for paper 2)

Chromosome.	№. of SNPs	Position range
1	759	149352-20374379
2	1,335	76-19845601
3	772	1077625-17481260
4	898	71647-17398735
5	794	91543-19761206
6	1,060	29814-19227608
7	1,070	20840-24022314
8	1,071	57151-21586336
9	781	100413-25111505
10	979	23203-23450698
11	1,021	25792-21709615
12	920	112108-20662796
13	877	76-20892663
14	1,315	205-23387655
15	803	13280-21968792
16	1,253	511-19155653
17	927	216620-16276634
18	734	146624-17276135
Total	**17,369**	

Appendix 7: Supplemental material for paper 2

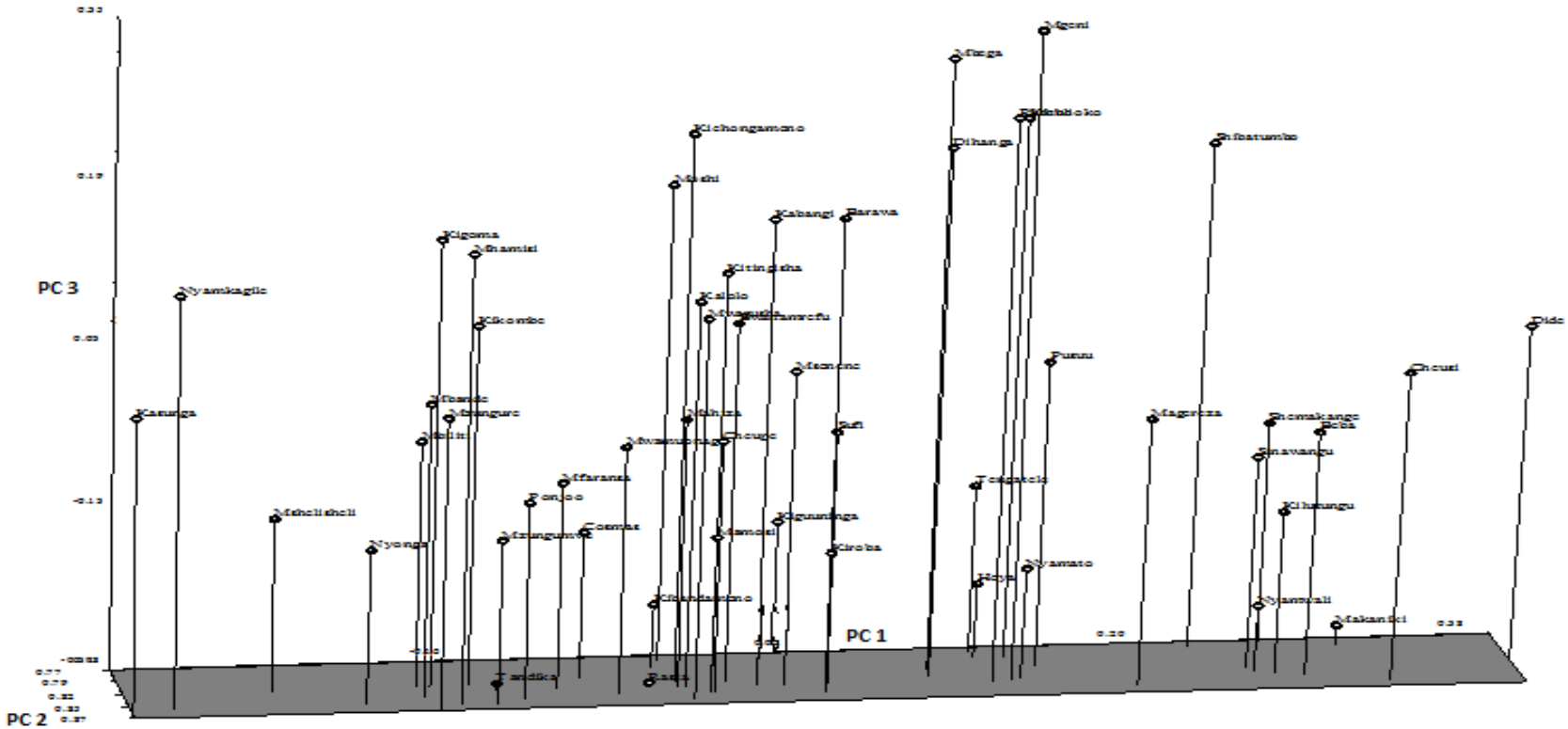


Figure S1. Principal component analyses (PCA) plot showing clustering of 52 cassava landraces using morphological descriptors

Appendix 8: Supplemental material for paper 2

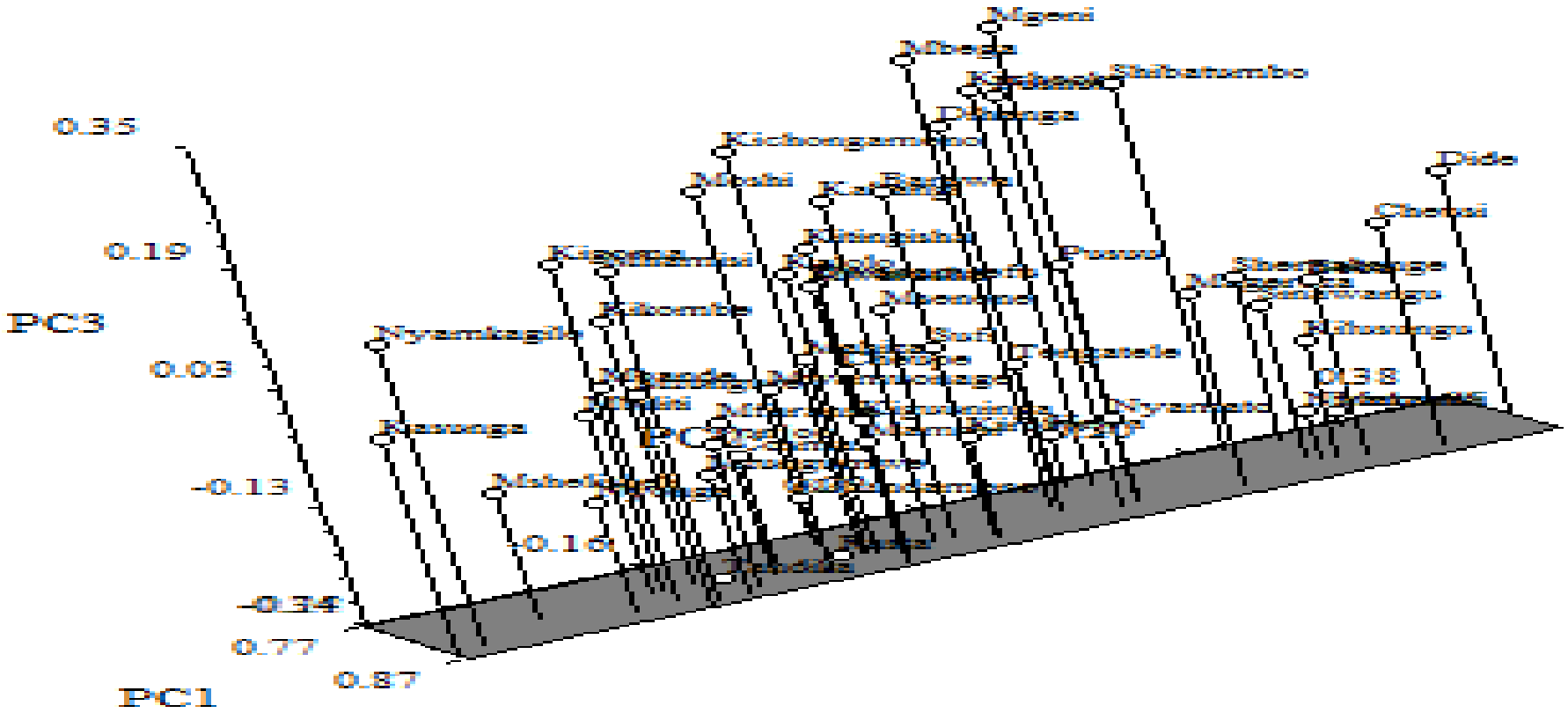


Figure S2: Principle component analysis (PCA) analysis plot showing differentiation among 52 cassava landraces using SNPs markers

Appendix 9: Figure for supplemental data Paper 3

Nyamkagile

Kibandameno

Msenene

Kilusungu

Kalolo

Kiroba

Supplemental Figure 1. Tubers of the six cassava landraces, from which starch was extracted for each analysis

Appendix 10: Sodium Dodecyl Sulfate – Polyacrylamide gel electrophoresis (SDS - PAGE) showing starch granule associated proteins

SDS PAGE analysis revealed only one band slightly below 63kDa. This suggest presence of GBSS which is 60kDa and is mainly found at the interior of the granule and is responsible for amylose synthesis

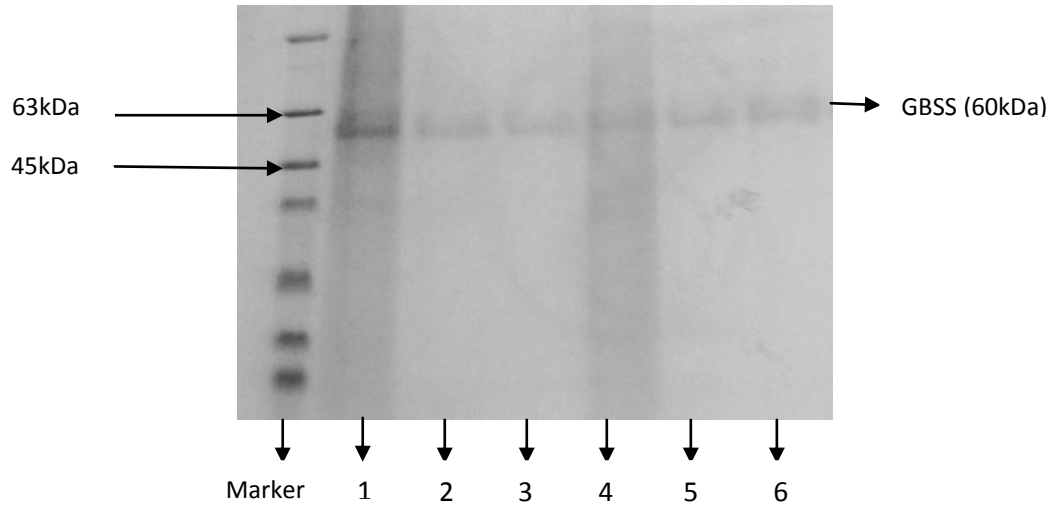
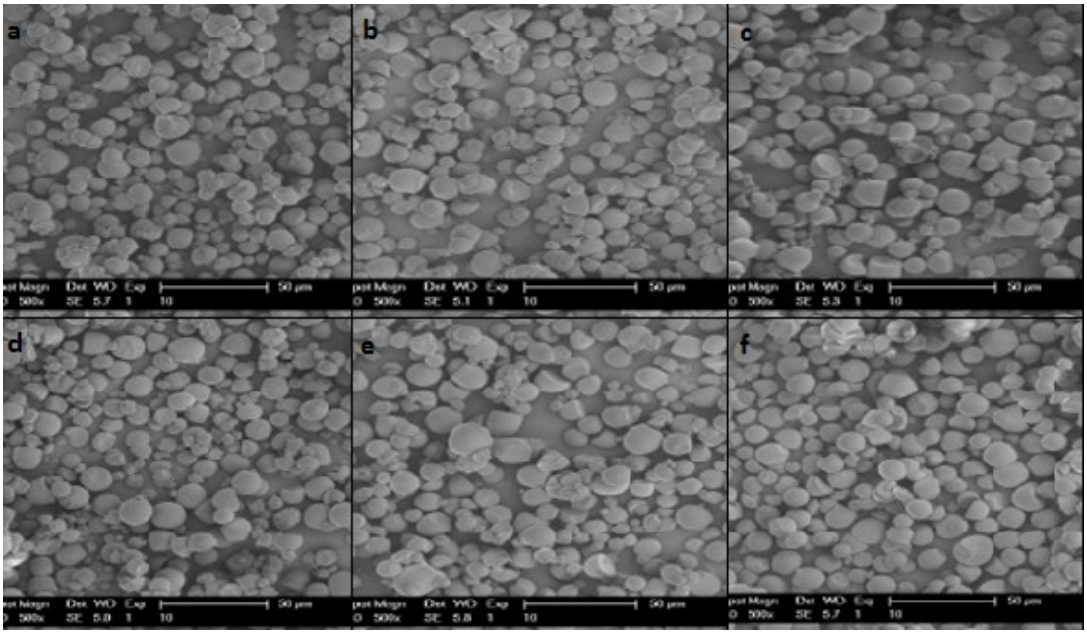


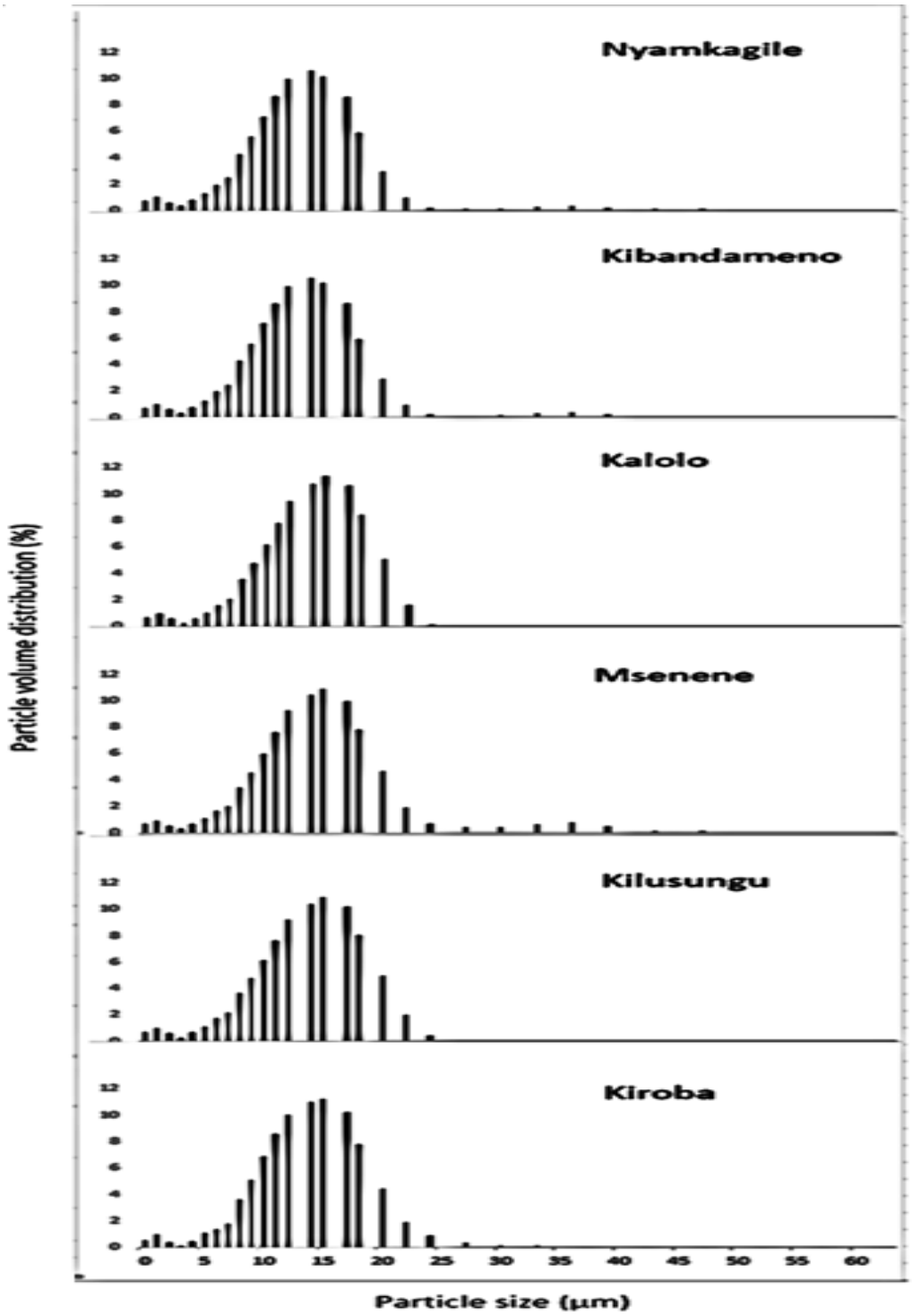
Figure 2: SDS PAGE showing starch granule associated proteins of cassava starches revealing one band at 60kDa implying presence of Granular Bound Starch Synthases (GBSS). This enzyme is responsible for amylose synthesis. 1= Nyamkagile, 2=Kibandameno, 3=Kalolo, 4=Msenene. 5=Kilusungu, 6=Kiroba.

Appendix 11: Supplemental Figure 3 for Paper 3.



Supplemental Figure 3. Scanning electron micrograph of purified cassava starches (bar=50 µm); a= Nyamkagile, b=Kibandameno, c= Kilusungu, d=Msenene, e=Kalolo, f=Kiroba landraces

Appendix 12: Supplemental Figure 4 for Paper 3.



Supplemental Figure 4: Starch particle size distribution of cassava landraces by laser diffraction. Values were derived from three biological replicates, where each replicate constituted one tuber.

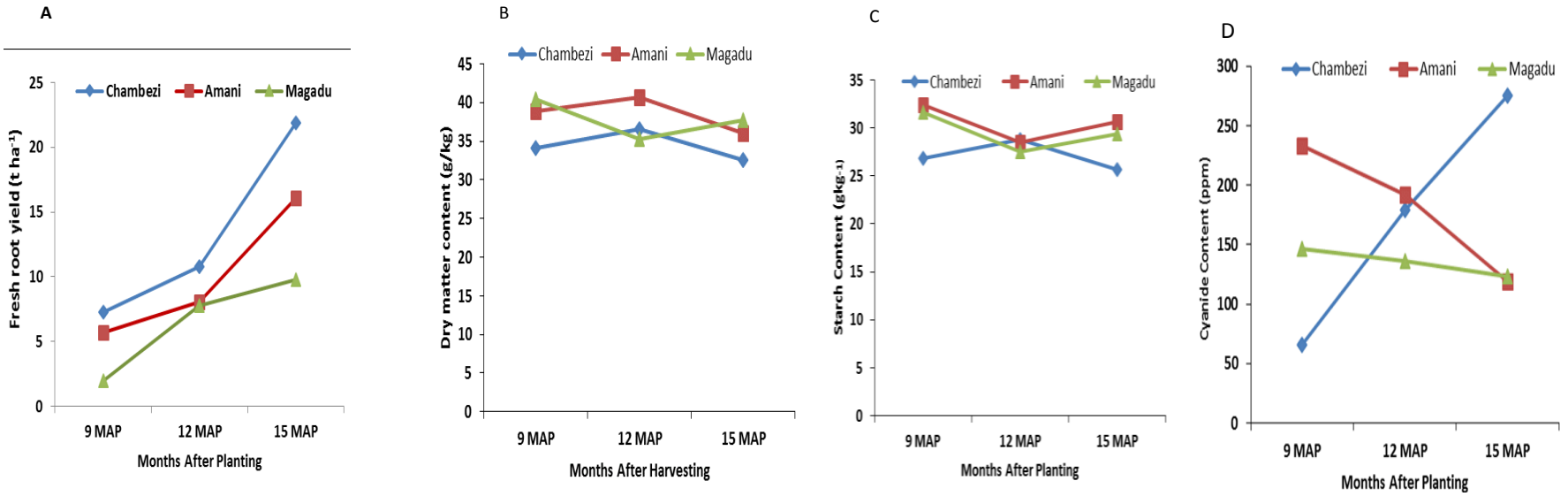
Appendix 13: Data sheet for assessment of effect of genotype and location on different agronomic importance traits- Harvesting data collection sheet

Duration: ……
Date:……..

Name of cultivar/landrace……..

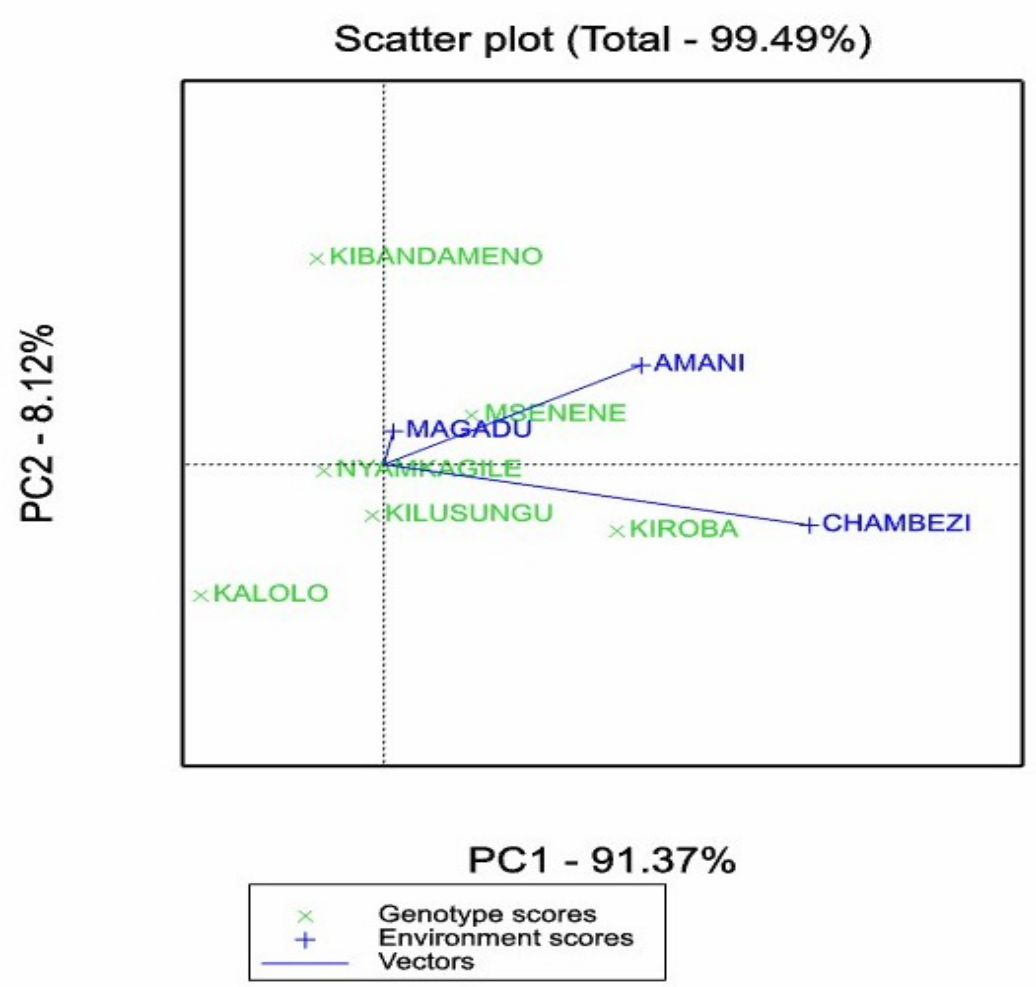
BAGAMOYO										
sn	**Parameters**	**Replications**								
		Rep 1			**Rep 2**			**Rep 3**		
		1	2	3	1	2	3	1	2	3
1	Weight of roots									
2	№ of commercial root									
3	Cyanide levels									
AMANI										
	Parameters	**Replications**								
		Rep 1			**Rep 2**			**Rep 3**		
		1	2	3	1	2	3	1	2	3
1	Weight of roots									
2	№ of commercial root									
3	Cyanide levels									
SUA										
	Parameters	**Replications**								
		Rep 1			**Rep 2**			**Rep 3**		
		1	2	3	1	2	3	1	2	3
1	Weight of roots									
2	№ of commercial root									
3	Cyanide levels									

Appendix 14: Supplemental figure 1 for paper 4



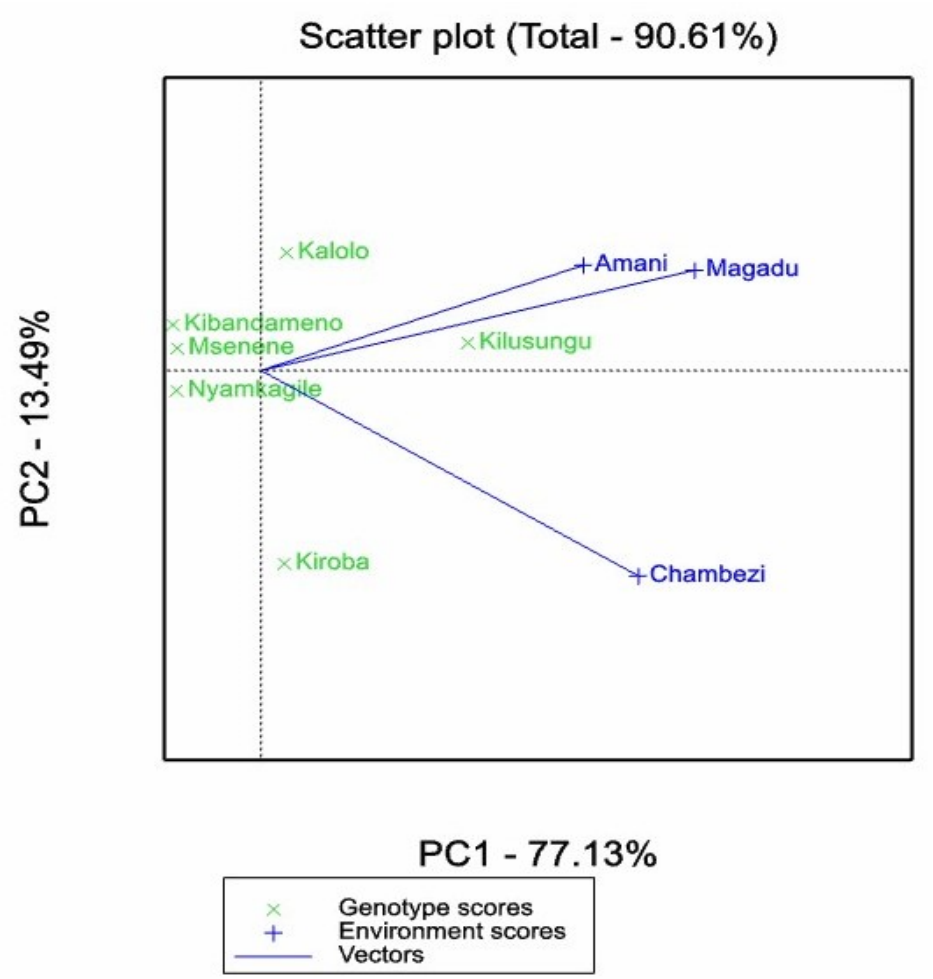
Supplemental Figure 1:Effect of time of harvesting (9, 12, 15 months after planting) on A) fresh root yield B) dry matter content C)starch content D) cyanide content, of six cassava landraces across three sites.

Appendix 15: Supplemental figure 2 for paper 4



Supplemental figure 2: Supplemental figure2. GGE Scatter plot showing discrimination and representativeness of the environments (sites) using the starch yield data of the six cassava landraces.

Appendix 16: Supplemental figure 2 for paper 4



Supplemental figure 3. GGE Scatter plot showing discrimination and representativeness of the environments (sites) using the cyanide content of the six cassava landraces.

Appendix 17: GGE plot for Starch Content

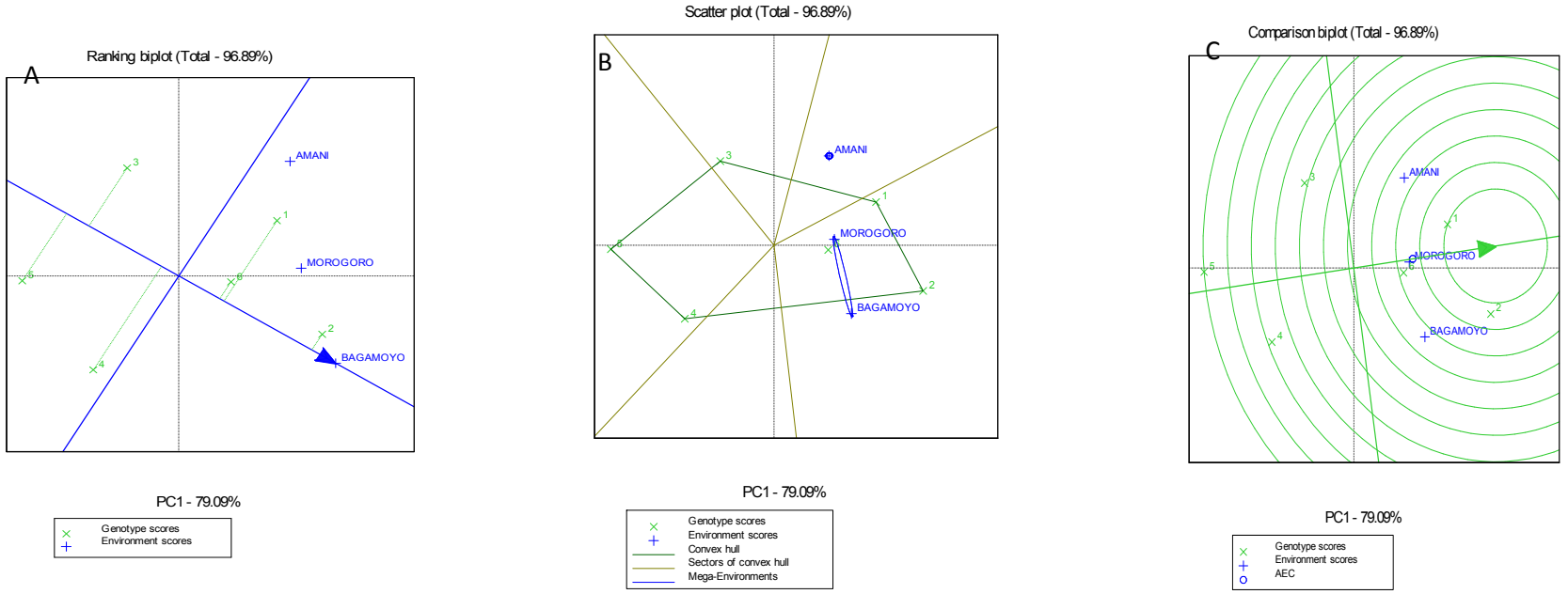


Figure 1: GGE biplots A) showing ranking of cassava landraces (genotypes) based on mean cassava starch content and stability performance across three environments B) scatter plot for which- won- where (superiority) showing the best landrace for each environment C) the average environment coordination (AEC) view to rank landraces relative to an ideal genotype.

Appendix 18: GGE biplot for cassava fresh yield

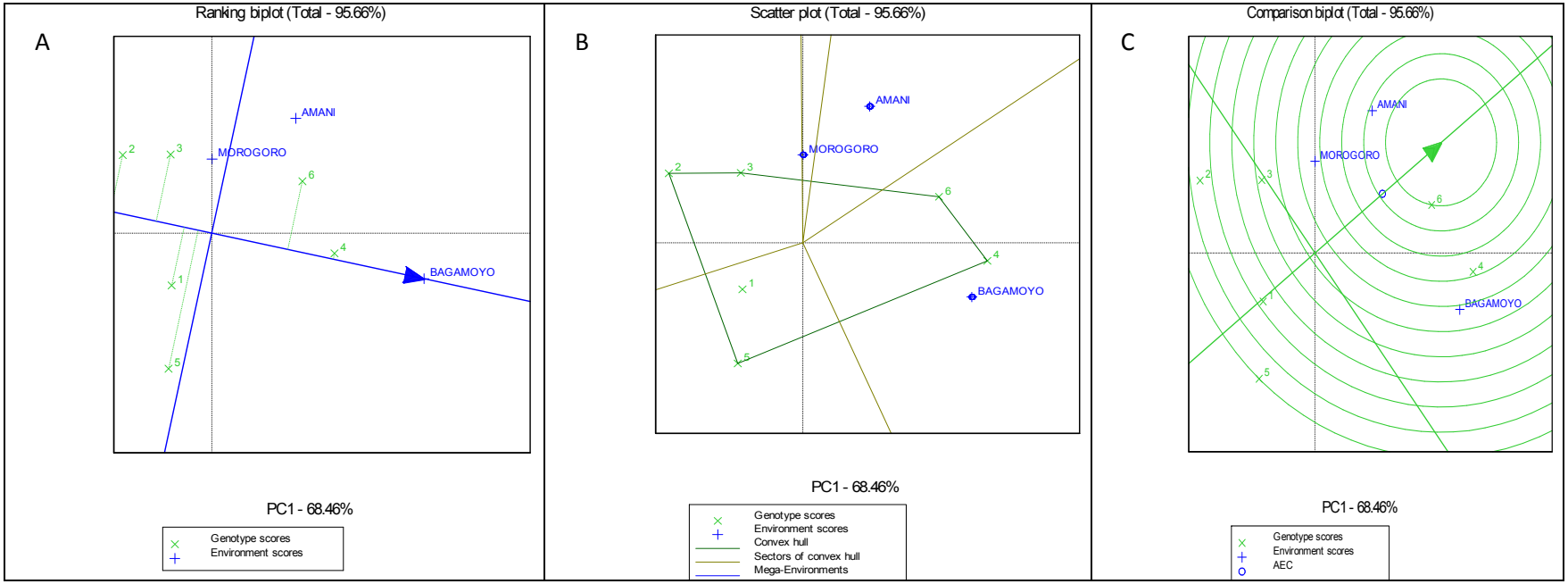


Figure 2: GGE biplots A) showing ranking of cassava landraces (genotypes) based on mean cassava yield content and stability performance across three environments B) scatter plot for which- won- where (superiority) showing the best landrace for each environment C) the average environment coordination (AEC) view to rank landraces relative to an ideal genotype.