EXPLOITATION OF HETEROSIS IN SWEETPOTATO (*Ipomoea batatas* L. (Lam)) VIA PROGENY TESTING AND USE OF MOLECULAR MARKERS

BY

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(Bsc Biological Sciences)

NOVEMBER, 2016

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI, GHANA

DEPARTMENT OF CROP AND SOIL SCIENCES

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A THESIS SUBMITED TO THE DEPARTMENT OF CROP AND SOIL SCIENCES, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY IN PLANT BREEDING

BY

NIKIEMA Jeanne

NOVEMBER, 2016

DECLARATION

I hereby declare that, except for specific references which I have duly acknowledged, this work is the outcome of my own research and that neither a part nor whole of this document has been submitted for any other degree at any other University.

…… ……..

NIKIEMA JEANNE DATE

(PG1631114)

We declare that we have supervised the above student to undertake the study submitted herein and verify that she has our permission to submit.

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PROF. RICHARD AKROMAH DATE

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(Head of Department)

ABSTRACT

Genetic diversity and heterosis (mid and better parent heterosis) was assessed in a West African breeding population by using 15 simple sequence repeat markers in 28 parents exclusively and other 10 parents with their 30 progenies in sweetpotato. The polymorphism information content (PIC) of the SSR primers used revealed that all of them were polymorphic except IB-297 and J116 A with PIC less than 50% for the 10

parents and their progenies and 6 primers (IB-248, IBS-10, IBS-18, IBR-12, IBR-16 and IBR-19) out of 15 were polymorphic for the 28 parents. These results mean that most of the primers used in this work can be used for parent and progeny diversity study in sweetptatoto. The progenies were produced from biparental crosses, and heterosis was estimated in some pre-harvest and harvest traits, harvest index and some quality traits. Parents PG12086-18, Nanungungungu and Apomuden occur most in the crosses. Some of the crosses exhibited high heterosis. Correlation among total yield and preharvest, harvest and quality traits revealed highly significant positive correlations between total yield and marketable roots, root size, number of marketable roots and harvest index. Vine vigour was also correlated significantly and positively with the weight of marketable roots, root size, number of plants harvested, percentage dry matter, iron and starch contents. Virus severity was significantly correlated negatively with the number of plants at maturity, percentage dry matter, iron content, percentage protein and starch content but not significant with total yield, weight of marketable roots, root size and number of marketable roots. The weevil damage was significantly correlated negatively with total yield, weight of marketable roots, root size, number of marketable roots and harvest index, and Percentage dry matter correlated significantly and positively with iron content, percentage protein, starch and zinc contents. The diversity study showed that the parents could be grouped into five clusters. The progenies from distantly related parent such as Nanungungungu x Bohye, Nanungungungu x Faara, Nanungungungu x Hi-starch, Faara x Nanungungungu, Nanungungungu x PG12086-18, Apomuden x Sauti and CIP440390 x PG12086-18 exhibited high heterosis for total yield, harvest index and the quality traits; and Nanungungungu x Otoo, Sauti x Nanungungungu, Apomuden x Hi-starch, PG1208618 x Apomuden, Apomuden x Faara and Apomuden x PG12086-18 exhibited high

heterosis for some yield related traits and quality traits. This study showed that all the progenies were not close to their parents due to the high somatic transformation in sweetpotato which is a source of genetic variation among genotypes.

DEDICATION

This work is dedicated to my lovely mother Mrs. Anastasie T. KABORE.

ACKNOWLEDGEMENTS

I am most grateful to the Almighty God for helping me to successfully undertake this research. I wish to also thank the Alliance for Green Revolution in Africa (AGRA) for sponsoring my studies and research work.

My sincere gratitude goes to my supervisor, Prof. Richard AKROMAH, Provost of the College of Natural Resources and Coordinator of the **AGRA program** "**Improved Msc. in Cultivar Development for Africa (IMCDA)",** for his direction and suggestions towards the success of this project. May the Almighty God continue to bless you

abundantly. I also wish to thank all the Lecturers of the Department of Crop and Soil Sciences and technicians of the biotechnology laboratory for their contributions and constructive criticisms, especially during seminars, and support in making this study successful.

My sincere gratitude also goes to the Director of Environmental and Agricultural Research Institute (INERA), and to the General Delegate of the National Centre for Science and Technology Research (CNRST), Burkina Faso, for the study leave given to me to complete this study.

I wish to express my profound gratitude to Dr. Edward, E. CAREY as supervisor at CRI-Fumesua, Dr. Marian QUAIN and Dr Mercy KITAVI for their numerous support and also for their valuable comments and suggestions throughout this study. I am grateful to all the staff of the International Potato Center (CIP) and biotechnology laboratory at Crops Research Institute (CRI) at Fumesua for their diverse contributions towards the success of this work.

I am very grateful to Dr. Koussao SOME, sweetpotato program at INERA, Burkinafaso, for his support during this study.

I would also wish to express my profound gratitude to my lovely mother, husband, children, Mrs. Anastasie KABORE, Frederic GAVI, Eliel and David GAVI and all the NIKIEMA family for their prayers, moral support, patience, encouragement and generous assistance for the execution of this research work.

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LIST OF ABBREVIATIONS

ABAM Associação Brasileira dos Produtores de Amido e Mandioca,

AFLP Amplified Fragment Length Polymorphism

AGRA Alliance for a Green Revolution in Africa

APS Ammonium Persulphate

AVRDC: Asian Vegetable Research and Development Centre CIP = International Potato Centre

BPH	Better Parent Heterosis
CIP	International Potato Center
CRI	Crops Research Institute
CSIR	Council for Scientific and Industrial Research
CV	Coefficient of Variation
DM	dry Matter
DNA	DeoxyriboNucleic Acid
F1	First Hybrid generation after fertilization
FAOSTAT	Food and Agriculture Organization of United Nation, statistic department

IBPGR International Board for Plant Genetic Resources:

IITA International Institut for Tropical Agriculture

INERA Institut de l‟Environnement et de Recherches Agricoles

LSD Least Significance Difference

MAGE MetaPhor agarose gel electrophoresis

MPH Mid-parent Heterosis

NARS National Agriculture Research System

NIRS Near-infrared reflectance spectroscopy

PAGE Polyacrylamide gel electrophoresis

PCR Polymerase Chain Reaction

PCR Polymerase Chain Reaction PIC Polymorphic Information Content

RAPD Random Amplified Polymorphic

SASHA Sweetpotato Action Security and Health in Africa

SASHA Sweetpotato Action Security and Health in Africa

SPCSV sweetpotato chlorotic stunt crinivirus

SPFMV Sweetpotato Feathery Mottle Potyvirus

SPHI Sweetpotato for Profit and Health Initiative

SPVD sweetpotato Virus Disease

SSA sweetpotato in Sub-Saharan

SSR: Simple Sequence Repeat

UPGMA Unweighted Pair Group Method using arithmetic Average

VAD Vitamin A Deficiency

WAAPP West Africa Agricultural Productivity Program

CHAPTER ONE

1.0 INTRODUCTION

Sweetpotato (*Ipomoea batatas* [L.] Lam) is a herbaceous dicotyledonous plant grown at latitudes ranging from 48°N to 40°S of the equator and altitudes from 0 to 3000 m above sea level (Woolfe, 1992; Vaeasey *et al*., 2008; Low *et al*., 2009; Troung *et al*., 2011). It belongs to the family *Convolvulaceae,* genus *Ipomoea*. This genus contains about 600 to 700 species (Vaeasey *et al*., 2008; Cao *et al*., 2009). Sweetpotato needs temperatures from 12 to 35^0 C for better growth and root production (Kuo, 1991; Woolfe, 1992). An annual rainfall of 600 to 1600 mm is required for its growth (Low *et al.*, 2009) and also a soil pH of 5.5 to 6.5 (Woolfe, 1992). It is usually considered that sweetpotato is of South or Central America origin according to Huaman, (1999).

This crop is one of the most economically important crops in the world. It is the seventh most important food crop in the world after rice, wheat, maize, potato, barley and cassava and, the third most important root and tuber crop in the world after potato and cassava (Belehu, 2003). The world production of sweetpotato was about 175,900,000 tons. China produced 75% of the global production and Africa produced about 14% of the world production led by Nigeria (3,300,000 tons in 2011) and Uganda (2,554,000 tons in 2011) (FAOSTAT, 2012). Annual production of sweetpotato in Africa has increased from 12.9 million tons in 2006 to 14.2 million tons in 2010 according to FAOSTAT, (2010).

In developing countries, sweetpotato is an important source of carbohydrate, vitamins A and C, fiber, iron, potassium and protein (Woolfe, 1992**).** The crop has flexibility in West Africa and is used in numerous food provisions in place of rice, cassava, yam, plantain and other basic foods (Ellis *et al*., 2001; Meludu *et al*., 2003; Zuraida, 2003).

Sweetpotato is very important in African agriculture for the prevention of food insecurity and malnutrition.

The Food and Agriculture Organization estimated that West, East, Central and Southern Africa had annual production of 4.2, 7.2, 1.2, and 0.5 million tons respectively in 2006. This proves that in-spite of its economic reputation the production of sweetpotato in Africa was very low because of lack of funds for its breeding. Also it is basically produced by smallholders especially the women for home consumption and to generate little incomes. It is often starred as orphan crop by many people as little breeding consideration is directed towards its improvement.

According to FAOSTAT, (2012) sweetpotato production in Ghana was low. Production was about 1.8 t/ha in 2011 compared to Nigeria 2.9 t/ha, Mali 18.8 t/ha and Burkina Faso 19.03 t/ha in the sub region. This low production can be attributed to various constraints, particularly viruses, weevils, lack of processing technology, poor availability of quality planting materials and inadequacy of improved cultivars with high and stable yield (Fuglie, 2007). It can also be because of limited market demand, with production mainly significant in the Northern and Coastal parts of Ghana where it is both a food security and cash crop (Otoo *et al.*, 2000; Otoo *et al.*, 2001).

Sweetpotato breeding has received less attention from plant breeders than many other crops, partly because of the genetic complexity of the crop: it is a polyploid. It is a hexaploid cross-pollinating crop with 2n=6x=90 (Austin and Huaman, 1996), and does not readily flower in some environments, it is self-incompatible and incompatible in some cross combinations. To boost productivity, new varieties of high yielding potentials must be created, which will incorporate both quantitative and qualitative traits and also resistance to viruses and weevils. With good crop management practices and improved varieties, high yield can be achieved in large areas such as China who produce about 75%

of the world production (FAOSTAT, 2012). However, in the past two decades, the yield of sweetpotato in Sub-Saharan Africa (SSA) were very low compared to the West pacific (China, Japan and Korea) and USA. The production per year was approximately 1.4, 2.1 and 1.2% for West pacific, USA and SSA respectively (FAOSTAT, 2011).

To improve yield in sweetpotato, breeding for new varieties with high and stable yield is very necessary. In this situation, the phenomenon of heterosis, which is the increase in yield or other traits in the hybrid, must be applied in sweetpotato breeding because it is an important way of increasing yield rapidly and improving quality by creating improved varieties (hybrids) from crosses between genetically diverse parents.

However, little is known about the use of heterosis to increase yield, resistance to stress and tuber quality in sweetpotato.The understanding and the use of heterosis in sweetpotato breeding will help identify better progenies that will produce high yield and perform well in termes of qualitative traits and resistance to stress. Applying heterosis in sweetpotato can help to solve the ever growing population demand in sweetpotato.
According to The breeding program in Ghana under the West Africa Agricultural Productivity Program (WAAPP) in collaboration with the Sweetpotato Action Security and Health in Africa (SASHA), one of the objectives is to develop high and stable yielding varieties. Therefore, heterosis can be applied in sweetpotato breeding to increase yield and boost Africa countries‟ economy and allow improvement of lives of several million families' lives by 2020 according to Sweetpotato for Profit and Health Initiative: SPHI, (Wasonga *et al.*, (2014).

Molecular markers have been used to study the genetic diversity in sweetpotato because they cover a large part of the genome and there is no environmental effect (Goulão and Oliveira, 2001). Many studies have shown that Simple Sequence Repeat (SSR) markers are more variable and provide an efficient means to recognize differences between

genotypes (Powell *et al.*, 1996). Therefore, heterosis will be exploited through development of mutual heterotic gene pools for the improvement of qualitative traits such as protein, beta-carotene, starch, dry matter, sugars and minerals (iron, zinc).

The main objective of this study was to develop heterotic sweetpotato gene pools for West Africa from regionally adapted advanced and released parents. The specific objectives were to:

i. identify progenies in the Ghanaian breeding program that exhibit heterosis increments from biparental crosses; ii. determine the phenotypic correlation among traits; iii. allocate the parents to separate gene pools based on molecular assessment of genetic distance

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and evolution

Sweepotato is from the Subgenus *Eriospermum*, section *Eriospermum*, series *Batatas* (Austin and Huaman, 1996). It is believed that the sweetpotato originated in America where most of the species are concentrated according to Huaman (1999). The series *batatas* contains 13 wild species closely related to sweetpotato. There are *I. trifida* and *I. tabascana* which are closely related to sweetpotato. It has been cultivated in America from where a lot of evidence show that it was spread broadly through the migration routes of people in the New World tropics (Huaman, 1999). The use of molecular markers has shown that the highest diversity of sweetpotato was established in Central America which can be the primary center of diversity and most probably the center of origin of sweetpotato (Huang and Sun, 2000; Zhang *et al.*, 2000). In accordance with Yen (1974), Peru-Ecuador, Papua New Guinea and the South Pacific are included in the secondary centers of origin. The evolution of *I. batatas* to the hexaploid level may have been

facilitated by sexual polyploidization via the production of unreduced gametes, but it is not yet known if *I. batatas* is an allopolyploid (Shiotani and Kawase, 1989) or an autopolyploid (Kriegner *et al*. 2003; Cervantes-Flores *et al*., 2006). It has been suggested by Nishiyama (1971) and Austin (1988) that *I. batatas* is of allopolyploid origin. According to Nishiyama, (1971) sweetpotato might have originated from I. *leucantha* (2n=2x), which produced *I. littoralis* (2n=4x). The crosses between these two species produced *I. trifida* (2n=3x), from which *I. trifida* (2n=6x) was derived from chromosome doubling. Different selection and domestication of these wild species might have produced *I. batatas* (2n=6x) which is the cultivated sweetpotato. According to Austin (1988) *I. triloba* and *I. trifida* contributed to the sweetpotato genome by using some morphological characters. Roullier *et al. (*2013) suggested that sweetpotato has been developed from two distinct autopolyploid happenings including different populations of an extinct ancestor species that was a progenitor of both *I. batatas* and *I. trifida*, which may be related to existing accessions of rough tetraploid *Ipomoea* accessions.

2.2 Biology and morphology

Sweetpotato is a perennial plant grown as an annual plant vegetatively by stem cuttings and storage roots. It can also be grown by true seeds. Its growth habit is mainly prostrate with a vine system expanding horizontally on the ground. The growth habit types are erect, semi-erect, spreading and very spreading (Huaman, 1999). Sweetpotato has fibrous roots absorbing nutrients, water and anchor the plant; the storage roots are lateral, fleshy, and thick and store photosynthetic products (Huaman, 1999). The commercial part of sweetpotato plant is the storage roots produced at the nodes of the mother stem cuttings that are underground. But some spreading cultivars produce storage roots at some of the nodes that come into contact with the soil. The storage roots can be in groups or scattered.

They can be long and tapered, some large and short with a smooth skin whose colour ranges from red, purple, brown and beige and the flesh colour ranges from white through yellow, orange and purple (Dapaah *et al.*, 2005).

The stem of sweetpotato is cylindrical and the overall length of the internodes depend on the growth habit of the cultivar and water availability. The stem colour depends on the cultivar and differs from green to entirely pigmented with anthocyanin (red-purple colours). The presence of hair in the apical shoots and stem of some cultivars varies from glabrous to very pubescent (Huaman, 1999). The branching depends on the cultivar (Yen, 1974) and vary in number and length. There are three types of branches in sweetpotato, primary, secondary and tertiary at different stages of growth. The branching intensity in sweetpotato is influenced by photoperiod, soil, spacing, moisture and nutrient supply (Somda and Kays, 1990; Sasaki *et al*., 1993).

The leaves of sweetpotato are simple and spirally arranged consecutively on the stem in a design identified as 2/5 phyllotaxis which means that there are 5 leaves spirally organized in 2 circles around the stem for any two leaves and are situated in the similar erect flat on the stem (Huaman, 1999). The total number of leaves per plant varies from 60 to 300 (Somda *et al*., 1991). The stomata are present on the upper and the lower surface of the leaves. According to Kubota *et al*. (1993), the cultivars that have a greater number of stomata on the abaxial surface and a lower number on the adaxial surface are identified among the high yielding cultivars. The petiole length also varies generally with genotype and may approximately range from 9 to 33 cm (Yen, 1974). The petiole length increases with the different stages of growth. Therefore, sweetpotato flower is physiologically complex because it is under the environmental effect such as photoperiod; it is open and receptive for a very short period of time; it is mostly incompatible and the variation in stamen length with respect to the style introduces more morphological problem into the pollination mechanism. It is a bisexual flower containing both the stamens and the pistil.

It has two small nectarines which attract the bees for the pollination. This pollination is mainly done early in the morning when the stigma is receptive. Sweetpotato fruit is a capsule turning brown when it is mature and is flat on one side and convex the other with a terminal end. Each capsule may contain one to four seeds with the colour rangin from brown to black. A thick, very rigid and impermeable to water or oxygen testa protect the embryo and endosperm making the seed germination very difficult. So scarification by mechanical abrasion or chemical treatment are require for the seed germination because the only dormancy present is the impermeability of the testa (Onwueme, 1978; Huaman, 1999).

2.3 Importance of sweetpotato

Sweetpotato is one of the most important global food crops (Loebenstein, 2009). Its importance as a food, feed, nutrition and income security crop is widely recognized (FAOSTAT, 2012). It is a useful crop that offers diverse utilities from consumption of fresh leaves or roots to processing into food, starch, candy, flour, natural colorants and alcohol (Lebot, 2010). It is also recognized as one of the healthy foods because of the presence of high beta-carotene and anthocyanins in the orange-fleshed and purplefleshed sweetpotato (Lin and Chang, 2005).

2.3.1 In human diet and animal feeding

Loebenstein, (2009) has reported that sweetpotato is one of the food crops in the world which yield more biomass and nutrients per hectare. It produces high root dry matter for human consumption and provides high calorie at 152 MJ ha^{-1} day^{-1}compared to other crops such as cassava, wheat, rice and maize with respectively 121, 135, 151, and 159 MJ ha^{-1} day^{-1} calories (Horton and Fano, 1985; Scott *et al*., 2000). However high dry matter content in sweetpotato is one of the characteristic preferred by consumers and processors. It has become a staple food in the highlands of Uganda, Rwanda, and Burundi (Lebot,

2010) and is also dried and eaten as a porridge in dry environments , including northeastern Uganda (Thottappilly, 2009). For example, in Indonesia, sweetpotato is used mostly as food while in Vietnam, it is for food and animal feed (Carpena, 2009). Sweetpotato is also rich in carbohydrates, sugars, vitamins B6, C, E, riboflavin, thiamin, niacin, copper, iron, potassium, zinc, aluminium, phosphate, ascorbic acid and folic acid (Woolfe, 1992). A total of 30 to 50 million tons of sweetpotato roots and vines are used in animal feeding every year in China (Zhang and Li, 2004), about 50% of their production.

2.3.2 In health

Sweetpotato has been recognized to be the first main food crop bio-fortified for provitamin A (Bouis and Islam, 2012; Hotz *et al*. 2012). The orange-fleshed and purplefleshed are very rich in Beta-carotene which is the precursor of vitamin A, an imperative micronutrient for the body and helps to combat vitamin A deficiency. The lack of Vitamin A can cause xerophthalmia which is cause of blindness, limit growth, poor immunity and increase mortality (Low *et al*, 2007). In the developing world, about 40 % of the children under five years of age have their body unable to fight against some diseases due to the lack of vitamin A, (Low *et al*, 2007). It has been reported by many researchers that sweetpotato can reduce the blood glucose content because of the antidiabetic compounds contained in the leaves and has also anticarcinogenicity properties (Islam, 2006). Some health benefits are shown in Table 2. 1.

Table 2.1 Certain health advantageous functions of sweetpotatoes and the different components

Health Beneficial Function	Components
Antioxidative activity	Polyphenol, vitamins, anthocyanin
Reduction of liver injury	Anthocyanin, ß-carotene
Antimutagenicity	Polyphenol, vitamins, anthocyanin
Anticarcinogenesis	Ganglioside

Antihypertension	Polyphenolics, vitamins, anthocyanin
Antimicrobial activity	Dietary fiber, polysaccharide
Anti-inflammation	Dietary fiber
Promotion of bowel movement	Dietary fiber
Anti-diabetic effect (WSSP)	Acidic glycoprotein
Anticaries effect	Dietary fiber
Ultraviolet protection effect	Polyphenolics, vitamins

Source : Islam *et al*. 2003 and Islam, 2006

2.3.3 In industries

It has been reported by Woolfe (1992) that starch is the main component constituting 70% of the dry weight of sweetpotato. The high quantity of starch biomass can be fermented and converted into ethanol for biofuel production. (Loebenstein and Thottappilly, 2009; Cervantes-Flores *et al*., 2010). Also the high quality starch from sweetpotato can be used in the chemical industry particularly, pharmaceutical and cosmetic according to the Brazilian Association of Producers of Cassava Starch (ABAM - Associação Brasileira dos Produtores de Amido e Mandioca, 2007).

2.4. Major pest and diseases of sweetpotato

Sweetpotato is affected by many pests and diseases which can cause a significant yield loss. The most devastating disease of this crop is the sweetpotato virus disease (SPVD) which is the simultaneous infection of the sweetpotato feathery mottle potyvirus (SPFMV) vectored by aphids (*Myzus persicae*, *Aphis gossypii* and *A. cracivora*) (Stubbs and McLean, 1958) and sweetpotato chlorotic stunt crinivirus (SPCSV) vectored by white flies (Schaefers and Terry, 1976; Cohen *et al*. 1992). SPVD is a constraint in about 90% in the East Africa area (Wasonga *et al*., 2014). Furthermore, sweetpotato weevils (*Cylas puncticollis* and *C. brunneus*) count as a major constraint in sweetpotato production

(Woolfe, 1992). Some (2013) reported that weevil infestation is the most important cause of sweetpotato storage root damage in the field. The damage caused by SPVD and weevil attacks have important negative effect on food safety by the reduction of the production due to important yield losses and can also affect the revenue of producers and consumers alike. So breeding for sweetpotato virus resistant varieties can be the best way to have high yield and it has been suggested to be a durable solution to sustainably regulate SPVD and other viral disease (Domola *et al*., 2008; Ngailo *et al*., 2013). Additionally, the use of virus-free sweetpotato planting materials has been suggested by Opiyo *et al*. (2010) to be a practical method to avoid the damages produced by viruses. The use of uncontaminated and virus free planting materials is feasible if the systems are well-organized (Carey *et al*., 1999; Feng *et al*., 2000).

2.5. Pest and disease management in sweetpotato

2.5.1 Integrated pest management

Insect pests constitute the major production problem for sweetpotatoes in the world (Horton and Ewell, 1991). Among them *Cylas* spp. are the number one pest problem in the world according to Jansson and Raman (1991). So breeding of sweetpotato must integrate pest management by associating many management practices to be successful. Therefore, Host plant resistance; Cultural practices; Biological control and Chemical control can be applied simultaneously.

In Host plant resistance, the use of resistant Plants offers a crucial role in the management of insect pests. The physical traits of the root (shape, length, neck length, colour of the skin, flesh colour and thickness) plays an important role in preference by *C. formicarius*. Teli and Salunkhe (1996) reported that round and oval roots of sweetpotato were more infested in the field by *C. formicarius* than long stalked, spindle and elongate ones. Pink and red coloured roots are considered less susceptible than white and brown coloured ones. Cultivars with thin foliage and lobed leaves with purple coloration at emergence

were also found less susceptible. Among the numerous genotypes screened for resistance against SPW, some of them such as CO-3 (Teli and Salunkhe, 1996); PI 508523, PI 54116, PI 564107 (Thompson *et al*., 1999) and CIPSWCA-2, S-594, 440038, SV-98 and Kamalasundari (Anonymous, 2009) were found resistant to *C. formicarius.*

Cultural practices include those interventions, which prevent the existence of the insect pests by advancing planting time, irrigation, planting of trap crops, adopting crop rotations, mixed or intercropping, sanitation, erecting birth perches.

Mixed cropping systems with sweetpotato and other crops (ginger, okra, maize, colocasia and yam) are practiced by farmers. Low incidence of *C. formicarius* was observed in these systems (Rajasekhara, (2005) and Rajasekhara *et al*. (2006)). SPW infestation reduced from 4.8 to 11.54 weevils kg^{-1}roots in sweetpotato intercropped with rice, cowpea or colocasia as compared with monoculture of sweetpotato (217.5 weevils) (Pillai *et al*., 1987). For example, in low land rice fields in India, the cropping systems such as rice-sweetpotato-cowpea; rice-rice-sweetpotato and rice-sweetpotatorice were effective in decreasing SPW infestation (Pillai *et al*., 1996).

Biological control consist of the use of sex pheromone to reduce weevil population. The sex pheromone alone contributed to significant reductions in weevil populations and root damage, which resulted in greater marketable root yield. The sex pheromone (Z)3dodecen-1-ol (E)-2-butenoate was demonstrated to be an effective reproduction disruptant for the control of *C. formicarius* (Mason and Jansson, 1991; Yasuda, 1995)

In chemical control, several insecticides were tested for the management of SPW by using them after planting, either by foliar spray or basal granular applications. Some of the insecticides are also used for vine dipping for successful control of SPW. Fenvalerate, permethrin and deltamethrin @ 0.003% were the most effective insecticides to *C.*

formicarius (Rajamma, 1990). Teli and Salunkhe (1994b) reported that dipping sweetpotato cuttings in insecticide solution before planting and spraying the crop 1 month after planting, and further 3 times, at 3-week intervals, then with cypermethrin or fenvalerate @ 375 g a.i. ha-1, was most effective in reducing damage caused by SPW.

2.5.2 Sweetpotato virus disease management

Cultural methods can be used during the first month after planting of the crop. Plants with SPVD should be removed. The early removal of infected plants can signifiantly reduce the probability of the remaining crop to develop the disease. In addition, new plantings should be at least 15 metres away from existing plantings where diseased plants might be present; this break will stop the whiteflies from spreading the virus to new plants.

Controlling sweetpotato viruses by host plant resistance involve either use of resistant cultivars or „clean seed". A graft transmission technique was used to screen sweetpotato clones for resistance to SPVD, where a high broad sense heritability for resistance was found (Hahn *et al*., 1981). Some SPVD-resistant cultivars were developed at Namulonge breeding programme in Uganda. These include NASPOT 1 to 6, NASPOT 11 (Grahame, 2015). New „Kawago" and „Sowola" were also found to be promising parents for improving resistance to SPVD (Mwanga *et al*. 2002, 2003).

For biological control, pepper spray and garlic juice have been evaluated and are effective for the reduction of insect population (Lawrence *et al*., 1999b, 2001), which can help to avoid virus infection. For chemical control, the insecticides Dursban can be sprayed to reduce termites and white flies" population and Cymethoate Super for thrips.

2.6 Heterosis

2.6.1 Definitions

Shull (1952) defined the phenomenon of heterosis as "the interpretation of increased vigor, size, fruitfulness and speed of development, resistance to disease and to insect pests, or to climatic rigors of any kind manifested by crossbred organisms as compared with corresponding inbreeds, as the specific results of unlikeness in the constitution of the uniting parental gametes". It means that the fundamental concept of heterosis is that, deleterious alleles persist in large random-mating populations, and the reduction in vigor of individuals or populations due to increase in homozygosity of deleterious alleles is made by inbreeding due to drift, population isolation, or consanguineous mating by plan or by chance. Genetic divergence of the two parental varieties is very necessary for the manifestation of heterosis according to Hallauer and Miranda (1988).

Heterosis is also defined by Falconer and Mackay (1996) as "the difference between the hybrid and the mean of its two parents". According to Shawn (2012), "heterosis is the increase in vigor that is observed in progenies of mating of diverse individuals from different species, isolated populations, or selected strains within species or populations". Heterosis is the occurrence of a superior offspring from mixing the genetic contributions of its parents. On the other hand, it is the superiority in performance (vigour or fitness) of a hybrid (F_1 individual) over the average performance of its inbred parents. It can be quantified in terms of the mid-parent heterosis which is the difference in the performance of the offspring relative to the average performance of the inbred parents and the high or better-parent heterosis which is the superiority of the offspring relative to the best parent. Heterosis can be estimated by using Fehr (1993) formula: Mid-parent heterosis = $[(F_1MP)/MP) *100]$ where $MP= (P_1+ P_2)/2$ and F_1 is the performance of the hybrid. The

better parent heterosis = [(F_1-BP)/BP*100] where BP is the performance of the best parent.

Heterosis is known as the increase in heterozygosity and is the opposite of inbreeding depression which is the reduction in performance due to the decrease in heterozygosity (Shawn, 2012) or the reduction in performance as a result of mating genetically alike parents. Exploitation of heterosis in sweetpotato can permit to identify the better progenies that will produce high yield and perform well in qualitative traits and resistance to biotic stress by the increase in inbreeding within mutually heterotic gene pools. Therefore, heterosis is better for the yield and yield stability in sweetpotato and is a key feature in the success of hybrid cultivars.

2.6.2 Genetic basis

Heterosis is based on some hypothesis. The theory of quantitative genetic stipulated that heterosis can come from dominance, overdominance, or epistasis. The dominance theory assumes that vigour in plants is conditioned by dominant alleles, the recessive alleles being neutral in effect. Consequently, crossing two parents with complementary dominant alleles will concentrate more favorable alleles in the hybrid than either parent. Overdominance theory is when the heterozygote is superior to the homozygote. It is an intra-allelic interaction where the presence of multiple alleles leads to greater performance than homozygosity for either allelic state (Shawn, 2012). In overdominance the hybrid performance is greater than the sum of the parents.

The epistasis hypothesis qualify heterosis to epistatic interactions between non-allelic genes (Schnell and Cockerham, 1992). Understanding of epistasis continues to be difficult in heterosis, but recent experiments have shown its importance. In some current studies, detection of epistatic interactions have been facilitated by using molecular markers and modern computational intensive statistical approaches. The role of epistasis

in hybrid performance has been provided by the generation means analysis. For example Wolf and Hallauer, (1997) have used a means-based analysis to support the role of epistasis in heterosis. The triple testcross analysis compares the relative performance of segregating progeny when testcrossed to both parents and to the F_1 hybrid. Deviation in performance of the F_1 testcross from the average of the parental testcrosses is consistent with epistatic gene action. If the major origin of heterosis is overdominance, it means that the hybrid heterozygote is better than one or other of the parents because phenotypically this hybrid is different and superior to the homozygous parents. Also, if it is dominance or epistasis the foundation of heterosis, breeding populations, and then individuals, will turn out to be stable for advantageous alleles and do similarly to any hybrid (Schawn, 2012)

2.7 Importance of heterosis in crop plants

Heterosis has been used as an important phenomenon in cultivar improvement because it is a major yield factor in plant breeding and has brought many changes in agriculture by improving key agronomic traits in crop plants. The use of heterosis in crop plants has been essential in agriculture and help to meet the world‟s food needs (Duvick, 1999). According to Duvick, (2001), heterosis has been successfully used in maize breeding and it has been exploited also in other crops such as wheat (Wang *et a*l., 2006; Qi *et al*., 2012), tomatoes (Krieger *et al*., 2010) and rice (Yu *et al*., 1997). It has been reported that the use of hybrid cultivars in rice has increased the production to about 15 tons per hectare in China. It is an important phenomenon for increasing yield rapidly and for improving quality in crops. Therefore, studies have shown that the production from heterotic hybrids are 10 to 30 percent greater than the ordinary varieties production. A study made by Luthra, (2006) in potato to identify superior parents and crosses based on progeny mean and heterosis (mid-parent and high-parent heterosis) has shown that progeny mean was

extremely related with heterosis and suggested that this can be used for potato improvement.

2.8 Heterosis in sweetpotato

Good results have been obtained by the use of heterosis in cereal crops the past years, but in the roots and tubers, heterosis has not been yet well exploited (Gruneberg, 2014). Until now only two studies have been done to evaluate heterosis gain in sweetpotato. The first study was done by Grüneberg *et al*. (2015) who have reported on mid-parent and mid-offspring storage root yield performance of 48 families which has shown heterosis increments in the range from -34% to 58%. The second study was done by Kivuva *et al*. (2015) in the F_1 and parental means for 6 parents and 15 families where mid-parent – midoffspring heterosis increments have been calculated for yield with the range from -43% to 92% under no drought and -54% to 82% under drought stress. However, in clonally propagated crops such as sweetpotato, the clone hybrids are the foundation to choose the best clone variety by using gene-pool separation and controlled crosses to increase yield and resistance to abiotic stresses by working with two mutually heterotic gene-pools which can cause an increase in yield from 20 to 40%. It has been assumed by Gruneberg *et al*. (2009) that crosses of parents of varied gene pools might produce more diverse progenies and possible heterosis increment.

2.9 Molecular characterization

Crop diversity characterization can be done morphologically because it is an important first step in diversity evaluation, but because of the limitations due to the phenotypic flexibility, little levels of polymorphism, late manifestation for certain traits, low repeatability (Yada *et al*., 2010b; Karuri *et al*., 2010), and the impact of the environment on morphological traits (Yada *et al*., 2010c), molecular characterization which is an important biotechnology tool in plant breeding programs (Favoretto *et al*., 2011) can be the best way of diversity characterization. It makes possible the identification of potential

heterotic gene pools within populations. Moreover, in many crop plants, molecular markers have been widely adopted as powerful tools for cultivar identification, germplasm characterization, phylogenetic studies and diversity analysis (Yang, 2014). Therefore, for heterosis exploitation in sweetpotato, the use of molecular approaches is very necessary for cultivar improvement (Yada *et al*., 2015). But, some makers based on PCR (Polymerase Chain Reaction) such as RAPD (Random Amplified Polymorphic DNA) and AFLP (Amplified Fragment Length Polymorphism) lack specific sequences which is a handicap for variety identification (Wang *et al*., 2011).

Microsatellites or simple-sequence repeats (SSR) markers were the first PCR-based markers to be broadly used for molecular fingerprinting (Arif *et al.*, 2011). They have many advantages such as, easy to reproduce, are co-dominant, have a frequent and random distribution, are highly polymorphic because they have a high mutation rate which influence the number of repeated units and are widely distributed all over the genome, easy to be amplified by PCR (La Rota *et al*., 2005; Arif *et al.*, 2011; Favoretto *et al*., 2011). Arif *et al*. (2011) have also reported that SSR markers are the most powerful genetic markers followed by AFLP and RAPD because they can simply distinguish among homozygotes and heterozygotes and be capable to identify the alleles, but AFLP and RAPD have two alleles per locus, so they are considered as dominant markers. It has been also reported by Jarret and Bowen (1994) that, simple sequence repeat markers are presently the most appropriate markers to determine the paternity and identify heterotic gene pools in sweetpotato because they occur all over the genome, are codominant and moderately easy to score.

In animals and plants, these markers are now one of the most useful molecular markers because of their great advantages. It has been reported by Favoretto *et al*. (2011) that SSR markers have been more efficient than RAPD markers because the use of three SSR primers permit to differentiate cultivars in comparison with six RAPD primers used.

Identification and characterization of the genetic diversity and relationship of sweetpotato have proved the efficiency of SSR markers according to Zhang *et al.* (2000); Hu *et al.* (2003); Yada *et al.* (2010).

According to Sun *et al.* (2001) the superiority of SSR markers have been demonstrated among other DNA markers and have also been used in evaluation of genetic diversity and germplasm identification. For example Karuri *et al*. (2009) have reported that SSR markers have been used in sweetpotato genotype characterization recently in the germplasm collection in Kenya for Sweetpotato Virus Disease (SPVD) resistance and high dry matter content. Therefore, because of the cross-pollination in sweetpotato allowing exchange of alleles between varieties, it is necessary to evaluate the genetic relationships among diverse cultivars and landraces for the exploitation of genetic diversity in germplasm (Yang, 2014).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. Experimental site

The study was carried out at the FOREST ecozone at the Council for Scientific and Industrial Research - Crops Research Institute (CSIR-CRI) at Fumesua (Kumasi, Ghana) locate at Latitude 7º24‟N, Longitude 1º21‟W and Altitude of 228.7m with a minimum annual rainfall of 1213.1 mm and maximum of 1807.3 mm. The annual minimum temperature ranges from 21.8ºC to 22.8ºC and maximum of 29.7ºC to 34.3ºc.

3.2. Germplasm source

Plant materials were from the Sweetpotato Action Security and Health in Africa (SASHA) crossing block (2014) and the CSIR-Crops Research Institute controlled

crosses (2013) at (Fumesua, Kumasi). Seeds of 15 families with more than 15 seeds per family involving 10 parents from some released varieties (Faara, Sauti, Apomuden, Histarch, Otoo, Ligri, and Bohye), advanced varieties (CIP-440390, PG12086-18) and Nanungungungu, a local variety from Burkina Faso were selected. Also eighteen parents from the crossing block were included for the molecular work. These were: PG12010-15, PG12151-73, Tupurple, PG11113-11, Blue Blue, CIP-442162, PG1216630, PG12164-26, PG12136-2, PG12164-21, PG11040-6, Jiti nada, AP3A, Patron, Dadanyuie, Santompona, Ogyefo and Okumkom. The fifteen families (crosses) and the twenty eight parents with their code are presented in Table 3.1.

Note; OP = Open pollinated

Table 3.1 Crosses of sweetpotato and the parents used in the study

Hybrid	Code	Code	Accessions	Pedigree	Origin	Type	Code	Accessions	Pedigree	Origin	Type
PG12086-18 X Apomuden	16001	1	PG12164-26	Faara x OP	Ghana	Advanced	16	Patron	-----	Burundi	Released
PG12086-18 X CIP440390	16002	2	CIP442162	-----	IITA	Advanced	17	PG1216630	Faara x OP	Ghana	Advanced
Nanungungungu X Ligri	16003	3	PG12136-2	Santom pona x Faara	Ghana	Advanced	18	PG1201015	440293 X OP	Ghana	Advanced
Nanungungungu X Otoo	16004	4	PG12164-21	Faara x OP	Ghana	Advanced	19	PG1208618	BM85/42 X OP	Ghana	Advanced
Nanungungungu X Bohye	16005	5	PG11113-11	Sauti x Otoo	Ghana	Advanced	20	Bohye	-----	------	Released
Faara X Nanungungungu	16006	6	PG12151-73	Apomuden x OP	Ghana	Advanced	21	Faara	-----	IITA	Released
CIP440390 X PG12086-18	16007	7	Tu-purple	-----	USA	Local	22	CIP440390	------	IITA	Advanced
Apomuden X Hi-starch	16008	8	PG11040-6	Beauregard x BOT03/036	Ghana	Advanced	23	Hi-starch	------	Japan	Released
Sauti X Nanungungungu	16009	9	Jiti nada	-----	Kenya	Local	24	Ligri	------	Cuba	Released
Apomuden X Faara	16010	10	AP 3A	Apomuden x OP	Ghana	Local	25	Sauti	------	Tanzania	Released
Apomuden X Sauti	16011	11	Blue blue	------	Ghana	Local	26	Otoo	------	Burundi	Released
Nanungungungu X PG12086-18	16012	12	Santompona	------	-----	Released	27	Nanungungung	------	Burkina Faso	Local
Apomuden X PG12086-18	16013	13	Okumkom	------	IITA	Released	28	Apomuden	------	Bangladesh	Released
Nanungungungu X Faara	16014	14	Ogyefo	------	Rwanda	Released					
Nanungungungu X Hi-starch	16015	15	Dadanyuie	------	IITA	Released					

3.3. Field experiment

3.3.1. Nursery preparation

The seeds were scarified mechanically using sand paper and sown into the seed boxes containing steam-sterilized soil (mixture of 3 wheelbarrow of top soil + 1 wheelbarrow

of sand + 3 shovels of manure). After germination, the seedlings were transferred into five litres pots filled with the steam -sterilized soil to allow good growth in the screen house for about two months. The seedlings were watered every day automatically with sprinklers.

A

Figure 3.1 Sweetpotato seeds germinating in the seed boxes A); seedlings being transferred into pots B) and seedlings nursery in the screen house C)

3.3.2 Land preparation and planting

The experiment was carried out in February to June under irrigation and rainfed on a total plot size of 550 m^2. The land was ploughed and harrowed by a tractor and the ridges were made manually using hoes. Some local tools such as cutlasses, garden lines and pegs were used to demarcate and make the plots. The field was laid out in a randomized complete block design (RCBD) with three replicates. Each block contained 25 rows and each row

was a plot of 6 m long. The planting materials for the progenies were the vine cuttings from the seedling nursery in the screen house. The planting materials for the parents were the vine cuttings taken from the crossing block. Each vine cutting was 30 cm with three or four nodes depending on the internode length. The distance between rows was one meter and two meters between blocks. The progenies and the parents were randomly planted together in the field. The planting was done the 10th February 2016 at a distance of 30 cm between plants. Twenty cuttings for the parents in each row and ten genotypes to seventeen for each family. The borders and the empty spaces were planted with the cultivar Sauti.

3.3.3. Soil sampling and analysis

Soil samples were taken at a depth of 15 cm in different three sides of the plot and bulked to get a composite of soil in each replicate for the determination of the soil pH, Nitrogen, Phosphorus, Potassium, soil organic carbon and the percent organic matter.

3.3.3.1 Determination of the soil pH

Soil pH was determined using Landon (1991) method in 1:1 suspensions of soil and water using a pH meter. Twenty grammes of each soil sample was weight into 100 ml polythene bottles. Fifty milliters distilled water was added and the bottle shaken for two hours. After calibrating the pH meter with buffer solutions of pH 4.0 and 7.0, the pH was read by immersing the glass electrode into the upper part of the suspension.

3.3.3.2 The total nitrogen

Total Nitrogen was determined by Kjeldahl (1883) method. For 14 g of N contained in

B = volume of standard HCl used in the blank titration

N = Normality of standard HCl

The weight of soil sample used, considering the dilution and the aliquot taken for distillation = 10 g (weight of soil) x 10 (aliquot) ml = l.0 g

100 ml (dilution)

Thus, the percentage of Nitrogen in the soil sample is: % N = [14 x (A _ B) x N x 100] / (1000x1). When N = 0. 1 and B = 0; % Nitrogen = A x 0.14

3.3.3.3 Determination of soil organic carbon

The amount of soil organic carbon was determined by the modified Walkley -Black Wet combustion method as described by Nelson and Sommers (1982) using this formula:

$$\%C = \frac{M \cdot V_{bl} \cdot V_s}{0.003 \cdot 1.33 \cdot 100 \cdot g}$$

Where: M = Molarity of $FeSO_4$; V_{bl} = ml $FeSO_4$ of blank titration; V_s = ml $FeSO_4$ of soil sample titration; g = mass of soil taken in gramme; 0.003= milli-equivalent weight of C in grammes (12/4000); 1.33 = correction factor used to convert the Wet combustion C one equivalent weight of NH_3, the weight of N in the soil = [14 x (A – B) x N]/1000

Where: A = volume of standard HCl used in the sample titration value to the true C value since the Wet combustion method is about 75 % efficient in estimating C value, (i.e. 100/75 = 1.33).

3.3.3.4. The percent organic matter

The percent organic matter was obtained by multiplying the value of the organic carbon by the correlation factor (1.724) to convert it to percent organic matter as described by Landon (1991). The soil organic matter content was then determined using the formula: % organic C x 1.724. (1.724 is the Conventional Van Bemellean factor).

3.3.3.5. Available phosphorus

The soil available phosphorus was determined by Bray-1 method (Bray and Kurtz, 1945). Phosphorus is removed from the soil using Bray No 1 solution as extractant. The extracted phosphorus was measured colourimetrically based on the reaction with ammonium

molybdate and development of „Molybdenum Blue" colour. The absorbance of the compound is measured at 882 nm in a spectrophotometer and was directly proportional to the amount of phosphorus extracted from the soil.

Available Phosphorus P (mg/kg) = (C x 14) / (ODW). Where: C = Phosphorus concentration from chart/equation (µg/2.5 mL); ODW = Oven-dry sample weight (g); 14 = Dilution factor (Bray and Kurtz, 1945).

3.3.3.6. Determination of exchangeable base: potassium (K+)

Potassium was determined in 1.0 M ammonium acetate extract (Black, 1986) and measured directly in the leachate by flame photometry at wavelength of 766.5.

Exchangeable K+ (cmol / kg)

a = mg/l K in the diluted blank percolate; b = mg/l K in the diluted blank percolate; w = weight (g) of air- dried sample; mcf = moisture correcting factor.

3.3.4 Harvesting

Harvesting was done at exactly 121 days after planting. The vines were cut with cutlasses for weighing leaving those at the border before uprooting the plants in the center of each plot. Hoes were used to uproot the storage roots. The following data were taken before harvest: number of vines per plot (NOVP); number of plants established (NOPE); first virus symptom at eight weeks after planting (VIR1) (1-9); second virus symptom one month before harvest (VIR2) (1-9) and vine vigour one month before harvest (1-9) (VV). The scoring was done by using the sweetpotato descriptors in a scale of one to nine (1 = very low; 3 = low; 5 = intermediate; 7 = high; 9 = very high) (CIP/AVRDC/IBPGR, Huaman, 1991).

During the growing season, irrigation, weeding and insecticide spraying were done when needed. The insecticides used were Dursban for termites and white flies and Cymethoate

Super for thrips. The following harvest data were taken: number of plants harvested (NOPH); number of plants with roots (NOPR); number of marketable roots (NOMR); number of non marketable roots (NONMR); weight of marketable roots (WOMR) (Kg); weight of non marketable roots (WORNM) (Kg); vine weight (Kg); root size (1-9); root form (1-9); root defects (1-9); weevil damage (1-9).

3.3.5 Quality traits

After harvest some quality parameters were measured such as: percent dry mater (% DM), fructose (%), glucose (%), sucrose (%), iron (mg/100g), zinc (mg/100g), starch (%) and protein (%). All these parameters except the % DM were determined using NearInfrared Reflectance Spectroscopy (NIRS) method (CIP, Ghana) with the NIRS machine XDS RAPID CONTENT ANALYZER (SN: 3013-0902) with milled samples of freezedried storage root in the Quality and Nutrition Laboratory (Q & N Lab) at CSRI-CRI Fumesua. Percent DM was determined using the formula % DM = (dry weight/fresh weight) × 100. After the harvest, five roots were taken as samples in both parents and progenies to the Q & N Lab for the quality traits analysis. The root samples were washed with tap water to remove all soil residue, rinsed with distilled water and dried with paper towel. Washed root samples were placed in white plastic trays and peeled with a ceramic peeler, washed with distilled water once more, dried again with paper towel and each root was cut longitudinally in four pieces. Fifty grammes of the samples (fresh weight) was taken from 3-4 slices of each of two opposite sections of each root. The samples were stored in a freezer at -20 ^{o}C and freeze-dried in 72 hours. The exact weight of the dried samples were obtained and used for dry matter content determination. The dried samples were milled in a stainless steel mill, placed in WhirlPak plastic bags and stored in a freezer

at -20 ºC and used later for scanning in the NIRS machine for the quality traits determination (Porras *et al*., 2014).

3.4. Molecular work

The genetic diversity study by molecular analysis with the use of fifteen SSR markers was carried out on twenty-eight parents and thirty progenies (2 progenies per family) from the crossing block involving ten parents.

3.4.1. DNA extraction

Young leaves of sweetpotato from 30 progenies and 28 parents were sampled, cleaned with 70% ethanol. The total DNA was isolated from the leaf tissue using CTAB method (Dellaporta *et al*. 1983) with slight modifications by Dr Mercy Kitavi (2016). About 0.2g of chilled leaf placed in 2.0 ml eppendorf microfuge tube was ground with mortars and pestles by adding 1ml of freshly prepared CTAB extraction buffer under hood to get a fine powder which was poured into 2.0 ml eppendorf tube. The tubes with the fine powder were incubated in the water bath at 65ºC for 25 mins with a gentle mixing by inverting the tubes. The samples were cooled at room temperature for 3 mins and centrifuged at 3500 rpm for 10 mins. Six hundred microlitres of the aqueous phase of the samples was transferred into a new 2ml tube and equal volume (600 µl) of Chloroform Iso-amyl alcohol (24:1 ratio) was added under hood and mixed gently by several inversions of the tube until mixture turned milky and centrifuged at 4000 rpm for 20 mins. The upper layer was collected carefully and put into a clean labelled 2.0 ml tube. One point five times volume of ice cold absolute ethanol (900 µl) and 0.05 µl/µl of 3M sodium acetate (90 µl) were added, mixed 10x by inverting the tubes and centrifuged at 4000 rpm for 20 mins. The precipitated DNA pellets were washed with 1ml of 70% ethanol and centrifuged at 4000 rpm for 20 mins. Ethanol was decanted and pellets were dried at 37ºC for 30 minutes. The pellets were dissolved in 500 µl low salt TE buffer. Six microlitres RNase A was added and incubated in the water bath at 37ºC for 1 hour with intermittent shaking

every 10 min. 400 µl of Chloroform: Iso-amyl alcohol (24:1 ratio) was added and mixed gently by several inversions (50x) of the tube and centrifuge at 3500 rpm for 10 minutes. The upper aqueous layer of the samples was transferred into a clean 1.5 ml tube. Fourty microlitres of 3M sodium acetate and 800 µl absolute ethanol were added and mixed gently by inverting the tubes. The samples were then incubated at -20^{o}C for 30 mins and later centrifuged at 4000 rpm for 20 mins to discard the supernatant. The pellets were washed with 800 µl of 70% ethanol and centrifuged at 3500 rpm for 20 mins. Ethanol was later decanted and pellets were dried at room temperature for about 30-60 minutes. The DNA pellets were finally dissolved in 100 µl of low salt TE buffer.

The quality of the DNA was checked on 1.0% Agarose gel with (5µl) 0.005% etidiumbromate. Ten microlitres sample (DNA) was added to 2µl loading dye (6X Bromophenol blue), short spun and loaded in the wells on gel submerged in 1X TAE loading buffer. The samples were then ran at 120 volts for forty five minutes (45 mins) and observed under UV light. The DNA concentration and the purity were checked by a Nanodrop (2000/2000C) spectrophotometer.

3.4.2. Simple sequence repeat (SSR) amplification

3.4.2.1 Polymerase Chain Reaction

The DNA from fifty eight samples were genotyped using SSR markers (Table 3.2) in a 10 µl/reaction. The PCR was set with a master mix of 8µl which contained 0.7 µl PCR grade water, 5µl 2X KAPA plant PCR buffer (2x dNTPs + 5 mM $MgCl_2$), 0.2µl of 25 Mm $MgCl_2$), 1.0µl forward and reverse primers, 1µl 100x KAPA plant PCR Enhancer and 0.1µl Kapa 3G DNA polymerase pipetted into 2 ml tube. After that, 2µl of 20ng/µl

DNA was added to make a total volume of 10µl. The reaction mixtures were short spun and placed in the thermal cycler SEE^{AMP} model I : TC _ 96/G/H(B) A with 96-well block and ran with the following programme: 5.0 minutes at 95ºC and 3 0 sec at 95ºC for denaturing, 1 min at (48 -63ºC) for annealing by using each of the specific annealing temperature of the primer, 2 mins at 72ºC and another 20 mins at 72ºC for extension, held at 4 ºC and run for 40 cycles. The reaction was completed withi n three hours thirty three minutes. The PCR products were stored at -20ºC until needed for loading.

The SSR primers used in this study for the genotyping are shown in Table 3.2.

Primer	Band size (bp)	Expected size (bp)	Primer sequence (F/R)	ling temperature (oC)
		164-177	F - GAG AGG CCA TTG AAG AGG AA R - AAG GAC CAC CGT AAA TCC AA	62
		194 -212	F - CTT CAC TCT GCT CGC CAT TA R - GTA CTT GGA CGG GAG GAT GA F-	48
		130-200	GCA ATT TCA CAC ACA AAC ACG R- CCT CAG CTT CTG GGA AAC AG F	58
		125-135	- AGA ACG CAT GGG CAT TGA R - CCC ACC GTG TAA GGA AAT CA F	53
		253-298	- CTA CGA TCT CTC GGT GAC G R - CAG CTT CTC CAC TCC CTA C F	60
		158-198	- CAG AAG AGT ACG TTG CTC AG R - GCA CAG TTC TCC ATC CTT	58
		232-242	F - CTG AAC CCG ACA GCA CAA G R - GGG AAG TGA CCG GAC AAG A F-	58
		Unknown	TCT TTT GCA TCA AAG AAA TCC A R- CCT CAG CTT CTG GGA AAC AG F	58
		140-155	- CAA ACG CAC AAC GCT GTC R - CGC GTC CCG CTT ATT TAA C	58
		140-153	F - CCC ACC CTT CAT TCC ATT ACT R - GAA CAA CAA CAA AAG GTA GAG CAG F'-	63
		243-263	GTA GAG TTG AAG AGC GAG CA R - CCA TAG ACC CAT TGA TGA AG	58
		331-393	F - GAT CGA GGA GAA GCT CCA CA R - GCC GGC AAA TTA AGT CCA TC F	60
		216-222	- CCT ATG GCA ATT CGG TCA CT R - GGA ACA TTG CCT ACA CTC TG F	58
		131-237	- GAC TTC CTT GGT GTA GTT GC	60

Ib-248	120-200
IbR20	150-300
Ib-297	150-250
IB-318	130-300
IbS10	200-330
IbS17	170-200
IbS18	200-300
J116A	200-300
IB-316	150-200
IBCIP-1	150-200
IB-R03	250-270
IB-R12	250-450
IbR14	170-250
IB-R16	150-270

1 B-R19	150-250	192-213	R - AGG GTT AAG CGG GAG ACT F - GGC TAG TGG AGA AGG TCA A R - AGA AGT AGA ACT CCG TCA CC	60	

Table 3.2

Names and some information of the fifteen Simple Sequence Repeats primers used for the genotyping of the sweetpotato accessions.

Note: The primers and their expected sizes were obtained from Buteler *et al*., (1999) and Ernest *et al*., (2015); F = Forward, R = Reverse

3.4.2.2 MetaPhor Agarose Gel Electrophoresis (MAGE)

MAGE was used to run all the twenty- eight parents. The MAGE has been used in SSR markers for good separation of the alleles according to Abdurakhmonov *et al.*, 2007. Three percent MetaPhor Agarose Gel was prepared by adding 9.6g of the Metaphor powder slowly into 320 ml 1x TBE (Tris-borate-ethylenediaminetetracetic acid) buffer with continuous swirling in a 500ml beaker. The Metaphor powder was soaked in the buffer for approximately 15 minutes to reduce the tendency of the agarose solution to foam during heating in the microwave. Sixteen microliters of 0.005% ethidium bromide was added to the gel and mixed before pouring it into a cast to solidify. After adding 2 µl 6x bromophenol blue (loading dye) to the 10 µl SSR PCR product, 8 µl of it was loaded on the MetaPhor agarose gel submerged in chilled 1x TBE buffer and electrophoresed for about 2 hours at 120 V using horizontal electrophoresis system. The gels were, after electrophoresis, visualized under UV transilluminator and photographed.

3.4.2.3 Polyacrylamide Gel Electrophoresis (PAGE)

PAGE has been known as the best and the most used for allele‟s separation (Cregan and Quigley, 1997). It was used for the ten parents and their thirty progenies. In this way 6% acrylamide gel was prepared by adding sterile water , 40% acrylamide solution, 10X TBE Buffer, 10% APS (Ammonium persulphate) and TEMED and gently swelled to mix. The mixture was then gently poured into the cassette with the comb. The gel was allowed to set and was placed in an electrophoresis chamber, the comb was removed and covered with enough running buffer. Six microliters of the PCR product, (amplicons) were loaded in the wells submerged in 1X TBE loading buffer. The gel was then ran at 120 volts for two hours. The gel was removed and placed in plastic bowls after electrophoresis, stained with etidium-bromide solution for 30 minutes, visualized under UV transilluminator and

photographed. The PAGE gave distinct and high resolution bands compared to that of the agarose bands.

3.4.3 Data analysis

3.4.3.1 Quantitative and qualitative data

The collected quantitative data and qualitative data were subjected to analysis of variance (ANOVA) using GenStat statistical package 12th edition. Means were compared using Least Significance Difference at 5% level of probability. Statistix 9th edition was used to determine the correlation among some parameters using Pearson correlation (Person, 1901). Heterosis was calculated as percentage increase or decrease over mid parent and better parent values and the „t"test was computed to determine the significant differences between the hybrid means by using Wynne *et al*. (1970) formula as follow: "t" for MPH $= \frac{F1-MP}{\sqrt{3}/2r(EMS)}$; "t" for BPH $= \frac{F1-BP}{\sqrt{2}/r(EMS)}$

Where, F_1 = Mean of F_1 hybrid

MP = mid parent value of the specific F_1 hybrid $[(P_1 + P_2)/2]$.

BP = better parent value in the specific F_1 hybrid (P_1 or P_2).

EMS = Error Mean square

3.4.3.2 Molecular data

AlphaImager HP Version: 3.4.0.0 (1993-2011) software was used to score the bands for the presence (1) or absence (0). Molecular data was analyzed using PowerMarker 3.25 (Liu and Muse (2005) and DARwin 5.0. The genetic distance (Dice dissimilarity

coefficient of presence and absence) was used to calculate the allelic diversity of the primers using this formula:

dij $= \frac{b+a}{2a+(b+c)}$ where: dij = dissimilarity among units i and j; xi, xj = variable values of units i and j; *a* = number of variables (xi = presence and xj = presence); b = number of variables (xi = presence and xj = absence); c = number of variables (xi = absence and xj = presence). The polymorphic information content (PIC) was calculated using Weir (1996) formula: ($PIC = 1 - \Sigma p_i^2$, Where p_i = allele frequency of the i^{th} allele); the number of alleles per primer, the heterozygosity and the gene diversity were used to determine the genetic diversity among the 28 parents and within the ten parents and their progenies. The factorial analysis (PCoA) was done to group the accessions according to their type using Dice dissimilarity index by DARwin 5.0 software (Perrier & Jacquemoud-Collet, 2009).

CHAPTER FOUR

4.0 RESULTS

4.1. Chemical properties of the experimental site

The soil analysis was carried out on the soil collected from the experimental site at Crops Research Institut, Fumesua. According to Landon (2014) interpretation of soils nutrient status, soil nutrients were generally low except for phosphorus (P) and potassium (K). The soil pH was also low indicating slight acidity level. The low level of organic carbon observed could have resulted in the low level of soil nitrogen as seen in the result. (Table

4.1.a and b).

Table 4.1. a: Chemical properties of the soil

PH	Phosphorus (mg/Kg)	% Total Nitrogen	Potassium (cmol/Kg)	% Organic Carbon	% Organic Matter
4.4	45.67	0.045	0.215	1.01	1.73

Table 4.1.b Soil nutrients status according to Landon (2014)

	Sample	pH	Total N	Avail P	K	Ca	Mg	Na	Org. C	Org. matter
Landon ratings	High	> 6.5	> 0.5	> 50.0	> 0.6	> 10.0	> 4.0	> 1.0	> 10.0	-
	Low	< 5.8	< 0.2	< 15.0	< 0.2	< 4.0	< 0.5	< 1.0	< 4.0	-

4.2 ANOVA for pre-harvest and harvest traits; and harvest index

The ANOVA revealed that significant differences existed among the parents and their progenies for weevil damage ($P<0.05$); vine vigour, virus severity and harvest index ($P<0.01$) and other traits ($P<0.001$) (Table 4.2). The co-efficient of variation ranged from 19.5 % for number of plant with roots to 69.1 % for total yield (Table 4.2). Among the parents and the progenies, significant effects ($P<0.001$) were observed for all the quality traits (Table 4.3). The highest co-efficient of variation recorded was 13.9 % compared to the ones noted for the pre-harvest and harvest traits (Table 4.3).

index.

Table 4.2: Mean square values from ANOVA for pre-harvest and harvest traits and harvest index for the parental clones and progenies.

Sources of variation	df	TY	NPH	NPWR	VV	VW	NMR	WMR	RS	WD	VS	HI
Entry	24	96.06***	53.42***	55.19***	2.88**	140.6***	32.22**	8.68***	11.95***	0.30*	2.17**	164.24**
Error	48	29.20	7.06	7.79	1.248	23.21	11.54	1.23	2.96	0.16	0.80	61.73
Grand mean		7.82	9.40	6.79	5.72	11.42	5.74	1.88	5.49	1.12	4.42	13.1
CV %		69.1	28.3	41.1	19.5	42.2	59.2	59.3	31.4	35.7	20.2	60.0

TY = Total yield, NPH = Number of plants harvested, NPWR = Number of plants with root, VV = Vine vigour, VW = Vine weight, NMR = Number of marketable roots, WRM = Weight of marketable roots, RS = Root size, WD = Weevil damage, VS = Virus severity and HI = Harvest

* Significant at P < 0.05; **Significant at P < 0.01 and ***Significant at P < 0.001

Source of variation	df	%DM	Fructose	Glucose	Sucrose	Iron	Protein	Starch	Zinc
Entry	24	172.60***	2.41***	4.34***	48.463***	0.826***	5.434***	497.437***	0.345***
Error	48	2.35	0.12	0.18	3.23	0.047	0.39	5.86	0.028
Grand mean		31.59	2.17	3.32	7.08	1.87	4.52	4.52	1.22
CV %		4.9	0.351	13	25.4	11.6	13.9	4.2	13.6

Table 4.3: Mean square values from ANOVA for quality traits for the parental clones and progenies.

%DM = Percentage Dry Matter

***Significant at P<0.001

The differences derived from the crosses are shown in the following root pictures of some crosses.

A)

B)

C)

D)

Figure 4.1 Roots from the crosses Apomuden x Faara A); Nanungungungu x Otoo B); Apomuden x PG12086-18 C) and PG12086-18 x CIP440390 D)

4.3 Heterosis estimates, means of parents and progenies for pre-harvest and harvest traits, harvest index and quality traits

4.3.1 Total yield

Mean values and heterosis estimates are presented on Table 4.4. The means of the total yield for the parents ranged from 21.25 t/ha for Otoo to 1.5 t/ha for Sauti. The mean of Otoo was not significantly different from that of Apomuden (17.07 t/ha). 16011 (17.09 t/ha) produced the highest total yield, but not significantly different from 16007 (17.02 t/ha), 16002 (11.94 t/ha), 16013 (9.43 t/ha), 16005 (8.80 t/ha) and 16001 (8.77 t/ha) at $P<0.05$.

The mid-parent heterosis (MPH) of the total yield for 16005 (361.94 %) was the highest, distantly followed by 16014 (202.89 %), 16007 (139.89 %) and 16011 (83.66 %); all these values were significant at varying level of probability. Some of the better parent heterosis (BPH) that were positive did not show significant effects.

4.3.2 Harvest index

Table 4.5 shows mean values and heterosis estimates for harvest index. The means of the harvest index for Apomuden (20.92 %), Hi-starch (19.75 %), Otoo (18.81 %), Ligri (15.58 %) and CIP440390 (12.1 %) were not significantly different from one another at $P<0.05$. CIP440390 (12.1) mean was not significantly different from the means of Bohye (6.10 %), Faara (4.86 %) and PG162086-18 (4.36 %). Progeny 16015 (28.84 %) had the highest mean for harvest index and it was not significantly different from the means of

16008 (20.72 %), 16013 (20.37 %), 16002 (18.48 %), 16007 (18.48 %), 16006 (17.92 %) and 16003 (16.22 %) at P<0.05.

The MPH of six progenies out of fifteen for harvest index were significant, they include 16006 (637.45 %), 16012 (611.42 %), 16005 (405.25 %), 16015 (192.05 %), 16002 (124.27%) and 16007 (124.27 %) (Table 4.4). Only 16006 (268.72 %) and 16012 (255.708 %) had positive and significant BPH.

Total yield (t/ha)							
P_1	Mean	P_2	Mean	F_1	Mean	MPH (%)	BPH (%)
Nanungungungu	0.00	Hi-starch	11.3	16015	3.67	-35.04	-67.52
Apomuden	17.07	Sauti	1.54	16011	17.09	83.66*	0.12
Nanungungungu	0.00	Ligri	4.18	16003	7.44	255.98	77.99
Faara	3.11	Nanungungungu	0	16006	1.29	-17.04	-58.52
Apomuden	17.07	Faara	3.11	16010	5.78	-42.71	-66.14**
Sauti	1.54	Nanungungungu	0	16009	5.13	566.23	233.11
Apomuden	17.07	PG12086-18	4.12	16013	9.43	-6.54	-44.75
Nanungungungu	0.00	Bohye	3.81	16005	8.8	361.94*	130.97
PG12086-18	4.12	CIP440390	10.07	16002	11.94	68.29	18.57
CIP440390	10.07	PG12086-18	4.12	16007	17.02	139.89**	69.01
PG12086-18	4.12	Apomuden	17.07	16001	8.77	-17.22	-48.90*
Nanungungungu	0.00	Otoo	21.25	16004	9.63	-9.364	-54.68**
Apomuden	17.07	Hi-starch	11.3	16008	7.18	-49.38*	-57.93*
Nanungungungu	0.00	Faara	3.11	16014	4.71	202.89*	51.44
Nanungungungu	0.00	PG12086-18	4.12	16012	1.28	37.89	-68.93
LSD (5%)	8.87		8.87		8.87		

Table 4.4: Mean values and he

16001= PG12086-18 x Apomuden; 16002 = PG12086-18 x CIP440390; 16003 = Nanungungungu x Ligri; 16004 = Nanungungungu x Otoo; 16005 = Nanungungungu x Bohye; 16006 = Faara x Nanungungungu; 16007 = CIP440390 x PG12086-18; 16008 = Apomuden x Hi-starch; 16009 = Sauti x Nanungungungu; 16010 = Apomuden x Faara; 16011 = Apomuden x Sauti; 16012 = Nanungungungu x PG12086-18; 16013= Apomuden x PG1208618; 16014 = Nanungungungu x Faara; 16015 = Nanungungungu x Hi-starch, P_1= Parent 1, P_2 = Parent 2, F_1 = Hybrid, MPH = Mid-parent heterosis and BPH

=Better parent heterosis

* Significant at $P < 0.05$, **Significant at $P < 0.01$

		Harvest index (%)					
P_1	Mean	P_2	Mean	F_1	Mean	MPH (%)	BPH (%)
Nanungungungu	0.00	Hi-starch	19.75	16015	28.84	192.05**	51.19
Apomuden	20.92	Sauti	0.68	16011	15.55	43.98	-25.67
Nanungungungu	0.00	Ligri	15.58	16003	16.22	108.22	4.11
Faara	4.86	Nanungungungu	0.00	16006	17.92	637.45**	268.72*
Apomuden	20.92	Faara	4.86	16010	10.04	-22.11	-52.01
Sauti	0.68	Nanungungungu	0.00	16009	4.97	1361.77	630..88
Apomuden	20.92	PG12086-18	4.38	16013	20.37	61.03	-2.63
Nanungungungu	0.00	Bohye	6.10	16005	15.41	405.25*	152.62
PG12086-18	4.38	CIP440390	12.10	16002	18.48	124.27*	-52.73
CIP440390	12.1	PG12086-18	4.38	16007	18.48	124.27*	52.73
PG12086-18	4.38	Apomuden	20.92	16001	12.89	1.90	-38.38
Nanungungungu	0.00	Otoo	18.81	16004	2.58	-72.57	-86.28
Apomuden	20.92	Hi-starch	19.75	16008	20.72	1.89	-0.96
Nanungungungu	0.00	Faara	4.86	16014	8.87	265.02	82.51

Nanungungungu	0.00	PG12086-18	4.38	16012		611.42*	255.71*
LSD (5%)	12.90		12.90		15.58 12.90		

4.5: Mean values and heterosis estimates for harvest index.

16001= PG12086-18 x Apomuden; 16002 = PG12086-18 x CIP440390; 16003 = Nanungungungu x Ligri; 16004 = Nanungungungu x Otoo; 16005 = Nanungungungu x Bohye; 16006 = Faara x Nanungungungu; 16007 = CIP440390 x PG12086-18; 16008 = Apomuden x Hi-starch; 16009 = Sauti x Nanungungungu; 16010 = Apomuden x Faara; 16011 = Apomuden x Sauti; 16012 = Nanungungungu x PG12086-18; 16013= Apomuden x PG1208618; 16014 = Nanungungungu x Faara; 16015 = Nanungungungu x Hi-starch, P_1= Parent 1, P_2 = Parent 2, F_1 = Hybrid, MPH = Mid-parent heterosis and BPH =Better parent heterosis

* Significant at P < 0.05, **Significant at P < 0.01

4.3.3 Vine vigour

Table 4.6 shows mean values and heterosis estimates for vine vigour. The means were not significantly different from one another. The vine of Nanungungungu (3.33) was less vigorous when compared to the ratings of other parents. The vigour scores for 16007 (7.67), 16002 (7), 16004 (6.33), 16010 (6), 16013 (6), 16005 (6), 16001 (6) and 16014 (6) were not significantly different from one another. PG12086-18 (7) was scored the most vigorous, followed by Hi-Starch (6.33), Faara (6) and Sauti (5.67).

The progenies 16005 (50 %), 16003 (39.16 %) and 16007 (31.45 %) had significant ($P<0.05$) and positive MPH for vine vigour. Significant ($P<0.05$) and positive BPH was recorded for 16012 (28.57 %).

4.3.4 Number of plants with roots

Mean values and heterosis estimates for Number of plants with roots were presented on Table 4.7. Bohye (17.67) had the highest number of plant with roots; its mean was not significantly different from that of Otoo (15.33) (Table 4.5). Sauti (1.33) had a mean number of plant with roots that was 16.34 less than the parental clone that had the highest. The number of plant with roots recorded for 16004 (13) was the highest and it was not significantly different at $P<0.05$ from the means of 16003 (10) and 16013 (8.67). Parent Nanungungungu was common to progenies 16004 and 16003.

The MPH (205.82 %) and BPH (136.24 %) of 16013 for the number of plants with roots were significant and positive. 16009 (752.63 %), 16003 (233.33 %) and 16004 (69.60 %) had significant and positive MPH. The BPH of 16008 (59.45 %) and 16005 (56.59 %) were also significant and positive.

Vine vigour							
P_1	Mean	P_2	Mean	F_1	Mean	MPH (%)	BPH (%)
Nanungungungu	3.33	Hi-starch	6.33	16015	4.67	-3.31	-26.22
Apomuden	4.67	Sauti	5.67	16011	5.67	9.67	0.00
Nanungungungu	3.33	Ligri	4.33	16003	5.33	39.16*	23.09
Faara	6.00	Nanungungungu	3.33	16006	5.67	21.54	-5.50
Apomuden	4.67	Faara	6.00	16010	6.00	12.46	0.00
Sauti	5.67	Nanungungungu	3.33	16009	5.67	26.00	1.80
Apomuden	4.67	PG12086-18	7.00	16013	6.00	2.83	-14.29
Nanungungungu	3.33	Bohye	4.67	16005	6.00	50.00*	28.48
PG12086-18	7.00	CIP440390	4.67	16002	7.00	19.97	0.00
CIP440390	4.67	PG12086-18	7.00	16007	7.67	31.45*	9.57
PG12086-18	7.00	Apomuden	4.67	16001	6.00	2.83	-14.29
Nanungungungu	3.33	Otoo	7.00	16004	6.33	22.56	-9.57
Apomuden	4.67	Hi-starch	6.33	16008	6.33	15.09	0.00
Nanungungungu	3.33	Faara	6.00	16014	6.00	28.62	0.00
Nanungungungu	3.33	PG12086-18	7.00	16012	5.00	-3.19	28.57*
LSD (5%)	1.83		1.83		1.83		

Table 4.6: Mean values and l

16001= PG12086-18 x Apomuden; 16002 = PG12086-18 x CIP440390; 16003 = Nanungungungu x Ligri; 16004 = Nanungungungu x Otoo; 16005 = Nanungungungu x Bohye; 16006 = Faara x Nanungungungu; 16007 = CIP440390 x PG12086-18; 16008 = Apomuden x Hi-starch; 16009 = Sauti x Nanungungungu; 16010 = Apomuden x Faara; 16011 = Apomuden x Sauti; 16012 = Nanungungungu x PG12086-18; 16013= Apomuden x PG12086-18; 16014 = Nanungungungu x Faara; 16015 = Nanungungungu x Hi-starch, P_1= Parent 1, P_2 = Parent 2, F_1 = Hybrid, MPH = Mid-parent heterosis and BPH =Better parent heterosis

* Significant at $P < 0.05$

Number of plants with roots							
P_1	Mean	P_2	Mean	F_1	Mean	MPH (%)	BPH (%)
Nanungungungu	0.00	Hi-starch	12.33	16015	4.33	-29.76	-64.88**
Apomuden	2.00	Sauti	1.33	16011	3.33	100.00	66.50
Nanungungungu	0.00	Ligri	6.00	16003	10.00	233.33**	66.67
Faara	7.67	Nanungungungu	0.00	16006	4.33	12.91	-43.55
Apomuden	2.00	Faara	7.67	16010	6.00	24.10	-21.77
Sauti	1.33	Nanungungungu	0.00	16009	5.67	752.63*	326.32
Apomuden	2.00	PG12086-18	3.67	16013	8.67	205.82**	136.24*
Nanungungungu	0.00	Bohye	17.67	16005	7.67	-13.19	56.59***
PG12086-18	3.67	CIP440390	7.00	16002	7.67	43.77	9.57
CIP440390	7.00	PG12086-18	3.67	16007	8.00	49.95	14.29
PG12086-18	3.67	Apomuden	2.00	16001	6.33	123.28	72.48
Nanungungungu	0.00	Otoo	15.33	16004	13.00	69.60*	-15.20
Apomuden	2.00	Hi-starch	12.33	16008	5.00	-30.22	59.45**

Nanungungungu	0.00	Faara	7.67	16014	4.00	4.30	-47.85
Nanungungungu	0.00	PG12086-18	3.67	16012	2.67	45.50	-27.25
LSD (5%)	4.58		4.58		4.58		

Table 4.7: Mean values and heterosis estimates for number of plants with roots.

16001= PG12086-18 x Apomuden; 16002 = PG12086-18 x CIP440390; 16003 = Nanungungungu x Ligri; 16004 = Nanungungungu x Otoo; 16005 = Nanungungungu x Bohye; 16006 = Faara x Nanungungungu; 16007 = CIP440390 x PG12086-18; 16008 = Apomuden x Hi-starch; 16009 = Sauti x Nanungungungu; 16010 = Apomuden x Faara; 16011 = Apomuden x Sauti; 16012 = Nanungungungu x PG12086-18; 16013= Apomuden x PG12086-18; 16014 = Nanungungungu x Faara; 16015 = Nanungungungu x Hi-starch, P_1= Parent 1, P_2 = Parent 2, F_1 = Hybrid, MPH = Mid-parent heterosis and BPH =Better parent heterosis

* Significant at P < 0.05, **Significant at P < 0.01 and ***Significant at P < 0.001

4.3.5 Vine weight

Table 4.8 shows mean values and heterosis estimates for vine weight. Otoo (29.87) had the highest mean and it was significantly different from all other parents used in this study. The vine weight of the progenies ranged from 3.6 to 19.6, 16002 had the highest and it was not significantly different from 16007, 16010, 16005, 16004 and 16003 at $P<0.05$.

Progenies 16003 (248.07 %), 16010 (76.57 %), 16002 (55.99 %) and 16007 (49.06 %) had positive and significant ($P<0.05$) MPH and only 16003 (174.71 %) had significant ($P<0.05$) and positive BPH.

4.3.6 Weight of marketable roots

Table 4.9 shows mean values and heterosis estimates for weight of marketable roots.

After the roots were harvested, the weight of marketable roots (WMR) was taken; the WMR varied greatly amongst the parental clones, the highest and the lowest were recorded for Otoo (7.21) and Nanungungungu (0), Otoo differed significantly at $P<0.05$ from other parents. 16005 (5.1) and 16002 (3.51) had means that were not significant from each other. The WMR was the lowest for progeny 16012.

The MPH and BPH of 16003 (544.44 %, 222.22 %), 16007 (347.37 %, 231.17 %), 16013 (293.29 %, 290.76 %) and 16002 (207.89 %, 127.92 %) for the weight of marketable roots were positive and significant except for the non-significance recorded for the BPH of 16003.

Vine weight							
P_1	Mean	P_2	Mean	F_1	Mean	MPH (%)	BPH (%)
Nanungungungu	2.47	Hi-starch	15.4	16015	4.33	-51.54	-71.88**
Apomuden	1.27	Sauti	20.2	16011	6.80	-36.66	-66.34**
Nanungungungu	2.47	Ligri	4.27	16003	11.73	248.07*	174.71*
Faara	15.8	Nanungungungu	2.47	16006	10.2	11.66	-35.44
Apomuden	1.27	Faara	15.8	16010	15.07	76.57*	-4.62
Sauti	20.2	Nanungungungu	2.47	16009	7.13	-37.10	-64.70*
Apomuden	1.27	PG12086-18	16.4	16013	9.93	12.39	-39.45
Nanungungungu	2.47	Bohye	17.27	16005	13.13	33.03	-23.97
PG12086-18	16.4	CIP440390	8.73	16002	19.6	55.99*	19.51
CIP440390	8.73	PG12086-18	16.4	16007	18.73	49.06*	14.21
PG12086-18	16.4	Apomuden	1.27	16001	4.67	-47.14	-71.52**
Nanungungungu	2.47	Otoo	29.87	16004	13.4	-17.13	-55.14***
Apomuden	1.27	Hi-starch	15.4	16008	3.6	-56.81	-76.62**
Nanungungungu	2.47	Faara	15.8	16014	9.07	-0.71	-42.59
Nanungungungu	2.47	PG12086-18	16.4	16012	6.4	-32.17	-60.98*
LSD (5%)	7.91		7.91		7.91		

Table 4.8: Mean values and l

16001= PG12086-18 x Apomuden; 16002 = PG12086-18 x CIP440390; 16003 = Nanungungungu x Ligri; 16004 = Nanungungungu x Otoo; 16005 = Nanungungungu x Bohye; 16006 = Faara x Nanungungungu; 16007 = CIP440390 x PG12086-18; 16008 = Apomuden x Hi-starch; 16009 = Sauti x Nanungungungu; 16010 = Apomuden x Faara; 16011 = Apomuden x Sauti; 16012 = Nanungungungu x PG12086-18; 16013= Apomuden x PG12086-18; 16014 = Nanungungungu x Faara; 16015 = Nanungungungu x Hi-starch, P_1= Parent 1, P_2 = Parent 2, F_1 = Hybrid, MPH = Mid-parent heterosis and BPH =Better parent heterosis

* Significant at P < 0.05, **Significant at P < 0.01 and ***Significant at P < 0.001.

Weight of marketable roots

P_1	Mean	P_2	Mean	F_1	Mean	MPH (%)	BPH (%)
Nanungungungu	0.00	Hi-starch	3.96	16015	1.17	-40.91	-70.45**
Apomuden	0.75	Sauti	0.17	16011	1.25	171.74	66.67
Nanungungungu	0.00	Ligri	0.81	16003	2.61	544.44*	222.22
Faara	0.81	Nanungungungu	0.00	16006	0.33	-18.52	-59.26
Apomuden	0.75	Faara	0.81	16010	2.36	202.56	191.36
Sauti	0.17	Nanungungungu	0.00	16009	1.22	1335.29	617.65
Apomuden	0.75	PG12086-18	0.74	16013	2.93	293.29*	290.67*
Nanungungungu	0.00	Bohye	1.17	16005	1.49	154.70	27.35
PG12086-18	0.74	CIP440390	1.54	16002	3.51	207.89*	127.92*
CIP440390	1.54	PG12086-18	0.74	16007	5.1	347.37***	231.17**
PG12086-18	0.74	Apomuden	0.75	16001	2.25	202.01	200.00
Nanungungungu	0.00	Otoo	7.21	16004	3.23	-10.40	-55.2**
Apomuden	0.75	Hi-starch	3.96	16008	1.13	-52.02	-71.46**
Nanungungungu	0.00	Faara	0.81	16014	0.9	122.22	11.11

Nanungungungu	0.00	PG12086-18	0.74	16012	0.26	-29.73	-64.86
LSD (5%)	1.82		1.82		1.82		

Table 4.9: Mean values and heterosis estimates for weight of marketable root

16001= PG12086-18 x Apomuden; 16002 = PG12086-18 x CIP440390; 16003 = Nanungungungu x Ligri; 16004 = Nanungungungu x Otoo; 16005 = Nanungungungu x Bohye; 16006 = Faara x Nanungungungu; 16007 = CIP440390 x PG12086-18; 16008 = Apomuden x Hi-starch; 16009 = Sauti x Nanungungungu; 16010 = Apomuden x Faara; 16011 = Apomuden x Sauti; 16012 = Nanungungungu x PG12086-18; 16013= Apomuden x PG12086-18; 16014 = Nanungungungu x Faara; 16015 = Nanungungungu x Hi-starch, P_1= Parent 1, P_2 = Parent 2, F_1 = Hybrid, MPH = Mid-parent heterosis and BPH =Better parent heterosis

* Significant at P < 0.05, **Significant at P < 0.01 and ***Significant at P < 0.001

4.3.7 Number of marketable roots

Mean values and heterosis estimates for Number of marketable roots were presented on Table 4.10. The means of the progenies ranged from 2.67 for 16014 to 11.33 for 16004. The progeny that produced the highest mean did not significantly differ from the means of 16011 (9), 16010 (7.33), 16011 (7.33), 16013 (7), 16003 (6.33) and 16005 (6.33). It was observed that Apomuden and Nanungungungu were the commonest to the progenies listed above. The mean for Hi-starch differed significantly at $P<0.05$ from the means of other parents and was the highest. Bohye had a mean of 9.67 and it was not significantly different from that of CIP440390 (4.67) and Ligri (4.33).

Most of the progenies had a positive MPH and BPH for number of marketable roots except for the significance observed for 16011 (260.00 %, 145.23%).

4.3.8 Root size

Mean values and heterosis estimates for root size were presented on Table 4.11. The means for the parents ranged from 7.67 (Otoo) to 0 (Nanungungungu). 16007 (8.67) had the highest root size, followed by 16010 (7.67), 16013 (7.67), 16002 (7.67), 16001 (7.33), 16005 (6.67), 16003 (6.33), 16009 (6.33) and 16004 (6) which were not significantly different from 16007 at $P<0.05$.

Sixty percent of the progenies evaluated showed positive and significant MPH for root size, they include 16009 (533%), 16005 (208.08 %), 16003 (137.52 %), 16014 (128.27 %), 16007 (62.51 %), 16010 (58.63 %), 16013 (58.63 %), 16001 (51.60 %) and 16002 (43.77 %). Out of these, 16009 (216.50 %), 16010 (53.40 %), 16013 (53.40 %) and 16007 (44.50 %) had significant and positive BPH.

Number of marketable roots							
P_1	Mean	P_2	Mean	F_1	Mean	MPH (%)	BPH (%)
Nanungungungu	0.00	Hi-starch	20.00	16015	3.67	-63.30**	-81.65***
Apomuden	3.67	Sauti	1.33	16011	9.00	260.00**	145.23*
Nanungungungu	0.00	Ligri	4.33	16003	6.33	192.38	46.19
Faara	6.00	Nanungungungu	0.00	16006	3.67	22.33	-38.83
Apomuden	3.67	Faara	6.00	16010	7.33	51.60	22.17
Sauti	1.33	Nanungungungu	0.00	16009	4.00	501.50	200.75
Apomuden	3.67	PG12086-18	3.00	16013	7.00	109.90	90.74
Nanungungungu	0.00	Bohye	9.67	16005	6.33	30.92	-34.54
PG12086-18	3.00	CIP440390	4.67	16002	5.33	38.98	14.13
CIP440390	4.67	PG12086-18	3.00	16007	4.43	15.51	-5.14
PG12086-18	3.00	Apomuden	3.67	16001	7.33	119.79	99.73
Nanungungungu	0.00	Otoo	15.00	16004	11.33	51.07	-24.47
Apomuden	3.67	Hi-starch	20.00	16008	5.00	-57.75**	-75.00**
Nanungungungu	0.00	Faara	6.00	16014	2.67	-11.00	-55.50
Nanungungungu	0.00	PG12086-18	3.00	16012	3.33	122.00	11.00
LSD (5%)	5.57		5.57		5.57		

Table 4.10: Mean values and h

16001= PG12086-18 x Apomuden; 16002 = PG12086-18 x CIP440390; 16003 = Nanungungungu x Ligri; 16004 = Nanungungungu x Otoo; 16005 = Nanungungungu x Bohye; 16006 = Faara x Nanungungungu; 16007 = CIP440390 x PG12086-18; 16008 = Apomuden x Hi-starch; 16009 = Sauti x Nanungungungu; 16010 = Apomuden x Faara; 16011 = Apomuden x Sauti; 16012 = Nanungungungu x PG12086-18; 16013= Apomuden x PG1208618; 16014 = Nanungungungu x Faara; 16015 = Nanungungungu x Hi-starch, P_1= Parent 1, P_2 = Parent 2, F_1 = Hybrid, MPH = Mid-parent heterosis and BPH =Better parent heterosis

* Significant at P < 0.05, **Significant at P < 0.01 and ***Significant at P < 0.001

Table 4.11: Mean values and heterosis estimates for root size.

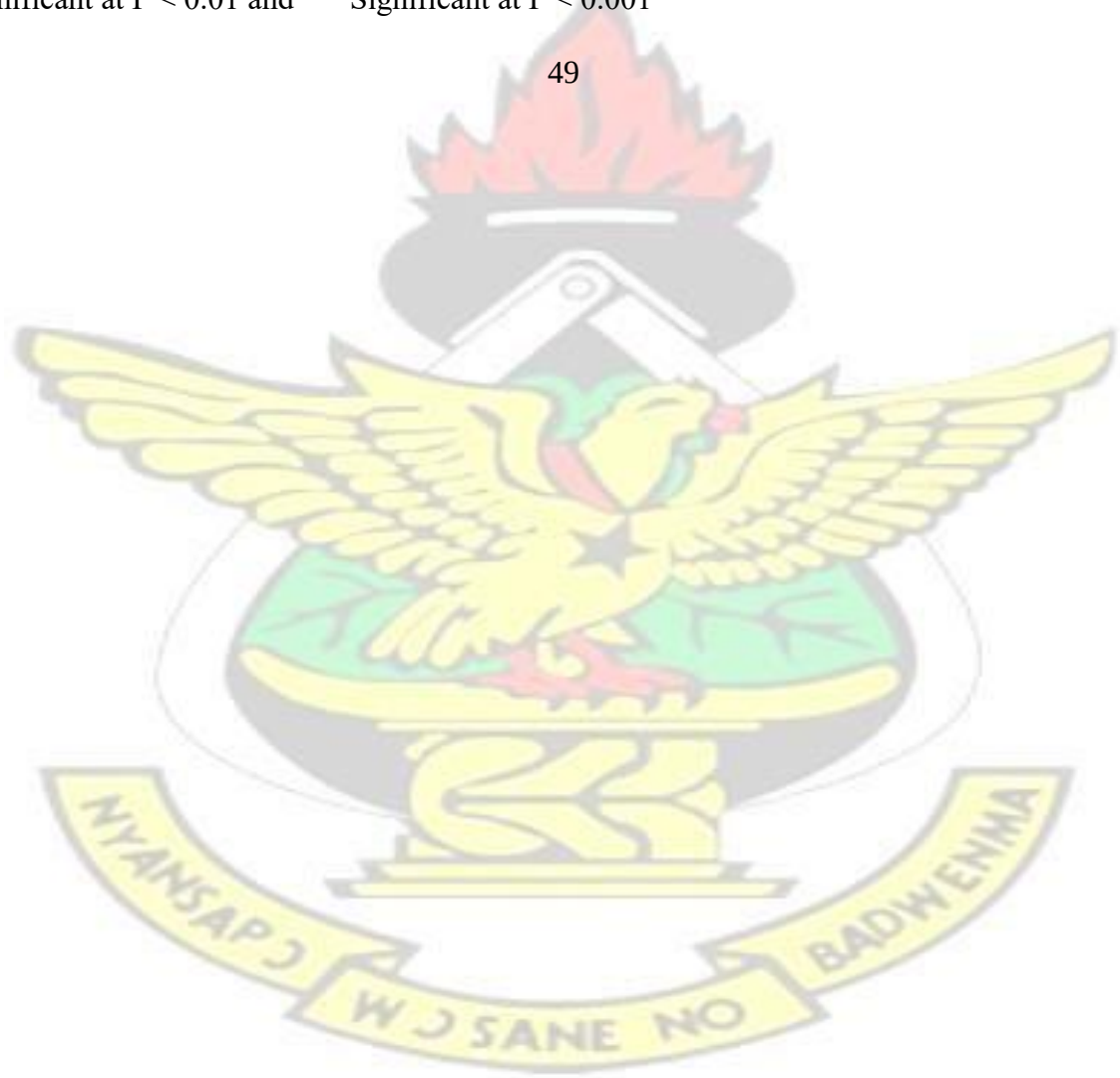
				Root size			
P_1	Mean	P_2	Mean	F_1	Mean	MPH (%)	BPH (%)
Nanungungungu	0.00	Hi-starch	7.00	16015	4.00	14.29	-42.86*
Apomuden	5.00	Sauti	2.00	16011	4.67	33.43	-6.60
Nanungungungu	0.00	Ligri	5.33	16003	6.33	137.52**	18.76
Faara	4.67	Nanungungungu	0.00	16006	4.33	85.44	-7.28
Apomuden	5.00	Faara	4.67	16010	7.67	58.63*	53.40*
Sauti	2.00	Nanungungungu	0.00	16009	6.33	533.00***	216.50**
Apomuden	5.00	PG12086-18	4.67	16013	7.67	58.63*	53.40*
Nanungungungu	0.00	Bohye	4.33	16005	6.67	208.08**	54.04
PG12086-18	4.67	CIP440390	6.00	16002	7.67	43.77*	27.83
CIP440390	6.00	PG12086-18	4.67	16007	8.67	62.51*	44.50*
PG12086-18	4.67	Apomuden	5.00	16001	7.33	51.60*	46.60
Nanungungungu	0.00	Otoo	7.67	16004	6.00	56.45	-21.77
Apomuden	5.00	Hi-starch	7.00	16008	5.33	-11.17	-23.86
Nanungungungu	0.00	Faara	4.67	16014	5.33	128.27*	14.13
Nanungungungu	0.00	PG12086-18	4.67	16012	2.67	14.35	-42.83
LSD (5%)	2.83		2.83		2.83		

16001= PG12086-18 x Apomuden; 16002 = PG12086-18 x CIP440390; 16003 = Nanungungungu x Ligri; 16004 = Nanungungungu x Otoo; 16005 = Nanungungungu x Bohye; 16006 = Faara x Nanungungungu; 16007 = CIP440390 x PG12086-18; 16008 = Apomuden x Hi-starch; 16009 = Sauti x Nanungungungu; 16010 = Apomuden x Faara; 16011 = Apomuden x Sauti; 16012 = Nanungungungu x PG12086-18; 16013= Apomuden x PG1208618;

BPH =Better parent heterosis

16014 = Nanungungungu x Faara; 16015 = Nanungungungu x Hi -starch, P1= Parent 1, P2 = Parent 2, F1 = Hybrid, MPH = Mid -parent heterosis and

* Significant at P < 0.05, **Significant at P < 0.01 and ***Significant at P < 0.001



4.3.9 Percentage dry matter

Table 4.12 shows mean values and heterosis estimates for dry matter. The percentage dry matter for most of the parents except for Nanungungungu (0) were high, ranging from 25.65 % for Apomuden to 37.99 % for Sauti. There was no significant difference observed between the means recorded for 16008 (39.68 %) and 16007 (38.83 %); although 16008 had the highest.

The MPH of all the progenies was highly signifcant and positive except for 16002 (7.98 %) and 16001 (-6.96 %) that were negative. Only the BPH of 16007 (7.80 %) was signifcant and positive.

4.3.10 Percentage protein

Table 4.13 shows mean values and heterosis estimates for percentage protein. The mean of protein content for Ligri (6.86) was the highest and it was significantly different from the others. Apomuden (5.65) was next to Ligri in magnitude, but not significantly different from that of CIP440390 (5.41), Faara (5.21) and Otoo (4.89). The mean of the progenies ranged from 6.55 (16001) to 3.42 (16011).

Progenies 16009 (186.23 %), 16005 (161.83 %), 16012 (104.95 %), 16004 (101.23 %), 16015 (82.54 %), 16006 (46.26 %), 16014 (45.49 %), 16001 (35.19 %), 16003 (30.32 %) and 16010 (13.44 %) had significant and positive MPH. Not more than 16009 (43.12 %), 16005 (30.91 %) and 16001 (15.93 %) had significant and positive BPH.

4.3.11 Starch content

Table 4.14 shows mean values and heterosis estimates for starch content. The starch content was very high in most of the parents (>50%) except for Nanungungungu (0) There was no significant difference observed between the means recorded for 16008 (66.89 %) and 16007 (64.18 %); although 16008 had the highest.

Starch content for mid parent heterosis was from –6.05% (PG12086-18 x CIP440390) to 85.51% (Sauti x Nanungungungu) with 9 crosses showing significant positive heterosis increment and for better parent heterosis increment was ranged from -21.45% (Faara x Nanungungungu) to 0.13% (Apomuden x Hi -starch) with none of the crosses showing significant positive heterosis increment.

Dry matter (%)							
P_1	Mean	P_2	Mean	F_1	Mean	MPH (%)	BPH (%)
Nanungungungu	0.00	Hi-starch	37.71	16015	33.2	76.08***	-11.96**
Apomuden	25.65	Sauti	37.99	16011	33.31	4.68***	-12.32**
Nanungungungu	0.00	Ligri	30.47	16003	28.71	88.45***	-5.78
Faara	37.41	Nanungungungu	0.00	16006	31.46	68.19***	-15.90***
Apomuden	25.65	Faara	37.41	16010	33.31	5.65***	-10.96**
Sauti	37.99	Nanungungungu	0.00	16009	34.07	79.36***	-10.32**
Apomuden	25.65	PG12086-18	36.02	16013	31.61	2.51***	-12.24**
Nanungungungu	0.00	Bohye	28.47	16005	27.85	95.64***	-2.18
PG12086-18	36.02	CIP440390	34.38	16002	32.39	-7.98***	-10.08*
CIP440390	34.38	PG12086-18	36.02	16007	38.83	10.31***	7.80*
PG12086-18	36.02	Apomuden	25.65	16001	28.69	-6.96***	-20.35***
Nanungungungu	0.00	Otoo	32.04	16004	28.21	76.09***	-11.95*
Apomuden	25.65	Hi-starch	37.71	16008	39.68	25.25***	5.22
Nanungungungu	0.00	Faara	37.41	16014	34.41	83.96***	-8.02*
Nanungungungu	0.00	PG12086-18	36.02	16012	35.48	97.00***	-1.5
LSD (5%)	2.52		2.52		2.52		

Table 4.12: Mean values and h

16001= PG12086-18 x Apomuden; 16002 = PG12086-18 x CIP440390; 16003 = Nanungungungu x Ligri; 16004 = Nanungungungu x Otoo; 16005 = Nanungungungu x Bohye; 16006 = Faara x Nanungungungu; 16007 = CIP440390 x PG12086-18; 16008 = Apomuden x Hi-starch; 16009 = Sauti x Nanungungungu; 16010 = Apomuden x Faara; 16011 = Apomuden x Sauti; 16012 = Nanungungungu x PG12086-18; 16013= Apomuden x PG1208618; 16014 = Nanungungungu x Faara; 16015 = Nanungungungu x Hi-starch, P_1= Parent 1, P_2 = Parent 2, F_1 = Hybrid, MPH = Mid-parent heterosis and BPH =Better parent heterosis

* Significant at P < 0.05, **Significant at P < 0.01 and ***Significant at P < 0.001

Protein (%)							
P_1	Mean	P_2	Mean	F_1	Mean	MPH (%)	BPH (%)
Nanungungungu	0.00	Hi-starch	3.78	16015	3.45	82.54***	-8.73
Apomuden	5.65	Sauti	3.85	16011	3.42	-28.00***	-39.47***
Nanungungungu	0.00	Ligri	6.86	16003	4.47	30.32**	-34.84***
Faara	5.21	Nanungungungu	0.00	16006	3.81	46.26**	-26.87**
Apomuden	5.65	Faara	5.21	16010	6.16	13.44*	9.03
Sauti	3.85	Nanungungungu	0.00	16009	5.51	186.23***	43.12**
Apomuden	5.65	PG12086-18	4.04	16013	4.2	-13.31	-25.66**
Nanungungungu	0.00	Bohye	4.27	16005	5.59	161.83***	30.91*
PG12086-18	4.04	CIP440390	5.41	16002	4.34	-8.15	-19.78*
CIP440390	5.41	PG12086-18	4.04	16007	4.98	5.40	-7.95
PG12086-18	4.04	Apomuden	5.65	16001	6.55	35.19***	15.93*
Nanungungungu	0.00	Otoo	4.89	16004	4.92	101.23***	0.61
Apomuden	5.65	Hi-starch	3.78	16008	3.75	-20.47*	-33.63**
Nanungungungu	0.00	Faara	5.21	16014	3.79	45.49**	-27.26**
Nanungungungu	0.00	PG12086-18	4.04	16012	4.14	104.95***	2.48
LSD (5%)	1.03		1.03		1.03		

Table 4.13: Mean values and h

16001= PG12086-18 x Apomuden; 16002 = PG12086-18 x CIP440390; 16003 = Nanungungungu x Ligri; 16004 = Nanungungungu x Otoo; 16005 = Nanungungungu x Bohye; 16006 = Faara x Nanungungungu; 16007 = CIP440390 x PG12086-18; 16008 = Apomuden x Hi-starch; 16009 = Sauti x Nanungungungu; 16010 = Apomuden x Faara; 16011 = Apomuden x Sauti; 16012 = Nanungungungu x PG12086-18; 16013 = Apomuden x PG1208618; 16014 = Nanungungungu x Faara; 16015 = Nanungungungu x Hi-starch, P_1= Parent 1, P_2 = Parent 2, F_1 = Hybrid, MPH = Mid-parent heterosis and BPH =Better parent heterosis

* Significant at P < 0.05, **Significant at P < 0.01 and ***Significant at P < 0.001

Table 4.14: Mean values and heterosis estimates for starch content.

			Starch content (%)				
P_1	Mean	P_2	Mean	F_1	Mean	MPH (%)	BPH (%)
Nanungungungu	0.00	Hi-starch	66.8	16015	58.13	74.04***	-12.98**
Apomuden	51.78	Sauti	64.31	16011	56.82	-2.11	-11.65**
Nanungungungu	0.00	Ligri	63.5	16003	58.27	83.53***	-8.24*
Faara	66.81	Nanungungungu	0.00	16006	52.48	57.10***	-21.45***
Apomuden	51.78	Faara	66.81	16010	62.41	5.25	-6.59
Sauti	64.31	Nanungungungu	0.00	16009	59.65	85.51***	-7.25*
Apomuden	51.78	PG12086-18	66.27	16013	59.4	0.64	-10.37**
Nanungungungu	0.00	Bohye	61.98	16005	53.51	72.67***	-13.67**
PG12086-18	66.27	CIP440390	62.41	16002	60.45	-6.05*	-8.78*
CIP440390	62.41	PG12086-18	66.27	16007	64.18	-0.25	-3.15
PG12086-18	66.27	Apomuden	51.78	16001	55.5	-5.97	-16.25***
Nanungungungu	0.00	Otoo	63.21	16004	56.66	79.28***	-10.36**
Apomuden	51.78	Hi-starch	66.8	16008	66.89	12.82***	0.13
Nanungungungu	0.00	Faara	66.81	16014	54.19	62.22***	-18.89***
Nanungungungu	0.00	PG12086-18	66.27	16012	58.43	76.34***	-11.83**
LSD (0.5%)	3.98		3.98		3.98		

16001= PG12086-18 x Apomuden; 16002 = PG12086-18 x CIP440390; 16003 = Nanungungungu x Ligri; 16004 = Nanungungungu x Otoo; 16005 = Nanungungungu x Bohye; 16006 = Faara x Nanungungungu; 16007 = CIP440390 x PG12086-18; 16008 = Apomuden x Hi-starch; 16009 = Sauti x Nanungungungu; 16010 = Apomuden x Faara; 16011 = Apomuden x Sauti; 16012 = Nanungungungu x PG12086-18; 16013= Apomuden x PG1208618; 16014 = Nanungungungu x Faara; 16015 = Nanungungungu x Hi-starch, P1= Parent 1, P2 = Parent 2, F1 = Hybrid, MPH = Mid-parent heterosis and BPH =Better parent heterosis

* Significant at P < 0.05, **Significant at P < 0.01 and ***Significant at P < 0.001

4.3.12 Iron content

Mean values and heterosis estimates for iron content were presented on Table 4.15 Apomuden (2.49 %) had the highest iron content and it was significantly different from that of Ligri (2.20 %) at P<0.05. 16001 (2.51 %) had the highest iron content; one of its parents was Apomuden, but not significantly different from that of 16005 (2.44 %) and 16006 (2.44 %).

Significant (P<0.001) and positive MPH was recorded for progenies 16005 (141.58 %), 16006 (141.58 %), 16009 (141.46 %), 16004 (119.59 %), 16015 (64.42 %), 16003 (63.64 %) and 16014 (51.49 %); only the first two 16005 (20.79 %) and 16006 (20.79%) had significant (P<0.05) and positive BPH.

4.3.13 Zinc content

Mean values and heterosis estimates for zinc content were presented on Table 4.16. Apomuden (1.59 %) had the highest zinc content as observed for its iron content, followed by Ligri (1.53 %), CIP440390 (1.38 %), Bohye (1.36 %) and Faara (1.35 %); their means were not significantly different. The zinc content of the progenies ranged from 1.6 % (16011) to 1.01 % (16008).

All the progenies had significant and positive MPH for zinc content except for 16010

(8.16 %), 16013 (2.54 %) and 16002 (2.33 %) which were not significant and 16008 (21.4 %) which was negative. Progenies 16012 (84.42 %), 16009 (65.38 %), 16007 (53.25 %) and 16015 (23.47 %) had significant and positive BPH, this order was also observed in the MPH.

Iron (%)							
P_1	Mean	P_2	Mean	F_1	Mean	MPH (%)	BPH (%)
Nanungungungu	0.00	Hi-starch	1.63	16015	1.34	64.42***	-17.79
Apomuden	2.49	Sauti	1.64	16011	1.41	-31.72***	-43.37***
Nanungungungu	0.00	Ligri	2.2	16003	1.8	63.64***	-18.18*
Faara	2.02	Nanungungungu	0.00	16006	2.44	141.58***	20.79*
Apomuden	2.49	Faara	2.02	16010	2.14	-5.10	-14.06
Sauti	1.64	Nanungungungu	0.00	16009	1.98	141.46***	20.73
Apomuden	2.49	PG12086-18	2.09	16013	1.95	-14.85*	-21.69**
Nanungungungu	0.00	Bohye	2.02	16005	2.44	141.58***	20.79*
PG12086-18	2.09	CIP440390	2.06	16002	1.85	-10.84	-11.48
CIP440390	2.06	PG12086-18	2.09	16007	1.995	-3.86	-4.55
PG12086-18	2.09	Apomuden	2.49	16001	2.51	9.61	0.80
Nanungungungu	0.00	Otoo	1.94	16004	2.13	119.59***	9.79
Apomuden	2.49	Hi-starch	1.63	16008	1.72	-16.50*	-30.92***
Nanungungungu	0.00	Faara	2.02	16014	1.53	51.49***	-24.26*
Nanungungungu	0.00	PG12086-18	2.09	16012	1.16	11.00	-44.50***
LSD (5%)	0.36		0.36		0.36		

Table 4.15: Mean values and heterosis for iron content

16001= PG12086-18 x PG12086-18 x CIP440390; Ligri; 16004 = Apomuden; 16002 = 16005 = Nanungungungu x Nanungungungu x Otoo; Nanungungungu; 16007 = Bohye; 16006 = Faara x 16008 = Apomuden x Hi- CIP440390 x PG12086-18; starch; 16009 = Sauti x Nanungungungu; 16010 = Apomuden x Faara; 16011 = Apomuden x Sauti; 16012 = Nanungungungu x PG12086-18; 16013= Apomuden x PG1208618; 16014 = Nanungungungu x Faara; Hi-starch, P_1= Parent 1, P_2 = 16015 = Nanungungungu x = Mid-parent heterosis and Parent 2, F_1 = Hybrid, MPH BPH =Better parent heterosis

* Significant at $P < 0.05$, **Significant at $P < 0.01$ and ***Significant at $P < 0.001$

Nanungungungu						94***	23.47*

Table 4.16: Mean values and heterosis for zinc content.

			Zinc content (%)				
P_1	Mean	P_2	Mean	F_1	Mean	MPH (%)	BPH (%)
	0.00	Hi-starch	0.98	16015	1.21		
	1.59	Sauti	0.78	16011	1.6		
		Ligri	1.53	16003	1.29		
	1.35	Nanungungungu	0.00	16006	1.32		
	1.59	Faara	1.35	16010	1.59		
	0.78	Nanungungungu	0.00	16009	1.29		
	1.59	PG12086-18	0.77	16013	1.21		
	0.00	Bohye	1.36	16005	1.38		
	0.77	CIP440390	1.38	16002	1.1		
	0.00	PG12086-18	0.77	16007	1.18		
	0.77	Apomuden	1.59	16001	1.49		
	0.00	Otoo	1.08	16004	1.3		
	1.59	Hi-starch	0.98	16008	1.01		
	0.00	Faara	1.35	16014	1.27		

Apomuden		35.02***	0.63
Nanungungungu	0.00	68.63***	-15.69
Faara		95.56**	-2.22
Apomuden		8.16	0.00
Sauti		230.77***	65.38**
Apomuden		2.54	-223.90*
Nanungungungu		102.94***	1.47
PG12086-18		2.33	-20.29*
CIP440390		206.49***	53.25**
PG12086-18		26.27*	-6.29
Nanungungungu		140.74***	20.37
Apomuden		-21.4*	-36.48***
Nanungungungu		88.15***	-5.93

Nanungungungu	0.00	PG12086-18	0.77	16012	1.42	268.83***	84.42***
LSD (5%)	0.27		0.27		0.27		

16001= PG12086-18 x Apomuden; 16002 = PG12086-18 x CIP440390; 16003 = Nanungungungu x Ligri; 16004 = Nanungungungu x Otoo; 16005 = Nanungungungu x Bohye; 16006 = Faara x Nanungungungu; 16007 = CIP440390 x PG12086 -18; 16008 = Apomuden x Hi -starch; 16009 = Sauti x Nanungungungu; 16010 = Apomuden x Faara; 16011 = Apomuden x Sauti; 16012 = Nanungungungu x PG12086-18; 16013= Apomuden x PG12086-18; 16014 = Nanungungungu x Faara; 16015 = Nanungungungu x Hi -starch, P_1= Parent 1, P_2 = Parent 2, F_1 = Hybrid, MPH = Mid -parent heterosis and BPH =Better parent heterosis

* Significant at $P < 0.05$, **Significant at $P < 0.01$ and ***Significant at $P < 0.001$.

4.4 Correlation among pre-harvest, harvest and quality traits.

Table 4.17 shows correlation coefficient values for pre-harvest, harvest and quality traits. Total yield had a significant ($P<0.001$) and positive correlation with the weight of marketable roots ($r = 0.91$), root size ($r = 0.53$), number of marketable roots ($r = 0.63$) and harvest index ($r = 0.61$). Also, vine vigour correlated significantly and positively with the weight of marketable roots ($r = 0.24$, $P<0.05$), root size ($r = 0.35$, $P<0.01$), number of plants harvested ($r = 0.33$, $P<0.01$), percentage dry matter ($r = 0.40$, $P<0.001$), iron content ($r = 0.29$, $P<0.05$) and starch content ($r = 0.40$, $P<0.001$). Significant and negative correlations were observed between virus severity and number of plants harvested ($r = -0.39$, $P<0.01$), percentage dry matter ($r = -0.33$, $P<0.01$), iron content ($r = -0.24$, $P<0.05$), percentage protein ($r = -0.25$, $P<0.05$) and starch content ($r = -0.35$, $P<0.01$), but not significant with total yield ($r = -0.12$), weight of marketable roots ($r = -0.11$), root size ($r = -0.17$) and number of marketable roots ($r = -0.16$). Weevil damage was correlated significantly and negatively with total yield ($r = -0.559$), weight of marketable roots ($r = 0.456$), root size ($r = -0.254$), number of marketable roots ($r = 0.423$) and harvest index ($r = -0495$) but not significant with virus severity ($r = -0.031$). Percentage dry matter correlated significantly and positively with iron content ($r = 0.36$, $P<0.001$), percentage protein ($r = 0.32$, $P<0.01$), starch content ($r = 0.93$, $P<0.001$) and zinc content ($r = 0.35$, $P<0.01$).

Table 4.17: Correlations among pre-harvest, harvest and quality traits.

	Total Yield	WRM	RS	NRM	NPH	%HI	VV	VS	WD	%DM	Iron	Protein	Starch
WRM	0.908***												
RS	0.533***	0.624***											
NRM	0.633***	0.628***	0.489***										
NPH	0.042	0.135	0.270*	0.504***									
%HI	0.612***	0.731***	0.625***	0.517***	0.034								
VV	0.185	0.236*	0.352**	0.136	0.330**	-0.0252							
VS	-0.124	-0.113	-0.167	-0.162	-0.387**	0.112	0.777***						
WD	-0.559***	-0.456**	-0.254*	-0.423***	0.0969	-0.495***	0.116	-0.031					
%DM	0.146	0.184	0.335**	0.152	0.225	0.183	0.402***	-0.334**	0.180				
Iron	0.152	0.12	0.296*	0.141	0.355**	0.151	0.294*	-0.236*	0.269*	0.361**			
Protein	0.106	0.058	0.184	0.094	0.235*	0.106	0.226	-0.248*	0.220	0.32**	0.822***		
Starch	0.201	0.224	0.366**	0.286*	0.392***	0.232*	0.396***	-0.345**	0.263*	0.929***	0.533***	0.491***	
Zinc	0.143	0.053	0.152	0.159	0.162	0.181	0.050	-0.175	0.296*	0.348**	0.697***	0.765***	0.474***

WRM = Weight of roots marketable, RS = Root size, NRM = Number of root marketable, NPH = Number of plant harvested, HI = Harvest index, VV = Vine vigour, VS = Virus severity, WD = Weevil damage, %DM = Percentage dry matter.

* Significant at P < 0.05, **Significant at P < 0.01 and ***Significant at P < 0.001

4.5 Diversity studies among sweetpotato genotypes with Microsatellites or Simple Sequence Repeats (SSR) markers.

For the primers used, all the genotypes gave good quality amplification products consisting predominantly of clear and sharp bands and some stutter bands, characteristic of SSR gels; all the polymorphic bands were scored for the diversity analysis. Out of the remaining primers, 1BS-07 was monomorphic and IB-242, IBCIP-13, IBR-21 and J10A had weak resolutions which made scoring of the bands difficult.

4.5.1: Genetic information by the SSR markers in the parents.

Table 4.18 shows the genetic information provided by the SSR markers in 28 genotypes of sweetpotato evaluated. Sixty-six polymorphic bands with an average of 4.4 bands per primer were scored. The number of alleles produced ranged from 2 for marker IBR-03 to 7 for marker IBR-12. The average heterozygosity value for all the markers was 0.60, marker IBR-03 (0.18) gave the lowest frequency and the highest was observed for J116A (0.96). It was observed that the marker with the highest number of alleles also had highest PIC value (0.69) but with a low allele frequency (0.45). On the other hand, the marker (IBS-17) with the lowest PIC value (0.23) had the highest allele frequency (0.86) with 3 alleles. The value of the gene diversity ranged from 0.25 for marker IBS17 to 0.73 for marker IBR-12, with a mean of 0.56. The genetic information provided in Table 4.18 revealed that the allele frequency for the 15 primers were less or equal to 0.86.

Table 4.18: Genetic information generated by 15 SSR markers in twenty-eight sweetpotato genotypes.

Primer	Band size (bp)	Expected size (bp)	AT (°C)	Number of alleles	Allele Frequency	Gene diversity	H_e	PIC	The
Ib-248	120-200	164-177	62	5	0.45	0.70	0.67	0.66	
IbR-20	150-300	194 -212	48	4	0.50	0.57	0.54	0.48	
Ib-297	150-250	130-200	58	4	0.54	0.55	0.71	0.45	
IB-318	130-300	125-135	53	4	0.55	0.52	0.79	0.42	
IbS-10	200-330	253-298	60	5	0.44	0.69	0.67	0.64	
IbS-17	170-200	158-198	58	3	0.86	0.25	0.29	0.23	
IbS-18	200-300	232-242	58	6	0.46	0.71	0.89	0.67	
J116A	200-300	Unknown	58	4	0.52	0.56	0.96	0.47	
IB-316	150-200	140-155	58	3	0.78	0.37	0.33	0.34	
IBCIP-1	150-200	140-153	63	4	0.50	0.55	0.82	0.45	
IBR-03	250-270	243-263	58	2	0.77	0.36	0.18	0.29	
IBR-12	250-450	331-393	60	7	0.45	0.73	0.36	0.69	
IBR-14	170-250	216-222	58	4	0.72	0.45	0.41	0.42	
IBR-16	150-270	131-237	60	6	0.45	0.72	0.79	0.68	
IBR-19	150-250	192-213	60	5	0.50	0.68	0.64	0.64	
Mean				**4.4**	**0.57**	**0.56**	**0.60**	**0.50**	

primers and their expected sizes were obtained from Buteler *et al*. (1999) and Ernest *et al*. (2015); AT = Annealing temperature, H_e = Heterozygosity, PIC = Polymorphic Information Content

4.5.2 Genetic information by the SSR markers in the ten parents and their progenies.

Table 4.19 shows the genetic information provided by the SSR markers in 40 genotypes of sweetpotato evaluated. Sixty-one polymorphic bands with an average of 4.1 bands per primer were scored. The number of alleles ranged from 3 for markers IBR-20, IB297, J116A, IB-316, IBS-17, IBCIP-1 and IBR-03 to 7 for marker IBR-12. The average heterozygosity value for all the markers was 0.80, marker IBR-14 (0.42) gave the lowest frequency and the highest was observed for IBS-10 (1.0). It was observed that the marker with the highest number of alleles also had highest PIC value of 0.78 but with a low allele frequency of 0.32, followed by markers IBR-16 (5 alleles, PIC value of 0.75 and allele frequency of 0.28) and IBS-18 (5 alleles, PIC value of 0.69 and allele frequency of 0.40). The markers that produced three alleles had varying PIC value and allele frequency. J116A had the lowest PIC value of 0.47 with an allele frequency of 0.51, IB-297 (PIC value of 0.49 and allele frequency of 0.58) and IBCIP-1 (PIC value of 0.50 and allele frequency of 0.53). The gene diversity varied from 0.56 for marker IB-297 to 0.81 for marker IBR-12 with a mean of 0.65. The genetic information provided in Table 4.18 showed that the allele frequency for the 15 primers were less or equal to 0.78.

Table 4.19: Genetic information generated by 15 SSR markers in forty sweetpotato genotypes.

Primer	Band size (bp)	Expected size (bp)	AT (°C)	Number of alleles	Allele Frequency	Gene diversity	H_e	PIC
Ib-248	120-200	164-177	62	5	0.48	0.68	0.90	0.63
IbR-20	150-300	194 -212	48	3	0.39	0.66	0.64	0.59
Ib-297	150-250	130-200	58	3	0.58	0.56	0.70	0.49
IB-318	130-300	125-135	53	5	0.51	0.59	0.92	0.51
IbS-10	200-330	253-298	60	4	0.38	0.71	1.00	0.66
IbS-17	170-200	158-198	58	3	0.51	0.59	0.85	0.51
IbS-18	200-300	232-242	58	5	0.40	0.73	0.72	0.69
J116A	200-300	Unknown	58	3	0.51	0.56	0.98	0.47
IB-316	150-200	140-155	58	3	0.51	0.62	0.98	0.55
IBCIP-1	150-200	140-153	63	3	0.53	0.58	0.90	0.50
IB-R03	250-270	243-263	58	3	0.47	0.63	0.62	0.56
IB-R12	250-450	331-393	60	7	0.32	0.81	0.79	0.78
IB-R14	170-250	216-222	58	4	0.50	0.65	0.42	0.59
IB-R16	150-270	131-237	60	5	0.28	0.78	0.95	0.75
IB-R19	150-250	192-213	60	5	0.56	0.60	0.70	0.54
Mean				**4.1**	**0.46**	**0.65**	**0.80**	**0.59**

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The primers and their expected sizes were obtained from Buteler *et al*. (1999) and Ernest *et al*. (2015); AT = Annealing Temperature, H_e = Heterozygosity, PIC = Polymorphic Information Content

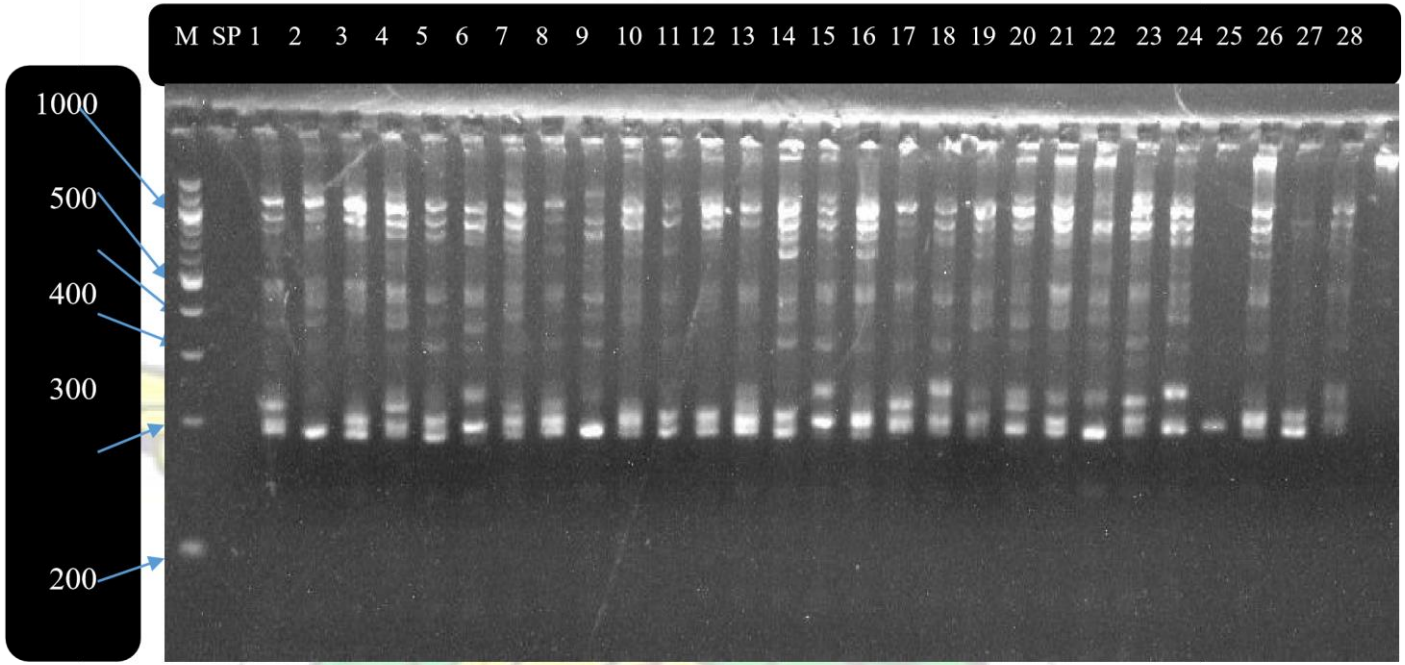

Figure 4.2: PCR amplification profiles of 28 sweetpotato genotypes with SSR primer IBR-19 in 3% metaphor agarose gel (MAGE)

M = 100bp ladder; SP = Negative control and 1 to 28 are the parental genotypes listed in Table 3.1

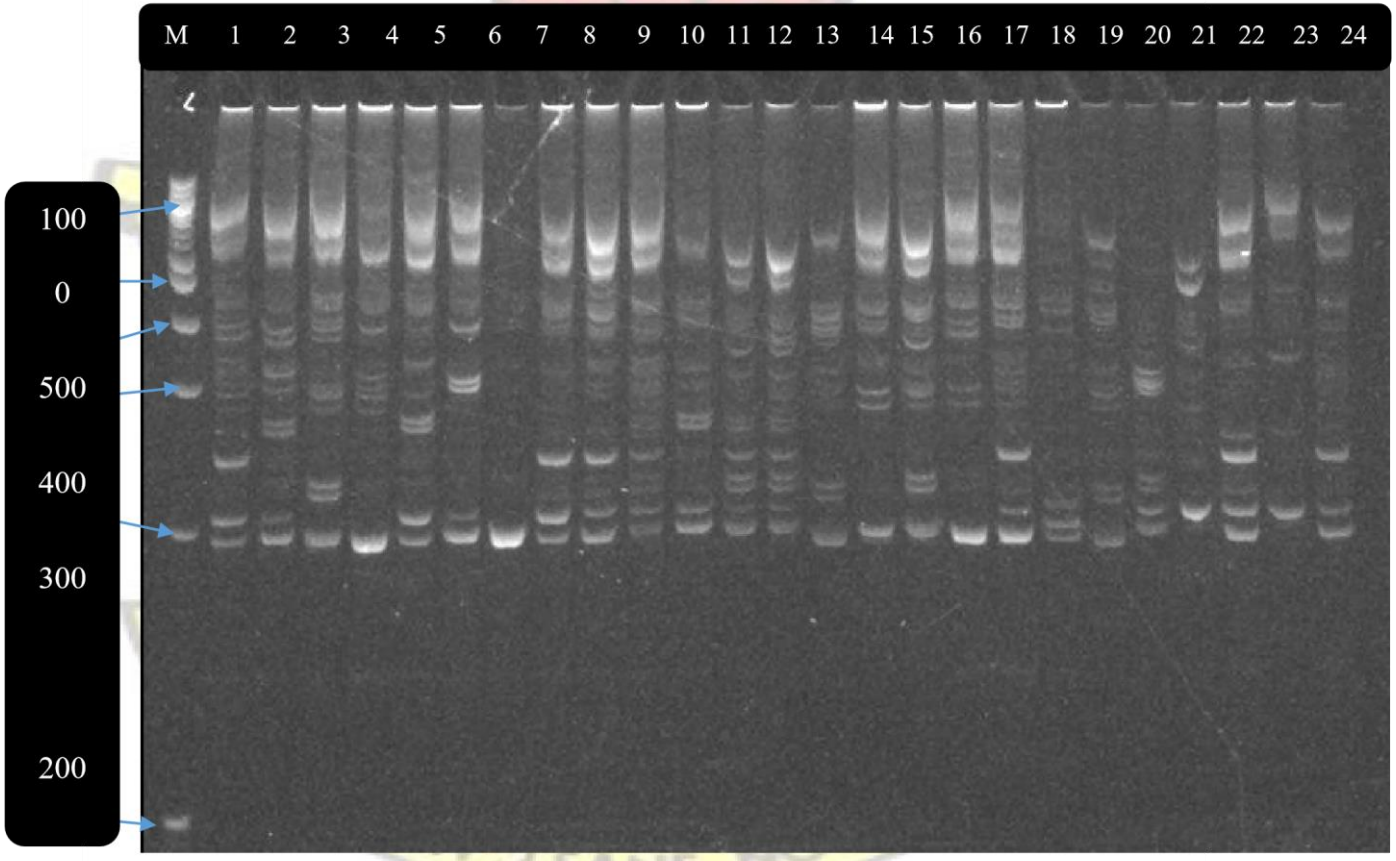
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
100
0
500
400
300
200

Figure 4.3: PCR amplification profiles of 25 sweetpotato genotypes with SSR primer IBR-19 in 6% Polyacrylamide gel (PAGE)

M = 100bp ladder; SP = Negative control, 1 to 10 are the parental genotypes and 11 to 25 are the progenies listed in Table 3.1

4.5.3 Factor analysis

4.5.3.1 Parents

Factor analysis is mostly used to give a global representation of diversity. In this study, principal coordinates analysis (PCoA) which is a multivariate method to examine a data table was done using Dice Dissimilarity Index to extract the main information from the table and symbolize it in an orthogonal variables named principal coordinates (Figure 4.3). Table 4.20 shows that the four coordinates (axes 1, 2, 3 and 4) had a total inertia of 43.91%, with positive eigen values that ranged from 0.00717 (axis 4) to 0.00935 (axis 1). The highest inertia percentage was observed for axis 1 (12.79), followed by axis 2 (11.47) and the lowest was for axis 4 (9.8).

Table 4.20: Factor coordinates calculated from dissimilarity index for the parents.

Axis	Eigen value	Inertia (%)
1	0.00935	12.79
2	0.00839	11.47
3	0.0072	9.85
4	0.00717	9.8

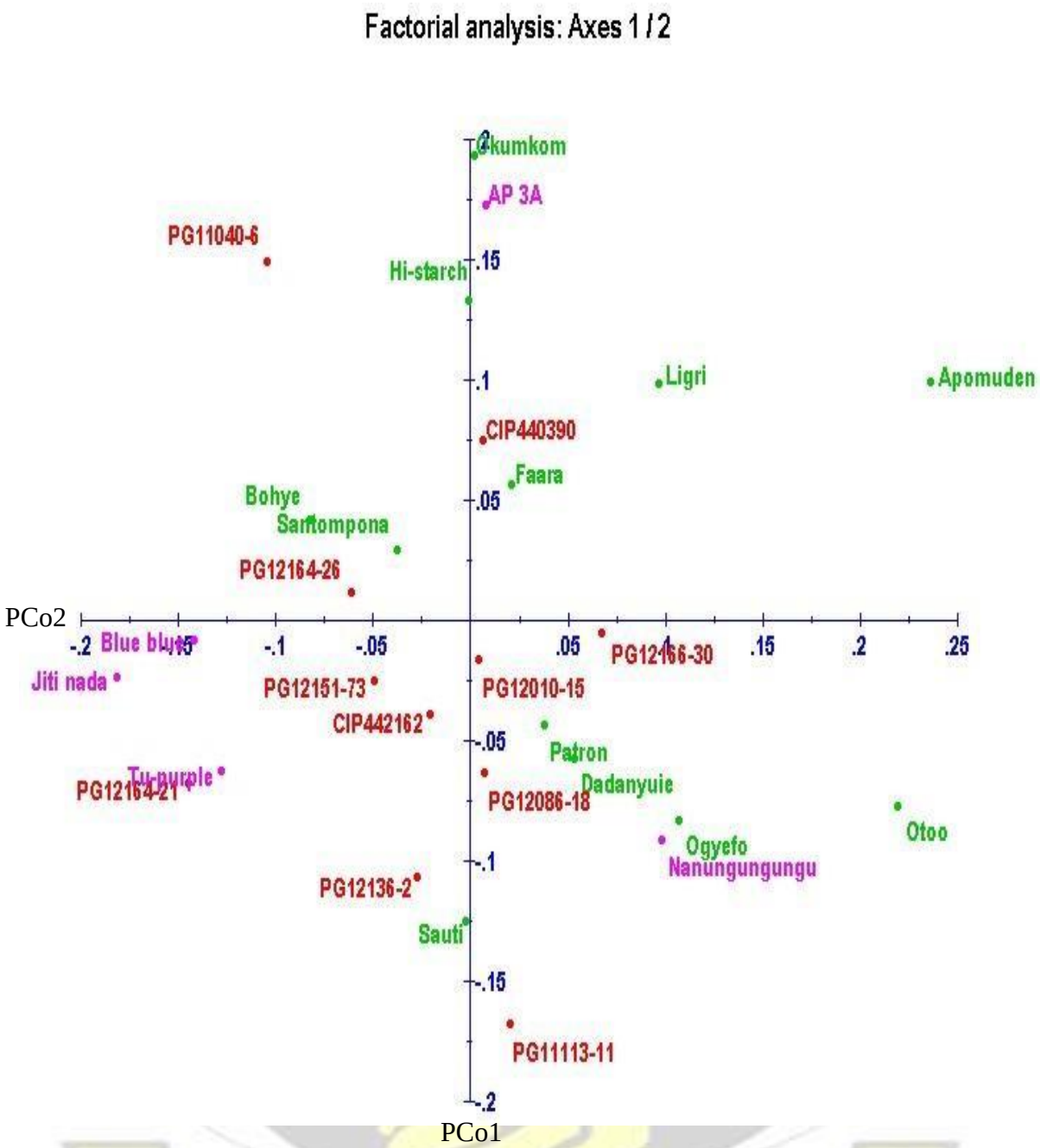

Figure 4.4: Principal coordinates of factor analysis for the twenty -eight genotypes of sweetpotato using Dice Dissimilarity Coefficient.

4.5.3.2 Parents and progenies

Table 4.21 shows that the four coordinates (axes 1, 2, 3, 4) had a total inertia of 38.51% with positive Eigen values that ranged from 0.00532 (axis 4) to 0.013 (axis 1). The highest inertia percentage was observed for axis 1 (16%) and the lowest was for axis 4 (6.55%).

The principal coordinates from the factor analysis is shown in figure 4.4.

Table 4.21: Factor coordinates calculated from dissimilarity index for the parent and progenies.

Axis	Eigenvalue	Inertia (%)
1	0.013	16
2	0.00697	8.58
3	0.006	7.38
4	0.00532	6.55

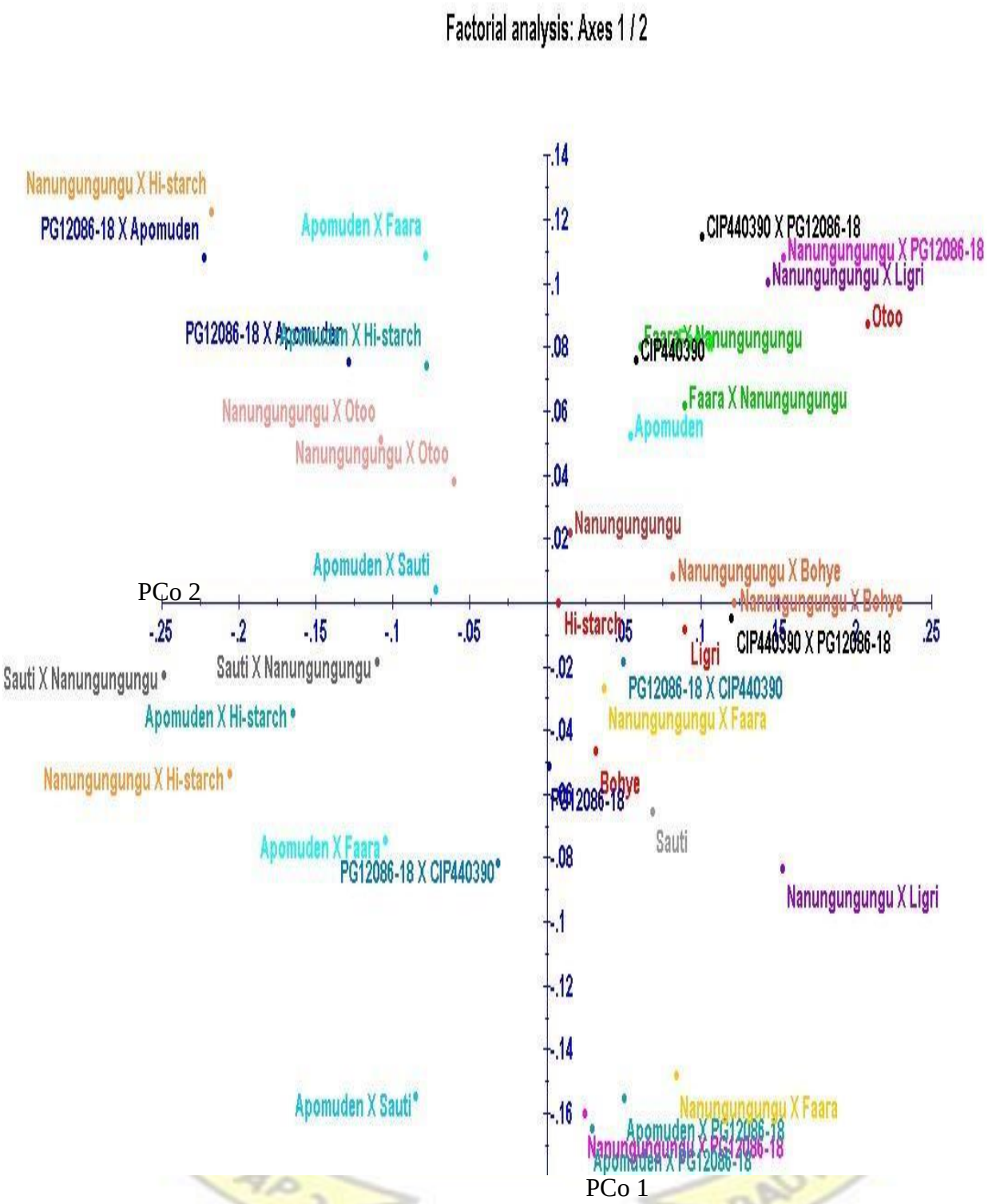

Figure 4.5: Principal coordinates of factor analysis for the ten parents and fifteen progenies of sweetpotato using Dice dissimilarity coefficient.

4.5.4 Cluster analysis

4.5.4.1 Parents

Cluster analysis of the distance matrix based on Unweighted Pair Group Method using arithmetic Average (UPGMA) was performed to reveal relationships among the 28 parents and also between the 10 parents and their progenies. Figure 4.5 shows the dendrogram of dissimilarity among the 28 parents. It revealed five main clusters A, B C, D and E at 0.01. Cluster A consisted of Hi-starch, PG11040-6, CIP440390, Faara, PG12086-18, Bohye, Okumkom and PG 12164-26. Cluster B consisted of PG 12010-15, PG12151-73, Apomuden and AP3A. Cluster C consisted of Sauti, PG12136-2, PG11113-11, PG12164-21 and CIP442162. Cluster D consisted of Blue blue, Tupurple, Jiti nada and Santompona and cluster E consisted of Nanungungungu, Ligri, PG12166-30, Ogyefo, Patron, Dadannyuie and Otoo. The lowest dissimilarity coefficient (0.11) was observed between Jiti nada and Blue blue in cluster D and the highest (0.93) between Apomuden (cluster B) and CIP440390 (cluster A).

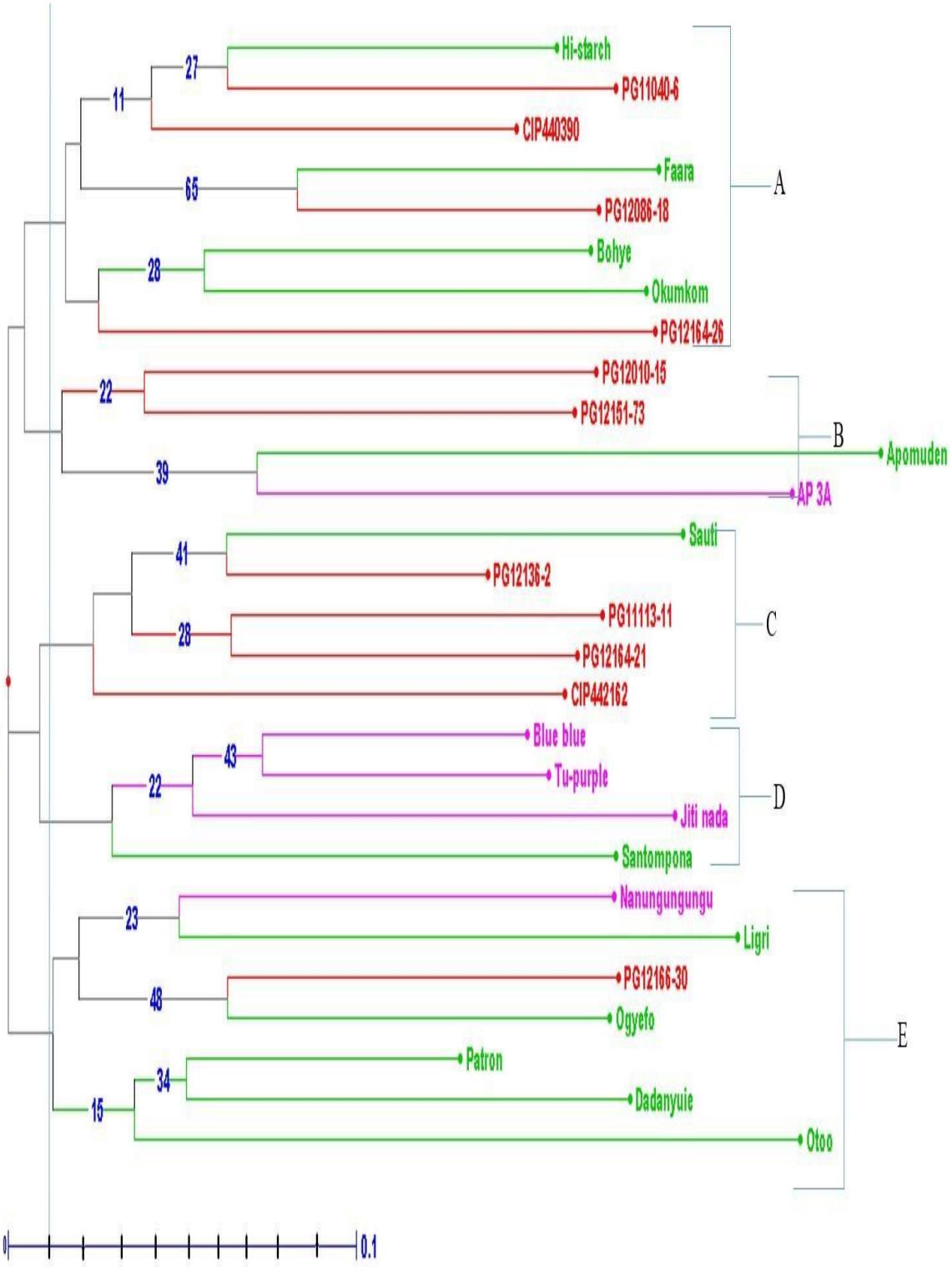

Figure 4.6: Dendrogram of dissimilarity among the 28 parents in sweetpotato using UPGMA.

4.5.4.2 Parents and progenies

Cluster analysis of the distance matrix based on Unweighted Pair Group Method using arithmetic Average (UPGMA) was performed to reveal relationships between the 10 parents and their progenies**.** The dendrogram (Figure 4.6) showed five main clusters with 1 to 21 accessions in a cluster. Cluster A was the biggest cluster and it comprised Nanungungungu x Bohye, Nanungungungu x Ligri, Faara x Nanungungungu, Apomuden, Nanungungungu x PG12086-18, CIP440390 x PG12086-18, Faara x Nanungungungu, Otoo, CIP440390 x PG12086-18, PG12086-18 x CIP440390, Faara, Nanungungungu x Faara, CIP440390, Sauti, Ligri, Bohye, Nanungungungu x Bohye, Apomuden x PG12086-18, Apomuden x PG12086-18, Nanungungungu x Ligri, and Histarch. Cluster B was made up of Nanungungungu x Faara, Apomuden x Sauti, PG12086-18 and Nanungungungu x PG12086-18. Cluster C was made up of Nanungungungu x Hi-starch, Nanungungungu x Hi-starch, Sauti x Nanungungungu, Apomuden x Hi-starch, PG12086-18 x Apomuden, Sauti x Nanungungungu, Nanungungungu x Otoo, Apomuden x Faara, PG12086-18 x Apomuden, Apomuden x Histarch, Apomuden x Sauti and Apomuden x Faara). Cluster D was made up of Nanungungungu x Otoo and PG12086-18 x CIP440390 and cluster E with only Nanungungungu. The lowest dissimilarity coefficient (0.11) was observed between the same progeny Nanungungungu x Hi-starch in cluster C and the highest (0.63) between Faara x Nanungungungu in cluster A and Nanungungungu x Faara in cluster B.

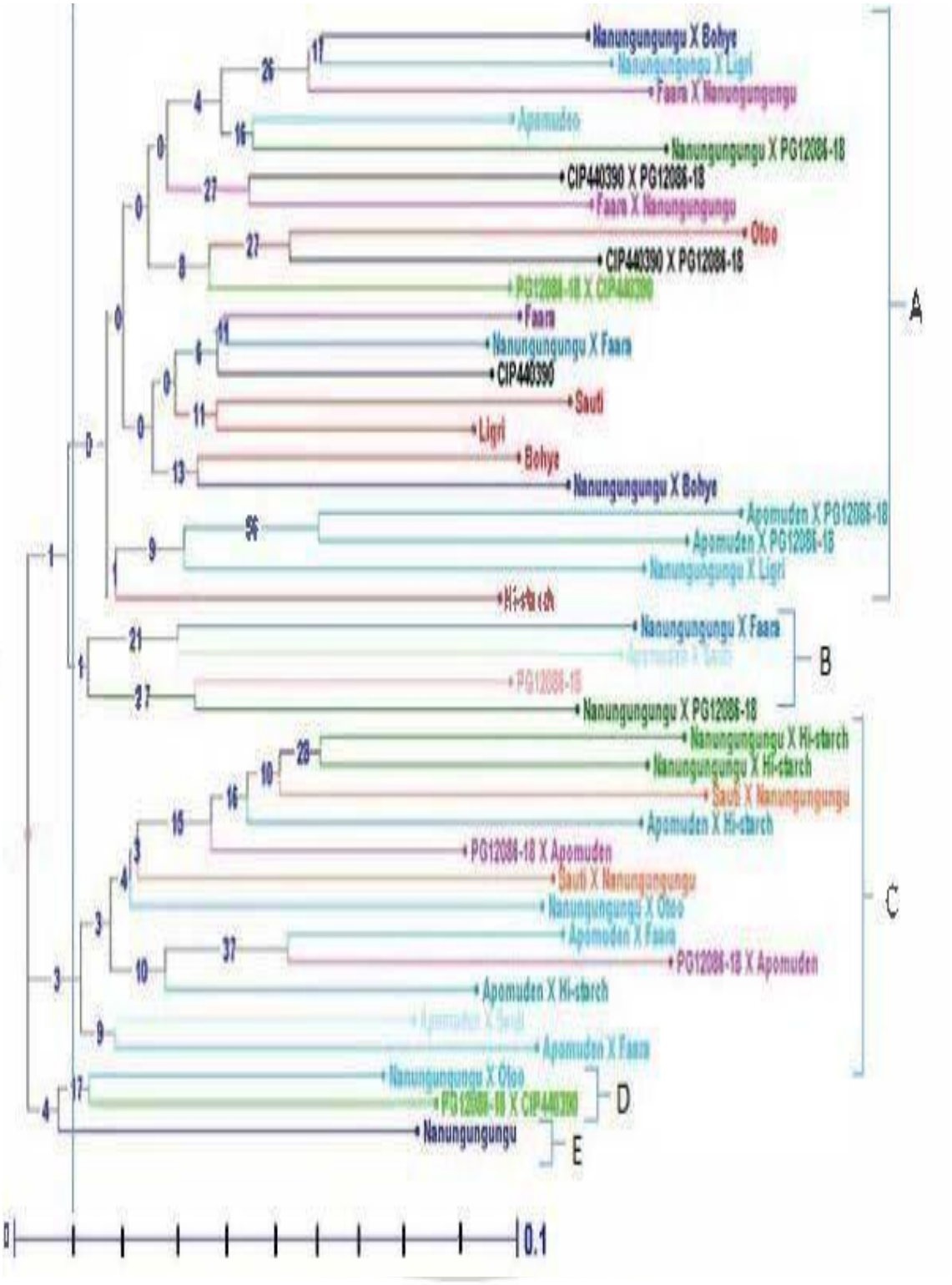

Figure 4.7: Dendrogram of dissimilarity between the 10 parents and their 30 progenies in sweetpotato using UPGMA

CHAPTER FIVE

5.0 DISCUSSION

5.1 Importance of soil pH and some nutrient in sweetpotato growth

Sweetpotato is a clonally propagated crop and requires a well-drained sandy loam soil, a sunshiny days and cool nights for a good growth. According to the North Carolina Sweetpotato Commission (2016), the crop can grow at a pH of 4.5 to 7.5 but the best pH range is from 5.5 to 6.2 (Woolfe, 1992). In this study the soil pH was 4.4 meaning that the soil was highly acidic and this could have affected crop development, making the plant very delicate to aluminium toxicity (Ames *et al*., 1996), resulting in the death of the plants by six weeks after planting.

Nitrogen (N), Phosphorus (P), and Potassium (K) are essential nutrients for sweetpotato growth and yield. A moderate amount of N, relatively high amount of P and high K lead to a good growth and high yield in sweetpotato. In this experiment the percentage of total Nitrogen was 0.045 and the potassium was 0.215 cmol/Kg and the available Phosphorus was 45.67mg/Kg. These values are relatively low particularly the nitrogen and phosphorus, and might have affected both root and foliage yield. The percentage Organic Carbon (1.01) and Organic Matter (1.73) were also low, but it has been reported by the North Carolina Sweetpotato Commission (2015) that a high organic soil exceeding 2% is not needed for sweetpotato production because it causes yield reduction.

5.2 Evaluation of some yield parameters and heterosis in sweetpotato

The analysis of variance revealed that there were highly significant differences at $P < 0.001$ and 0.01 among the parents and their progenies in most of the yield parameters.

Only the weevil damage was significant at $P < 0.05$. The mean squares values observed in all the traits and the harvest index showed that there was a large variability among the parents as well as progenies. This large variability could be explained by the polyploidy

nature of sweetpotato (2n=6x=90) (Shiotani and Kawase, 1989). Sweetpotato is a hexaploid cross-pollinated species that will experience a high level of genomic repetition during meiosis resulting in a high variability among genotypes.

5.2.1 Severity of weevils and virus infestation in sweetpotato

According to the ANOVA table, the mean square of the weevil damage was 0.30 with a mean value of 1.12. This result means that the weevil infestation was low according to the sweetpotato descriptors (CIP/AVRDC/IBPGR, Huaman, 1991). This low infestation can be explained by the fact that most of the planting materials (vine cuttings) were from the nursery in the screen house and were clean. This result agrees with the report of Stathers *et al*., (2003) who suggested that weevils can be avoided by using uninfested planting materials and avoid planting in weevil infested fields. The low weevil infestation could also be due to the low Nitrogen content in the soil as it was revealed by the soil analysis. No fertilizer has been applied. Nitrogen has been known to boost the protein and starch content in sweetpotato and can attract the weevils. According to Bartolini, (1982) and Li, (1982), Protein and Starch helped sweetpotato weevils in their nutritious aspect.

Nevertheless, virus infestation was intermediate and the symptoms were showing in most of the parents accessions which contaminated some progenies. The parent planting materials were taken from the field (crossing block) and were already infested.

Sweetpotato Virus Disease (SPVD) is the greatest worry in sweetpotato cultivation (Tairo *et al*., 2004). For example in this study the most affected cultivar was Nanungungungu which produced no roots and had a very low foliage yield. Again the parents and progenies that were more infested produced very low yields. For example Wasonga, *et al*. (2014) has reported that SPVD is a limitation in almost 90% of sweetpotato production in the East Africa area.

5.2.2 Exploitation of heterosis by progeny testing in sweetpotato

Heterosis was defined by Shawn (2012) as "an increase in vigor that is observed in progenies of mating of diverse individuals from different species, isolated populations, or selected strains within species or populations". It can also be defined as the incidence of a superior offspring from mixing the genetic contributions of its parents. The results from this study in vine vigor and weight; number of plant with roots; number and weight of marketable roots and root size among the F_1 hybrids and their parents showed that 25 crosses produced positive and significant heterosis and 2 crosses have revealed negative and significant heterosis for all the traits in the mid-parent heterosis. For better parent heterosis, only 9 crosses showed positive and significant heterosis and 15 crosses exposed negative and significant heterosis for all the traits.

The low heterosis increment observed in vine vigor and weight can be explained by the fact that the parents involved in these crosses were very closely related (same cluster) or distantly related (different cluster) and the incompatibility of allele"s combinations can result on low heterosis as was reported by Manosh *et al*. (2008) that two extremely different parental population crosses got from very nearly or far linked parents can reveal low heterosis.

Heterosis results occurred in number of plant with root, number of marketable roots, weight of marketable roots and root size showed a high heterosis for mid parent and better parent. The parents involved in the successful crosses were moderately distant in the same or different cluster as was reported by Manosh *et al*. (2008) that crosses created from parents that have medium dissimilarity can dispose the hybrid to higher heterosis. In a contrary report, Moll *et al*. (1965) suggested that it is desirable to choose distantly linked parents to attain higher heterosis. However the high heterosis observed can be due to an intra-allelic interaction at one or multiple loci leading to greater performance in the hybrid. It was reported by Shawn (2012) that the over-dominance can be the basis of heteosis

observed. Nevertheless, the results of all the crosses generated by one of the parent being Hi-starch gave very low heterosis. This may be because Hi-starch was a heterozygote and was crossed with a homozygote parent which might have generated hybrids homozygote instead of hybrids heterozygote which can perform well.

The crosses which can be selected for mid parent heterosis can be: (Nanungungungu x Ligri = 5 traits, CIP440390 x PG12086-18 = 4 traits, Apomuden x PG12086-18 = 3 traits, PG12086-18 x CIP440390, Apomuden x Faara, Nanungungungu x Bohye and Sauti x Nanungungungu with 2 traits, Nanungungungu x Otoo, Apomuden x Sauti and Nanungungungu x Faara with one trait). And for better parent heterosis: (Apomuden x PG12086-18 with 3 traits, CIP440390 x PG12086-18 with 2 traits, Nanungungungu x Ligri, Nanungungungu x PG12086-18, Sauti x Nanungungungu, Apomuden x Faara, Apomuden x Hi-starch and PG12086-18 x CIP440390 with one trait).

5.2.3 Heterosis in the total root yield and for harvest index of yield in sweetpotato

The total root yield is the weight of marketable roots and non-marketable and the harvest index is the proportion of economic yield in the total biological yield (root and foliage) of the crop expressed in percentage.

Heterosis gain was very high in root yield and the harvest index of yield. In this study only 4 crosses showed positive and significant heterosis for mid parent in root yield and 6 crosses for harvest index. The crosses Faara x Nanungungungu and Nanungungungu x PG12086-18 showed both positive and significant mid parent and better parent heterosis. This was because the progenies were presented in a separating population and required later higher differences for all the characters related to yield. In a similar work by Luthra (2006) who studied 120 hybrids and 29 parents of potato based on progeny mean, heterosis and specific combining ability, only 4 progenies exhibited significant positive heterosis.

The harvest index is known as an essential yield factor in sweetpotato. In summary the following crosses can be selected as high yielding

[Apomuden x Sauti (83.66%), Nanungungungu x Bohye (361.14%), CIP440390 x PG12086-18 (139.89%), Nanungungungu x Faara (302.89%)] and economically profitable [Nanungungungu x Hi-starch (192.05%), Faara x Nanungungungu (637.45%), Nanungungungu x Bohye (405.25%), PG12086-18 x CIP440390 (124.27%), CIP440390 x PG12086-18 (124.27%) and Nanungungungu x PG12086-18 (611.42%)] and used for hybrid breeding.

5.3 Evaluation of some quality parameters and heterosis in sweetpotato

A good variety in sweetpotato is not only a high yielding but must also incorporate some quality traits. In this study some quality traits such as dry matter, protein, starch, glucose, sucrose, fructose, iron and zinc were evaluated. The analysis of variance showed a very high significant differences at $P < 0.001$ among the parents and their progenies in all the quality traits. The mean squares observed showed that there was a large variability among them. This large variability can be explained by the hexaploid nature of sweetpotato (2n=6x=90) (Shiotani and Kawase, 1989).

Heterosis was estimated in 6 quality traits such as dry matter, protein, starch, glucose, iron and zinc. The results showed that there was 58 crosses that revealed significant positive heterosis and 12 crosses revealed significant negative heterosis for all the traits in mid-parent heterosis. For better parent heterosis only 14 crosses showed significant positive heterosis gain and 43 crosses showed significant negative heterosis gain for all the traits.

Mid parent heterosis in dry matter was from -7.98% (PG12086-18 x CIP440390) to 97% (Nanungungungu x PG12086-18) with all the crosses exhibiting significant positive heterosis except 2 crosses. For better parent, heterosis ranged from –20.35% (PG12086-

18 x Apomuden) to 7.80% (CIP440390 x PG12086-18) the only cross with significant positive heterosis.

Starch content for mid parent heterosis was from –6.05% to 85.51% with 9 crosses showing significant positive heterosis and for better parent heterosis ranged from 21.45% to 0.13%. None of the crosses showed any significant positive heterosis. The progeny CIP440390 x PG12086-18 with low heterosis of 7.80% in dry matter content was better than his best parent and 13 crosses showed low (2.51%) to high (97%) heterosis for mid parent in dry matter content and 9 crosses showed low (12.82%) to high (83.53%) heterosis in starch content for mid parent heterosis. These results mean that the hybrids were performing better than their 2 parents put together instead of the best parent alone.

According to the results there was high heterosis (mid parent) in most of the crosses for dry matter. The high heterosis can be due to over dominance or epistatic gene interaction that involve dominance and additive gene effect as reported by Shawn (2012). The transformation of photosynthesis products from the leaves to the roots can also cause starch accumulation through some combinations. According to Woolfe, (1992) starch is the main constituent of sweetpotato storage root dry matter, and the high dry matter varieties are favored by consumers in sub-Saharan Africa (Tumwegamire *et al*., 2011).

High heterosis in iron and zinc content observed can be explained by the additive or nonadditive gene action involved in the manifestation of these traits. Moreover, the results of heterosis in the quality traits revealed that most of the superior hybrids were generated by distantly related parent confirming the large genetic diversity in sweetpotato quality parameters. These results agree with the findings of Moll *et al*.
(1965) and corroborated by Grüneberg *et al*. (2009) that it is necessary to choose distantly linked parents to attain higher heterosis. In contrast, Manosh *et al*. (2008) suggested that higher heterosis can be achived by crosses produced from parents that have moderate

dissimilarity.

The crosses which can be selected for mid parent heterosis can be Nanungungungu x Hi-starch, Nanungungungu x Ligri, Faara x Nanungungungu, Nanungungungu x Bohye, Nanungungungu x Otoo, Nanungungungu x Faara successful in all the 6 quality traits; Sauti x Nanungungungu and Nanungungungu x PG12086-18 (5 traits); Apomuden x Histarch (3 traits); PG12086-18 x Apomuden, Apomuden x Sauti, Apomuden x Faara, Apomuden x PG12086-18, CIP440390 x PG12086-18 (2 traits). In better parent heterosis, the performing crosses were Sauti x Nanungungungu, Nanungungungu x Bohye and CIP440390 x PG12086-18 (2 traits); Nanungungungu x Hi-starch, PG1208618 x Apomuden, Nanungungungu x Ligri, Faara x Nanungungungu, Apomuden x PG1208618, Nanungungungu x Otoo and Nanungungungu x PG12086-18 (1 trait).

5.4 Diversity among the sweetpotato genotypes using simple sequence repeat makers.

Simple Sequence Repeat markers (SSR) were used to study diversity among the 28 parents and within ten parents and their progenies. These markers have been used in diversity studies by several authors (La Rota *et al*., 2005; Arif *et al.*, 2011; Favoretto *et al*., 2011) who reported that SSR markers were the first and the most powerful genetic markers for diversity study because of their numerous advantages such as the easy reproduction, the co-dominancy, their frequent and random distribution and the high polymorphism because of their high mutation rate.

A moderate to high polymorphism was detected in this study with the 15 polymorphic SSR primers used which showed an average of 4.4 polymorphic alleles per SSR primer and a mean PIC value of 0.50 in the 28 parents and a PIC from 0.47 to 0.78 with a mean of 0.59 and an average of 4.1 polymorphic alleles per SSR in the parent and progenies. These results were obtained by calculation using allele‟s frequencies and estimated heterozygosity information‟s as was reported by Norman *et al*. (2012). A work done by

Otoo *et al*. (2009) showed diverse PIC values that ranged from 0 .0 to 0.89 with a mean of 0.53 by using 13 SSR markers on Yam genotyping. According to Xie *et al*. (2010), a PIC < 0.25 is measured as low; 0.25 < PIC < 0.50 for example is moderate and PIC > 0.50 is measured as high. Knowing the usefulness of the SSR primers in identifying a small genetic dissimilarity (Fikiru *et al*., 2010), the primers used were able to differentiate the accessions at a PIC from 0.23 to 0.69 with the highest PIC of 0.69 having the highest number of allele (7 alleles). These results confirmed the findings of Norman *et al*. (2012) who reported earlier that PIC value is related to the number of alleles and their frequencies and establishment of the effectiveness of the PIC as a measure of the capacity of a marker to differentiate between closely linked individuals (Prevost and Wilkinson., 1999). Moreover gene diversity ranged from 0.25 to 0.73 with a mean of 0.56 and a heterozygosity from 0.18 to 0.96 with a mean of 0.60 revealed as well the moderate diversity among the 28 parents. Yada *et al*. (2010) supported that SSR primers can reveal a moderate genetic diversity values. The high diversity observed within the 10 parents and their progenies can be explained by the fact that the progenies mixed with their parents presented a high heterozygosity and most of them were generated by parents distantly related. This was in accordance with Chaurasiya *et al*. (2013) who reported that some studies in India have confirmed that the hybrids from more varied parents showed higher genetic diversity. According to the polymorphism information content of the SSR primers used, all the primers were polymorphic except IB-297 and J116 A with PIC less than 50% for the parents and progenies evaluation and

6 primers (IB-248, IBS-10, IBS-18, IBR-12, IBR-16 and IBR-19) out of 15 were polymorphic for the 28 parents evaluation. These results mean that the primers listed above can be used for parents and progenies diversity study in sweetpotato.

5.5 Diversity study using factor and cluster analysis

A global representation of diversity was done by the principal coordinate analysis and the genotypes were grouped into different clusters by cluster analysis. Nei‟s genetic distance (Nei, 1983) was used to determine the relationship among the 28 parents and also between the ten parents and their 30 progenies based on their type (released, advanced or local variety). Genetic dissimilarity between genotypes were evaluated based on Dice‟s genetic dissimilarity coefficient.

For the 28 parents, the released accessions (7), advanced (5) and local (1) contributed more to the PCo1, however the advanced accessions (5), released (2) and local (3) contributed more to the PCo2. This implied that the grouping of the accessions was not based on their types but revealed the way all the accessions were related to each other. The dendrogram indicated more the relationship between all the accessions and showed that the released genotypes were more genetically different from one another than the advanced and local ones. The high variability is as a result of the nature of pollination in sweetpotato in which recombination and rearrangement of alleles occur when genotypes are intercrossed. The high genetic dissimilarity observed among the parents is an important factor that assist breeders in selecting parental genotypes for an increased heterosis in sweetpotato breeding programs. This agrees with Tumwegamire *et al.* (2011).They reported that genetic differences among genotypes is essential for heterosis exploitation in sweetpotato breeding programmes.

For the 10 parents and their progenies, it was revealed that the progenies contributed more to the PCo because of the small number of the parents. Some of the progenies were not grouped with their parents and all the parent were in the quadrant 3 and 4 with the maximum in quadrant 3. The results showed that the grouping of the genotype was not based on their types. The dendrogram revealed the relationship between the parents and

their progenies. The most distantly related progenies were Faara x NanungungunguNanungungungu x Faara (0.63) and the closest was between the same progeny Nanungungungu x Hi-starch. It was expected from this study that all the progenies should be close to their respective parents but the factor analysis and the dendrogam showed that some of the progenies that have common parent were not in the same cluster and also some progenies were not close to their parent. This could be as a result of the random reassortment of alleles in the various biparental cross (Yada *et al*., 2015) and also the high somatic transformation in sweetpotato which is a source of genetic variation among genotypes (Hernandez *et al*., 1964). The diversity observed can be due to the nature of pollination combined with the self-incompatibility in sweetpotato and its hexaploid nature (Tumwegamire *et al*., 2011)

CHAPTER SIX

6.0 CONCLUSION AND RECOMMANDATONS

6.1 Conclusion

Sweetpotato is considerated as a staple food and a cash crop in Sub-Saharan Africa. In view of its importance, breeding for high yielding and resistance to biotic and abiotic must be incorporated in its breeding programs. This current study revealed high heterosis in the number of plants with roots, number and weight of marketable roots, and root size for mid parent and better parent heterosis. The parents involved in the successful crosses were moderately distant in the same or different cluster.

The crosses which can be selected for mid parent heterosis are: Nanungungungu x Ligri (5 traits), CIP440390 x PG12086-18 (4 traits), Apomuden x PG12086-18 (3 traits), PG12086-18 x CIP440390, Apomuden x Faara, Nanungungungu x Bohye and Sauti x Nanungungungu with 2 traits, Nanungungungu x Otoo, Apomuden x Sauti and Nanungungungu x Faara with one trait. And for better parent heterosis: Apomuden x

PG12086-18 (3 traits), CIP440390 x PG12086-18 (2 traits), Nanungungungu x Ligri, Nanungungungu x PG12086-18, Sauti x Nanungungungu, Apomuden x Faara, Apomuden x Hi-starch and PG12086-18 x CIP440390 with one trait.

Four progenies (Apomuden x Sauti, Nanungungungu x Bohye, CIP440390 x PG1208618 and Nanungungungu x Faara) showed significant positive heterosis for mid parent in total yield and six progenies in harvest index with two for better parent.

However, to meet the consumers‟ preference, heterosis was estimated in some quality traits such as percentage dry matter, protein content, iron and zinc contents in which high heterosis were detected. The crosses which can be selected for mid parent heterosis were Nanungungungu x Faara; Nanungungungu x PG12086-18; Sauti x Nanungungungu; Nanungungungu x Bohye; Faara x Nanungungungu; Nanungungungu x Hi-starch with 4 traits; Nanungungungu x Otoo; Nanungungungu x Ligri; Apomuden x Hi-starch with 3 traits; PG12086-18 x Apomuden; Apomuden x Sauti; CIP440390 x PG12086-18 with 2 traits and Apomuden x Faara with 1 trait. In better parent heterosis, the performing crosses were Sauti x Nanungungungu, Nanungungungu x Bohye and CIP440390 x PG12086-18 with 2 traits; Nanungungungu x Hi-starch, PG12086-18 x Apomuden, Nanungungungu x Ligri, Faara x Nanungungungu, Apomuden x PG1208618, Nanungungungu x Otoo with 1 trait. From the heterosis results 10, 4 and 6 progenies out of fifteen exhibited significant and positive heterosis in the six pe-harvest and havest traits, total yield and harvest index respectively.

The correlation between pre-harvest, harvest and quality traits results revealed high significant positive correlation between total yield and root marketable (r =0.91), root size (r = 0.53), number of marketable roots (r = 0.63) and harvest index (r = 0.61). Also, vine vigour correlated significantly and positively with the weight of marketable roots (r =

0.24), root size (r = 0.35), number of plants harvested (r = 0.33), percentage dry matter (r = 0.40), iron content (r = 0.29) and starch (r = 0.40). Weevil damage was significantly correlated negatively with total yield, harvest index and yield related traits. Virus severity was significantly correlated negatively with the number of plants harvested (r = -0.39), percentage dry matter (r = -0.33), iron (r = -0.24), protein content (r = -0.25) and starch (r = -0.35), but not significant with total yield (r = -0.12), weight of marketable roots (r = 0.11), root size (r = -0.17) and number of marketable roots (r = -0.16). Percentage dry matter correlated significantly and positively with iron (r = 0.36,), protein content (r = 0.32), starch (r = 0.93) and zinc content (r = 0.35).

Virus infection was intermediate and the symptoms were showing in most of the parental accessions which contaminated some progenies and was one of the causes of the low yield production.

According to the polymorphism information content of the 15 SSR primers used, all the primers were polymorphic except IB-297 and J116 A with PIC less than 50% for the parents and progenies evaluation, and 6 primers (IB-248, IBS-10, IBS-18, IBR-12, IBR16 and IBR-19) out of 15 were polymorphic for the 28 parents evaluation. These results mean that the primers listed above can be used for parent and progeny diversity study in sweetptatoto.

The diversity analysis revealed that the 28 parents were clustered into five groups. The most distantly related accessions were (Apomuden - CIP440390 (0.93), Apomuden - Bohye (0.92), Apomuden - Okumkom (0.90), Apomuden - Blue blue (0.88) and Apomuden - Santompona (0.75). All these accessions were in different clusters and the wide distance between them must be related to their geographical origin because they all came from different countries all over the world. The closest accessions were Jiti nada -

Blue blue and Jiti nada - PG12164-21 (0.11), PG12151-73 - PG12010-15 (0.15), Santompona - Blue blue (0.17), and Tu-purple - Blue blue (0.22) mostly in the same cluster and were all advanced or local accessions except Santompona.

For the 10 parents and their progenies, it was revealed that some of the progenies did not group with their parents and this result can be because of the random reassortment of alleles in the various biparental cross and the high somatic transformation in sweetpotato which is a source of genetic variation among genotypes. The most distantly related accession was Faara x Nanungungungu - Nanungungungu x Faara with genetic distance of 0.63 and the closest was between two progenies Nanungungungu x Hi-starch with 0.11 as genetic distance. Most of the progenies which produced high heterosis were from distantly parents related and were in different clusters except a few whose parents were close and in the same cluster. Genetic study is essential in the selection of parental genotypes for development of significant traits in sweetpotato.

6.2 Recommandations

- More work should be directed to heterosis study in sweetpotato using the same SSR markers by increasing the parents and crosses under rainfed condition to be able to do a good evaluation.
- More effort should be made to improve sweetpotato crosses by increasing the crossing blocks for more seed production for further heterosis studies.
- Virus resistant and early maturing cultivars should be cultivated to avoid yield losses in sweetpotato.

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