NUTRITIONAL, PHYTOCHEMICAL, ANTIOXIDANT AND GENETIC CHARACTERIZATION OF JACKFRUITS (*ARTOCARPUS HETEROPHYLLUS*) FOUND IN SELECTED REGIONS OF KENYA AND UGANDA

BY

REDEMTOR AWUOR OJWANG (MSc)

H80/98390/2015

A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD) IN BIOCHEMISTRY, SCHOOL OF MEDICINE, UNIVERSITY OF NAIROBI

AUGUST 2020

DECLARATION

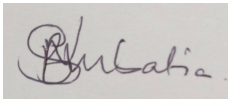
I, the undersigned, declare that this is my original work and that it has not been presented
to any institution of learning for academic credit. All the sources used herein are duly acknowledged.

Redemtor Awuor Ojwang

Signature  Date 25/08/2020

This thesis has been submitted with our approval as the University Supervisors.

Dr Edward. K. Muge  Date 25/08/2020
Department of Biochemistry
School of Medicine
University of Nairobi

Dr Betty. N. Mbatia  Date 25/08/2020
School of Pharmacy and Health Sciences
United States International University

Prof Dorington.O. Ogoyi  Date 25/08/2020
Technical Department
National Biosafety Authority

DEDICATIONS

I dedicate this research work to the almighty God for his faithfulness throughout the research work and for enabling me to obtain funding and overcome numerous challenges that I faced during the study. Additionally, I dedicate it to my loving husband Samson Omondi Oiro, and my sons Jaydon Kue, and Lemuel Gweth for being my support system. Similarly, I dedicate the work to my wonderful mother Margret Akumu Ojwang for her prayers and encouragement as the journey was quite challenging. Likewise, I dedicate it to my brother Oscar Okinyi, my sister Linsey Atieno, my dad Churchill Ojwang and the entire extended family for their support. God bless you.

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to the following individuals for their immense support towards the research work. My supervisors Dr Edward Muge, Dr. Betty Mbatia, and Prof. Dorington Ogoyi for the advice and guidance throughout the research work from drafting of the proposal, to seeking funds, carrying out the laboratory work, drafting the manuscripts for publication and compiling this thesis. Additionally, I would like to thank the International Foundation for Science (IFS) for funding the research work, as well as Prof Ogoyi and Dr Muge for purchasing some of the consumables I used in the laboratory.

Profound thanks and appreciation to Dr. Evans Nyaboga for his guidance and input especially in genetic characterization work. Similarly, am grateful to the director CEBIB, for allowing me to conduct part of my research in their laboratories. Likewise, I truly appreciate Asma Adan for her immense support and Tiberius Oboge for his assistance. Lastly, I thank the laboratory technicians Benson Mwanza, Mr Thomas Arani, Mrs Phyllis Mwai, and Mrs Ann Owiti their assistance.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

CTAB:	Cetyl trimethylammonium bromide
SDS:	Sodium dodecyl sulfate
DPPH:	1, 1-Diphenyl-2-picrylhydrazyl
PCoA:	Principal coordinate analysis
UPGMA:	Unweighted pair group method with arithmetic mean
PVP:	Polyvinylpyrrolidone
EDTA:	Ethylenediaminetetraacetic acid
PIC:	Polymorphism information content
RFLP:	Restriction fragment length polymorphisms
RAPD:	Rapid amplified polymorphic DNA
AFLP:	Amplified fragment length polymorphisms
SSR:	Simple Sequence Repeats
POX:	Peroxidase markers
PCR:	Polymerase Chain Reaction
SRAP:	Sequence-related amplified polymorphism

ABSTRACT

Some sections of the work included in the thesis have been published in peer reviewed journals and they include Chapter three '(*European Journal of Medicinal Plants*)' and chapter four '(*Journal of Advances in Biology and Biotechnology*).' Additionally, Chapter four has been accepted with corrections in *Journal of Crop Improvement*.

According to Food and Agriculture Organization (FAO) of the United Nations, 821 million people were affected by food and nutrition insecurity in 2017 worldwide of which 3.4 million people were in Kenya. This was attributed to overdependence on a few food crops for nutritional needs. Therefore, there is need to find alternative sources of nutrition in Kenya to minimize food and nutrition insecurity. Jackfruit is an underutilized plant and it is a potential alternative nutritional source in Kenya. However, no study has been conducted to determine the nutritional, phytochemical, antioxidant and genetic characterization of Jackfruit in Kenya. This study sought to (i) Determine the nutritional profile, phytochemical content, and antioxidant activities of jackfruit seeds and pulp in selected regions of Kenya and Uganda. (ii) Compare the phytochemical content and antioxidant activities of jackfruit roots, leaves and bark, found in selected regions of Kenya and Uganda and (iii) Compare the effectiveness of various pre-treatment protocols for seed germination and DNA extraction from leaves, and (iv) determine the genetic diversity of jackfruits from selected sites in Kenya and Uganda.

The nutritional analysis was conducted using fresh weight, whereas mineral analysis was conducted using dry weight. The moisture contents in the pulp and seeds were (62.67-70.42%) and (44.76-50.54%) respectively. The ash, lipid, protein and carbohydrate content values were all higher in the seeds than in the pulp. The values were: seeds (1.12 -1.64%) and pulp (0.34 -0.48%) for ash content, seeds (0.41-0.50%) and pulp (0.09-0.12%) for lipid content, seeds (14.11 to 16.26%) and pulp (10.56 to 13.67%) for protein content and seeds (31.41%-34.95%) and (21.65 to 24.91%) pulp for carbohydrate content. Both jackfruit seeds and pulp were rich in essential minerals such as potassium, sodium, calcium, magnesium, zinc and iron.

Phytochemical analysis revealed that the phenolic and flavonoid contents, as well as DPPH scavenging activity, and the reducing power values were higher in the seeds than in the pulps. Moreover, the roots had the highest phenolic, flavonoids and tannin content, while the bark had the least. The phenolic contents were roots (67.37- 59.00 mg/g) and bark (30.88 - 16.30 mg/g). Similarly, the flavonoids compositions were roots (10.74 - 7.31 mg/g) and bark (3.09 – 1.49 mg/g). The tannin contents were roots (3.88 - 2.69 mg/g), and bark (0.93 -0.52 mg/g). Notably, the DPPH scavenging activity was also highest in the roots (66 -72%), while that of the bark was the least (24 - 40%). The reducing power of the roots, leaves and bark were from (114.38 – 93.62 µg/ml), (71.63-67.04 µg/ml) and (54.16-33.15 µg/ml) respectively.

In genetic characterization, the initially sampled young leaves from mature trees, yielded degraded DNA, which is attributable to high concentration of phytochemicals in the leaves. A resampling of the fruits was done and the seeds germinated using an optimal protocol. The fresh seeds that were pre-soaked in 3% hydrogen peroxide took the shortest time to germinate compared to those that were pre-soaked in 3%

hydrochloric acid and distilled water. The seeds pre-soaked in 3% sulphuric acid did not germinate. The extraction of DNA from leaves obtained from the seedlings was also unsuccessful using five different protocols. Subsequently, modification of one of the techniques resulted in high yield and good quality DNA. The optimised CTAB protocol had additional steps of phenol chloroform (1:1 w/v), chloroform isoamyl alcohol (24:1) and chloroform, which ensure complete removal of secondary metabolites. Additionally, it did not employ liquid nitrogen and beta-mercaptoethanol; hence, it is suitable for research labs in low resource settings.

Jackfruit samples from different regions were characterized using Simple sequence repeats (SSR) and Sequence-related amplified polymorphism (SRAP) markers. The average values for the polymorphic SSR markers were gene diversity (0.55), PIC (0.48), Simpson's diversity index (0.48) and observed heterozygosity (0.42). The average of the genetic diversity parameters using polymorphic SRAP markers were gene diversity (0.61), PIC (0.56), Simpson's diversity Index (0.61) and observed heterozygosity (0.47). The PCoA analysis for the combined SSR and SRAP markers revealed that most samples from the same region, were closely related and the correlation coefficient of their genetic diversity parameters was 0.78. Jackfruit was found to have moderate genetic diversity and all parts of the tree studied were beneficial. The pulp and seeds are potential good alternative sources of nutrition, while the roots, barks, leaves, seeds and pulp can be used as natural antioxidants sources.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

The world's population is projected to reach 9.8 billion by the year 2050 and Africa is predicted to account for 50% of this population (UN, 2017). Similarly, the food demand, is expected to rise by 70% to meet the nutritional needs of the growing population. Nutrition and Biodiversity have been proposed as ways of minimizing food and nutrition insecurity (FAO, 2009). The current world's population is over-dependent on a few crops including rice, maize, wheat and potatoes for daily calorie needs. The overreliance on these crops increases the prevalence of food and nutrition insecurity, as they contain only some of the nutrients required by the human body (proteins, carbohydrates and fats). Moreover, they may lack other important nutrients and micronutrients such as vitamins and essential minerals. Additionally, the overreliance on these plants reduces the biodiversity of the crops. There is need to find alternative nutrition sources, as this will improve plant biodiversity, which is part of the Sustenance Development Goals (SDGs) of 2030 (Goal 2) (FAO, 2017).

A biodiversity awareness program initiated by 'International Plant Genetic Resources Institute (IPGRI)' and 'Food and Agriculture Organization (FAO)' has been ongoing, with the objective of minimizing food and nutrition insecurity (IPGRI, 2006; FAO, 2005; FAO and UNEP, 2020). A number of underutilized plants have been listed as potential alternative nutrition sources including Jackfruit. Terms that have been used to describe the plants include: 'promising,' 'underutilized,' and 'neglected' (Hunter and Fanzo, 2011). The plants are viewed as great potential alternative nutrition sources, however their potential has not been fully exploited (Frison et al., 2011). The popularization of these plants would therefore provide the required alternative nutrition sources. Jackfruit (*Artocarpus heterophyllus*) has been listed as one of the

plants that is ‘underutilized.’ The popularization of this fruit in areas where it can be cultivated, would be beneficial especially to the developing countries that are heavily affected by food and nutrition insecurity (Williams and Haq, 2002; Gajanana et al. 2010; Khan et al. 2010; Jagtap and Bapat 2010; Swami et al. 2012).

Jackfruit *(Artocarpus Heterophylus)* is of key significance in the genus *Artocarpus*. The fruit of the tree is the largest in the world, with a mass of up to 80 kg under optimal production conditions (Nakintu et al., 2019). Limited studies have been conducted on the compositional profile of jackfruits from other regions mainly India. The pulp and seeds of the fruits have been reported to have proteins, starch, vitamins, lipids, and essential minerals (Mukprasirt et al., 2004; Ajayi et al., 2008). However, no available studies that have been reported so far, on the nutritional profile and elemental analysis of jackfruit found in Kenya and Uganda. The study is important in determining heterogeneity in nutritional composition of fruits from these regions for the purpose of initiating breeding programs that will contribute in promoting food and nutrition security in the region.

Studies reveal that jackfruit seeds and pulp have moderate content of phytochemical compounds (Abu Bakar et al., 2015; Sirisha et al., 2014; Shanmugapriya et al., 2011; Gupta et al., 2011). The components exhibit antioxidant properties, which are effective in alleviating the symptoms of chronic diseases. Notably, illnesses such as cancer, diabetes and cardiovascular illnesses are among the major causes of death worldwide (WHO, 2017). The conditions are mediated by mechanisms that involve the action of free radicals and peroxides that lead to the damage of DNA, proteins and lipids. The free radicals are produced by the body as a by-products of the cells’ metabolic activities and they cause oxidative stress when there is a major imbalance between them and the defense system of the antioxidants (Beer et al., 2002; Lobo et al., 2010). Antioxidants reduce the formation of free radicals through enzyme inhibition or chelation to trace elements (Shanmugapriya, 2011). The most important and abundant

phytochemicals include phenolic compounds and flavonoids and others include saponins, tannins and alkaloids (Sudha et al., 2011).

Studies reveal that Jackfruit has various phytochemicals and it is a potent antioxidant. Sirisha et al. (2014), Shanmugapriya et al. (2011) and Gupta et al. (2011) suggest that jackfruit seeds contain phenolics, flavonoids, tannins and saponins as well as antioxidant activities. Abu Bakar et al. (2015) revealed that the fruit peel of some *Artocarpus* species have significantly higher phytochemical composition and antioxidant activities compared to pulp and seeds. Goswami et al. (2011) suggested that the phytochemical composition of Jackfruit pulp region varied depending on the pulp and the region where it is planted. Therefore, the phytochemical composition varies in the different parts of the plant.

Previous research on jackfruit phytochemical properties have mostly focused on the pulp and the seeds and limited studies on the roots, leaves and bark have been reported. Research has shown that the roots, leaves and bark of other plants contain a high quantity of phytochemicals and antioxidant properties. The leaves of Himalayan Cobra Lily (*Arisaema jacquemontii*) was found to have significantly higher antioxidant activities compared to its fruit and tubers (Sudan et al., 2014). Moreover, medicinal plants *Hymenocardia lyrata*, *Garcinia lucida* and *Acalypha racemosa* had high antioxidant activities in their roots (Slyvie et al., 2014), whereas *Azadirachta indica* roots, had significantly high levels of phenolic content and antioxidant activities (Hossain et al., 2014). Jackfruit is food for people of the lower social class in Asia where it is dominant (Ocloo et al., 2010; Shyamalamma et al., 2008). Parts of the tree such as the roots, bark, and leaves have multiple medicinal characteristics (Arung et al., 2006). Therefore, the medicinal properties indicate potentially high phytochemical content and antioxidant activities.

Studies demonstrate that the optimization protocols for seed germination and DNA extraction of plants vary from one plant to another. Travlos and Economou (2006),

demonstrated that Sulphuric acid can be effectively used to optimize seed germination in *Medicago arborea*. Abubakar and Maimuna et al. (2013) on the other hand, demonstrated that 50% HCl can optimize seed germination in *Parkia biglobosa* species. Hydrogen peroxide solution on the other hand, was also found to be the highly effective in the optimization of seed germination in Prunus species (Imani et al., 2011). Notably, no study has been reported so far in optimization of jackfruit seed germination. The information would be important for faster development of jackfruit seedlings that can be used for propagation of the plant and also preserve genetic diversity, which is not possible with techniques such as grafting. The information would also be useful in jackfruit genetic characterization studies as the fresh leaves from the seedlings, have less accumulation of secondary metabolites, which interfere with the DNA extraction (Sudan et al., 2017).

Numerous studies show there is diversity in jackfruits, however, they are mostly based on morphological characteristics. Two types of jackfruit have been identified based on their fruit characteristics (soft and hard) (Odoemelam, 2005). The cultivar identification and genetic diversity determination using morphological information is limited as the environmental conditions may affect the phenotypic traits of interest (Shyamalamma et al. 2008). Molecular markers have reduced these disadvantages because they are highly polymorphic and are more accurate (Sensoy et al., 2007; Prashanth et al., 2002). Therefore, they are suitable for characterization of genotypes, which are supplemented by phenotypic traits. A few studies have been conducted on genetic characterization of jackfruit (Schnell et al., 2001; Gopalsamy et al., 2012). However, studies on genetic characterization of jackfruit using SRAP and SSR markers have not been reported yet in Kenya. A comprehensive characterization of Jackfruit is crucial in initiating breeding programs of jackfruit in Kenya to minimize food and nutrition insecurity.

1.2 Problem statement

The number of people affected by malnutrition worldwide are 1.9 billion, and those who are underweight are 462 million. Notably, under-nutrition accounts for the death of 45% of children under five annually, whereby majority are from developing countries (WHO, 2017). Moreover, most individuals in these countries have no access to balanced diet (FAO, 2014; FAO et al., 2012). In Africa, the food insecurity prevalence is 333.2 million, and in Kenya, the number of those affected is 3.4 million (FAO, 2017). Therefore, there is need to diversify the sources of nutrition by including neglected plants such as the jackfruit.

Despite the multiple health benefits of jackfruit, its commercial use is very low (Ocloo et al., 2010). In Kenya, the cultivation of this plant is conducted in small scale in very few regions (Ojwang et al., 2015). Moreover, there are no studies that have been reported so far on the nutritional profile of jackfruit found in Kenya and Uganda. Notably, studies from other regions (India, Malaysia, and Ghana) indicate that the plant contains carbohydrates, proteins, and minerals (Shafiq et al., 2017; Ocloo et al., 2010; Abedin et al., 2012; Gupta et al., 2011, Ocloo et al., 2010; Gupta et al., 2011). Furthermore, limited studies have reported phytochemical and antioxidant activities of the roots, backs, leaves, pulp and seeds. Notably, jackfruit seeds and pulps from other regions have moderate phytochemical contents and high antioxidant activities (Abu Bakar et al., 2015; Sirisha et al., 2014; Shanmugapriya et al., 2011; Gupta et al., 2011). Hence, there is need to determine the phytochemical composition of these tissues in Kenya and Uganda.

Studies suggest that the efficiency of seed pre-treatments, varies from one seed to another depending on its physical properties (Abubakar and Maimuna et al., 2013; Travlos and Economou, 2006). A number of protocols have also been developed for optimization of seed germination, however no protocol has been reported so far for jackfruit. There is therefore need to optimize the germination of jackfruit seeds to facilitate the propagation of the plant in areas where it can be cultivated. Moreover,

genetic diversity studies are crucial for propagation of superior varieties of Jackfruits. Numerous protocols have been developed for DNA extraction (Murray and Thompson, 1980, Allen et al., 2006), but most of the protocols do not yield good quality DNA for plants high in phytochemicals (Sudan et al., 2017; Sika et al., 2015). Hence, there is need to optimize a DNA protocol for Jackfruit since the secondary metabolites interfere with the extraction process.

The Simple Sequence Repeat (SSR) markers are some of the most popular genetic markers used in DNA fingerprinting. The markers have been used to successfully characterize mango cultivars (Kumar et al., 2013), watermelon cultivars (Hwang et al., 2011), Potato cultivars (Rocha et al., 2010), and Mulberry (Mathithumilan et al., 2013). Additionally, the Sequence Related Amplified Polymorphism (SRAP) primers are efficient and they have been successfully used in characterization of *Brassica oleracea* plants (Li and Quiros, 2001), cucumber (Meng et al., 2012) and Cucurbits (Ferriol et al., 2003; Qian et al., 2006). However, no studies have been reported so far on genetic diversity of the fruit in Kenya using SSR and SRAP markers.

1.3 Justification

The nutritional value of the fruit and its economic value are important in ensuring food and nutrition security (Gupta, 2011; Shyamalamma et al. 2008). Moreover, the knowledge of the nutritional profile of jackfruit seeds and pulp found in Kenya and Uganda will promote its utilization. Furthermore, the consumption of phytochemicals and antioxidants can potentially reduce the risk of illnesses such as heart diseases, diabetes mellitus, cancer and neurodegenerative diseases (Lobo et al., 2010). Studies on other plants have revealed higher levels of phytochemicals in roots, bark and leaves compared to the edible region in other plants (Sudan et al., 2014; Slyvie et al., 2014; Hossain et al., 2014). Thus, the determination of phytochemical composition of jackfruit roots, bark and leaves may potentially provide alternative sources of natural antioxidants. Goswami et al. (2011) suggested that the phytochemical levels vary depending on the geographical location. Hence, there need to investigate the

phytochemical compositions of the jackfruit tissues found in Kenya and Uganda. This will facilitate the sustainable utilization of the tree as a source of nutrition and natural antioxidants in Kenya and Uganda.

Common techniques of breaking seed dormancy include soaking in water or other chemicals for a specific duration of time to break the seed coating and stimulate germination. Some of the chemicals used in breaking seed dormancy include Sodium hypochlorite (NaOCl) bleaching agent, hydrogen peroxide (H_2O_2), dilute sulphuric acid (H_2SO_4) and hydrochloric acid (HCl) (Abubakar and Maimuna et al., 2013; Travlos and Economou, 2006). Notably, the techniques used in stimulating seed germination vary from one plant to another. The knowledge of effective seed pre-treatment protocol for jackfruit will facilitate the cultivation and propagation of more jackfruit seedlings in areas where it can be cultivated.

A few protocols have been proposed for plants high in phytochemicals. The methods may however not be effective in some plants (Sudan et al., 2017). Moreover, the approaches require the use of highly volatile liquid nitrogen and beta-mercapto-ethanol, which may not be readily available in labs in low resource settings. In this study, the SSR primers markers were preferred because (i) they are multiallelic codominant markers, (ii) transferable, (iii) highly reproducible and (iv) have a wide genome coverage (Kumar et al., 2013), whereas the Sequence Related Amplified Polymorphism (SRAP) primers were chosen because they are (i) simple, (ii) target the coding regions, (iii) reliable and (iv) reproducible.

1.4 Broad objective

To study the nutritional profile, phytochemical content, antioxidant activities and genetic diversity of jackfruits (*Artocarpus Heterophyllus*) from selected regions in Kenya and Uganda, with the aim of providing a potential alternative source of nutrition

and natural antioxidants that will minimize overreliance on a few crops, and understand its genetic diversity for future identification of superior varieties for breeding programs

1.41 Specific Objectives

1. To determine the nutritional profile, phytochemical content, and antioxidant activities of jackfruit seeds and pulps found in selected regions of Kenya and Uganda.
2. To compare the phytochemical content and antioxidant activities among the jackfruit roots, leaves and bark found in selected regions of Kenya and Uganda.
3. To determine effective protocols for jackfruit seed germination and DNA extraction from leaves.
4. To determine the genetic diversity of jackfruits found in selected regions of Kenya and Uganda

1.5 Hypotheses

1. The Kenyan and Ugandan jackfruit pulp and seeds are rich in nutrients, essential minerals and have high phytochemical composition and antioxidant properties
2. The Kenyan and Ugandan jackfruit roots, bark and roots have high phytochemical composition and antioxidant properties
3. The Kenyan and Ugandan jackfruits have significant genetic diversity

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Jackfruit (*Artocarpus heterophyllus*) description, origin and distribution

Jackfruit tree is medium in size and grows to a height of up to 25 metres and it has a trunk diameter of up to 80 cm. The shape of a young tree is either pyramidal or conical, while the older trees are either domed or spread in shape. The trees normally produce a whitish substance called latex when cut (Elevitch and Manner, 2006). The fruit of the plant is the largest globally as it may weigh up to 80kgs (Nakintu et al., 2019). The plant belongs to the *Artocarpus* genus, which emanates from the greek words 'artos' and 'karpos' that mean 'bread' and 'fruit' respectively (Sundarraj and Ranganathan, 2018). Other plants from the same genus include 'Mulberries (*Morus* spp.), Breadfruit (*Artocarpus altilis*), Breadnut (*A. altilis* 'Seminifera'), Champedak (*A. integer*), Lakoocha (*A. lakoocha*), Marang (*A. odoratissimus*) and African Breadfruit (*Treculia african*)' (Morton, 1987).

Jackfruit is a tropical plant and the probable origin is the Indian Western Ghats rainforests. The trees are mainly found in South Eastern Asia, South America and some parts of Africa (SCUC, 2006). In East African region, the fruits were introduced by Arab traders and Asians. Notably, they are highly distributed in Uganda compared to Kenya. In Uganda, they were introduced in the 1890s; however, the taxonomic information of the plant with regards to the subspecies present is limited and it is mainly classified morphologically based on the pulp region (soft and hard). The classification of the crop varieties in on cultural basis and the naming system is in vernacular (Nakintu et al., 2019). The Jackfruits in Kenya have not been classified; however, in Uganda, Nakintu et al. (2019) classified the fruits into four ethno-varieties namely firm white (Namata), firm red (Namusaayi), firm yellow (Kanaanansi), and soft (Serebera).

2.2 Botany of Jackfruit

Flowering twigs grow from the main branches and trunks and the flowers are monoecious, they have the male and female spikes on the same tree. The flowers are small and light green in colour when young and turn darker as they age. The leaves are green, glossy and large up to 16 cm in length (Elevitch and Manner, 2006). The fruit is heavy and a central fibrous core holds it together. The fruit has an oblong cylindrical shape and the length is up to 40 cm or more. The fruit weighs around 2–80 kgs and the seeds are light brown in color around 2 -3 cm (Abu Bakar et al., 2015; Nakintu et al., 2019). There are around 100 to 500 seeds per fruit and the roots are taproot (Morton, 1987). The seeds may be eaten raw, fried or roasted and it can also be ground to make flour that can be used for baking (Morton, 1987).

2.3 Health benefits and other uses of Jackfruit

The fruit is of high nutritional value both in the edible pulp region and the seeds. It is rich in minerals and vitamins such as vitamin A that aids in good vision. Vitamin B complex that facilitates the conversion of food to energy. Iron which forms part of the hemoglobin and takes part in the transportation of oxygen to the metabolic cells (SCUC, 2006). The ripe-fleshy pulp contains high quantities of proteins and carbohydrate (Odoemelam, 2005). Fresh seeds are high in starch and good sources of vitamin B1 and B2 (Morton, 1987). The oil from the seeds also has high composition of unsaturated lipids like Omega-6 and Omega-3, which are important in reducing the risk of cardiovascular illnesses (Ojwang et al., 2015).

The unripe pulp region is used to make curry or it is also be dried and stored in tins. The ripe one can make jam and ice cream, the fruit can also be fried and eaten like potato chips. The dried seeds can be ground into powder and used for baking (El-Zaher 2008). Tender leaves can be cooked or used as fodder for cattle and the timber obtained

from the tree is of high quality and can be exported. It is also suitable for making furniture, as it is highly similar to mahogany. The bulbs and seeds are considered nutritious, cooling, tonic and can be used to overcome the influence of alcohol. The roots on the other hand, can be used to treat skin diseases and the bark contains tannins that can be used to make clothes and cordage (ropes) (Ashrafuzzaman, 2012).

Jackfruit is burnt alongside coconut shells and corns and its ash is used to heal ulcers. The dried latex yields a compound with marked androgen action and the latex can also be mixed with vinegar to promote healing of snakebites, abscesses and granular swellings. The roots also treats diarrhea apart from skin diseases (Gopalsamy et al., 2012). Jackfruit is rich in potassium which can aid in regulation of blood pressure it also contains' lignans, isoflavones, saponins, which are phytochemicals. Their health benefits are numerous and they include: anti-ulcer, antioxidant, anti-aging and antihypertensive (Mui et al., 2001).

2.3.1 Jackfruit nutritional and mineral content

A study comparing the seeds of *Treculia Africana* and *Artocarpus heterophyllus* was conducted to find out their phytochemical and mineral composition. The aim was to examine if they can be used for human or animal consumption. The two seeds were found to have more protein composition compared to those in animal proteins such as fish and beef. They were also found to be rich in carbohydrate and lipid content**.** The lipid content was found to be 11.4 % and 18.5 % for the seeds of the two species respectively (Ajayi, 2008). Abedin et al. (2012) carried out an investigation on the nutritive content of jackfruit seeds using proximate and mineral composition. The examination of the seeds properties were conducted using three types of fruits called Khaja, Gala and Durosha. The moisture content was found to be high (57.8-78.9%). The ash content on the other hand, was (2.1% -4.1%), whereas the protein content was (13-18%), respectively. The seeds were also found to be rich in mineral, starch and fibre.

The lipid content in the jackfruit seeds has been reported to be 0.45 ± 0.24 % of dry weight with Omega-3 and Omega-6 PUFAs at 9.94 ± 0.99% and 31.19 ± 0.82 %, respectively.

The amount of polyunsaturated lipids were found to reduce in dried and boiled samples. The oil would also not be appropriate for frying food but can be used for cake icing and creams because of its sweet scent (Ojwang 2012). Omega-3 PUFA is vital in the body as it enhances the vascular endothelial function and plays a role in the reduction of blood pressure. It also reduces the sensitivity of the platelets, triglycerides in serum and susceptibility to cancer (Berquin et al., 2007).

2.3.2 Comparison of Jackfruit nutritional profile and main calorie sources in Kenya

The nutritional profile of Jackfruit from other regions is comparable to those of the domestic calorie sources. Firstly, the protein composition is higher compared to maize (7.6%), which are the main source of calorie in Kenya. Notably, the composition is comparable to wheat in Kenya, which is 16.1% (De Groote et al., 2010; Abong et al., 2009). Moreover, the carbohydrate composition of Jackfruit seeds is comparable to maize and potatoes, which range from 16-29%. Additionally, the moisture content is comparable to that of potatoes, which ranges from 57-77%., whereas the mineral composition is comparable to that of maize (De Groote et al., 2010; Abong et al., 2009). Therefore, the plant is promising as an alternative nutrition source that can supplement the available crops to reduce overreliance on a few crops.

2. 4. Phytochemical composition of jackfruit seeds and pulp region

A study by Burci et al. (2015), examined the phenolic composition and antioxidant activities of jackfruit seeds. Phenolic composition was examined utilizing folin reagent and antioxidant activities were determined using ferric thiocyanate method (FTC) and

total reductive capability technique. The DPPH and hydrogen peroxide scavenging activity was also determined. The study also examined the phenolic content and antioxidant properties through various extraction solvents. Ethanolic extract was found to have the highest activity, followed by the hexanic and chloroform extracts. Ethyl acetate extract, had the least antioxidant activity. The total phenolic content ranged from 3.034 to 5.72 µg GAE/g for all extracts.

Arina and Azrina (2016) compared the phenolic content and antioxidant properties of fresh and fried cempedak, breadfruit and jackfruits. The samples were extracted using methanol and folin's reagent was used to determine the amount of phenolic content present. The beta-carotene and FRAP assay were employed in evaluating the antioxidant properties. The fried jackfruit was found to have the highest phenolic composition (76.836+ 0.619 mg GAE/100g) followed by the fried cempedak and bread fruit had the least phenolic content (54.042 + 0.596 mg GAE/100g). The fresh sample however were found to have high antioxidant activity compared to the dried samples. Fresh cempedak had the highest antioxidant activity followed by fresh breadfruit and jackfruit had the least composition. Using beta-carotene assay, fried cempedak had the highest composition, followed by fresh jackfruit and no correlation was found between TPC and antioxidant activity determined using FRAP technique and beta-carotene assay.

Shanmugapriya et al. (2011), examined the phytochemical and antioxidant properties of jackfruit seeds. The phenolic content and flavonoid content was examined using spectrophotometric methods. The reducing power and DPPH scavenging activities were also determined using spectrophotometric assays. The results of the study showed that jackfruit phenolic content ranged from 4.16-1.18 mg/g and the flavonoid content ranged from 4.05-0.86 mg/g. The reducing power ranged from 13.12 to 9.56mg/g, while the DPPH scavenging activity had an IC_{50} of 50.09%. A study by Sirisha et al. (2014) examined the phytochemical composition of the different *Artocarpus* species,

which included the *A. heterophyllus, A. integrifolia, A. hircitus, A. incisus,* and *A. integer*. The study found that the different *artocarpus* species had moderately high levels of phenolics, flavonoids and tannin levels. The *A. incises and A. heterophyllus* were found to have the highest phenolic content, while *A. heterophyllus* had the highest flavonoids content. In all samples the tannin content was found to be generally lower compared to the phenolic content.

2.5 Seed germination strategies

Tropical species that are found in regions that have high wet and dry seasons cycle mostly have dormant seeds. The extent of seed dormancy usually varies from one tree to another. Most of the dormancy exhibited by these seeds results from physical features, which include a thick seed coat that prevents water and oxygen from moving into the cell. Most seeds of the tropical species usually germinate after several years depending on the habitat of the species. Other than physical dormancy, some species also experience chemical dormancy. This is where, the seeds usually contain some germination inhibitors that makes them to take prolonged periods of time before germination (Abubakar and Maimuna et al., 2013).

A study by Travlos and Economou (2006), optimized the seed germination of the *Medicago arborea.* The hot water treatments, immersion in Sulphuric acid for 2 minutes and immersion in water for 20 hours was found to greatly increase the rate of seed germination. Immersion of the seeds in high temperatures and prolonged soaking of the seeds in Sulphuric acid was found to be harmful to the seeds and the germination process. A different study by Abubakar and Maimuna et al. (2013), showed that wet heat, mechanical stratification and 50% HCl induced germination of *Parkia Biglobosa* seeds.

2.6 DNA extraction in plants high in secondary metabolites studies

Jackfruit is one of the plants that has been found to have high medicinal value. The leaves of the plant have high content of secondary metabolites such as phenolic compounds, flavonoids and tannins amongst others (Ojwang et al., 2017). The secondary metabolites, potentially interfere with DNA extraction from the plant by either being co-extracted with the DNA or binding to the DNA, which affects the quality of the DNA extracted. The plant has also been found to have high concentration of essential oils and polysaccharides (Ajayi et al., 2008, Ojwang, 2015), which also interfere with the DNA extraction process.

Doosty et al. (2012), investigated four reported DNA extraction methods in order to determine the one that worked for *Satureja khuzistanica*, a plant that has high medicinal value. The study compared the protocols that had been previously reported for DNA extraction in plants. The study found that modified protocol of Kang and Young et al. (2004), gave high quality DNA, while the other protocols yielded poor quality DNA. Some of the modifications that had been done on the protocol to optimize DNA extraction include: increasing the concentration of NaCl, changing the incubation time, changing temperatures during centrifugation, using proteinase K and harvesting of the young leaves before flowering.

Aruda et al. (2017) compared eight different protocols that had been previously reported for DNA extraction in plants. The study utilized the leaves of *Mimosa tenuiflora*, which are high in phytochemicals. The plant had previously been found to have high phenolics, flavonoids and tannins contents. The CTAB based protocol was optimized through inclusion of a phenol step, increase in concentration of CTAB and NaCl, reduced incubation periods and use of lower temperatures for incubation. The protocol was found to yield high quality DNA and Polymerase Chain Reaction (PCR) analysis using the technique was successful.

2.7 Genetic diversity studies of Plants

2.7.1 Significance of genetic diversity studies

Genetic diversity can be defined as the variation of genetic makeup within the same species of organisms or different closely related species. Genetic variability mostly takes place in organisms for adaptation to various environmental conditions. Genetic diversity studies of organisms is useful in determining the evolutionary relationships. High genetic diversity of organisms such as plants has been associated with benefits such as high ability to tolerate extreme environmental conditions, fight diseases and predators. On the contrary, low genetic diversity is viewed as a threat to the plants existence, since they have low tolerance to harsh environments and diseases and can easily become extinct. There are a number of factors that have been found to affect the genetic diversity of plants and they include: mutation, population size, inbreeding and spatial distribution (Amos and Harwood, 1998).

The knowledge of genetic diversity in plants, allows the plant breeders to establish novel or enhance the existing cultivars with superior traits. These may be the farmer's or breeder's desired characteristics. The genetic diversity in plants is currently being exploited to improve the agricultural yield that will help in meeting the food demand for the ever-growing population (Narain, 2000). Developing countries in the mid-1960s attempted to meet the growing food demand through large plantations of high yielding crops and those that had high response to fertilizers popularly termed as 'Green revolution'. This led to large tracks of land getting covered by plants that have similar genetic makeup, which led to extinction of primitive and adaptive genes from the plant populations that mostly consisted of wheat and rice (Evenson and Gollin, 2003). The promotion of genetic diversity in plants therefore ensures that there is food security for generations to come.

2.7.2 Factors affecting genetic diversity

The population size affects the genetic diversity of plants in that, highly populated plants are likely to have a high genetic variation compared to plants that have a small population, if all other factors are held constant (Govindaraj et al., 2015). Inbreeding can be defined as reproduction in organisms that have a close genetic relationship. Inbreeding has been found to result in low genetic diversity and fitness, which is termed as 'inbreeding depression.' The offspring of inbreeding, have been found to be less tolerant to extreme environmental conditions and biotic stress. The high susceptibility has been attributed to the fact that the organisms have less genetic variability, which means that they may not have genotypes that are more tolerant to environmental and to biotic stress (Govindaraj et al., 2015).

Mutation is the other factor that is likely to affect genetic variability. Mutation can be defined as changes in the DNA sequence that results from insertion or deletion of one or more nucleotides from the DNA sequence or gene. Mutation is triggered by a number of factors, which include: electromagnetic radiations, reactive oxygen species and some organic and inorganic compounds. This leads to genetic variation of organisms that can either lead to development of superior traits or less desirable traits (Narain, 2000).

Spatial distribution of plants, is where the plant species are fragmented in different habitats. The distance between the separations may be very high such as the existence of the same species of plants in different continents. The separation of plants in spatial distribution, may also be relatively moderate and this may allow gene flow to occur from one plant to another through cross pollination. This may lead to plants in different regions behaving in a similar manner (Govindaraj et al., 2015). In instances where the separation of the plants is wide, no gene flow occurs in the different populations and hence may promote the occurrence of rare alleles leading to genetic diversity. Limited studies have been carried out on the effect of spatial distribution of plants on genetic diversity. However, it is hypothesized that spatial distribution may promote genetic

diversity through preservation of rare alleles that are mostly lost in large populations through gene flow (Amos and Harwood, 1998).

2.7.3 Techniques used for genetic diversity of plants

There are a number of techniques that can be used to examine the genetic variation in plants. The techniques include: Morphological, biochemical and DNA based molecular markers. The morphological techniques are mostly based on difference in phenotypic traits. The visual traits include: size, colour, growth habits and pigmentation amongst others. The morphological characterization does not require any sophisticated instrumentation. However, the phenotypic traits are sometimes influenced by environmental factors such as water availability, quality of soil used, presence of light and availability of fertilizers amongst others (Govindaraj et al., 2015). This may lead to inaccurate conclusion of the genetic diversity of some plants.

The biochemical techniques on the other hand, are mostly based on allozymes and they were mostly used in pre-genetic error. The biochemical markers are enzymes called isozymes that show allelic variation in plants. The variation in the enzymes can be detected through electrophoresis. The isozyme markers are co-dorminant and they detect variation at functional gene levels. The use of the markers is however challenging because its resolution in determination of diversity is low. The genetic markers are widely utilized in assessing the genetic diversity of plants. A large number of DNA markers that are used in assessing genetic diversity of plants (Govindaraj et al., 2015).

Molecular markers have the ability to detect DNA variations that arise from mutation in the chromosomes. The markers have no effect on the phenotypic trait of the plants in question. These markers are usually located near the genes that code for the trait of interest. They are usually inherited in both dominant and co-dominant manner. They are usually genetic loci and can be used to distinguish traits in the genome and provide numerous advantages over the other characterization techniques. They have high

stability and can be detected in any tissue and in all stages of development. Their accuracy may not be affected by environmental factors and they are also relatively fast and more precise compared to the other techniques (Winter and Kahl, 1995).

2.7.4 Types of molecular markers used in genetic studies

Molecular markers can be grouped into three categories namely: DNA sequence based, PCR based and hybridization based. The Restriction fragment length polymorphisms (RFLPs) are markers that are based on hybridization and were initially developed in the 1980s. The technique is based on the variation of lengths produced by the DNA fragments that are usually produced by restriction enzymes, which digests DNA at particular sites. The RFLP technique has been used in genetic characterization of wheat, rice, barley and maize (Garcia et al., 2004; Devos et al., 1993). The advantages of this technique is that it has an unlimited number of loci and is reliable. The technique is however not widely used because it is labour intensive, time consuming, requires large amount of DNA and expensive. The PCR based techniques are therefore preferred because they are faster and need less genetic material for analysis (Govindaraj et al., 2015).

Rapid amplified polymorphic DNAs (RAPDs), were the initial PCR based markers to be established. The technique was found to be relatively simpler compared to RFLP and less costly. The technique was also found to have the challenge of low reproducibility (Jobin-Décor, 1997). Amplified fragment length polymorphisms (AFLPs), were then developed to overcome the challenge. The markers were a combination of PCR and RFLP and were found to be highly reproducible and highly effective in identification of polymorphisms (Govindaraj et al., 2015). Other PCR markers have since been developed and successfully used in genetic characterization of plants. The markers are namely: Simple sequence repeats (SSR), Peroxidase markers (POX), Start codon targeted (SCoT) and SRAP markers amongst others (Hwang et al., 2011; Collard and Mackil, 2009; Gulsen et al., 2010).

2.7.5 Studies on genetic characterization of plants using molecular markers

The phenotypic and genetic analysis of different mango types in Mozambique using AFLP genetic markers revealed that the genetic diversity of mangoes was different in different geographical locations (Mussane et al., 2011). The study characterized thirty varieties of mangoes from different regions. The genetic diversity was then determined and the similarity was found to range from 60-89.4%. The mangoes percent polymorphism was found to be 74.9%. A combination of both phenotypic and genetic traits in a dendrogram was found to be precise in grouping the mangoes on the basis of their origin (Mussane et al. 2011).

Genetic variation of 32 papaya genotypes were examined using the AFLP markers. The findings of the study showed five clusters in the dendrogram generated. The genetic relationship between the 'Sunrise' and the inbreed line was found to be closer compared to the rest. The improved germplasm and landrace varieties on the other hand, were found to have a distant relationship. The major papaya varieties that are produced for commercial purposes in Brazil and some of the both inbred lines and the improved germplasm were also clustered in the same group but not in the same clade. The study demonstrates that AFLP markers were successfully used to assess genetic diversity in Papaya (Oliviera et al., 2011).

Hwang et al. (2011), examined the genetic diversity of 27 watermelons (*Citrullus lanatus* var. lanatus) obtained from various countries. The plants had different physical features and four subtypes were assessed using AFLP and SSR markers. The percent polymorphism was found to be 93.3% using the AFLP markers and the average polymorphic alleles in SSR was 6.4 in each primer. The high polymorphism in the samples was attributed to the wild type species as lower level of diversity was observed among the adapted cultivars. A total of 965 polymorphic bands were obtained in the study from AFLP and SSSR markers and used to construct a phylogenetic tree. Two water melon samples were grouped into two main clusters. The first cluster was mainly composed of the adapted cultivars, while the second one mostly contained the wild

type species. The genetic dissimilarity among the two clusters was found to be approximately 0.63. The low diversity in adapted species implies that the genetic diversity of the water melons were not taken into consideration during the breeding program (Hwang et al., 2011).

Collard and Mackil (2009) proposed a new method for generation of plant DNA markers called Start codon targeted (SCoT) markers. The markers were then used to genetically characterize different rice genotypes. The markers were found to be highly reproducible and dominant. The characterization of *Tinospora cordofila*, a medicinal plant, has also been done using SCoT markers. Twenty one accessions were collected from the wild habitat Delhi and Uttar Pradesh states of India. Thirty five SCoT primers were screened to determine the diversity and out of this number, 19 amplified reproducible bands. The primers were then chosen for molecular characterization of 21 accessions of the plant. A total of 102 bands were generated with 90 being polymorphic. The resolving power of the SCoT markers was from 5.14 to 0.95 and the primer average was 2.6. The polymorphic information content was found to range from 0.49 to 0.19 and the average per primer was 0.35. The estimation of Jaccard's coefficient of genetic similarity revealed that the values were ranging from 1 to 0.68, which implies that the plants were genetically diverse. The UPGMA cluster analysis showed two distinct sub-clusters in the dendrogram generated. In the first cluster, 8 accessions were grouped together, while second cluster had thirteen accessions.

Guo et al. (2014) used Start codon targeted polymorphism (SCoT) to characterize 37 whip grass (*Hemarthria compressa* L.) and to determine their population structure and genetic diversity. A number of parameters were analyzed using the markers and twenty five primer sets produced 368 clear bands and out of which 282 (77.21%) were found to be polymorphic. The allele number per primer was found to range from 5 to 21 and the polymorphism information content (PIC) was found to be 0.358. The accessions had a similarity coeffient of 0.563-0.872 and no association was found between the genetic relationships and place of origin.

A study by Gulsen et al. (2010) successfully used peroxidase enzyme (POX) based markers to define the relationship between buffalo grass and other grasses obtained from various geographical regions. The POX enzymes in plants are grouped into two categories depending on their catalytic activities and structure. The first category is called 'intracellular POX (Class I)' and are associated with bacterial POX. The second one is the class III POX which acts as enzymes in plants. The peroxidase gene-based markers were also successfully used to estimate the genetic diversity of Turkish apples (Gulsen et al., 2010).

A study by Nemli et al. (2013) successfully used peroxidase markers to carry out the genetic diversity of common beans. The beans were obtained from different regions and the polymorphic information content was found to be 0.4, where the maximum variation was found to be 93% and the minimum variation on the other hand, was found to be zero (Nemli et al., 2013). The amino acids of peroxidase enzymes of different organisms have been found to be nearly 90% similar in sequence. The size of the POX genes is about 50 kDa and are present in many plant tissues. The plant POX are involved in a series of reactions that are important in numerous physiological processed. POX is used as model enzymes and are used in studying the self-defense molecular mechanism in plants. The POX primers have been successfully used in genetic characterization of many plants which include cotton, sugarcane, arabidopsis and rice (Gulsen et al., 2010).

2.7.6 Genetic characterization of plants using SSR markers

Research by Mathithumilan et al. (2013) developed mulberry SSR markers and tested ability to be transferred to other species in the *Moracea* family including jackfruit. The study identified 358 sequences, which were used in developing suitable microsatellite pairs that represented 136 EST and 222 genomic regions. The use of the developed primers both in the amplification of mulberry species and closely related species indicated that there was 100% in mulberry. However, 79% was found to amplify genes

in other species of the same family namely: Jackfruit, Fig and Ficus. The markers recorded high degree of polymorphism with a polymorphic information content (PIC) average of 0.559 and the values ranged between 0.076 and 0.943, which makes them suitable in analysis of genetic diversity and can also be used in detection of heterozygosity.

A study by Rocha et al. (2010) utilized SSR and RAPD markers to estimate the genetic diversity of sixteen potato cultivars. Twenty five RAPD primers were used to analyze 16 potato cultivars which produced 92 bands that were polymorphic. Twenty SSR primers used yielded 136 bands that were polymorphic and a dendrogram was generated and used to distinguish the cultivars genetically. The identity of the potatoes was done based on three SSR primers and six RAPD primers. The studies clearly demonstrate that SSR markers are effective in determination of genetic diversity.

2.7.7 Genetic characterization of plants using Sequence Related Amplified Polymorphism (SRAP) markers

The sequence-related amplified polymorphism (SRAP) are DNA markers that amplify the open reading frames (ORFs). The amplification is usually carried out using both the forward and the reverse primer combinations. The primers are 17 to 18 nucleotide long, where the main sequences have a length of 13 to 14 base pair. The first 10 to 11 nucleotides are attached at the 5' terminal. The sequence CCGG is then followed and AATT is usually in the reverse primer. The selective sequences are then attached at the end of the core sequences at the 3' end. The other nucleotides in the forward and reverse sequences are usually different from each other (Li and Quiros, 2001)

Li and Quiros et al. (2001) successfully used the SRAP primers to characterize the *Brassica oleracea* plants. The study found that the 45% of the bands matched those of the genes present in the gene bank after sequencing. Twenty percent (20%) of the primers were also found to be co-dorminant. The primers were also used to

successfully tag the glucosinolate desaturation gene *BoGLS-ALK* and the study also used the markers to successfully amplify DNA in rapeseed, cabbage, lettuce, rice and potatoes (Li and Quiros et al., 2001).

The SRAP markers were successfully used in identification of markers that were linked to the round shape of cucumber. The study used 130 plants of F2 generation that displayed segregation and two marker combinations were found to display a higher correlation to the round fruit shape phenotype. Marker ME21/EM18M600 showed 49.5% of phenotype variation, while ME21/EM18M600 was responsible for 33.1% of the variation. The markers were found to be simple and efficient and the study suggested that they can be used for genetic characterization of many plants (Meng et al., 2012).

A study by Mirajkar et al. (2017) used SRAP and Target region amplified polymorphism (TRAP) markers to assess the genetic variability of sugar cane (*Saccharum officinarum L.*). The study used 57 markers that comprised of 30 SRAP and 27 TRAP markers to assess genetic variability in mutant sugarcanes. A total of 260 amplicons were produced in the PCR, where 147 of them were polymorphic. The PIC value was up to 0.39 and low diversity among the parent sugarcane. The mutant sugarcane had a PIC value that was up to 0.82. The SRAP markers have also been used successfully for characterization of cucurbits (Ferriol *et al*., 2003; Qian *et al*., 2006).

2. 7.8 Morphological and genetic characterization studies on jackfruits

The fruit occurs naturally in two textual forms, in one, the periath is soft and pulpy when it is ripe, while the periath remains firm in the other form (Rahman et al., 1988). Variations of jackfruits have can be characterized using numerous features such as the structure and size of the tree, shape of the leaf and fruit, quality of fruit and bearing age, fruit colour, taste, texture and odour of the pulp region (Singh *et al*., 1996; Tulyathana et al., 2002).

The AFLP markers have been used successfully in a number of jackfruit studies. Twenty-six jackfruit accessions were analyzed using AFLPs to estimate genetic diversity in Fairchiki Tropical Garden (FTG) germplasm collection in USA. The garden consisted of germplasm from different continents and countries. Thirty primer pairs were evaluated and 12 were selected for the analysis based on the amplification ability. One hundred and eighty-seven bands were produced by the 12 primers. Forty-nine percent were found to be polymorphic. The AFLP markers showed that there was moderate genetic diversity in this collection (Schnell et al., 2001).

The characterization of 50 jackfruit varieties were studied in India using AFLP markers. Sixteen markers were examined and eight were chosen for the analysis based on their ability to amplify the DNA samples. A total of 5976 bands were produced and 22% were found to be polymorphic. The similarity coefficient ranged from 0.978 - 0.137 and the fruits were categorized into three main clusters. The first group mainly consisted of fruits grown in dry areas. The second one contained fruits that are grown in heavy to medium rainfall and the last group consisted of fruits from distant locations. A relationship was found between the clusters and the geographical locations and a moderate genetic diversity was also observed in the plants (Shyamalamma et al., 2008).

A study done by Gopalsamy et al (2012), where OPC7 (GTCCGACGACGA) RAPD markers were used to determine the genetic diversity and examine the relationship between five jackfruit accessions present in different regions in the Southern part of India. There was moderate genetic diversity among the five jack fruit varieties. Three major clusters were identified with one cluster having 2 varieties, while the rest had one. The individuals in a cluster shared common phylogenetic characteristics (Gopalsamy et al., 2012).

2.7.8.1 Genetic diversity studies on jackfruit using SSR markers

The study on genetic diversity in Kenya has not been reported yet using SSR and SRAP markers. However, SSR markers have been used for characterization of Ugandan and Indian Jackfruits. Kavya et al. (2019) examined genetic diversity in Jackfruit using 22 SSR markers for purple colour, whereby six exhibited polymorphism and the similarity coefficient ranged from 0-0.96. The UPGMA analysis clustered the fruits into three major clusters, where fruits with the cream and yellow pulp were in clusters I and II, whereas the orange and red pulps were grouped in cluster III. Therefore, the different colours can be applied in breeding programs and diversity analysis.

Nakintu et al. (2019) examined genetic diversity of Jackfruit trees from 12 districts. Ten SSR markers were used to determine the genetic relationship of the fruits. A STRUCTURE and PCoA analysis revealed two major clusters. The trees in cluster 1 were from central and eastern districts of Uganda, while cluster 2 were from western and central districts. Mbarara district had the highest diversity (Heterozygosity (He) = 0.79, (gene diversity) I = 1.71), while Pallissa and Kamuli were the lowest with (He = 0.59, I = 1.12) and (He = 0.61, I = 1.08), respectively. Notably, genetic diversity was higher within the populations and moderate variation was observed among the geographical zones. Therefore, the preservation of the genetic differences in Jackfruit is critical for future improvement of the crop.

CHAPTER THREE

3.0 Compositional, elemental, phytochemical and antioxidant characterization of Jackfruit (*Artocapus heterophyllus*) pulps and seeds from selected regions in Kenya and Uganda

3.1. Introduction

Food and nutrition insecurity is considered one of the world's greatest challenges that results from the ever-growing food demand, due to increase in population (Fanzo, 2014). One problem that is directly linked to under-nutrition is starvation (FAO, 2017). It is estimated that a total of 800 million people in the world today suffer from starvation. This means that one out of every nine people in the world today is hungry (FAO, 2017). The World Health Organization puts at 1.9 billion the number of individuals worldwide, who suffer from malnutrition. People from developing countries are the most affected by under nutrition, which accounts for 45% of deaths for children under the age of five years (WHO, 2017). This has been attributed to the fact that majority of the people from these countries, have no access to balanced diet, while some have no food at all and end up dying of starvation (FAO, 2017). In Kenya, USAID (2018) puts at 3.5 million, the number of people who are nutritionally insecure.

Improved nutrition and food security are part of the Sustenance Development Goals (SDGs) of 2030. Only three plant species, namely maize, rice and wheat account for half of the daily calories, consumed by the world's population (FAO, 2017). In Kenya, there is over dependence on a few crops for nutrition, with maize accounting for over 70% of the populations' calorie needs. There is need to find alternative nutrition sources (Madegwa, 2015). Terms such as neglected, underutilized, promising amongst others are usually used to describe potentially useful plant species that have not been fully exploited (Fanzo, 2014). These crops are normally viewed as great potential contributors to the improvement of food and nutrition security (Frizon et al., 2011). Jackfruit (*Artocarpus heterophyllus*) has been listed as one of the plants that is

'underutilized.' The knowledge of its nutritional profile, is beneficial to countries where it can be potentially cultivated and are affected by malnutrition and economic depression (Jagtap, 2010; Swami et al., 2012; Morton, 1987).

Jackfruit tree belongs to the genus *Artocarpus*, the fruit of the plant is considered to be the largest in the world, with mass of up to 50-80 kg (Abu Bakar et al., 2015; Mukprasirt and Sajjaanantakul, 2004; Nakintu et al., 2019). The consumption of the plant is low in Kenya and its cultivation, is mostly done in small scale. In Uganda, the consumption of jackfruit is moderately high, however limited studies have been done on characterization of the plants from that region (Ojwang et al., 2015). Studies from other regions have shown that jackfruit pulps and seeds contain carbohydrates, proteins, lipids and minerals (Mukprasirt and Sajjaanantakul, 2004, Ojwang et al., 2015; Ajayi, 2008). Studies on characterization of jackfruits in Kenya and Uganda, have been done on the seed lipid profile (Ojwang et al., 2015). There is however, no attempt that has been made so far to study the nutritional profile, mineral composition, phytochemical and antioxidant activities of the seeds and pulp from this region. This study sought to determine the nutritional profile, mineral composition, phytochemical and antioxidant activities of jackfruits seeds and pulp found in selected regions of Kenya and Uganda. Results obtained will be used to promote the use of jackfruit, as an alternative nutrition and natural antioxidant source.

3.2. Materials and Methods

3.2.1. Fruit sampling and Preparation

A total of 30 fruits were collected from Mombasa, Kwale and Ugenya counties in Kenya and Kampala, Mukono and Jinja districts in Uganda. Five mature fruits were picked per region and the distance between the trees were a difference at least 2-5 Km. However, in some regions the distance was longer due to scarcity of the trees and unavailability of mature fruits in some trees. The fruits were then sliced and the seeds and pulps were removed and used for the analysis. The samples were analysed in

triplicate and the results were expressed as percentage weight of fresh samples except for mineral analysis, where dry weight was used.

3.2.2 Determination of moisture and ash content

Jackfruit seeds and pulp were cut into small pieces, weighed, and dried in an oven at 105 °C for 17 hours. Then, the dried seeds were also weighed and the moisture content was determined using the formula described by Gupta et al., (2011).

$$\%\ \text{Moisture content} = \frac{\text{Original mass} - \text{oven dried}}{\text{Original mass}} \text{ x } 100 \ldots\ldots \text{(i)}$$

The ash content was analysed by weighing the samples before and after burning in a fiery furnace at 500 ºC for 24 hours. The moisture and ash content were calculated as shown below (Gupta et al., 2011).

$$\%\ \text{Ash content} = \frac{\text{Original mass} - \text{ash mass}}{\text{Original mass}} \text{ x } 100 \ldots\ldots\ldots\ldots \text{(ii)}$$

3.2.3 Determination of total nitrogen and crude protein content

The total nitrogen and crude protein determination were done for both the pulp and seed samples. Determination of the crude protein of the the seeds and the pulp parts was done by micro Kjedahl according to AOAC 928.08 (AOAC, 1995). The total crude protein was calculated from total nitrogen using the formula:

$$\text{Protein content} = \text{Nitrogen content x } 6.25 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(iii)}$$

3.2.4. Determination of crude lipid content

The fat content of the samples was determined using chloroform/methanol as a solvent according to AOAC method 991.36 (AOAC, 1995)**.**

3.2.5 Determination of total carbohydrate content

Total carbohydrate content to the seeds and the pulp parts was calculated using the formula:

$$\text{TC}\,\% = \{100 - \text{MC}\,\% - \text{PC}\,\% - \text{LC}\% - \text{AC}\,\%\} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (\text{iv})$$

Where TC % is Total carbohydrates (% fresh weight),

MC% is moisture content (% fresh weight)

LC% is lipid content (% fresh weight)

AC% is ash content (% fresh weight)

3.2.6. Determination of mineral composition

A mass of 1 g of the sample was oven dried and digested using 10 ml of concentrated nitric acid in beaker, while heating in a hot plate until the solution was clear. The solution was then transferred to a 50 ml volumetric flask and topped up to the mark. The mineral composition of jackfruit seed and pulp, were determined on aliquots of the digested solutions of the dried samples using 'atomic absorption flame emission spectrophotometer' according to Gupta et al., 2011). The elements that were analysed include calcium (Ca), iron (Fe), magnesium (Mg), potassium (K), sodium (Na), zinc (Zn), and copper (Cu).

3.2.7 Phytochemical analysis

3.2.7.1 Sample preparation and methanolic extraction

The jackfruit seeds and pulps were air-dried for a day and then ground into finer particles using mortar and pestle. A mass of 10 g of the samples was then mixed with methanol to make up to 100 ml volume in conical flasks, plugged with cotton wool and allowed to stand for 24 hours before evaporating the solvents. The crude extracts obtained were stored at 4 C˚until use.

3.2.7.2 Total phenolic compound determination

The Folin-Ciocalteu method was used following protocol by Tambe and Bhambar (2014) with slight modifications. About 1 ml of the methanolic extract from jackfruit seed and pulp were mixed separately with 9 ml of distilled water and 1 ml of Folin-Ciocalteu phenol reagent. The mixture was mixed well by shaking. After 5 minutes, 10 ml of 7 % Sodium carbonate (Na_2CO_3) was then added and the final volume made to 25 ml with distilled water. A set of standard solutions were also prepared using different concentrations of gallic acid solution (20, 40, 60, 80, 100 µl). Both the samples and the standards were incubated for 90 minutes at room temperature. The absorbance readings were obtained using an Ultraviolet (UV)/visible spectrophotometer (UV mini 1240, Shimadzu, Japan) at 765 nm with methanol as blank. The phenolic content was determined and expressed as mg of GAE/g of extract using the following formula:

$$C = \frac{z \text{ mg GAE/ml x vol. of chemical used in assay (ml)}}{\text{Mass of sample (mg)}} \ldots\ldots\ldots\ldots\ldots (v)$$

Where:

z = concentration value obtained from the calibration curve

GAE = gallic acid equivalent

3.2.7.3. Determination of total flavonoids

About 100 µl of methanolic extract was mixed with an equal volume of 20 % aluminum trichloride and a drop of acetic acid. Methanol was then used to top up the mixture to 5 ml and the samples allowed to incubate for 40 minutes. The absorbance of the samples, were then read at 415 nm against the blanks prepared in a similar way, as the test samples but without aluminum trichloride. Different concentrations of rutin solution in methanol was used to prepare a standard curve. All samples were analysed in triplicate (Kumaran and Karunakaran, 2006).

3.2.8 Antioxidant activities

3.2.8.1. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH scavenging activity was done according to the Blois (1958) method with a few modifications. About 300 µl of extract was mixed by shaking with 3 ml of methanolic solution of 0.02 mM DPPH and incubated at room temperature for 30 minutes before taking absorbance values at 517 nm in a spectrophotometer (UV mini 1240, Shimadzu, Japan). The standard, blank and positive control were ascorbic acid, methanol reagent and DPPH, respectively. The DPPH scavenging activity was determined using the equation:

$$\text{Scavenging activity (\%)} = \frac{(\text{Control absorbance} - \text{Absorbance of sample})}{\text{Control absorbance}} \text{x}100 \ldots . \text{(vi)}$$

3.2.8.2. Reducing power assay

The assay was carried out following protocol by Hossain et al. (2014), with a few modifications. A mixture of 1 ml of extract, 2.5 ml of potassium ferricyanide [$K_3Fe(CN)6$] (1%, w/v) and 0.2 M phosphate buffer (pH 6.6) was prepared and incubated at 50 °C for 20 minutes before addition of 2.5 ml of trichloroacetic acid (10%, w/v) to stop the reaction. The samples were then centrifuged at 1000 g for 10 minutes and 2.5 ml of the supernatant mixed with 2.5 ml of distilled water and 0.5 ml

ferric chloride (0.1%, w/v). The absorbance of the solution was then read at 700 nm against distilled water as the blank and ascorbic acid of different concentrations used as standards.

3.2.9 Statistical analysis

The means and standard deviation of the data were calculated from three independent experiments of each sample from the different regions. The five samples obtained from each region were analyzed in triplicates, and the means were analyzed for variation among the regions using one way analysis of variance (ANOVA) at P=0.05. Additionally, the variation in means of the nutritional, phytochemical content and antioxidant activities in the pulp and seeds were analyzed using ANOVA at P=0.05. The data analysis was carried out using SPSS 16 statistical tool and Microsoft excel 13.

3.3. Results

3.3.1 Nutritional profile

3.3.1.1 Moisture content and Ash content

The moisture content was found to be higher in the edible pulp region (62.67-70.42%), compared to the seeds (44.76-50.54%) as shown in Figure 3.1. There was no significant variation at (*P=.05*) in moisture content of pulp and seeds from different regions. However, there was a significant variation in composition of moisture content of seeds and pulp at (*P=.05*). The percentage ash content of the seeds (1.12 -1.64%) was significantly higher than that of the pulp (0.34 -0.48%). The levels were however found to have no significant variation at (*P=.05*) across the regions as shown in Figure 3.2.

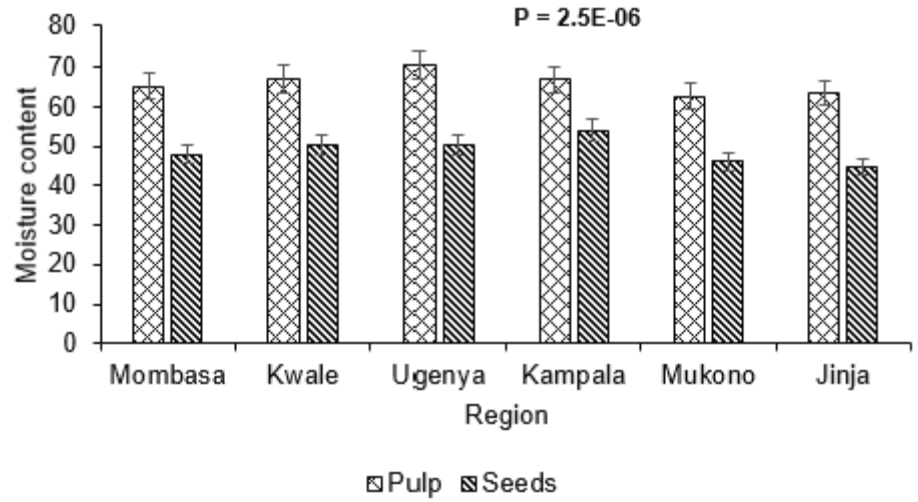


Figure 3.1. Percentage moisture of seeds and pulps (fresh weight). The pulp content was significantly higher (P=0.05) compared to the seeds in all regions.

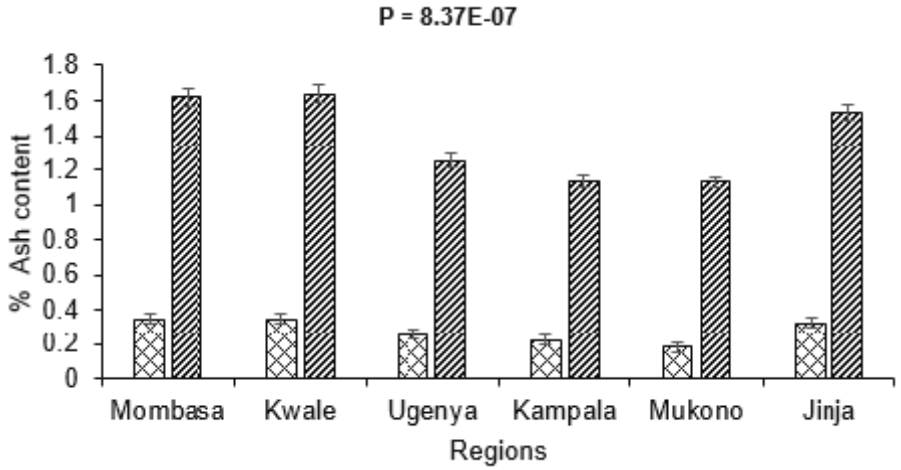


Figure 3.2. Percentage ash content of seeds and pulps. The content was significantly higher in the seeds compared to the pulp region.

3. 3.1.2 Lipid content

The jackfruit seeds were found to have a significantly higher lipid content (0.41-0.50%) compared to the pulp (0.09-0.12%) as shown in Figure 3.3.The variation in lipid content was however not significant at *P=.05* in pulp and seeds from the different regions.

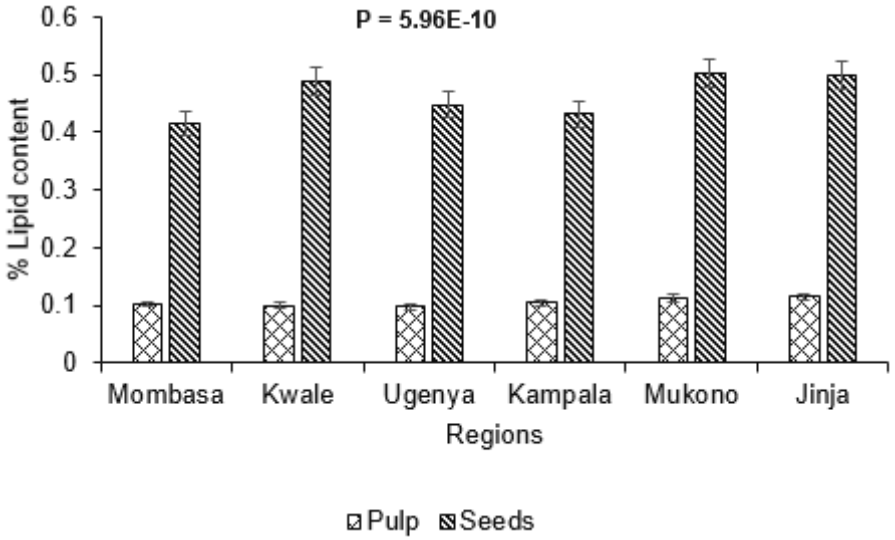


Figure 3.3. Percentage lipid content of seeds and pulps (fresh weight). The content was significantly higher (P=0.05) in the seeds compared to the pulp in all regions.

3.3.1.3 Protein and carbohydrate contents

The protein and carbohydrate content of the pulp was ranged from 10.56 to 13.67% and 21.65 to 24.91%), respectively, while that of the seeds was from 14.11 to 16.26% and 31.41% -34.95%, respectively (Figure 3.4 and 3.5). The seeds had the highest carbohydrates and proteins content, while the pulps had the least. There was a significant variation at *P=.05* in levels of proteins in the seeds and pulp within and across the regions. There was also a significant variation (*P=.05*) in levels of carbohydrates present in both the pulp and the seeds (Figure 3.5).

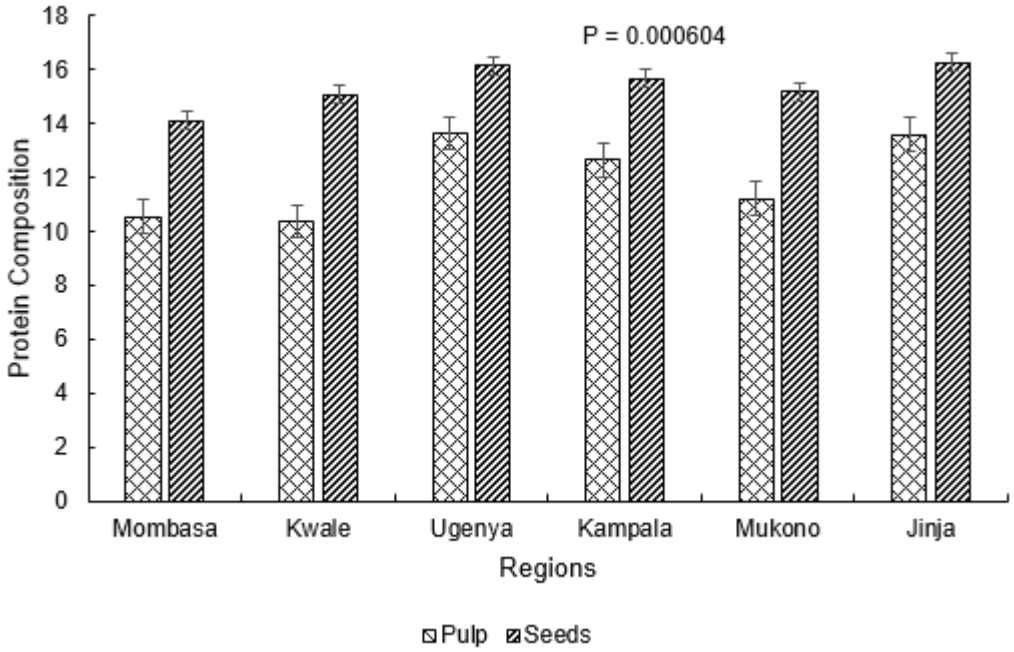


Figure 3.4. Percentage protein content of seeds and pulps (fresh weight). The seeds had a higher content compared to the pulps in all regions and the difference was significant at p=0.05.

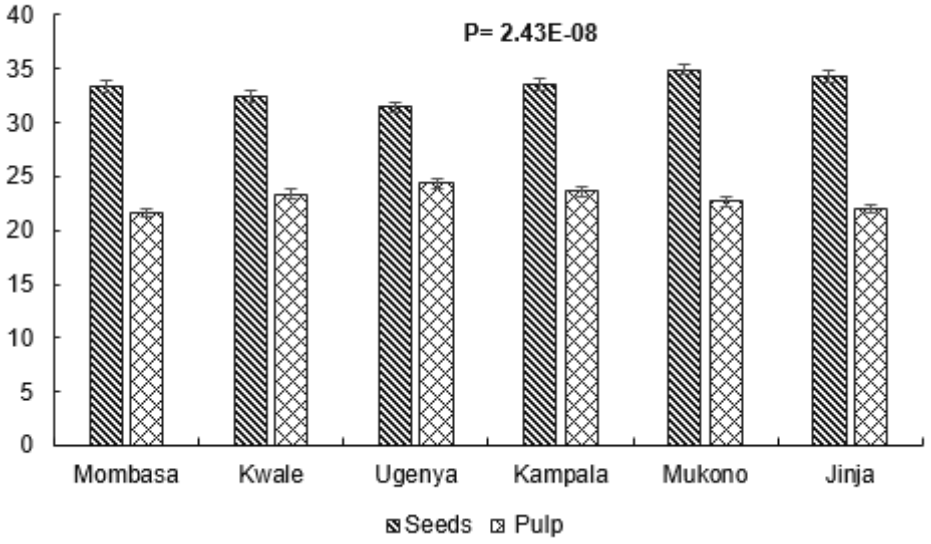


Figure 3.5. Percentage of carbohydrate contents of seeds and pulps (fresh weight).The content was comparable in the pulps and seeds of the different regions; however, the levels were higher in the seeds than in the pulp.

3.3.1.4 Jackfruit seeds and pulps nutritional profile

In both seeds and pulp, the moisture content had the highest composition, followed by carbohydrate, which had the second highest composition and then protein. Lipid and ash contents were the least as shown in Table 3.1. The seeds generally had a higher nutritional content compared to the pulp region.

Table 3.1. Jackfruit seeds and pulps nutritional profile in Kenya and Uganda

Nutritional Profile	Jackfruit tissues	Kenya	Uganda
%Moisture	Pulp	66.55±2.63	64.40±2.24
	Seeds	49.24±1.45	48.27±5.01
%Ash	Pulp	0.32±0.05	0.24±0.07
	Seeds	1.50±0.22	1.26±0.21
%Lipid	Pulp	0.10±0.002	0.11±0.005
	Seeds	0.45±0.037	0.47±0.04
% Protein	Pulp	11.53±1.86	12.50±1.19
	Seeds	14.04±19	16.19±1.75
%Carbohydrates	Pulp	22.43±1.80	19.38±1.19
	Seeds	32.39±0.98	34.15±1.18

3.2.2 Elemental analysis

In jackfruit seeds and pulps mineral analysis, potassium had the highest K composition that was more than 10 times greater than those of those of all the other minerals, while Cu and Zn had the least concentrations. The elemental analysis of the both seeds and pulp had the following trend K>Na>Ca>Mg>Fe with Cu and Zn having the least concentrations as shown in table 3.2. The composition of sodium, calcium, magnesium, zinc, copper and iron in the pulp were lower compared to that those of the seeds. The concentration of potassium was however higher in the pulp compared to the seeds. Generally, the seeds had higher mineral composition compared to the pulp region.

Table 3.2. Average mineral contents of seeds and pulps in mg/100g of dry weight.

Minerals	Pulp	Seeds
Sodium	185.33±21.02	193.75±17.60
Calcium	141.67±28.40	176.73±30.16
Iron	14.05±1.40	23.09±1.60
Magnesium	76.28±12.64	84.25±6.63
Potassium	2836.66±193.58	2297.5±129.44
Zinc	5.31±1.64	7.12±2.27
Copper	2.21±0.16	2.84±0.58

3.3.2 Determination of phytochemical composition

3.3.2.1 Phenolic content

The phenolic content of jackfruit pulp were lower than those of seeds. The content obtained ranged from 12.10 -14.55 mg/g and 17.37-18.69 for pulp and seeds, respectively (Figure 3.6). There was no significant difference at (*P=.05)* in the phenolic content of the seeds and pulp across the different regions, however there was a significant difference at (*P=.05*) in the phenolic composition of the seeds and pulp.

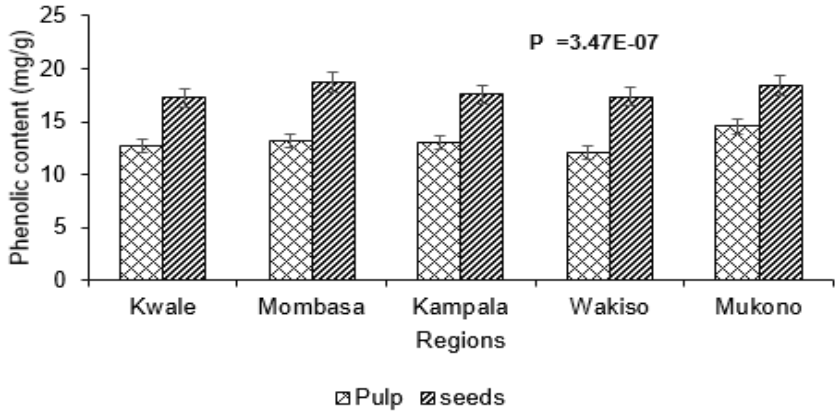


Figure 3.6. Phenolic composition of seeds and pulps in different regions. The phenolic content was significantly higher (P=0.05) compared to the pulps in all regions.

3.3.2.2 Flavonoids content

The flavonoids content was found to be lower in the pulp (0.29 - 0.18 mg/g) than in the seeds (0.89 - 0.5 mg/g) as shown in Figure 3.7. There was no significant difference (*P=.05*) in flavonoid content of the seeds and pulp across the different regions. There was however a significant variation (*P=.05*) in levels of flavonoids in the seeds and pulp.

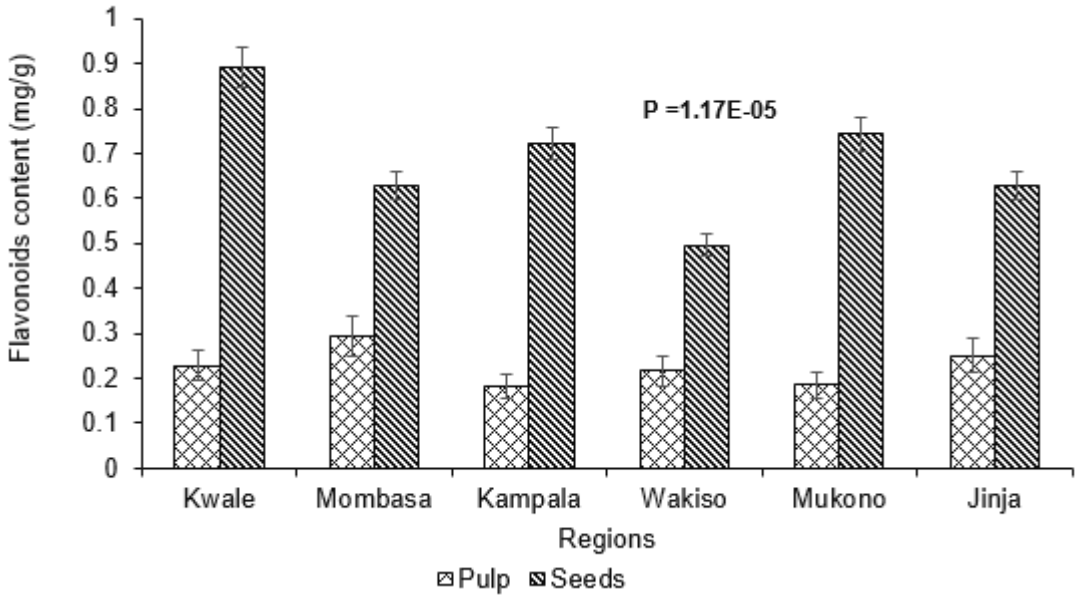


Figure 3.7. Flavonoids content of seeds and pulps. The contents were significantly higher (P=0.05) in the seeds than in the pulps.

3.3.3 Determination of antioxidant activity

3.3.3.1 DPPH scavenging activity

A calibration curve for DPPH scavenging activity using ascorbic acid was generated. The DPPH scavenging activity of the pulp (15.49-17.47%) was lower than that of the seeds (21.70-24.44%) (Figure 3.8). The variation in scavenging activity of the pulp and seeds from the different regions was statistically significant (*P=.05*). However, the scavenging activity of the seeds, in the different regions showed no significant variation at *P=.05*.

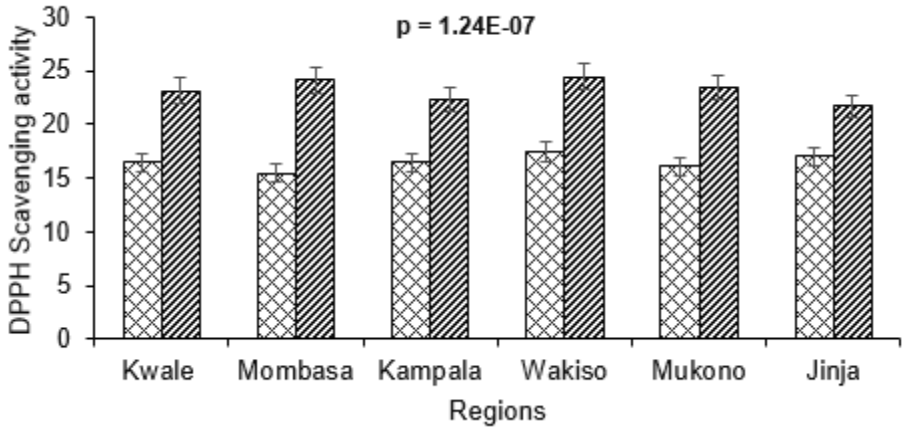


Figure 3.8. DPPH scavenging activities of pulps and seeds. The scavenging activity was significantly higher (p=0.05) in the seeds than in the pulps.

3.3.3.2 Reducing power

The reducing power of the seeds ranged from 51.05 to 58.00 µg/ml with Kwale having the least and Mukono the highest. The pulp ranged from 43.54 to 45.38 µg/ml, with Kampala having the highest and Kwale the least. The variation in reducing powers was found to be statistically significant using ANOVA *(P=.05)* for the seeds and pulp. There was however no significant variation in reducing power of seeds and pulp across the regions (Table 3.3).

Table 3.3. Reducing power of pulps and seeds from different regions

Regions	Reducing power Pulp (µg/ml)	Seeds (µg/ml)
Kwale	43.54[a]±2.19	51.05±2.69
Mombasa	44.58 [a] ± 2.51	52.87± 2.83
Kampala	45.38 [a] ± 1.10	57.57 ± 2.06
Wakiso	44.56 [a] ±1.44	52.56 ± 1.78
Mukono	43.44 [a] ± 1.73	58.00 ± 2.7
Jinja	44.24 [a] ± 1.61	56.12 ± 2.18

[a]: values in the same column are statistically the similar at P=.05

3.4. Discussion

3.4.1 Nutritional profile

The ash content is the measure of the inorganic material present in the fruit, which are minerals in most cases. The percentage ash content of the seeds (1.12 -1.64%) and pulp (0.34 -0.48%) of jackfruit, indicate they are rich in minerals. The composition of minerals in the seeds was however higher than that of the pulp region. These finding is consistent with that of Eke-Ejiofor and Owuno (2013), who also found that the ash content of jackfruit pulp is 0.43% and inconsistent with that of Goswami et al. (2011), who got pulp ash content of 0.7 -1.0%. The ash content of the seed is consistent with that of Awal *et al.* (1991), who found the ash content to be 1.8%. The lipid content was found to be (0.41-0.50%) in the seeds and (0.09-0.12%) in the pulp region (Ojwang et al., 2015). There are a number of health benefits of lipids in the body such as synthesis of cell membranes, which ensures integrity of the cell and enables it to carry out a number of vital body processes. The consumption of lipids is therefore important to ensure proper functioning of the body. The jackfruit was found to have moderate lipid content with values similar to those of Madrigal-Aldana et al. (2011).

High moisture content reduces the shelf life of fruits as moisture increases the microbial activity. The moisture content of the pulp and seeds of jackfruit were ranged from 62.67-70.42% and 44.76-50.54% respectively implying that both fresh jackfruit seeds and pulp cannot be stored for long in fresh state. Jackfruit therefore need to be preserved by techniques such as drying amongst others to increase their shelf life.

Proteins are important nutrients in the body, as they help in repairing worn out tissues and are a source of amino acids required for protein synthesis. The findings of this study where the pulp region and the seeds had 10.56 to 13.67% and 14.11 to 16.26% fresh weight respectively, indicate that jackfruit pulp and seeds are good sources of protein. These findings are consistent with those of Abedin et al.(2012), who found

jackfruit seeds protein content of 13 -18% and close to those of Gupta et al.(2011), who obtained 11.85%. Both jackfruit seeds and pulp have very high carbohydrate levels (31.41% -34.95%) and 21.65 to 24.91%) for seeds and pulp were respectively. This means that both jackfruit seeds and pulp are good sources of energy. The values obtained are close to those of Gupta et al. (2011), who obtained 26.2% for the seeds.

The nutritional profile was different from that of Shafiq et al. (2017), who analysed jackfruit pulps from Pakistan and found that the carbohydrates, proteins and crude fat content were 13.08± 0.31%, 1.48±0.11% and 5.63±0.18 %. The values were lower than the ones obtained in this study. However, the moisture contents of the pulps were similar to the ones obtained in this study. Moreover, the protein content of the seeds was close to that obtained by Ocloo et al. (2010), who analysed the nutritional profile of jackfruit found in Ghana, whereby, the fat and ash contents were 1.27% and 2.7%, respectively. However, the carbohydrate content was significantly higher (79.34) compared to the one obtained in this study.

3.4.2 Mineral composition

Jackfruit was found to be rich in essential minerals, with the highest being potassium. The mineral helps in strengthening of bones and teeth in the human body. It also facilitates the contraction and relaxation of muscles in the body. Potassium together with other electrolytes such as Mg, Ca and Na also conducts impulse in the body, which is important for proper functioning of the heart and other body organs (Soetan et al., 2010). Calcium was also in high composition and it also helps in strengthening of bones and teeth. Inadequate intake of Ca has been associated with illnesses such as osteoporosis (Soetan et al., 2010).

Magnesium levels were also found to be moderately high. The mineral plays a role in the regulation of blood sugar levels. It is used as a cofactor by many enzymes such as

insulin in glucose metabolism (Takaya et al., 2004). The fruit was found to contain moderate levels of iron, which is an important mineral that is used by the body to form hemoglobin. The concentration of red blood cells in the body is highly determined by the presence of iron (Soetan et al., 2010). Zinc on the other hand, is required by the body in small quantities and also plays a major role in a number of metabolic processes. It is also used as a cofactor by a number of enzymes (Soetan et al., 2010).

Copper was also found to be in low levels in the seeds and in the pulp. The mineral is required in the body in small quantity and it is crucial in formation of connective tissues. It is also used alongside iron, in the formation of red blood cells (Soetan et al., 2010). The findings of this study were consistent with those of Gupta et al. (2011), who also found that jackfruit seeds had very high potassium contents in the seeds. The study also found that jackfruit seeds had high calcium and sodium contents. The study was however inconsistent with that of Ejiofor and Owuno (2013), whose calcium levels were very low.

The findings by Ocloo et al. (2010), were different in composition from those obtained in the study, however the trend was similar. The study showed that potassium content was the highest, which was similar to the findings of this study the contents obtained, although they were considerably lower compared to those obtained in this study. The potassium, calcium, sodium, iron and magnesium content in the study was 1478.1 mg/100g, 308.7 mg/100g, 6.07 mg/100g and 13.07 mg/100g. Notably, the iron levels (14.05±1.40) were almost similar; however, the calcium levels obtained in this study were much lower. Conversely, the sodium levels were much higher compared to the findings of the study. Therefore, Jackfruit seeds and pulp are promising alternatives of mineral sources.

3.4.3 Phytochemical composition

In this study, the pulp had the least phenolic content (14.5 - 12.10 mg/g) and the seeds had the highest (18.69 - 17.37 mg/g). The flavonoids content was found to be lower in the pulp (0.29 - 0.18 mg/g) than in the seeds (0.89 - 0.5 mg/g). Phytochemicals are important in the body, because they react with oxygen species and hence prevent the formation of free radicals in the body. The free radicals have been associated with the damage of proteins in the body including DNA (Sudha et al., 2011). This difference in composition of seeds and pulp, may be attributed to genetic factors, the genes that express the phenolic and flavonoid contents may have been higher in the seeds than in the pulp (Andrew et al., 2010). These current findings are consistent with that of Shanmugapriya et al. (2011), who found flavonoid content of 4.05 mg/ml using ethanolic extract. The phenolic content (4.16 mg/g) was however lower compared to the ones obtained in this study. This may be due to difference in the extraction solvents as methanol and ethanol have been found to have different efficiencies in extraction of phytochemicals (Nahak et al., 2010). The study therefore shows that the seeds are richer sources of phytochemicals compared to the pulps.

The phenolic content of the pulp (14.5 -12.10 mg/g) obtained in this study were much higher than that of Shafiq et al. (2017), who obtained 2.39 mg/g. Conversely, the flavonoid content (1.09 mg/g) was substantially lower (0.29 - 0.18 mg/g). The difference may be attributed to the extraction solvent used, which was acetone. Moreover, the phenolic content was significantly higher that of Gupta et al, who obtained 1.45 mg/g in the seeds, while the flavonoid content was much higher than the one obtained in this study, which was 290.6 mg/g. The variation may be due to difference in the environmental conditions as well as genetic factors. However, the findings were consistent with Abu Bakar et al. (2015), who analysed the flesh, seeds and pulp of *Artocarpus* species using methanolic extracts in Malaysia and found that the phenolic contents (11.67-13.72 mg/g) in the seeds, while the flavonoid content 0.82-1.23 mg/g in the pulp. The peel values were also close to those obtained in the

bark and leaves in this study, as they were 21.29-42.38 mg/g phenolic content. Therefore, the Jackfruit seeds and pulp are rich in phytochemicals.

3.4.4 Antioxidant activity

Antioxidants help in reducing the damage that results from the action of free radicals in the body. The free radicals have been associated with symptomatic characteristics of diseases such as cancer, hypertension, diabetes mellitus and neurodegenerative illness (Abu Bakar et al., 2015). The current study found the DPPH scavenging activity of the jackfruit pulp and seeds ranged from 15.49-17.47% and 21.70-24.44%, respectively. The study was also consistent with that of Abu Bakar et al. (2015), who found that the pulp of various *Artocarpus* plants had relatively low DPPH activity. The seeds and the pulp had relatively lower DPPH activity, compared to those of the barks, roots and leaves of jackfruits (Ojwang et al., 2017). This could be attributed to the fact that the leaves, roots and barks are more exposed to the environment and therefore require more phytochemicals to help them fight pathogens compared to the seeds and pulp that are enclosed inside the fruit. The reducing power of the seeds was (51.05 ± 2.69 µg/ml) and that of the pulp was (43.54-45.38 µg/ml). The reducing power obtained for both the seeds and roots were relatively high. The values were much higher compared to those obtained by Gupta et al. (2011), who found the reducing power to be (14.02 -16.68 µg/ml). The variation may be attributed to difference in the extraction solvent as the study used acetone and dichloromethane: methanol (1:1).

3.5. Conclusion

The compositional analysis of jackfruit seeds and pulp reveal that there is a significant variation in composition of the two tissues. The two regions are however both rich in proteins and carbohydrates. The fruit can therefore be used as an alternative source of both carbohydrates and proteins. The fruit was also found to be rich in minerals such as potassium, calcium, sodium and iron, which are essential in the body. Jackfruit is

therefore a good source of essential minerals. Jackfruit seeds and pulp were found to have moderate levels of phytochemicals and antioxidant activities hence a potential source of natural antioxidants. The knowledge of jackfruit nutritional profile, may contribute in reducing the overdependence on a few crops for nutritional needs and biofortification of maize, with jackfruit flour. It may also help in the popularization of jackfruit in Kenya, where it is underutilized. This will reduce the cases of under nutrition in Kenya and in turn contribute towards the Sustainable Development Goals (SDGs) of 2030, of ensuring food and nutrition security. The information on phytochemical and antioxidant activity, may also help in providing alternative sources of natural antioxidants.

CHAPTER FOUR

4.0 Comparative analysis of phytochemical profile and antioxidant activities of methanolic extracts of leaves, roots and bark of jackfruit (*Artocarpus heterophyllus*) from selected regions in Kenya and Uganda

4.1 Introduction

The search for natural antioxidants derived from the plant, has been on the increase in the recent past (Slyvie et al., 2014, De Beer et al., 2002, Lobo et al., 2010, Shanmugapriya et al., 2011). Antioxidants have been found to minimise the negative effects of free radicals in the body. Free radicals are usually produced, as by-products of the cells' metabolic activities (De Beer et al., 2002). These radicals cause oxidative stress, when there is a major imbalance between them and the antioxidants defence system. This results in damage of DNA, proteins and lipids through oxidative stress (Lobo et al., 2010). Chronic diseases such as heart diseases, diabetes mellitus, cancer and neurodegenerative diseases, are also mediated by mechanisms that involve the action of free radicals (Abu Bakar et al., 2015). These diseases are among the major causes of death worldwide (WHO, 2017).

Phytochemicals have antioxidant properties and can be used both in the prevention and treatment of these illnesses (Abu Bakar et al., 2015). They have hydroxyl groups that are good hydrogen donors, which react with reactive oxygen species to prevent the formation of new radicals. They can also chelate or reduce metals that are involved in production of free radicals (Sudha et al., 2011). Plants such as jackfruit, have been found to have relatively high phytochemical composition and antioxidant activities (Shanmugapriya et al., 2011). Jackfruit (*Artocarpus heterophylus*) is a medium-sized tree that is evergreen and has a height that ranges from 8 to 25m. The diameter of the stem is 30 to 80cm and the tree has a dense shade (Elevitch and Manner, 2006). It is considered the largest fruit in the world with weight of up to 50kg (Abu Bakar et al., 2015). Jackfruit has been listed as an underutilised plant and is usually considered to

be for people from lower social class in Asia, where it is dominant (Ojwang et al., 2015, Ocloo et al., 2010, Shyamalamma et al., 2008). Parts of the tree such as the roots, bark and leaves have been found to have a number of medicinal properties (Hakim et al., 2005, Arung et al., 2006).

Studies have shown that the jackfruit seeds and pulp have relatively high content of phytochemical compounds and antioxidant activities (Shanmugapriya et al., 2011, Abu Bakar et al., 2015). Studies by Shanmugapriya et al. (2011) and Gupta et al. (2011), showed that jackfruit seeds contains important phytochemicals such as phenolic compounds, flavonoids, tannins and saponins. The studies also found that jackfruit has high antioxidant activities, which include: high DPPH and ABTS scavenging activity. They were also found to have high iron reducing power and chelating activities (Gupta et al., 2014). Abu Bakar et al. (2015), revealed that the fruit peel of some *Artocarpus* species had significantly higher phytochemical composition and antioxidant activities such as DPPH, ABTS scavenging activity and reducing power compared to pulp and seeds. Goswami et al. (2011), suggested that jackfruits' pulp region phytochemical composition, varied depending on the pulp and the region where it is planted.

Previous research on jackfruit phytochemical properties have mostly focussed on the pulp and the seeds (Shanmugapriya et al., 2011, Abu Bakar et al., 2015, Gupta et al., 2011, Goswami et al., 2011). Studies from other plants, have shown that the roots, leaves and barks also contain a high quantity of phytochemicals and antioxidant properties (Slyvie et al., 2014, Sudan et al., 2014). The leaves of Himalayan Cobra Lily (*Arisaema jacquemontii*) was found have significantly higher antioxidant activities, compared to the fruit and tubers (Sudan et al., 2014). Medicinal plants *Hymenocardia lyrata*, *Garcinia lucida* and *Acalypha racemosa* were also found to have high antioxidant activities in their roots (Slyvie et al., 2014). *Azadirachta indica*

roots, were found have significantly high levels of phenolic content and antioxidant activities (Hossain et al., 2014).

Despite the fact that numerous studies on other plants have shown higher levels of phytochemicals in roots, bark and leaves, compared to the edible region (Sudan et al., 2014), there is limited information with regards to phytochemical composition of these tissues in jackfruits. Furthermore, there has been no attempt made so far, to determine the phytochemical composition of the various tissues of jackfruits found in Kenya and Uganda. A knowledge of the phytochemical composition of the different tissues, will help in providing more alternatives of natural antioxidants.

This study sought to determine, the phytochemical composition and antioxidant activities of jackfruit roots, leaves and bark found in selected regions of Kenya and Uganda. The knowledge of the phytochemical composition, will facilitate in the identification of the part of jackfruit tree that are suitable for harvesting of phytochemical compounds.

4.2 Materials and Methods

4.2.1 Sampling design and preparation

A total of 192 samples of roots, leaves and barks from 64 jackfruit trees, were collected from Mombasa (8) and Kwale (17) Counties of Kenya, and Kampala (12), Wakiso (8), Mukono (10) and Jinja (9) districts in Uganda. The samples were selected based on the availability of the trees and a distance of 2 to 5 km apart, although in some regions the distance between the Jackfruit trees was longer due to scarcity of the trees, especially in Kenya. The samples were cleaned thoroughly with water and air dried for three weeks under a shade. About 10g of each sample, was cut into small pieces and further crushed using a grinder to fine particles. A volume of 100 ml methanol was then added to the samples in conical flasks, plugged with cotton wool and left to stand

for 24 hours. The supernatants were then collected and the solvents evaporated to make the crude extract that was then stored at 4 ^{o}C until use.

4.2.2 Phytochemical composition

4.2.2.1 Determination of total phenolic compounds

The concentration of phenolic compounds in the plant extracts, was determined using spectrophotometric method following protocol by Sirisha et al. (2014) and Tambe and Bambar (2014), with a few modifications. Folin-Ciocalteu assay method was used for the determination of the total phenol content. The reaction mixture consisted of 1 ml of extract and 9 ml of distilled water in a volumetric flask (25 ml). One millilitre of Folin-Ciocalteu phenol reagent was added and the mixture shaken well. After 5 minutes, 10 ml of 7 % Sodium carbonate (Na_2CO_3) solution was added to the mixture. The volume was then made up to 25 ml with distilled water. A set of standard solutions of Gallic acid (20, 40, 40, 60, 80 and 100 µg/ml) were also prepared. The mixture and the standards, were then incubated for 90 minutes at room temperature before taking their absorbance readings against the reagent blank (methanol) at 765 nm with an Ultraviolet (UV) / visible spectrophotometer (UV mini 1240, Shimadzu, Japan). Total phenolic content was expressed as mg of GAE/g of extract using the equation (vii) below.

$$C = \frac{\text{z mg GAE/ml x vol. of chemical used in assay (ml)}}{\text{Mass of sample (mg)}} \quad \ldots\ldots\ldots\ldots\ldots (vii)$$

Where

z = concentration value obtained from the calibration curve

GAE = Gallic acid equivalent,

4.2.2.2 Determination of total flavonoids

The method is based on the formation of the flavonoids – aluminium complex (Kumaran and Karunakaran, 2006). The complex has a maximum absorption at a wavelength 415 nm. An aliquot (100 µl) of the plant extract in methanol (10 mg/ml)

was mixed with 100 µl of 20% aluminium trichloride in methanol and a drop of acetic acid and then diluted with methanol to 5 ml. The absorption at 415 nm was then read after 40 minutes against a blank. Blanks were prepared from 100 µl of plant extracts and a drop of acetic acid and then diluted to 5 ml with methanol. The absorption of standard rutin solution (0.5 mg/ml) in methanol at different concentrations was then measured under the same conditions. All determinations were carried out in triplicate.

4.2.2.3 Determination of total tannins

The tannins were determined by Folin -Ciocalteu method. About 0.1 ml of the sample extract was added to a 10 ml volumetric flask containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteuphenol reagent. A volume of 1 ml of 35 % Na_2CO_3 solution was then added to the volumetric flask and the mixture was diluted to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 minutes. Sets of reference standard solutions of Gallic acid (20, 40, 60, 80 and 100 µg/ml) were also prepared. Absorbance for test and standard solutions were then measured against the blank, which was the reagent solution (methanol) at 725 nm, in a UV/Visible spectrophotometer. The tannins content were expressed in terms of mg of GAE /g of extract (Sirisha et al., 2014; Tambe and Bhambar, 2014).

4.2.3 Determination of Antioxidant activity:

4.2.3.1 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The principle behind DPPH free radical assay holds that the antioxidants react with the stable DPPH radical and convert it into 1, 1-diphenyl-2-picryl hydrazine. Scavenging activity on DPPH was carried out using the method by (Blois, 1958) with a few modifications. A volume of 300 µl extracts (0.1-0.5 mg/ml) was mixed with 3 ml of methanolic solution of 0.1 mM DPPH. The mixture was shaken and incubated at room temperature for 30 minutes and absorbance was then measured at 517 nm in a spectrophotometer. Ascorbic acid was used as the standard, methanol and DPPH

reagent were used as control and methanol was used as blank. The experiment was performed in triplicate and the percent inhibition was calculated from control using the equation (viii) below.

$$\text{Scavenging activity (\%)} = \frac{(\text{Control absorbance} - \text{Absorbance of sample})}{\text{Control absorbance}} \text{x100} \ldots \text{(viii)}$$

4.2.3.2 Reducing power assay

The protocol by Hossain et al. (2014), was used to determine the reducing power of extracts of jackfruit roots, leaves and barks with a few modifications. One ml of methanolic extract was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5ml of potassium ferricyanide [$K_3Fe(CN)6$] (1% w/v). The mixture was incubated for 20 minutes at 50°C after which, the reaction was terminated by addition of 2.5 ml of trichloroacetic acid (10%, w/v). The mixture was then centrifuged for 10 minutes at 1077g and 2.5 ml of the supernatant was then mixed with 2.5 ml of distilled water and 0.5ml ferric chloride (0.1% w/v) solution. The absorbance was measured at 700 nm and an increase in absorbance indicated an increase in reducing power. Ascorbic acid was used as the standard and distilled water was used as blank

4.2.4 Statistical analysis

The data analysis was carried out using Microsoft Excel 2013 data analysis tool and SPPS 16 statistical software. Firstly, the means were calculated based on data collected from three independent experiments for all samples from the different regions. Then, the variation in samples per region was analysed using one-way analysis of variance (ANOVA, P=0.05). Subsequently, the variation of the phytochemical content and antioxidant activities was analysed for the different tissues of the Jackfruit tree. A correlation matrix was also generated to determine the relationship between phenolic, flavonoid, tannins, DPPH and reducing power.

4.3 Results

4.3.1 Calibration curves

The absorbance readings of different concentrations of the standards (Gallic acid and rutin) were used to construct calibration curves. The graphs obtained for the determination of total phenolic compounds, flavonoids and tannins contents had R^2 values of 0.9971, 0.9965 and 0.9985 respectively, (table 4.1). The values obtained from the calibration curves were used to quantify phenolic compounds, flavonoids and tannins content present in the jackfruit leaves, roots and barks. The composition was expressed as mg of Gallic acid equivalents per g of dry weight for phenolic compounds and tannins. The flavonoid content was expressed as mg of Rutin equivalents per g of dry weight.

Table 4.1. Equations and R2 values of the calibration curves

Standard Curve	Equation	R^2 -value
Phenolics	y = 0.0359x -0.2101	0.9971
Flavonoids	y = 0.0011x + 0.0639	0.9965
Tannins	y = 0.0099x + 0.0368	0.9988

4.3.2 Phenolic content of jackfruit leaves, roots and barks from different regions

The phenolic content of leaves, barks and roots were as shown in figure 4.1. Generally, the roots had the highest phenolic content (67.37- 59.00 mg/g), followed by leaves (37.39-30.92 mg/g) and then barks (30.88 - 16.30 mg/g). The samples from Kwale and Jinja regions recorded the highest phenolic content, while Kampala had the least as shown in Figure 4.1. There was a significant variation at ($P = .05$) in levels of flavonoids in the roots, bark and leaves. There was however no significant variation at ($P = .05$) in phenolic content across the different regions. The *P* values are as shown in table 2.

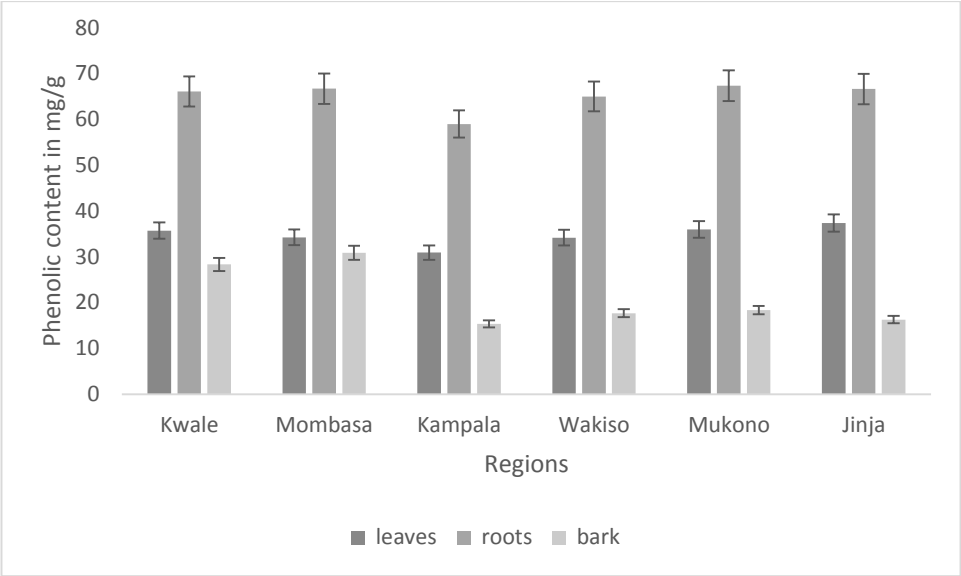


Figure 4.1. Phenolic content of leaves, barks and roots from different regions of Kenya and Uganda. The roots from all regions had higher content compared to the leaves and barks.

4.3.3 Flavonoid composition of jackfruit leaves, roots and barks from different regions

The flavonoid composition of the different tissues was as shown in figure 4.2. There was significant difference (*P =.05*) in flavonoid content of the leaves, roots and bark. Flavonoids content was highest in the roots (10.74 - 7.31 mg/g) followed by leaves (6.70 - 5.02 mg/g) and the bark had the lowest (3.05 - 1.49 mg/g). Roots from Kwale had the highest flavonoid content, while those from Wakiso had the least. Similarly, leaves from Mombasa had the highest flavonoids content, while those form Mukono had the lowest. The barks from Kwale had the highest content, while those from Jinja had the lowest. There was a significant difference (*P =.05*) in levels of flavonoids in the roots, bark and leaves. There was however no significant variation (*P =.05*) in phenolic content across the different regions. The *P* values are as shown in table 4.2.

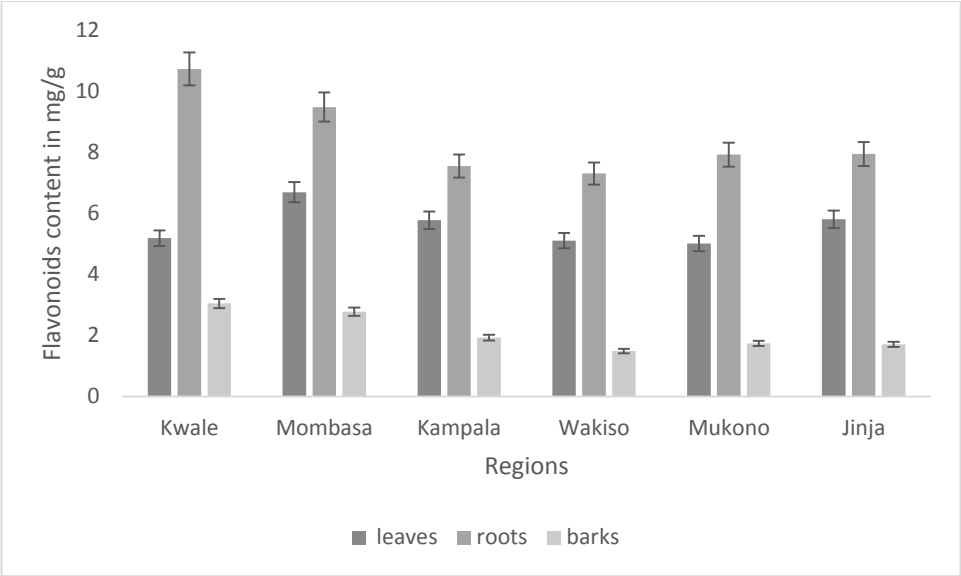


Figure 4.2. Total flavonoids content of leaves, roots and barks from different regions of Kenya and Uganda. The roots from all regions had higher contents compared to the leaves and barks.

4.3.4 Tannin composition of jackfruit leaves, roots and barks from different regions

The tannin content from different tissues were as shown in figure 4.3. The roots had the highest level of tannins content in all regions (3.88 - 2.69 mg/g), followed by the leaves (2.56 – 1.97mg/g) and the barks had the least content (1.93 -0.52 mg/g). Roots from Kwale region had the highest tannin content, and roots from Jinja had the least. The highest tannins content were in leaves collected from Kwale region and the least were those from Mombasa. The barks from Kwale had the highest content, while those from Jinja had the least content. There was a significant variation at ($P = .05$) in tannin levels of roots, bark and roots. There was however no significant variation at ($P = .05$) in tannin levels in the different regions as shown in table 4.3.

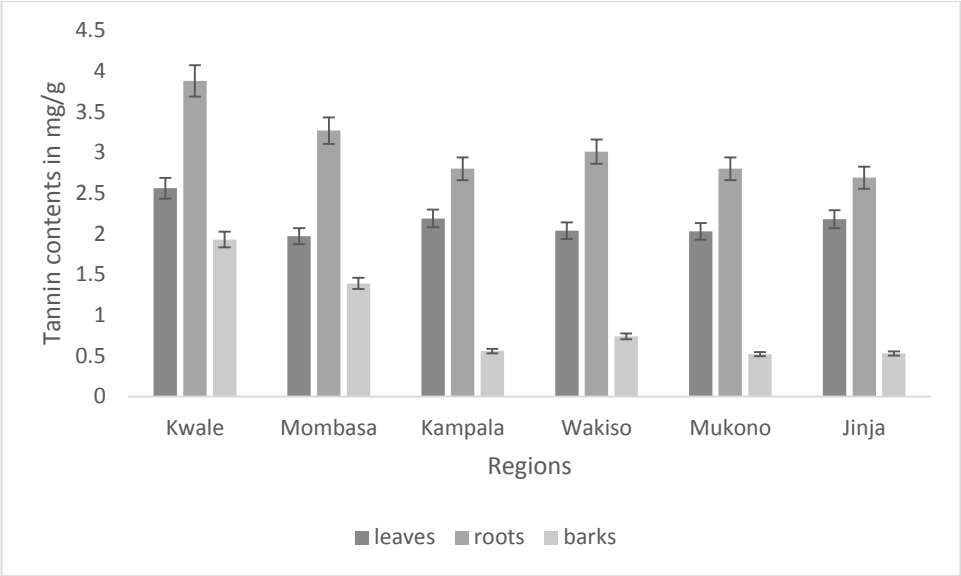


Figure 4.3. Phenolic, flavonoids and tannins content in the roots of jackfruit trees from different regions. The roots from all regions had higher contents compared to the leaves and barks.

Table 4.2. P values of phenolic, flavonoid and tannin contents analysed at (P =.05), within the different parts and across the regions.

Phytochemical composition	*P* value In the different tissues	*P* value Across the regions
Phenolic content	5.3293E-09	0.997
Flavonoids content	2.3247E-08	0.975
Tannins content	2.2084E-06	0.852

4.3.5 Comparison of phytochemical content of leaves, bark and roots of jackfruit from the different regions

The composition of phenolics, flavonoids and tannins in the jackfruits within the same tissues had significant variation at (*P=0.05)*. There was however no significant variation across the regions as shown in table 4.3. The roots had the highest composition of all the phytochemicals, followed by the leaves and the bark had the least. The variation in the different parts was much higher compared to those of the different regions.

Table 4.3. P values of phenolic, flavonoids and tannin contents of different parts of the tree

Phytochemical composition variation in different parts of the tree	*P* values Within the plant	*P* values Across the regions
Leaves	6.57E-17	0.999
Bark	1.99E-07	0.977
Roots	1.22E-18	0.999

4.3.6 Overall jackfruit phytochemical composition in different parts of the tree

The average phenolic content of the leaves, roots and bark were 35.18±2.33 mg/g, 65.56±3.05 mg/g and 23.59±8.84 mg/g respectively. The flavonoid contents of the leaves, roots and bark were 5.74 ± 0.68mg/g, 8.73±1.3mg/g and 2.24±0.67mg/g respectively. The tannins contents, which were the least, included: 2.18±0.2mg/g, 3.2±0.57mg/g and 1.02±0.58mg/g as shown in table 4. Overall, the average phenolic composition of all the parts was 41.44±4.74 mg/g, the flavonoid composition was 5.57±0.91 mg/g and tannins composition is 2.14±0.45 mg/g as illustrated in figure 4.4.

Table 4.4. Average composition of phenolic, flavonoid and tannin content of leaves, barks and roots.

	Leaves (mg/g)	Barks (mg/g)	Roots (mg/g)
Phenolic content	35.18±2.33	23.59 ±8.84mg/g	65.56±3.05mg/g
Flavonoid content	5.74±0.68mg/g	2.24±0.67mg/g	8.73±1.3mg/g
Tannin content	2.18±0.2mg/g, and	3.2±0.57mg/g	1.02±0.58mg/g

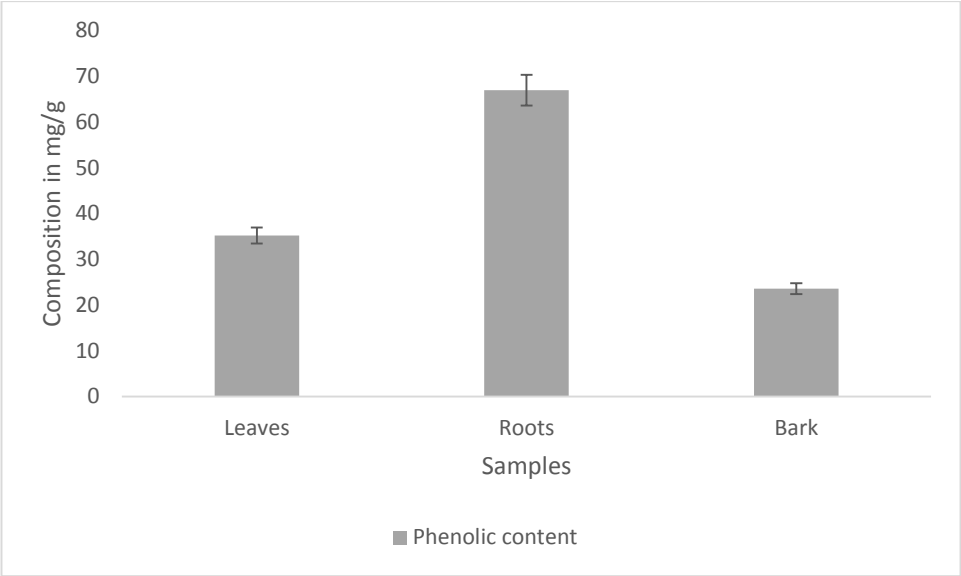


Figure 4.4. Overall composition of phenolics present in the roots, barks, and leaves of Jackfruits found in Kenya and Uganda

4.3.7 DPPH scavenging activity

The percentage scavenging activity of the different extracts were as shown in Figure 6. The roots showed a significantly higher DPPH activity of up to 72.91±2.58%, while the stem bark showed the least DPPH scavenging activity of 24.39±1.86%. The variation in scavenging activity of the samples was significant at (*P=.05*), implying that there is a significant difference in scavenging activities of the leaves, roots and barks. The scavenging activity of the standards (ascorbic acid) was found to be 82%. In overall, the roots from all regions had the highest antioxidant activity, followed by the leaves, while the bark had the least activity (Figure 4.5). The DPPH scavenging activity was found to increase, with increase in concentration in both the roots, bark and leaves. The roots still showed the highest activity at different concentrations, followed by the leaves and then the barks. The IC_{50} for the roots, barks and leaves were calculated using the equations of the linear curves (Figure 4.6). The IC_{50} for the roots, leaves and the bark were found to be 92.61µg/ml, 425. 83µg/ml and 568.40 µg/ml respectively.

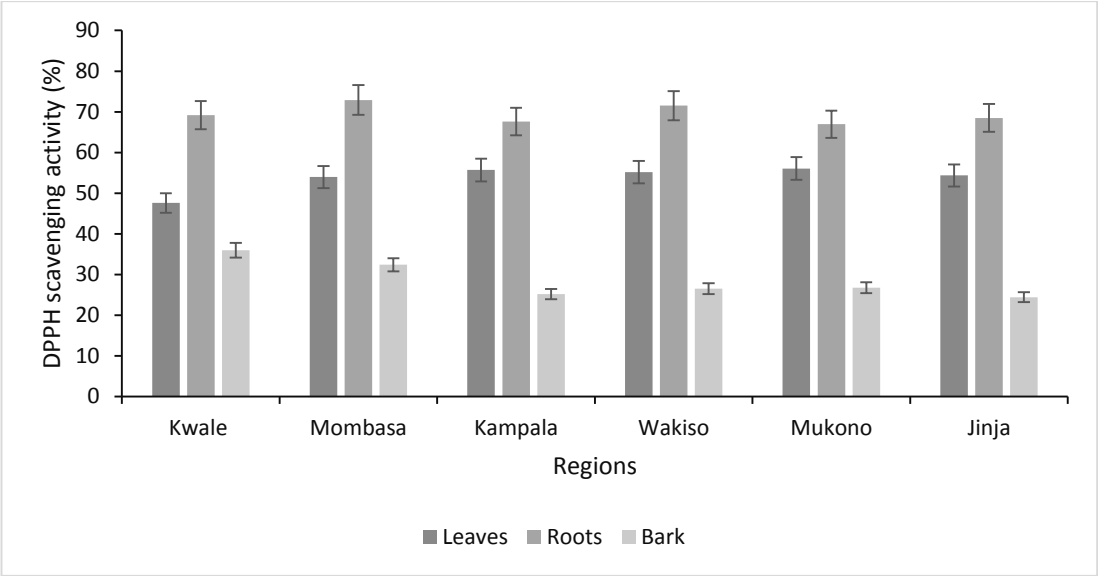


Figure 4.5. DPPH scavenging activity of Jackfruit leaves, roots, and bark samples from different regions. *The barks had significantly lower vales compared to the roots and leaves.*

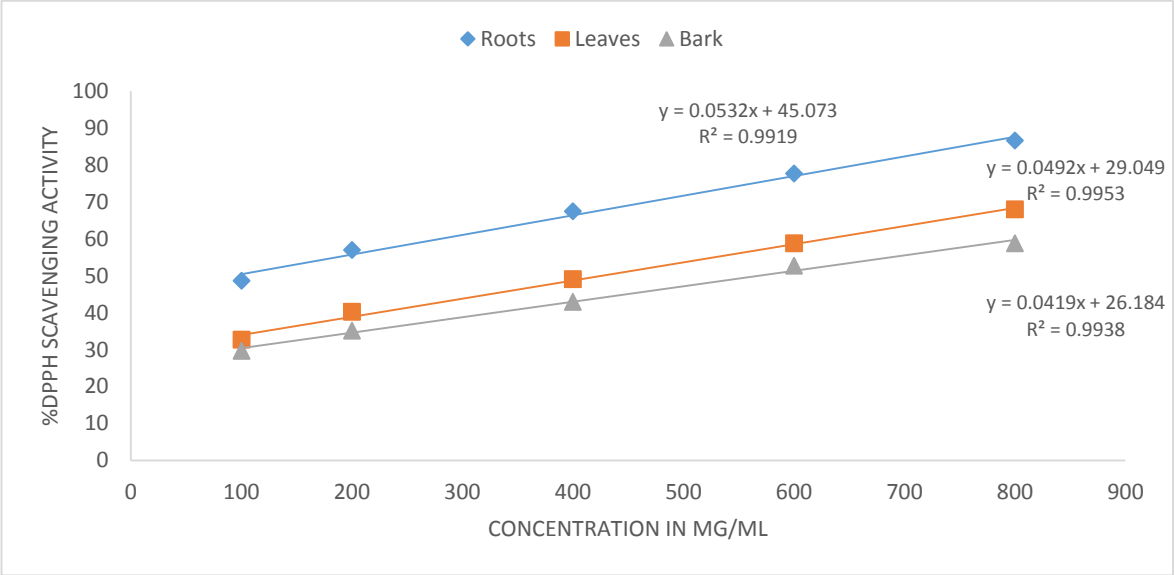


Figure 4.6. %DPPH scavenging activity of jackfruit barks, roots and leaves at different concentrations. The roots had higher scavenging activity, followed by leaves and the bark had the least activity.

4.3.8 Reducing power assay

The calibration graph for ferric reducing assay was generated, the R^2 value is 0.9917 and the equation for the graph is y = 0.0212x - 0.224. The reducing power was found to be significantly high in the roots, with values of up to 114.38 ± 6.92 µg/ml, the leaves had values of up to 71.63 ± 4.07µg/ml. The bark on the other hand, had the least values that ranged from 31.47 to 54.16 µg/ml. The reducing powers were also found to be significantly different at (*P=.05*) for the leaves, roots and bark. The roots had the highest values of up to 123 ± 2.39 µg/ml. Followed by leaves, which were up to 71.63 ± 4.07 µg/ml and the bark had the least, which were up to 31.47 ± 0.88 µg/ml as shown Figure 4.7**.**

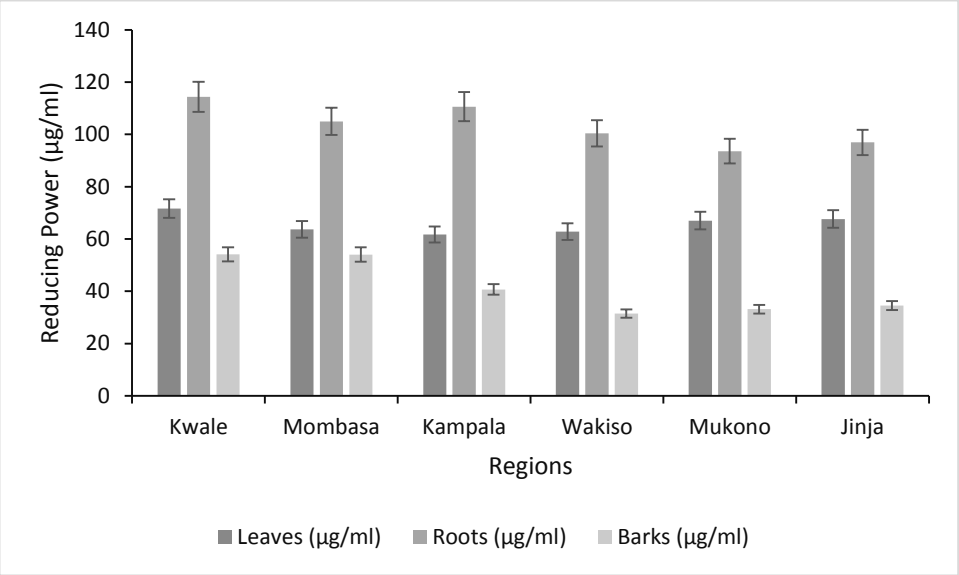


Figure 4.7. The reducing power values of Jackfruit leaves, roots and bark extracts**.** The roots had significantly higher reducing power compared to the leaves and bark.

4.3.9 Correlation matrix of phenolic, flavonoids, tannins, DPPH and reducing power

A correlation matrix showing the inter-correlation between phenolic, tannins, flavonoid content, DPPH activity and Ferric reducing is as shown in table 4.5. The

correlation between phenolic content and reducing power had a higher value compared to that of flavonoid, tannins and DPPH. The correlation between flavonoid content and DPPH scavenging activity also showed a higher correlation compared to that of phenolic and tannins. Phenolic compounds, flavonoid and tannin contents also showed a lower correlation with each other. Reducing power showed least correlation with DPPH compared to the rest, though the correlation values were still significantly high implying that the two are correlated. In general all the values were above 0.9 indicating high inter-correlation between phytochemical composition and antioxidant activities.

Table 4.5. Correlation matrix of phenolic, flavonoids and tannins, DPPH and reducing power

	Phenolic	Flavonoids	Tannins	DPPH	Reducing power
Phenolic	1	0.9624	0.9584	0.9319	0.995
Flavonoids	0.9562	1	0.9999	0.9972	0.9804
Tannins	0.9584	0.9999	1	0.9967	0.9819
DPPH	0.9319	0.9972	0.9967	1	0.9632
Reducing power	0.9951	0.9804	0.9819	0.9632	1

4.4 Discussion

4.4.1 Phytochemical composition of the jackfruit leaves, barks and roots from different regions

The roots had the highest composition of phytochemicals and antioxidant activities, followed by leaves, and the barks had the lowest. This could be because, the roots are more exposed to the pathogens and microorganisms in the soil. The phytochemical compounds may therefore be higher in roots for 'self-protection' purposes against micro-organisms, predators and pathogens (Abu Bakar et al., 2015). The difference in phytochemical composition was also found to vary in some regions. This may be attributed to difference in altitude of the regions. Altitude has been found to play a role

in influencing the composition of the phytochemicals. The phytochemical composition has been found to increase with increase in altitude (Owuor et al., 1990).

The difference in phytochemical composition and antioxidant properties of the different parts, may also be attributed to genetic factors. The genes of some plants may tend to code for more phytonutrients in the roots, while others may express more compounds in the leaves and other parts (Andrew et al., 2010). This may explain why in all regions, the phytochemical composition of the roots was much higher compared to those of the leaves and the fleshy parts. In an environment, where there are many pathogens that affect the leaves, they are likely to have more phytochemical properties compared to the other parts (Andrew et al., 2010).

The phytochemical composition values, were much higher compared to those found in the seeds and fruits of jackfruit from other studies. A study by Shanmugapriya et al. (2008), found the total phenolic and flavonoid content of the seeds to be 4.16mg/g and 4.05mg/ml in ethanolic extract. This could be attributed to the fact that the roots, stem bark and leaves, form the outer parts of the plant and are more exposed to micro-organisms, predators and pathogens (Abu Bakar et al., 2015). The seeds on the other hand, are covered inside the fruit hence physically protected. They however also contain relatively high levels of phytochemicals that protect the plants from internal damage (Andawulan et al., 1999).

The findings of the current study are consistent with those of Hossein et al. (2014), who found that the ethanolic extract of the roots of *Azadirachta indica*, a medicinal plant also had very high phenolic content of 123.81 mg/g. The study was also consistent with that of Eneji et al. (2011), who found that the levels of flavonoids and tannins, were higher in the root bark compared to the stem bark (twig) of *Gongronema latifolium* medicinal plant. The flavonoid content in the roots, were found to be

3.5mg/g, while the tannins content was found to be 0.25 mg/g. The twig extracts on the other hand, had 0.52 mg/g of flavonoid and 0.24 mg/ml of tannins. The study also showed that the tannins levels were generally lower compared to the flavonoid content (Eneji et al., 2011). A study by Sylvie et al. (2014) on three medicinal plant extracts *Hymenocardia lyrata*, *Garcinia lucida* and *Acalypha racemosa*, were however found to be inconsistent, as the levels of phenolic contents were found to be highest in the bark, followed by roots and then leaves. The phenolic content of the leaves was 169.782 mg/g, however the flavonoid content was consistent with the findings of this study, as the roots had the highest flavonoid content of 217.17 mg/g.

A study by Abu Bakar et al. (2015) revealed that the peels of different *Artocarpus* species had significantly higher phytochemical composition compared to the fruit and seeds. The phenolic content of *A. odoratissimus* peel was found to be 42.38 mg/g, which was significantly higher compared to that of the seed, which was 13.72 mg/g and fleshy part, which was 3.53mg/g. A study by Vinha et al. (2017), also showed that the plant peel of *Persea Americana Mill* had more phenolic and flavonoid content compared to the pulp. The phenolic and flavonoid content of the peels were 410 mg/100 g and 21.9 mg/100 g respectively. In the study the levels of phenolic compounds were also found to be significantly higher than those of flavonoid compounds, which is consistent with the findings of the study. These studies demonstrate that the phytochemical composition of different parts of the plant vary both within the same plant and from one species to another.

4.4.2 DPPH scavenging activity

The DPPH scavenging activity of the roots were found to be much higher compared to those of the leaves and barks. The roots had a DPPH scavenging activity of up to 72.91 ± 2.58%, while the stem bark showed the least DPPH scavenging activity of 26.48 ± 1.90%. The high DPPH activity of the roots can be attributed to the high composition of phytochemical compounds such as phenolics, flavonoids and tannins.

The scavenging activity of the leaves, were also relatively high. The results are consistent with those of Slyvie et al. (2014), who also found that the roots of different medicinal plants exhibited more DPPH scavenging activity compared to the bark. The scavenging activity of the roots, barks and the seeds were found to increase with increasing extract concentration. IC_{50} for the roots, barks and leaves were found to be 92.61 µg/ml, 425. 83 µg/ml and 568.40 µg/ml respectively. This shows that the roots, leaves and bark of jackfruit tree are natural antioxidants.

4.4.3 Reducing power

The reducing power of jackfruit bark, leaves and roots were found to be up to 123.21 ± 2.39 µg/ml in the roots, 71.63 ± 4.07 µg/ml in the leaves and 31.47 to 54.16 µg/ml in the bark extracts. The roots were found to have the highest activity, followed by the leaves with the bark showing the least reducing power. This can be attributed to the fact that phytochemical compounds are powerful reducing agents and since the composition of phytochemicals is high in the roots, it is likely to have high anti-oxidant properties compared to the leaves and bark. The values are much higher than those found in the seeds of jackfruit extracted in different solvents, which ranged from 10.13 to 13.12 µg/ml. This can also be attributed to the fact that the phenolics and flavonoids were much higher in the roots, barks and leaves from this study compared to the composition of the seeds in Shanmugapriya et al. (2011)

4.4.4 The correlation between phytochemical composition and antioxidant properties

The correlation matrix between phenolic, tannins, flavonoid content, DPPH activity and Ferric reducing power, showed that, there is a correlation between phenolic content and DPPH scavenging activity. The findings are similar to those of Vinha et al. (2017), who found a high level of correlation between phenolic content and DPPH scavenging activity. However, the flavonoids showed a higher correlation with DPPH

scavenging activity implying that plants that have high flavonoid contents are likely to have a higher radical scavenging activity. The findings are consistent with the study by Abu Bakar et al. (2015), which showed that there is a high level of correlation between phytochemical composition and antioxidant activities.

4.5 Conclusion

Jackfruit roots, bark and leaves have relatively high composition of phytochemicals and antioxidant properties especially in the roots, followed by leaves and then bark. The distribution of the phytochemicals and antioxidants, also vary in the different tissues as phenolics and flavonoids compositions are significantly higher than those of tannins. The different parts of the plant can therefore be used as natural antioxidants. Future studies should focus on finding the genes responsible for the difference in expression of phytochemicals in the leaves, barks and roots of jackfruit.

CHAPTER FIVE

5.0 Comparison of seed germination and DNA extraction from jackfruit leaves

5.1 Introduction

The seed dormancy of most trees from tropical regions, present a major challenge in propagation of tree seedlings in the fields. One of the factors that contributes to seed dormancy is the thick coatings that prevents, the entry of water and oxygen into the seeds (Luna et al., 2009). A number of seed pre-treatments techniques have been developed to break the seed dormancy and facilitate the propagation of the tropical trees species (Imani et al., 2011). The efficiency of the pre-treatment techniques varies, depending on the species of the seeds. Therefore, there is need to optimize the seed pre-treatment protocol for individual species.

Studies by Travlos and Economou (2006), demonstrated that Sulphuric acid can be effectively used to optimize seed germination in *Medicago arborea*. Abubakar and Maimuna (2013), demonstrated that 50% HCl can break seed dormancy and induce germination in *Parkia biglobosa* species. Hydrogen peroxide solution on the other hand, was also found to be the effective in the induction of seed germination in *Prunus* species (Imani et al., 2011). There are two ways that are commonly used in seed germination and they include planting them in pots containing soil in the green house or using moistened paper towels (Phaneendranath, 1980). The two techniques have been effectively used for germinating other seeds such as corn and sorghum (Phaneendranath, 1980).

There is no study has been reported so far in optimization of jackfruit seed germination. The information would be important for faster development of jackfruit seedlings that can be used for propagation of the plant and also preserve genetic diversity, which is not possible with techniques such as grafting. The information would also be useful in jackfruit genetic characterization studies as the fresh leaves

from the seedlings, have less accumulation of secondary metabolites, which interfere with the quality of DNA (Sudan et al., 2017).

One of the major challenges that limits Jackfruit genetic characterization studies is the difficulty in DNA extraction from the tissues because of high concentrations of secondary metabolites in the leaves (Ojwang et al., 2017; Sudan et al., 2017). The metabolites react with DNA and in turn cause shearing of the DNA. Some of the secondary metabolites also bind to the *Taq* polymerase and hence inhibit the PCR reaction process (Sudan et al., 2017). One standard technique of DNA extraction from plants has also been found not to work for most of these crops (Doosty et al., 2012). There is therefore need for optimization of DNA protocol for individual plants especially those high in secondary metabolites. Furthermore, most protocols suggested also employ the use of liquid nitrogen and β-mecarptoethanol, which are volatile, costly and hazardous (Sika et al., 2015). Liquid nitrogen on the other hand, has been associated with Asphyxiation, asthma attacks and cryogenic burns (burns that are caused by liquids that are extremely cold) (McBride, 2003).

Doosty et al. (2012) compared the efficiency of four DNA extraction protocols for a medicinal plant called *Satureja khuzistanica*. The study found that only one of the protocols, yielded good quality DNA when the protocol was optimized with some modifications, while the rest yielded degraded DNA. A different study by Arruda et al. (2017), optimized the extraction of DNA from *Mimosa tenuiflora* plants, which are high in phytochemicals. The study used 8 different protocols and only one protocol was found to yield good quality DNA after a few modifications. This means that the efficiency of DNA protocols varies depending on the plant and there is therefore need to optimize DNA extraction in plants high in phytochemicals. This study sought to optimize the protocol for seed germination and DNA extraction of jackfruits.

5.2 Materials and Methods

5.2.1 Initial sampling for DNA extraction

Leaves for DNA extraction were collected from different regions of Kenya and Uganda. In Kenya, the initial sampling was done in Kwale, Mombasa, Siaya and Kilifi counties and a total of 41 trees were sampled. In Uganda, the sampling was done in Kampala, Wakiso, Mukono and Jinja districts and a total of 79 trees were sampled. The Kenyan regions were selected because they were areas where jackfruits are mostly grown. The sampling from the trees was done at a distance of at least 2 to 5 km apart, though in some regions, the distance covered was longer due to scarcity of the trees. The young leaves obtained from mature trees were quickly placed in airtight zip-lock polythene bags containing silica gel and transported for storage at -80 ºC until use. The study was carried out at the Nairobi University, Chiromo campus, Biochemistry department and at the Centre for Biotechnology and Bioinformatics (CEBIB).

The leaves from the different regions were then used for DNA extraction using a number of DNA extraction techniques. The leaves collected yielded degraded DNA, which necessitated resampling of the fruits with aim of using the seeds for germination and acquisition of young leaves. The germination of the seeds was also a challenge and this necessitated the development of an optimization protocol for the seeds Extraction of DNA from the young leaves using different protocols also yielded poor quality DNA. The optimization of the extraction protocol was also carried out, which eventually yielded good quality DNA.

5.2.2 Re-sampling and Germination of jackfruit seeds

A total of 3 - 4 fruits were sampled from selected regions in Kenya and Uganda. The fruits were sampled from Kampala, Wakiso and Mbale regions of Uganda. In Kenya, the fruits were sampled from Kwale (Ukunda, Lunga lunga, Kikoneni, Msambweni), Mombasa, Muranga, Busia and Ugenya counties. Obtaining mature and ripe fruit from

the trees was a major challenge compared to sampling the leaves because of the drought that affected some areas causing the fruits not to grow. The mature fruits were also quickly harvested and sold to the vendors and it was therefore difficult to find them on the trees. Mature fruits were cut and seeds obtained and grouped into four categories. The study also sought to include more regions in Kenya that had not been initially included during the first sampling to get a clearer picture of the genetic diversity of the fruits in Kenya, which was the main focus of the study. Some of the jackfruit samples used (Figure 5.1).

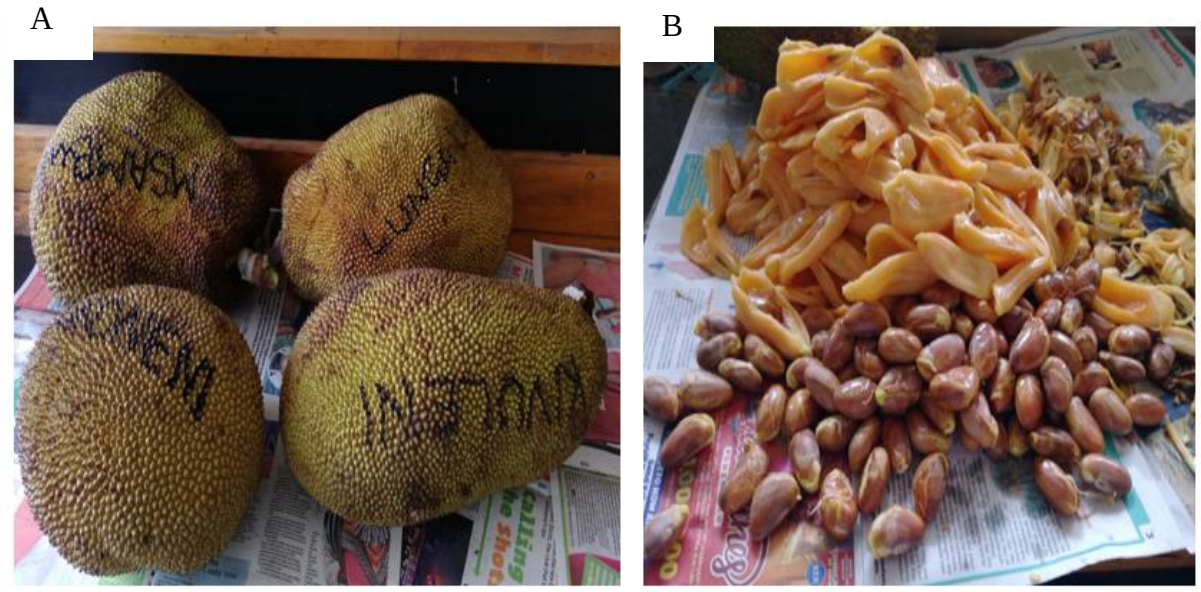


Figure 5.1. The jackfruit samples, seeds and fleshy part. Figure A shows some of the samples collected from the different regions, Figure B shows the fleshy pulp region and the seeds obtained from the plants.

Germination experiments were conducted in four different groups of seeds under different treatments. The first group was soaked overnight in 3% Sulphuric acid, the second category was soaked in 3% HCl, the third one was soaked in 3% hydrogen peroxide and the last group was soaked in distilled water overnight. In each of the four categories, the seeds were further divided into two sub-groups. The first group was planted in triplicate in the green house in pots using autoclaved soil. The other group was wrapped in paper towels and inserted in zip lock bags and then moistened with:

3% Sulphuric acid (group 1), 3% HCl (group 2), Hydrogen peroxide (Group 3) and distilled water (Group 4) (Figure 5.2) and placed in an adequately lit location and allowed to germinate until the leaves and roots had formed.

Figure 5.2. An illustration of the seeds wrapped in paper towels and moistened with different solutions

The plants were examined for several weeks and the duration taken by each group of seeds to germinate recorded. The germinated plants were then collected and transplanted in the green house. The leaves for DNA extraction were then collected from the samples that were germinated using distilled water for all the regions. The results obtained were then compared to those of the leaves sampled from mature trees and the results were noted.

5.2.3. DNA extraction protocols

5.2.3.1 Protocol 1 (DNA extraction kit)

DNA from young leaves sampled from mature trees was extracted using Primeprep genomic DNA extraction kit for plants (GeNet Bio, Korea) following the protocol outlined by the manufacturer. Briefly, 0.1g of sample was ground using pestle and mortar. A volume of 400 µl of TL buffer and 40 µl of the enzyme was then added and the mixture incubated at 65^{o}C. A volume of 100 µl of PPT buffer was then added and the mixture incubated at 4^{o}C for 5 minutes. The mixture was then centrifuged at 18098 g and 400 µl of lysate transferred to new eppendorf tubes. Two volumes of GB buffer was then added and mixed and 700 µl of the mixture. They were then put in spin columns inside a collection tube and centrifuged for 1 minute at 18098 g. The flow through was discarded and 500 µl of GW1 buffer was then added and the mixture spanned for 1 minute at 11084 g. The flow through was again discarded and a GW 2 buffer was then added and the mixture spanned for 1 minute at 11084 g rpm. A volume of 100 µl of GE buffer was then added to the spin column and the mixture spanned for 1 minute at 11084 g. The DNA extracts collected were then run on 1% agarose gel electrophoresis and viewed under UV light to determine the presence and quality of DNA extracted.

5.2.3.2 Protocol 2: CTAB-SDS based DNA extraction method

The leaves obtained from the field and those from seeds germinated in the green house, were used for DNA extraction. The CTAB extraction buffer (100 mM Tris-HCl (pH 8), 1.4 M NaCl, 0.2 M EDTA (pH 8), 4% (w/v) PVP and 2% (w/v) CTAB) was heated at 60°C for 20 minutes in a water bath. A mass of 100 mg of deveined leaves were then added to pre-chilled mortar and pestle. A volume of 650 µl of CTAB buffer and 15 µl of 20% (w/v) SDS were then added and the de-veined leaves crushed using pestle and mortar. The samples were then transferred to 1.5 ml microtubes and incubated at 55 °C in a water bath. The mixture was then allowed to cool for 10 minutes and an equal volume of chloroform-isoamyl alcohol (24:1) added. The mixture was then

centrifuged at 16060 g for 15 minutes and the upper layer transferred into a new microtube. An equal volume of chloroform-isoamyl alcohol (24:1) was again added and mixture re-centrifuged for 15 minutes at 16060 g. The upper phase was removed and 50 µl of 7.4 M ammonium acetate and 2 volumes of ice cold absolute ethanol added. The samples were incubated at -20 °C overnight for precipitation of DNA and centrifuged at 9503 g for 10 minutes. The supernatant was discarded and 70% ethanol added to the DNA pellets and the mixture centrifuged at 9503 g for 5 minutes and the washing step repeated. The samples were then dried by invertion in paper towels for two hours and the pellet of DNA dissolved in 50 µl of sterile distilled water. A volume of 1 µl of RNase was then added to each of the DNA samples and incubated for 30 minutes. The samples were then run on a 1% gel electrophoresis at 60V for 1 hour and the gel viewed under UV light. The protocol used was based on (Dellaporta et al., 1983) and (Doyle and Doyle, 1990) with a few modifications on volumes of reagents used for CTAB buffer, and duration of centrifugation.

5.2.3.3. Protocol 3: SDS- LiCl based protocol

Deveined leaves from mature trees and from germinated seedlings were weighed and 0.2g of the leaves crushed in 750 µl of extraction buffer (50mM Tris-HCL pH8, 10mM EDTA pH8, 2% SDS and 100mM LiCl) and 10 µg/ml of Proteinase K using pestle and mortar. The mixture was then transferred to 1.5 ml microtubes and vortexed for 5 minutes. The samples were then centrifuged at 16558 g at 4 ^{o}C for 15 min and 500 µL of the resultant supernatant transferred in a 1.5 ml microtube. An extra 750 µl of extraction buffer and 10µg/ml of Proteinase K was again added to the pellet and centrifuged at 16558 g at 4 ^{o}C for 15 min. The supernatant was transferred to a new microtube and 5µl of 20µg/ml RNase added. The sample was then incubated for 30 min at 37 ^{o}C before addition of 1ml of phenol (pH 8) and the mixture vortexed and centrifuged at 16558 g at room temperature. The aqueous upper layer was removed and mixed with 1 ml Phenol: Chloroform 1:1 v/v, in a new microtube, vortexed and centrifuged for 5 minutes. This was then followed by addition of 20µL, 10M

ammonium acetate and 1.5mL of cold absolute ethanol and the tube inverted severally. The samples were then incubated at -20ºC for 20 minutes and centrifuged at 16558 g at 4 ºC for 30 min. The pellets were washed twice in 70% ethanol, dried and dissolved in distilled water. The protocol was adapted from (Arida et al., 2010), with a few modifications on volumes of reagents used.

5.2.3.4 Protocol 4: Sucrose based protocol for plants high in secondary metabolites

A volume of 850 µl of SGS buffer {Tris HCl (100 mM, pH 8.0), EDTA (75 mM, pH 8.0), NaCl (0.5 M), sucrose (0.5 M), glycerol (2%), and SDS (2%); double distilled water} that had been previously warmed in a water bath at 65ºC for 20 minutes was transferred to a microfuge tube. A mass of 100 mg of fresh leaves were and crushed using pestle and mortar into fine particles and transferred into microfuge tubes containing the extraction buffer. The tubes were then vortexed and incubated at 65°C in a water bath for 40 minutes with mixing done after every 5 minutes. The tubes were then removed from the water bath and cooled for 2 minutes before addition of 700 µl Chloroform isoamyl alcohol (24:1), mixed and the sample centrifuged for 10 minutes and the process repeated. The supernatant was then transferred to a clean microfuge tube and 5 µl of RNase added. The mixture was incubated in a water bath for 40 min. Approximately 700 µl of chloroform isoamyl alcohol (24:1) was again added and the mixture centrifuged at 16060 g for 5 minutes. The supernatant was then transferred to a clean tube and 1 ml of chilled ethanol added. The tube was inverted 4 times and stored at -20ºC for 10 minutes. The samples were then centrifuged at 6082 g for 12 minutes and the supernatant discarded. The DNA was then washed twice using 75% ethanol and air dried before dissolving the DNA pellets in 100 µl of TE buffer (Sudan et al, 2017).

5.2.3.5. Protocol 5a: Modified CTAB-SDS based protocol for DNA extraction of plants high in phytochemicals

The CTAB extraction buffer (100 mM Tris-HCl (pH 8), 1.4 M NaCl, 0.2 M EDTA (pH 8), 4% (w/v) PVP and 2% (w/v) CTAB) was heated at 60°C for 20 minutes in a water bath. A mass of 0.1g of leaves were then added to a pre-chilled pestle and mortar. A volume of 650 µl of CTAB buffer and 150 µl of 20% (w/v) SDS were then added and the de-veined leaves crushed. The samples were then transferred to 1.5 ml microfuge tubes and incubated at 60 °C in a water bath for 20 minutes. The mixture was allowed to cool for 10 minutes and 1.5 times the volume of phenol chloroform was then added and the mixture inverted severally to mix well. The mixture was centrifuged at 16060 g for 10 minutes and the supernatant transferred to a new micro-centrifuge tube and the process repeated two more times. Chloroform-isoamyl alcohol (24:1) was then added and the mixture inverted severally to mix well. The mixture was centrifuged at 16060 g for 10 minutes and the supernatant transferred to a new microfuge tube and the process repeated two more times. The supernatant was again transferred into a new tube and 1.5 volumes of isopropanol was then added to the mixture inverted severally to mix and then kept at -20^{o}C overnight for DNA precipitation. The samples were then centrifuged for 5 minutes and air dried. A volume of 500 µl of NaCl and 1 µl of RNase were then added to the sample and incubated at 37^{o}C for 30 minutes. Isopropanol was again added to the sample and DNA allowed to precipitate for 30 minutes. The mixture was then washed and dried. The extracted DNA was dissolved in 50 µl of double distilled water. The samples were then electrophoresed on a 1% agarose gel for 1 hour and the gel viewed under UV light.

5.2.3.6 Protocol 5b

In this protocol everything was similar to that of protocol 5a and the adjustment were made in the number of times and volume of phenol chloroform and chloroform isoamyl was used in each extraction as shown in Table 5.1.

Table 5.1. Difference in the CTAB-SDS method and the modified protocols for DNA extraction from jackfruit leaves

Steps	Protocol 2 **CTAB-SDS based DNA extraction method**	Protocol 5a: **Modified CTAB-SDS based protocol for DNA extraction of plants high in phytochemicals**	Protocol 5b
Phenol chloroform step	Not used	Repeated 3x (x1.5 volume of sample)	Repeated 4x (x1.0 volume of sample)
Chloroform Isoamyl step	Used twice (x1.0 volume of sample)	Repeated 3x (x1.5 volume of sample)	Repeated 4x (x1.0 volume of sample)
Plain chloroform	Not used	Used once (x1.5 volume of sample)	Used once (x1.0 volume of sample)

5.2.4. Polymerase Chain Reaction (PCR) amplification

Primers were used in evaluating the quality of DNA extracted. The PCR reactions were performed using a thermocycler (Veriti Applied Biosystems, United States). Each PCR reaction consisted of 4 µl of 5× PCR reaction buffer, 1 µl of $MgCl_2$, 0.5 µl of 10 mM dNTPs, 1 µl of each forward and reverse primer, 0.2 µl of Taq DNA polymerase (Biolabs, England), 2 µl of 10 ng DNA and sterile water was added to a final reaction volume of 20 µl. The PCR reaction profile was: initial denaturation at 94 °C for 5 minutes followed by 35 cycles of denaturation at 94 °C for 1 minute, primer annealing ranged from 46 -50 °C (depending on the primer) for 45 seconds, extension at 72 °C for 1 minutes and final extension at 72 °C for 7 minutes. The PCR products were separated through electrophoresis in 1.0% agarose gels stained in ethidium bromide and viewed in UV light and gel doc XR (Bio-Rad, USA).

5.3. Results

5.3.1 Jackfruit seed germination

The results from the 4 sets of germination experiments were as shown in Table 5.2. The paper towel wrapped and green house planted samples took almost the same duration to germinate. However, the samples in the green house produced leaves faster than the paper towel wrapped samples, which were then transplanted into the glasshouse after germination. The hydrogen peroxide samples showed a faster rate of germination compared to the samples soaked overnight in 3% distilled water and 3% HCl. The samples that were soaked in 3% sulphuric acid did not germinate. The leaves obtained from the distilled water seedlings were then harvested and used for DNA extraction. Both the young and mature leaves of the seedlings were as illustrated in Figure 5.3 and 5.4.

Table 5.1. Germination period of pre-soaked Jackfruit seeds in different solutions

Sample	**Germination**	**Duration**
3% Sulphuric acid	No	12 weeks
3% HCl	Yes	12 weeks
3% Hydrogen peroxide	Yes	4 -6 weeks
Distilled water	Yes	6-8 weeks

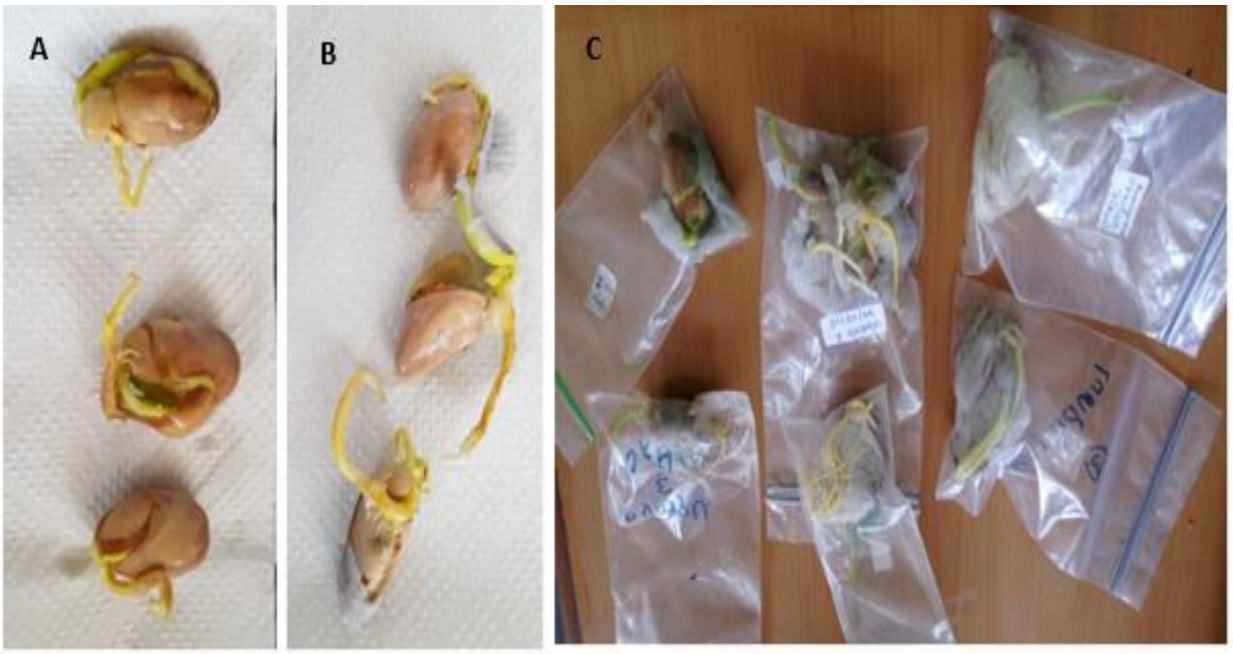


Figure 5.1. Germination of seeds wrapped in paper towel. A and B are the germinated seeds that were soaked in distilled water and H_2O_2 respectively. C represents germinated samples while still inside the zip lock bags.

Figure 5.2. Germinated Jackfruit seeds in the green house. 4A shows the initial germination process, while (B), (C) and (D) shows the kind of leaves used for DNA extraction.

5.3.2 Comparison of DNA extraction protocol

DNA extraction was carried out using five different techniques namely: Extraction kit, CTAB-SDS based, SDS-LiCl based, Sucrose based and Modified CTAB-SDS based and the extracted DNA profiles are shown in Figure 5.3. The first four protocols yielded sheared DNA, which appeared as smears on the electrophoresis gel, while the modified CTAB-SDS based DNA protocol yielded sharp distinct bands. PCR analysis using the DNA from protocols with sheared DNA did not show any bands, when the PCR products were run on a 1 % gel electrophoresis while PCR amplification of DNA from modified protocols yielded amplicons (Figure 5.4). The DNA extracted using Protocol 5 (a) was chosen for further PCR analysis because it had brighter DNA bands

compared to protocol 5 (b) (Figure 5.5). Additionally, the 5 (a) modified protocol was further used for PCR analysis of jackfruit samples from different regions using SSR AH 31 marker and the results were as shown in Figure 5.6.

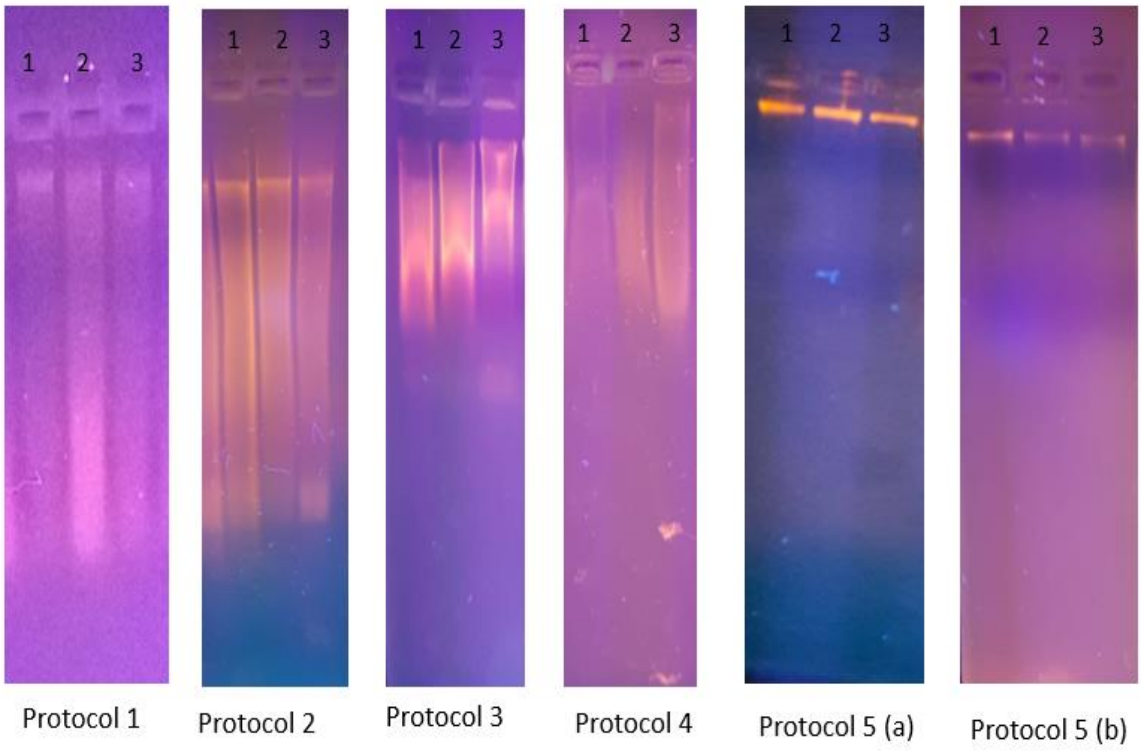


Figure 5.3. Jackfruit DNA analysis. DNA extraction results for different protocols; Protocol 1 DNA extraction Kit, Protocol 2 CTAB-SDS, Protocol 3 SDS-LiCl, Protocol 4-sucrose based and protocol 5 a and b (Modified CTAB-SDS based protocol)

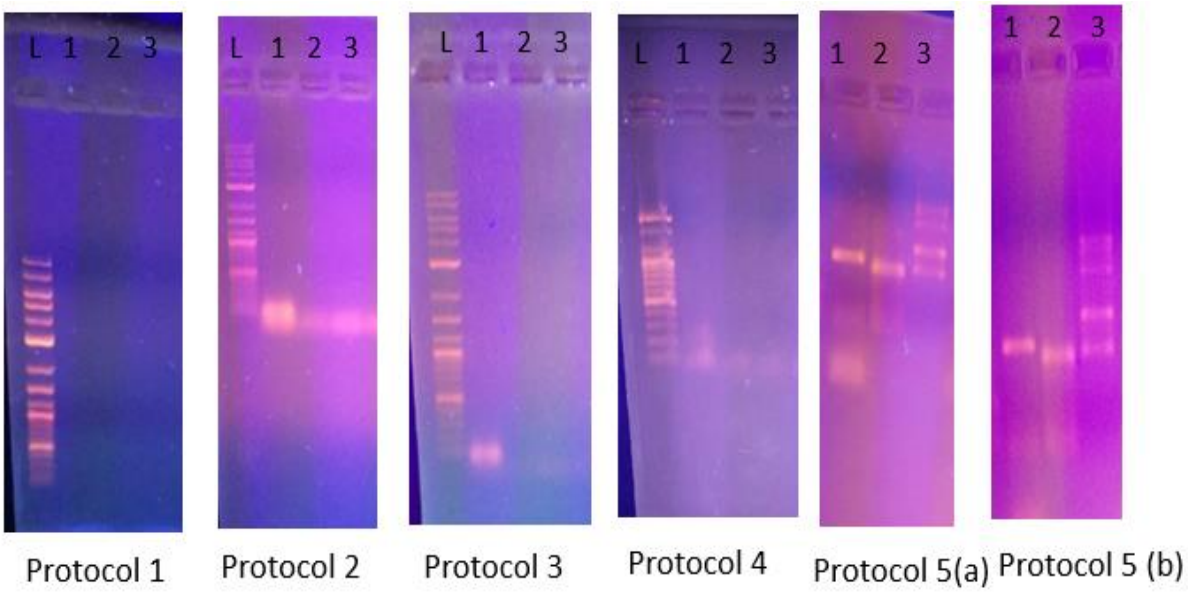


Figure 5.4. PCR analysis results for Jackfruit DNA templates extracted using five different protocols with SRR primers and Muranga samples. *Protocol 1 (DNA extraction Kit), Protocol 2 (CTAB-SDS), Protocol 3 (SDS-LiCl), Protocol 4 (sucrose based) and protocol 5 (Modified CTAB SDS based protocol).*

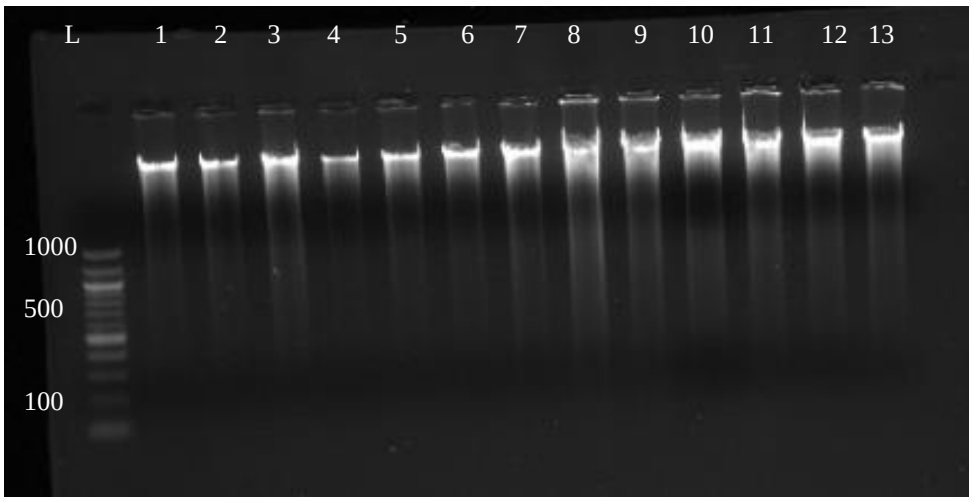


Figure 5.5. Jackfruit DNA templates extracted from five different regions. Muranga (1-3), Ukunda (4-6), Kampala (7-9), Mbale (10-12) and Ugenya (13) using modified CTAB-SDS based protocol 5 (a), and L is ladder.

Figure 5.6. PCR amplification of Jackfruit DNA templates. Muranga (1-3), Ukunda (4-6), Kampala (7-9), Mbale (10-12) and Ugenya (13-15) extracted using protocol 5 (a) and amplified using SSR AH 31, and L is ladder.

5.4 Discussion

5.4.1. Jackfruit seed germination protocols

The samples that were wrapped in moistened paper towels and those planted in the green house after soaking were found to germinate at almost the same time. This implies that both techniques have the same efficiency in stimulating the germination of jackfruit seeds**.** The samples in the green house produced leaves faster than the paper towel wrapped samples. This may imply that planting the plants in the soil provided more optimum conditions for seedling development than the zip lock bags. Soil contains more nutrients that are required for plant growth and it is therefore advisable to transplant them immediately after germination. The wrapped paper towel, is a good alternative for laboratories with low resource settings without a greenhouse. The young leaves can be used for DNA extraction in genetic characterization analysis.

Amongst all the solutions that were used in seed pre-treatment, 3% hydrogen peroxide was found to be the most effective as seeds derived from the same fruit took a shorter time to germinate compared to those pre-treated using distilled water, 3% HCl and water. Hydrogen peroxide acts as a signaling molecule that stimulates germination

through reactivation of metabolic pathways and regulation of phytohormones (Wojtyla et al., 2016). The solution is also mild and hence did not cause cell death while increasing the seed permeability enabling cell multiplication and germination (Imani et al., 2011).

Distilled water was also found to facilitate seed germination, however it took more time compared to hydrogen peroxide. This may be attributed to the fact that the seeds pre-treated with distilled water are more susceptible to fungi. Infestation of the seeds with fungi, reduces the capacity of the seeds to germinate and may also lead to abnormal seedlings (Szopinska, 2014). Hydrogen peroxide has been found to reduce the infestation of the seeds with fungi, high concentration however has been found to have a negative effect on plant growth (Szopinska, 2014). Hydrogen peroxide also has high oxidative reactivity that may play a role in increasing the permeability of water and oxygen in the seeds. The compound is also naturally produced by plants during distress, which makes it ideal compared to other solutions used to stimulate germination and it also has antimicrobial properties (Szopinska, 2014).

Seeds pre-treated with 3% HCl took much longer time to germinate, which implies that 3% HCl is less effective in stimulating germination of the jackfruit seeds. This may be because, the solution is a strong acid that may have caused denaturation or death of some of the cells present in jackfruit seeds and this may have reduced their ability to germinate at a faster rate. Seeds that were treated with 3% Sulphuric acid on the other hand did not germinate completely. This may be because this acid, may have caused seed death and hence no germination took place (Imani et al., 2011). The use of these acids in optimization of jackfruit germination is therefore not a suitable option.

Hydrogen peroxide and distilled water are suitable for jackfruit seed germination. The optimization of germination of jackfruit seeds has not been reported yet but the findings were consistent with Imani et al. (2011), who optimized germination of *Prunus* species seeds hydrogen peroxide. The findings were however inconsistent with

Travlos and Economou (2006), who used Sulphuric to optimize seed germination in *Medicago arborea* and Abubakar and Maimuna et al. (2013) who used HCl to optimise seed germination in *Parkia biglobosa* seeds. Conversely, sulphuric acid and HCl, have been effectively used in optimization of seed germination of other plants such as *Medicago arborea* (Travlos and Economou, 2006), and *Parkia biglobosa* (Abubakar and Maimuna, 2013). Therefore, the protocols of seed germination, vary from one seed to the other depending on the type of plant.

5.4.2. Comparison of DNA extraction methods

The Extraction kit, CTAB-SDS based, SDS-LICL based and Sucrose based technique yielded degraded DNA for both young leaves obtained from mature trees and those obtained from the seedlings of jackfruit tree. This implies that the techniques were not effective in removing secondary metabolites and essential oils, which are abundant in jackfruit leaves and are known to interfere with the quality of the DNA extracted. The DNA pellets were mostly characterized by brown pigmentation during precipitation implying a co-extraction of DNA with phytochemicals (Sudan et al., 2017).

The modified CTAB-SDS based protocol was found to produce very good quality DNA that yielded good results in PCR analysis for the leaves obtained from the seedlings. The modified protocol included the use of additional steps of sample extraction in phenol chloroform (3-4 times) and chloroform isoamyl alcohol (3-4 times) and finally an extraction in plain chloroform. The repeated steps of phenol chloroform and chloroform isoamyl alcohol may have facilitated the removal of the secondary metabolites and essential oils. The protocol also does not employ the use of beta-mercapto-ethanol and liquid nitrogen.

A few studies have been conducted on genetic characterization of jackfruit (Schnel et al., 2001; Shyamalamma et al., 2008; Gopalsamy et al., 2012). However, the protocols used required the use of liquid nitrogen and beta-mecapto-ethanol. Liquid nitrogen is quite costly and very volatile and may not be available in labs that have low funding.

The use of beta-mecarpto-ethanol for plants in DNA extraction is also quite uncomfortable as the reagent is highly volatile and has a very strong repulsive smell. The reagent has also been found to be toxic, when inhaled. The DNA extraction protocol for jackfruit extraction that does not employ the use of these reagents and therefore minimizes the exposure of the individual carrying out DNA extraction to hazardous chemicals and also reduces the cost of DNA extraction.

5.5 Conclusion

The use of 3% hydrogen peroxide in seed pre-treatment of jackfruit is therefore more effective compared to the use of 3% sulphuric acid and 3% HCl in optimization of jackfruit seed germination. The SDS-CTAB based protocol for DNA extraction of jackfruit with more repeated steps of phenol chloroform and chloroform iso-amyl is effective in extraction of DNA, without using beta-mecarptoethanol and liquid nitrogen.

CHAPTER SIX

6.0 Genetic characterization of jackfruit using Simple Sequence Repeats (SSR) and Sequence-Related Amplified Polymorphism (SRAP) markers

6.1 Introduction

Biodiversity can potentially contribute in minimizing the current food and nutrition insecurity challenges especially in developing countries. One of the problems facing the modern-day farming is the focus is mostly on crop yield and little attention is paid to the adaptive capacity of plants to harsh environment. Genetic diversity studies can enhance the monitoring of how genetic variation is affected by environmental factors. Urbanization and overpopulation are some of the factors that have heavily contributed to loss of biodiversity in plants. The knowledge of genetic diversity of plants can facilitate the preservation and propagation of superior species that are at risk of extinction (Govindaraj et al., 2015). Jackfruit (*Artocarpus heterophyllus*) is one of the underutilized plants that has the potential to alleviate food and nutrition insecurity especially in developing countries (Williams and Haq, 2002; Gajanana et al. 2010; Khan et al. 2010; Jagtap and Bapat 2010; Swami et al. 2012). To help in the popularization of the fruit and its improvement programs, knowledge about the extent of genetic diversity of the crop is an important pre-requisite.

Evaluation of genetic diversity and genetic relationships within the plant can provide useful information for utilization in breeding programs (Mathithumilan et al., 2013). The untapped valuable genetic diversity of jackfruit is yet to be fully utilized for improved food and nutrition security. A few studies have been conducted on genetic characterization of jackfruits. These studies have been carried out based on amplified fragment length polymorphism (AFLP) and randomly amplified polymorphic DNA (RAPD) markers and SSR markers. The findings showed that the jackfruits, have moderate genetic diversity (Schnell et al., 2001; Gopalsamy et al., 2012; Nakintu et al., 2019). However, the RAPD markers have been found to be non-reproducible and

are not transferable, whereas AFLP requires more time and high quantity of DNA. These challenges can be overcome by using sequence-related amplified polymorphism (SRAP) markers.

Simple sequence repeat (SSR) markers are one of the most effective molecular markers used in DNA fingerprinting. These markers are mostly used to amplify specific genes in the genome and are highly conserved in most plants. The markers are preferred for genotyping of plants because: they are multiallelic codominant markers, transferable, highly reproducible and have a wide genome coverage (Kumar et al., 2010). SSR markers have been previously used for genetic characterization studies of plants including mango, watermelon, potato, Mulberry and Jackfruits (Kumar et al., 2013; Hwang et al., 2011; Mathithumilan et al., 2013; Rocha et al., 2010); Nakintu et al., 2019). However, the characterization of Jackfruits in Kenya using SSRs has not been reported. Another molecular marker that is simple and cheaper to use for high-throughput diversity analysis is Sequence-related amplified polymorphism (SRAP). It is also one of the highly recognized markers because of its codominant nature, reliability, amplifies the open reading frames (ORFs) in a genome with reasonable throughput rate (Li and Quiros, 2001). The SRAP markers have been successfully used in genetic characterization of *Brassica oleracea,* cucumber, sugar cane and Cucurbits (Li and Quiros, 2001; Ferriol et al., 2003; Meng et al., 2012; Qian et al., 2006; Shiram et al., 2017).

Genetic characterization of jackfruit using SRAP markers has not been reported in Kenya and few studies have been conducted using SSR in Uganda. This study sought to determine the genetic diversity of jackfruits cultivated in Kenya and Uganda using SSR and SRAP markers. The information obtained from this research, will be useful in future identification of superior genotypes that can be used in breeding programs to minimize food and nutrition insecurity in Kenya and Uganda.

6.2 Materials and Methods

6.2.1 Plant materials

A total of 30 fruits samples of jackfruit were obtained from selected regions in Kenya and Uganda. The fruits were sampled from Kampala, Wakiso and Mbale regions of Uganda. In Kenya, the fruits were sampled from Kwale (Lunga, Kikoneni, and Msambweni), Mombasa (Kisauni and Ukunda), Muranga, Busia and Siaya Counties of Kenya. The samples were collected from different trees with a distance of at least 2 to 5 km apart within the same region, which was also dependent on the fruit availability. Seeds were then extracted from the fruits, soaked overnight in distilled water and germinated in the green house. The leaves from the seedlings were then used for DNA extraction.

6.2.2 Extraction of genomic DNA

Young leaves were used for genomic DNA extraction according to CTAB protocol described by (Dellaporta et al., 1983) and (Doyle and Doyle, 1990) with modifications. The CTAB extraction buffer (100 mM Tris-HCl (pH 8), 1.4 M NaCl, 0.2 M EDTA (pH 8), 4% (w/v) PVP and 2% (w/v) CTAB) was heated at 60 °C for 20 minutes in a water bath. Approximately 0.1 g of leaves without midrib were grinded in 650 µl of pre-warmed CTAB buffer and 150 µl of 20% (w/v) SDS in a pre-chilled mortar and pestle. The homogenates were transferred to 1.5 ml microfuge tubes and incubated at 60 °C in a water bath for 20 minutes. The mixture was allowed to cool for 10 minutes, 1.5 times volume of phenol-chloroform added and the mixture inverted five to mix well. The mixture was centrifuged at 16060 g for 10 minutes and the supernatant was transferred to a new micro-centrifuge and the process repeated twice. Chloroform-isoamyl alcohol (24:1) was added and the mixture inverted five times to mix well. The mixture was centrifuged at 16060 g for 10 minutes and the supernatant was then transferred to a new microfuge tube and the process repeated twice. The supernatant was again transferred into a new tube and 1.5 volumes of chloroform was added and the mixture was inverted five to mix well. The mixture was centrifuged at 16060 g for

10 minutes and the supernatant transferred to a new microfuge tube followed by addition of 1.5 volumes of isopropanol and the mixture kept at -20 ºC overnight for DNA precipitation. The samples were then centrifuged for 10 minutes at 16060 g, the supernatant was discarded and the DNA pellets were air dried on a paper towel. The DNA pellets were dissolved in 500 µl of NaCl followed by addition of 1 µl of RNAse and the mixture incubated at 37 ºC for 30 minutes. After incubation, 1.5 volumes of isopropanol was added to the DNA samples and DNA allowed to precipitate for 30 minutes at -20 ºC. The DNA pellets were then washed twice using 70% ethanol and dried. The extracted DNA was dissolved in 50 µl of sterile water.

6.2.3 Agarose gel electrophoresis

The agarose gel was prepared by weighing 0.5 g of agarose, which was made up to 50 ml using the TBE buffer in a 100 ml conical flask. The mixture was then heated on a hot plate until all the agarose had dissolved. It was then allowed to cool for 3 minutes and 1 µl of ethidium bromide was added and the mixture swirled gently. The contents were then poured on a casting tray that had a sixteen toothed comb in place. The gel, was then allowed to cast for 20 minutes and the tray was put into an electrophoresis tank filled with the TBE buffer. A volume of 5 µl of sample was then mixed with the 2 µl of the dye and loaded into each well. A volume of 2 µl, A 100 bp ladder was also added to the first well and the electrophoresis was run at 60V for one and half hours and the gel was viewed in a gel doc XR (Bio-Rad, USA).

6.2.4 Molecular analysis

6.2.4.1 SSR-PCR amplification

6.2.4.1.1 Optimization of PCR

The sequences of the SSR primers used in this study were obtained from (Mathithumilan et al., 2013) and (Liu et al., 2016). A total of 20 SSR primers were initially optimized using two samples and only the ones that showed amplification

were selected. The optimization was done using a gradient PCR machine (Veriti Applied Biosystems, United States) by examining suitable temperatures that yielded clear and repeatable bands at 5^{o}C below and above the Tm. Six SSR markers that amplified clear and distinct bands were then selected (Table 9.1 Apendix).

6.2.4.1.2 SSR PCR protocol

The PCR reactions were performed using a thermocycler (Veriti Applied Biosystems, United States). Each PCR reaction consisted of 4 µl of 5× PCR reaction buffer, 1 µl of $MgCl_2$, 0.5 µl of 10 mM dNTPs, 1 µl of each forward and reverse primer, 0.2 µl of Taq DNA polymerase (Biolabs, England), 2 µl of 10 ng DNA and sterile water was added to a final reaction volume of 20 µl. The PCR reaction profile was: initial denaturation at 94 °C for 5 minutes followed by 35 cycles of denaturation at 94 °C for 1 minute, primer annealing ranged from 46 -50 °C (depending on the primer) for 45 sec, extension at 72 °C for 1 minutes and final extension at 72 °C for 7 minutes. The PCR products were separated through electrophoresis in 1.0% (w/v) agarose gels stained in ethidium bromide and viewed in a gel doc XR (Bio-Rad, USA).

6.2.4.2 SRAP-PCR amplification

6.1.4.2.1 SRAP-PCR optimization

The analysis of SRAP markers was done according to the procedure described by Li and Quiros (2001). The optimization was done using a total of 16 SRAP primer combinations, which were screened using two DNA samples. Nine SRAP primer combinations (Table 9.2 Apendix) with clear and distinct bands were used for analysis of samples.

6.2.4.2.2 PCR amplification using SRAP markers

PCR reactions consisted of 4 µl of 5 × PCR reaction buffer, 1 µl of $MgCl_2$, 0.5 µl of 10 mM dNTPs, 1 µl of each forward and reverse primer, 0.2 µl of Taq DNA

polymerase (Biolabs, England), 2 µl of 10 ng DNA and sterile water was added to a final reaction volume of 20 µl. The PCR reaction profile was: initial denaturation at 94 °C for 5 minutes followed 5 cycles of denaturation at 94 °C for 1 minute, primer annealing was 35 °C for 45 seconds, extension at 72°C for 1 minute. The other 30 cycles were ran at an annealing temperature of 50 °C using the same conditions and a final extension was done at 72 °C for 5 minutes. Amplification products were separated through electrophoresis in 1.0% (w/v) agarose gels stained in ethidium bromide and viewed in a gel doc XR (Bio-Rad, USA).

6.2.5 Data analysis

Only the bands that were clear, distinct and reproducible from SSR and SRAP-PCR amplifications were scored. The presence and absence of a band was scored as (1) and (0), respectively. Gene diversity, polymorphic information content (PIC), Simpson's diversity index and Heterozygosity for each SSR and SRAP markers were calculated using the PowerMarker V3.25 software. The DendroUPGMA server (Garcia-Vallvé et al., 1999) was used in calculating matrix distances (Jaccard similarity and dissimilarity coefficients). The dendrograms were constructed using Fig Tree software (Version 1.4.2). The correlation between SSR and SRAP markers genetic diversity parameters, were determined using SPSS version 19, and the Principle Coordinate analysis (PCoA) was performed using the GenAlex 6.5 software.

6.3 Results

6.3.1 Genetic diversity analysis using SSR markers

6.3.1.1. Polymorphism detected by SSRs

A total of 234 bands were amplified by the 6 SSR primers and the percentage of polymorphic bands was 40.89%. Generally, the genetic diversity values were moderate as they were found to range from 0 to 0.62. The highest genetic diversity of 0.6289 was obtained with marker AH 76, followed by marker AH 46 (0.5889). The markers AH 77 and AH 31 both had no genetic diversity as they were 0. The two markers were

consistently found to give monomorphic bands. The SSR primer's polymorphic information content (PIC) values ranged from 0 to 0.5661. The highest PIC value was obtained with marker AH 76 (0.5661), followed by AH 46 (0.5284). The PIC values for both primers/markers AH 77 and AH 31 was 0. The Simpson's diversity index was moderate and it ranged from 0 to 0.52. The observed heterozygosity ranged from 0 to 0.5 and the SSR primer AH 76 had the highest value (0.5), while AH 77 and AH 31 had the least values of 0 (Table 6.1).

Table 6.1. Characteristics of SSR markers including gene diversity, PIC, Simpson's index, observed heterozygosity and percent polymorphism among the jackfruit samples.

Marker	Gene diversity	PIC	Simpson's diversity index	Observed Heterozygosity	Polymorphism (%)
AH14	0.49	0.3705	0.43	0.433	50
AH 76	0.63	0.5661	0.52	0.500	66.67
AH 59	0.48	0.4456	0.46	0.300	66.67
AH 46	0.59	0.5284	0.49	0.433	66.67
AH 77	0.0000	0.0000	0.00	0.000	0
AH 31	0.0000	0.0000	0.00	0.000	0
Average (polymorphic)	0.546675	0.47765	0.48	0.4165	62.50%

6.3.2 Jaccard's similarity and dissimilarity coefficient using SSR markers

Jackfruit samples evaluated had high similarity coefficients that ranged from 0.46 to 1.0 (Table 6.2). Samples from the same region showed higher similarity values compared with those from different regions. For instance, Muranga 1 sample was found to be highly similar to Muranga 2 and Muranga 3 with Jaccard's similarity coefficient of 0.89 in both of them. Busia 3 sample was also found to be highly correlated to Busia 4 with a Jaccard's similarity value of 1. Kampala 2 and Kampala 3 were found to have a coefficient value of 0.91. Some samples from different regions were also found to share high similarity values. For instance, Kikoneni 1 sample was highly similar to Kivulini, Msambweni, Lunga 1 and Lunga Lunga 2, which all had a

coefficient of 0.86. The sample was also found to be highly similar to Mombasa samples with Jaccard similarity coefficient of 1. The Busia samples were also found to show high similarity with the Mbale samples and the Jaccard's similarity coefficient in this case was also 1. Busia 1 sample was also found to have high similarity to Wakiso 1 sample with similarity coefficient of 1 (Table 6.2).

The dissimilarity coefficients were moderately lower as they ranged from 0.5 to 0. Generally, more samples from the same region had lower dissimilarity values compared to samples from different regions. For instance, Kampala 2 had 0.09 dissimilarity to Kampala 3, Busia 3 had 0 dissimilarity to Busia 4. Ugenya 3 had 0.11 dissimilarity to Ugenya 4. On the other hand, Kikoneni sample 1 was found to be highly dissimilar to Wakiso 1 sample and the dissimilarity coefficient was 0.4. Lunga Lunga 2 was found to be highly dissimilar to Kampala 3 and the dissimilarity coefficient was 0.46. Samples from different regions were also found to have low dissimilarity, for Instance the Kivulini sample 1 was found to have low dissimilarity with the Mombasa samples, which were 0.14 and 0.2. Lunga Lunga samples were also found to have low dissimilarity with samples from Msambweni and Kivulini, which all had dissimilarity coefficient of 0.14 (Table 6.3).

Table 6.2. Jaccard's similarity coefficient using SSR markers for Jackfruit samples from different regions

	MU1	MU2	MU3	UK1	UK2	UK3	KA1	KA2	KA3	MB1	MB2	MB3	BU1	BU2	BU3	BU4	UG1	UG2	UG3	UG4	WA1	WA2	WA3	MO1	MO2	LU1	LU2	KIV1	M5	W1	KIK1
MU1	1.00	0.89	0.89	0.90	0.55	0.89	0.89	0.73	0.82	0.73	0.67	0.80	0.67	0.58	0.67	0.67	0.67	0.67	0.80	0.89	0.80	0.70	0.67	0.67	0.64	0.60	0.78	0.67	0.60	0.60	0.67
MU2		1.00	1.00	0.80	0.60	1.00	0.78	0.80	0.73	0.64	0.75	0.70	0.75	0.64	0.75	0.75	0.75	0.75	0.70	0.78	0.70	0.60	0.56	0.75	0.55	0.67	0.67	0.67	0.67	0.67	0.75
MU3			1.00	0.80	0.60	1.00	0.78	0.80	0.73	0.64	0.75	0.70	0.75	0.64	0.75	0.75	0.75	0.75	0.70	0.78	0.70	0.60	0.56	0.75	0.55	0.67	0.67	0.67	0.67	0.67	0.75
UK1				1.00	0.64	0.80	0.80	0.67	0.75	0.67	0.60	0.90	0.60	0.54	0.60	0.60	0.60	0.60	0.73	0.80	0.73	0.64	0.60	0.60	0.58	0.70	0.55	0.70	0.55	0.55	0.60
UK2					1.00	0.60	0.60	0.50	0.46	0.64	0.75	0.70	0.75	0.64	0.75	0.75	0.75	0.75	0.55	0.60	0.70	0.46	0.56	0.75	0.70	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.75
UK3						1.00	0.78	0.80	0.73	0.64	0.75	0.70	0.75	0.64	0.75	0.75	0.75	0.75	0.70	0.78	0.70	0.60	0.56	0.75	0.55	0.67	0.67	0.67	0.67	0.67	0.75
KA1							1.00	0.64	0.73	0.80	0.75	0.70	0.75	0.50	0.75	0.75	0.75	0.75	0.70	0.78	0.70	0.60	0.75	0.60	0.58	0.73	0.67	0.88	0.88	0.67	0.60
KA2								1.00	0.91	0.67	0.60	0.58	0.60	0.82	0.60	0.60	0.60	0.60	0.73	0.64	0.58	0.64	0.46	0.60	0.58	0.70	0.55	0.55	0.70	0.60
KA3									1.00	0.75	0.55	0.67	0.55	0.75	0.55	0.55	0.55	0.55	0.82	0.73	0.67	0.73	0.55	0.55	0.67	0.64	0.64	0.64	0.64	0.55
MB1										1.00	0.60	0.58	0.60	0.67	0.60	0.60	0.60	0.60	0.73	0.64	0.73	0.50	0.60	0.60	0.90	0.55	0.70	0.70	0.70	0.70	0.60
MB2											1.00	0.67	1.00	0.60	1.00	1.00	1.00	1.00	0.67	0.75	0.67	0.56	0.71	1.00	0.67	0.86	0.86	0.86	0.86	0.86	1.00
MB3												1.00	0.67	0.58	0.67	0.67	0.67	0.67	0.80	0.89	0.80	0.70	0.67	0.60	0.64	0.86	0.78	0.78	0.78	0.60	0.67
BU1													1.00	0.60	1.00	1.00	1.00	1.00	0.67	0.75	0.67	0.56	0.71	1.00	0.67	0.86	0.86	0.86	0.86	0.86	1.00
BU2														1.00	0.60	0.60	0.60	0.60	0.73	0.64	0.73	0.64	0.46	0.60	0.73	0.70	0.55	0.55	0.70	0.60	
BU3															1.00	1.00	1.00	1.00	0.67	0.75	0.67	0.56	0.71	1.00	0.67	0.86	0.86	0.86	0.86	0.86	1.00
BU4																1.00	1.00	1.00	0.75	0.75	0.67	0.56	0.71	1.00	0.67	0.86	0.86	0.86	0.86	0.86	1.00
UG1																	1.00	1.00	0.67	0.75	0.67	0.56	0.71	1.00	0.67	0.86	0.86	0.86	0.86	0.86	1.00
UG2																		1.00	0.67	0.75	0.67	0.56	0.71	1.00	0.67	0.86	0.86	0.86	0.86	0.86	1.00
UG3																			1.00	0.89	0.80	0.70	0.67	0.67	0.80	0.60	0.78	0.78	0.78	0.78	0.67
UG4																				1.00	0.89	0.78	0.75	0.75	0.70	0.67	0.88	0.88	0.88	0.67	0.75
WA1																					1.00	0.70	0.67	0.67	0.80	0.60	0.78	0.78	0.78	0.60	0.67
WA2																						1.00	0.75	0.56	0.55	0.67	0.67	0.67	0.67	0.50	0.56
WA3																							1.00	0.71	0.67	0.63	0.86	0.86	0.86	0.63	0.71
MO1																								1.00	0.67	0.86	0.86	0.86	0.86	0.86	1.00
MO2																									1.00	0.60	0.78	0.78	0.78	0.78	0.67
LU1																										1.00	0.75	0.75	0.75	0.75	0.86
LU2																											1.00	1.00	0.75	0.86	0.86
KIV1																												1.00	0.75	0.86	
M5																													1.00	0.86	
W1																															1.00
KIK1																															

Table 6.3. Jaccard's dissimilarity coefficient using SSR markers for jackfruit samples from different regions

	MU1	MU2	MU3	UK1	UK2	UK3	KA1	KA2	KA3	MB1	MB2	MB3	BU1	BU2	BU3	BU4	UG1	UG2	UG3	UG4	WA1	WA2	WA3	MO1	MO2	LU1	LU2	KIV1	MS W1	KIK1
MU1	0.00																													
MU2	0.11	0.00																												
MU3	0.11	0.00	0.00																											
UK1	0.10	0.20	0.20	0.00																										
UK2	0.46	0.40	0.40	0.36	0.00																									
UK3	0.11	0.00	0.00	0.20	0.40	0.00																								
KA1	0.11	0.22	0.22	0.20	0.40	0.22	0.00																							
KA2	0.27	0.20	0.20	0.33	0.50	0.20	0.36	0.00																						
KA3	0.18	0.27	0.27	0.25	0.54	0.27	0.27	0.09	0.00																					
MB1	0.27	0.36	0.36	0.33	0.36	0.36	0.20	0.33	0.25	0.00																				
MB2	0.33	0.25	0.25	0.40	0.25	0.25	0.25	0.40	0.46	0.40	0.00																			
MB3	0.20	0.30	0.30	0.10	0.30	0.30	0.30	0.42	0.33	0.42	0.33	0.00																		
BU1	0.33	0.25	0.25	0.40	0.25	0.25	0.25	0.40	0.46	0.40	0.00	0.33	0.00																	
BU2	0.42	0.36	0.36	0.46	0.36	0.36	0.50	0.18	0.25	0.33	0.40	0.42	0.40	0.00																
BU3	0.33	0.25	0.25	0.40	0.25	0.25	0.25	0.40	0.46	0.40	0.00	0.33	0.00	0.40	0.00															
BU4	0.33	0.25	0.25	0.40	0.25	0.25	0.25	0.40	0.46	0.40	0.00	0.33	0.00	0.40	0.00	0.00														
UG1	0.33	0.25	0.25	0.40	0.25	0.25	0.25	0.40	0.46	0.40	0.00	0.33	0.00	0.40	0.00	0.00	0.00													
UG2	0.40	0.30	0.30	0.46	0.30	0.30	0.30	0.40	0.42	0.30	0.40	0.10	0.30	0.46	0.30	0.30	0.30	0.00												
UG3	0.20	0.30	0.30	0.22	0.40	0.30	0.22	0.40	0.27	0.30	0.33	0.20	0.33	0.27	0.33	0.33	0.33	0.33	0.00											
UG4	0.11	0.22	0.22	0.20	0.40	0.22	0.22	0.36	0.18	0.27	0.33	0.11	0.25	0.36	0.25	0.25	0.25	0.25	0.11	0.00										
WA1	0.20	0.30	0.30	0.27	0.30	0.30	0.30	0.42	0.33	0.27	0.33	0.20	0.33	0.27	0.33	0.33	0.33	0.33	0.20	0.11	0.00									
WA2	0.30	0.40	0.40	0.36	0.55	0.40	0.40	0.36	0.27	0.50	0.44	0.30	0.44	0.36	0.44	0.44	0.44	0.44	0.30	0.22	0.30	0.00								
WA3	0.33	0.44	0.44	0.40	0.44	0.44	0.25	0.55	0.46	0.40	0.29	0.33	0.29	0.55	0.29	0.29	0.29	0.29	0.33	0.25	0.33	0.25	0.00							
MO1	0.33	0.25	0.25	0.40	0.25	0.25	0.25	0.40	0.46	0.40	0.00	0.33	0.00	0.40	0.00	0.00	0.00	0.00	0.33	0.25	0.33	0.44	0.25	0.00						
MO2	0.36	0.46	0.46	0.42	0.30	0.46	0.30	0.42	0.33	0.10	0.33	0.36	0.33	0.27	0.33	0.33	0.33	0.33	0.20	0.30	0.20	0.46	0.33	0.33	0.00					
LU1	0.40	0.33	0.33	0.46	0.33	0.33	0.33	0.30	0.36	0.33	0.14	0.40	0.14	0.30	0.14	0.14	0.14	0.14	0.40	0.35	0.40	0.33	0.38	0.14	0.40	0.00				
LU2	0.22	0.33	0.33	0.30	0.33	0.33	0.13	0.46	0.36	0.30	0.14	0.22	0.14	0.46	0.14	0.14	0.14	0.14	0.22	0.13	0.22	0.33	0.14	0.14	0.22	0.25	0.00			
KIV1	0.22	0.33	0.33	0.30	0.33	0.33	0.13	0.46	0.36	0.30	0.14	0.22	0.14	0.46	0.14	0.14	0.14	0.14	0.22	0.13	0.22	0.33	0.14	0.14	0.22	0.25	0.00	0.00		
MS W1	0.40	0.33	0.33	0.46	0.33	0.33	0.33	0.30	0.36	0.30	0.14	0.40	0.14	0.30	0.14	0.14	0.14	0.14	0.22	0.33	0.40	0.50	0.38	0.14	0.22	0.25	0.25	0.14	0.00	
KIK1	0.33	0.25	0.25	0.40	0.25	0.25	0.25	0.40	0.46	0.40	0.00	0.33	0.00	0.40	0.00	0.00	0.00	0.00	0.33	0.25	0.33	0.44	0.29	0.00	0.33	0.14	0.14	0.14	0.14	0.00

6.3.3 Cluster analysis of jackfruit samples using SSR markers

The dendrogram of genetic diversity analysis of the samples using SSRs revealed three major clusters (Figure 6.1). Cluster 1, cluster II and cluster III comprised of 2, 15 and 13 samples respectively. Most samples from the same geographical region were grouped under the same cluster though a few samples from the same regions were grouped in different clusters. Cluster 1 contained Msambweni 1 and Mombasa 2 samples. Cluster 2 had the highest number of samples and most samples were from Busia, Ugenya, Lunga Lunga and Mbale. Kampala 1 sample was found to have high genetic relatedness to Lunga Lunga 2 and Kivulini1 samples. Cluster 3 mostly consisted of samples from Muranga and Kampala. Kampala 2 and Kampala 3 samples were found to be in the same clade. Muranga 1, 2 and Ukunda 3 samples were also found to be in the same clade. Busia 2 was in the same clade as Kampala 2 and Kampala 3, while Wakiso 2 was in its own clade. Wakiso 1 and Ugenya 3 were also in their own clade and Muranga 1, Ukunda1, Mbale 3 and Ugenya 4, were found to have a high genetic relationship, where Muranga 1 and Ukunda 1 were in the same clade, while Mbale 3 and Ugenya 4 were in the same clade. Cluster 1 contained samples from Mombasa and Mbale (Figure 6.1).

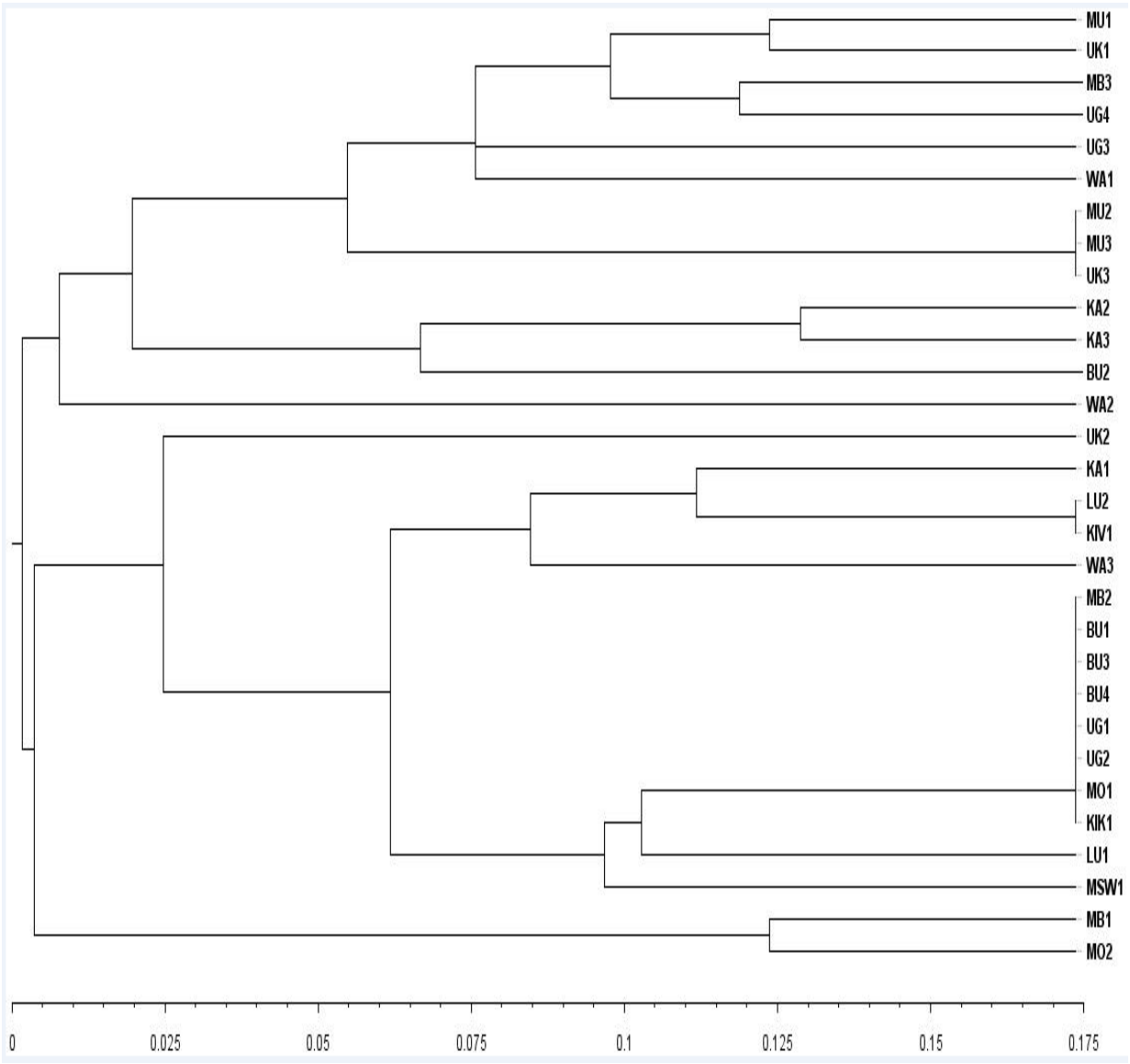


Figure 6.1. Genetic relatedness of samples using SSR markers, where Mu – Muranga, Ug-Ugenya (Siaya), Mb-Mbale, Wa-Wakiso, Lu – Lunga Lunga, Bu – Busia, Mo- Mombasa, Kik-Kikoneni, Msw-Msambweni, Kiv-Kivulini and Uk-Ukunda.

6.3.4 Analysis of genetic diversity of jackfruit samples using SRAP markers

6.3.4.1 Polymorphism detected by SRAP markers

A total of 426 bands were amplified and the percentage polymorphism was found to be 64.79%. The genetic diversity values were high and they ranged from 0 to 0.81. The primer combination ME5- EM10 showed the highest genetic diversity (0.8111),

while that of ME8-EM7 and ME11-EM7 had no genetic diversity. Other primer combinations that had high genetic diversity were ME11-EM9 (0.7133), ME 8-EM 10 (0.6244) and ME11-EM10 (0.6244). The PIC values ranged from 0 to 0.78 and the primer combination ME5- EM10 had the highest PIC value (0.7847), while that of ME8-EM7 and ME 11-EM7 had the lowest PIC value of 0. The other primer combinations that had high PIC values were ME 11-EM 9 (0.6718), ME 8-EM 10 (0.5875) and ME11-EM 10 (0.6259). The Simpson's diversity index ranged from 0 to 0.87. The combinations that had high values were ME 5-EM10 (0.8733), ME 11-EM 9 (0.6569), ME5-EM7 (0.6545) and ME11-EM 10 (0.6261). The ME8-EM7 and ME 11-EM 7 combinations on the other hand, had the lowest diversity index of 0. The heterozygosity values ranged from 0 to 0.767. The primer combinations that had high heterozygosity values were ME 5- EM10 (0.767), ME 11-EM 9 (0.567), and ME11-EM 10 (0.533). Two primer combinations namely ME8-EM7 and ME 11-EM 7 had the lowest heterozygosity value of 0 (Table 6.4).

Table 6.4. Genetic diversity parameters of SRAP markers

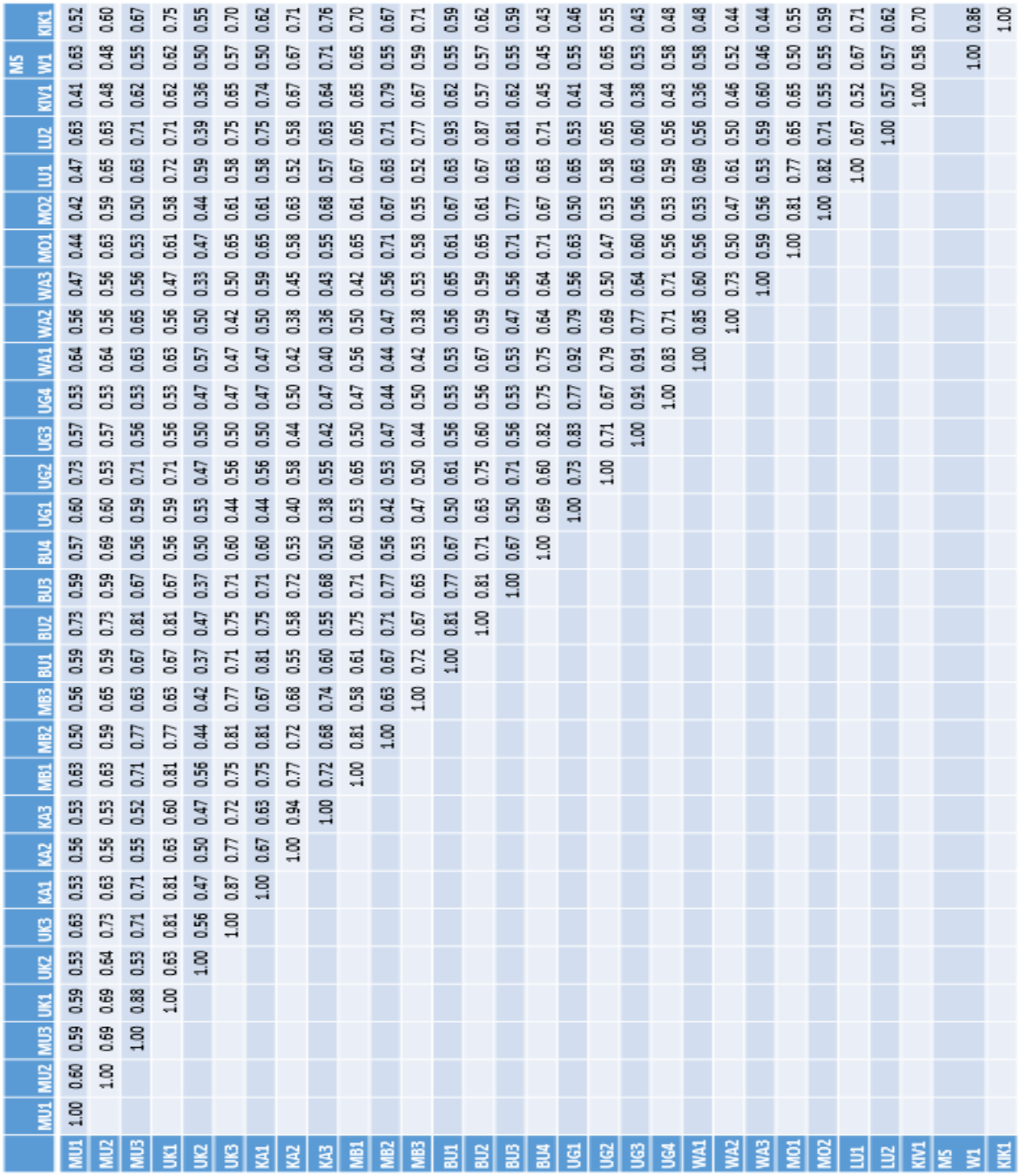
Marker	**Gene diversity**	**PIC**	**Simpson's Diversity index**	**Observed Heterozygosity**	**Polymorphism (%)**
ME 5- EM10	0.81	0.78	0.87	0.77	100
ME5-EM9	0.54	0.50	0.48	0.37	66.67
ME5-EM7	0.48	0.36	0.65	0.40	66.67
ME8-EM7	0.00	0.00	0	0.00	
ME8-EM9	0.39	0.37	0.42	0.23	66.67
ME8-EM 10	0.62	0.59	0.58	0.43	100
ME 11-EM 7	0.00	0.00	0	0.00	
ME 11-EM9	0.71	0.67	0.67	0.56	75
ME11-EM 10	0.68	0.63	0.63	0.53	100
Average diversity of polymorphic markers	0.61	0.56	0.61	0.471	82.14%

6.3.5 Jaccard's similarity and dissimilarity coefficients using SRAP markers

The similarity values ranged from 0.38 to 0.81. Generally, the samples from the same geographical region had higher similarity coefficient compared to those from different regions. For instance, Mbale 1 and Mbale 2 had a similarity coefficient of 0.81. The Busia 1 sample, was also found to have high similarity to Busia 2 and Busia 3 with Jaccard's similarity coefficient values of 0.81 and 0.77 respectively. Ugenya 1 was also found to have high similarity coefficient with Ugenya 2, Ugenya 3 and Ugenya 4 and the values were 0.73, 0.83 and 0.77, respectively. The similarity coefficient between Mombasa 1 and Mombasa 2 samples was found to be 0.81. Kampala 2 sample was also found to have high similarity to Kampala 3 sample. Some samples from different geographical regions were also found to have high similarity such as Kikoneni and Kivulini, Kikoneni and Mswambweni, and Mombasa 2 and Lunga Lunga 1. The similarity coefficients were found to be 0.86, 0.70 and 0.71, respectively as shown in Table 6.5.

The dissimilarity values ranged from 0.14 to 0.67. Low dissimilarity coefficients were found in most samples from the same region. For instance: Kampala 2 and Kampala 3 had a dissimilarity coefficient of 0.06, Ugenya 2 with Ugenya 3 and Ugenya had dissimilarity coefficients of 0.09 and 0.09 respectively. Mombasa 1 and Mombasa 2 had a similarity coefficient of 0.19. Other samples from the same region that had a small dissimilarity value were Wakiso 1 and Wakiso 2, Mbale 1 and Mbale 2 as well as Ukunda 1 and Ukunda 3 with dissimilarity coefficient values of 0.15, 0.19 and 0.19, respectively. There were also samples from different geographical regions that had low dissimilarity and they included Kampala 1 and Ukunda 1, Lunga Lunga 2 and Busia 3 and Kivulini and Msambweni with dissimilarity coefficient values of 0.19, 0.13 and 0.14, respectively. The samples that were found to have the highest dissimilarity values were Wakiso 3 and Ukunda 2, whose dissimilarity coefficient was 0.67.

Table 6.5. Jaccard's similarity coefficient using SRAP markers for Jackfruit samples from different regions



	MU1	MU2	MU3	UK1	UK2	UK3	KA1	KA2	KA3	MB1	MB2	MB3	BU1	BU2	BU3	BU4	UG1	UG2	UG3	UG4	WA1	WA2	WA3	MO1	MO2	LU1	LU2	KIV1	MS	W1	KIK1
MU1	1.00	0.60	0.59	0.59	0.53	0.63	0.53	0.56	0.53	0.63	0.50	0.56	0.59	0.73	0.59	0.57	0.60	0.73	0.57	0.53	0.64	0.56	0.47	0.44	0.42	0.47	0.63	0.41		0.63	0.52
MU2		1.00	0.69	0.69	0.64	0.73	0.63	0.56	0.53	0.63	0.59	0.65	0.67	0.73	0.59	0.69	0.60	0.53	0.57	0.53	0.64	0.56	0.56	0.63	0.59	0.65	0.63	0.48		0.48	0.60
MU3			1.00	0.88	0.53	0.71	0.71	0.55	0.52	0.71	0.77	0.63	0.67	0.81	0.67	0.56	0.59	0.71	0.56	0.53	0.63	0.65	0.56	0.53	0.50	0.63	0.71	0.62		0.55	0.67
UK1				1.00	0.63	0.81	0.81	0.63	0.60	0.81	0.77	0.63	0.67	0.81	0.67	0.56	0.59	0.71	0.56	0.53	0.63	0.63	0.47	0.61	0.58	0.72	0.71	0.62		0.62	0.75
UK2					1.00	0.56	0.47	0.50	0.47	0.56	0.44	0.42	0.37	0.47	0.37	0.50	0.53	0.47	0.50	0.47	0.57	0.50	0.33	0.47	0.44	0.59	0.39	0.36		0.50	0.55
UK3						1.00	0.87	0.77	0.72	0.75	0.81	0.77	0.71	0.75	0.71	0.60	0.44	0.56	0.50	0.47	0.47	0.42	0.50	0.65	0.61	0.58	0.75	0.65		0.57	0.70
KA1							1.00	0.67	0.63	0.75	0.81	0.67	0.81	0.75	0.71	0.60	0.44	0.56	0.50	0.47	0.47	0.50	0.59	0.65	0.61	0.58	0.75	0.74		0.50	0.62
KA2								1.00	0.94	0.77	0.72	0.68	0.55	0.58	0.72	0.53	0.40	0.58	0.44	0.50	0.42	0.38	0.45	0.58	0.63	0.52	0.58	0.67		0.67	0.71
KA3									1.00	0.72	0.68	0.74	0.60	0.55	0.68	0.50	0.38	0.55	0.42	0.47	0.40	0.36	0.43	0.55	0.68	0.57	0.63	0.64		0.71	0.76
MB1										1.00	0.81	0.58	0.61	0.75	0.71	0.60	0.53	0.65	0.50	0.47	0.56	0.50	0.42	0.65	0.61	0.67	0.56	0.65		0.65	0.70
MB2											1.00	0.63	0.67	0.71	0.77	0.56	0.42	0.53	0.47	0.44	0.44	0.47	0.56	0.71	0.67	0.63	0.71	0.79		0.55	0.67
MB3												1.00	0.72	0.67	0.63	0.53	0.47	0.50	0.44	0.50	0.42	0.38	0.53	0.58	0.55	0.52	0.77	0.67		0.59	0.71
BU1													1.00	0.81	0.77	0.67	0.50	0.61	0.56	0.53	0.53	0.56	0.65	0.61	0.67	0.63	0.93	0.62		0.55	0.59
BU2														1.00	0.81	0.71	0.63	0.75	0.60	0.56	0.67	0.59	0.59	0.65	0.61	0.67	0.87	0.57		0.57	0.62
BU3															1.00	0.67	0.50	0.71	0.56	0.53	0.53	0.47	0.56	0.71	0.77	0.63	0.81	0.62		0.55	0.59
BU4																1.00	0.69	0.60	0.82	0.75	0.75	0.64	0.64	0.71	0.67	0.63	0.71	0.45		0.45	0.43
UG1																	1.00	0.73	0.83	0.77	0.92	0.79	0.56	0.63	0.50	0.65	0.53	0.41		0.55	0.46
UG2																		1.00	0.71	0.67	0.79	0.69	0.50	0.47	0.53	0.58	0.65	0.44		0.65	0.55
UG3																			1.00	0.91	0.91	0.77	0.64	0.60	0.56	0.63	0.60	0.38		0.53	0.43
UG4																				1.00	0.83	0.71	0.71	0.56	0.53	0.59	0.56	0.43		0.58	0.48
WA1																					1.00	0.85	0.60	0.56	0.53	0.69	0.56	0.36		0.58	0.48
WA2																						1.00	0.73	0.50	0.47	0.61	0.50	0.46		0.52	0.44
WA3																							1.00	0.59	0.56	0.53	0.59	0.60		0.46	0.44
MO1																								1.00	0.81	0.77	0.65	0.65		0.50	0.55
MO2																									1.00	0.82	0.71	0.55		0.55	0.59
LU1																										1.00	0.67	0.52		0.67	0.71
LU2																											1.00	0.57		0.57	0.62
KIV1																												1.00		0.58	0.70
MS																														1.00	0.86
W1																															1.00
KIK1																															

Table 6.6. Jaccard's dissimilarity coefficient using SRAP markers for Jackfruit samples from different regions

	MU1	MU2	MU3	UK1	UK2	UK3	KA1	KA2	KA3	MB1	MB2	MB3	BU1	BU2	BU3	BU4	UG1	UG2	UG3	UG4	WA1	WA2	WA3	MO1	MO2	LU1	LU2	KIV1	MS	W1	KIK1
MU1	0.00	0.40	0.41	0.41	0.47	0.38	0.47	0.44	0.47	0.38	0.50	0.44	0.41	0.27	0.41	0.43	0.40	0.27	0.43	0.47	0.36	0.44	0.53	0.56	0.58	0.53	0.38	0.59	0.37	0.48	
MU2		0.00	0.31	0.31	0.36	0.27	0.38	0.44	0.47	0.38	0.41	0.35	0.41	0.27	0.41	0.31	0.40	0.47	0.43	0.47	0.36	0.44	0.44	0.38	0.41	0.35	0.38	0.52	0.52	0.40	
MU3			0.00	0.13	0.47	0.29	0.29	0.45	0.48	0.29	0.24	0.37	0.33	0.19	0.33	0.44	0.41	0.29	0.44	0.47	0.38	0.35	0.44	0.47	0.50	0.37	0.29	0.38	0.46	0.33	
UK1				0.00	0.38	0.19	0.19	0.37	0.40	0.19	0.24	0.37	0.33	0.19	0.33	0.44	0.41	0.29	0.47	0.47	0.38	0.44	0.53	0.35	0.42	0.28	0.29	0.38	0.43	0.25		
UK2					0.00	0.44	0.53	0.50	0.53	0.44	0.56	0.58	0.63	0.53	0.63	0.50	0.47	0.53	0.53	0.53	0.43	0.50	0.67	0.53	0.56	0.41	0.61	0.64	0.50	0.45			
UK3						0.00	0.13	0.24	0.28	0.25	0.19	0.24	0.29	0.25	0.29	0.40	0.56	0.44	0.50	0.53	0.53	0.58	0.50	0.35	0.39	0.42	0.25	0.35	0.43	0.30			
KA1							0.00	0.33	0.37	0.25	0.19	0.33	0.19	0.25	0.29	0.40	0.56	0.44	0.50	0.53	0.53	0.50	0.41	0.35	0.39	0.42	0.25	0.26	0.50	0.38	
KA2								0.00	0.06	0.24	0.28	0.32	0.45	0.42	0.28	0.47	0.60	0.42	0.56	0.50	0.58	0.62	0.55	0.42	0.37	0.48	0.42	0.33	0.33	0.29	
KA3									0.00	0.28	0.32	0.26	0.40	0.45	0.32	0.50	0.62	0.45	0.58	0.53	0.60	0.64	0.57	0.45	0.32	0.43	0.37	0.36	0.47	0.24	
MB1										0.00	0.19	0.42	0.39	0.25	0.29	0.40	0.47	0.35	0.50	0.53	0.44	0.50	0.58	0.35	0.39	0.33	0.35	0.35	0.42	0.30	
MB2											0.00	0.37	0.33	0.29	0.24	0.44	0.58	0.47	0.53	0.56	0.56	0.53	0.44	0.29	0.33	0.37	0.29	0.21	0.46	0.33	
MB3												0.00	0.28	0.33	0.37	0.47	0.53	0.50	0.56	0.50	0.58	0.62	0.47	0.42	0.45	0.48	0.24	0.33	0.41	0.29	
BU1													0.00	0.19	0.24	0.33	0.50	0.39	0.50	0.56	0.47	0.44	0.35	0.39	0.33	0.37	0.07	0.38	0.46	0.46	
BU2														0.00	0.19	0.29	0.38	0.25	0.40	0.44	0.33	0.41	0.41	0.35	0.39	0.33	0.13	0.43	0.43	0.43	
BU3															0.00	0.33	0.50	0.29	0.44	0.47	0.47	0.53	0.44	0.29	0.24	0.37	0.19	0.38	0.46	0.46	
BU4																0.00	0.31	0.40	0.18	0.25	0.25	0.36	0.36	0.29	0.33	0.38	0.29	0.55	0.55	0.57	
UG1																	0.00	0.27	0.17	0.23	0.08	0.21	0.44	0.38	0.50	0.35	0.47	0.59	0.45	0.55	
UG2																		0.00	0.29	0.33	0.21	0.31	0.50	0.53	0.47	0.42	0.35	0.57	0.35	0.46	
UG3																			0.00	0.09	0.09	0.23	0.36	0.40	0.44	0.38	0.40	0.62	0.47	0.57	
UG4																				0.00	0.17	0.29	0.29	0.44	0.47	0.41	0.44	0.57	0.42	0.52	
WA1																					0.00	0.15	0.40	0.44	0.47	0.31	0.44	0.64	0.42	0.52	
WA2																						0.00	0.27	0.50	0.53	0.39	0.50	0.55	0.48	0.57	
WA3																							0.00	0.41	0.44	0.47	0.41	0.40	0.55	0.57	
MO1																								0.00	0.19	0.24	0.35	0.35	0.50	0.46	
MO2																									0.00	0.18	0.29	0.46	0.46	0.41	
LU1																										0.00	0.33	0.48	0.33	0.29	
LU2																											0.00	0.43	0.43	0.38	
KIV1																												0.00	0.42	0.30	
MS																													0.00	0.14	
W1																														0.00	
KIK1																															

6.3.5. Cluster analysis of jackfruit samples using SRAP markers

The samples were grouped into 3 major clusters where Cluster 1, cluster II and cluster III had 6, 14 and 9 samples, respectively and one sample was an out-group. Most samples from the same geographical region were clustered together. Cluster 1 consisted of Kivulini, Msambweni, Kikoneni and Kampala samples. The Kampala 2 and 3 samples were in the same clade, while Msambweni and Kikoneni were also in the same clade. Cluster 2 consisted of samples from Lunga Lunga, Mombasa, Busia, Mbale, Muranga and Ukunda. Busia 1, 2 and 3 samples were also found to be closely related to Lunga Lunga 2, while Mombasa 1 and Mombasa 2, were closely related to Lunga Lunga 1. Muranga 3 and Ukunda 1 shared the same clade, while Mbale 2 sample was found to be more closely related to Kampala 1 and Ukunda 3 compared to the rest. Those that were grouped in cluster 3, were mostly samples from Ugenya and Wakiso. Ugenya 1, 2 and 3 samples were found to be closely related to Wakiso 1 and Wakiso 2. Muranga 1 sample on the other hand, was closely related Ugenya 2 as shown in Figure 6.2.

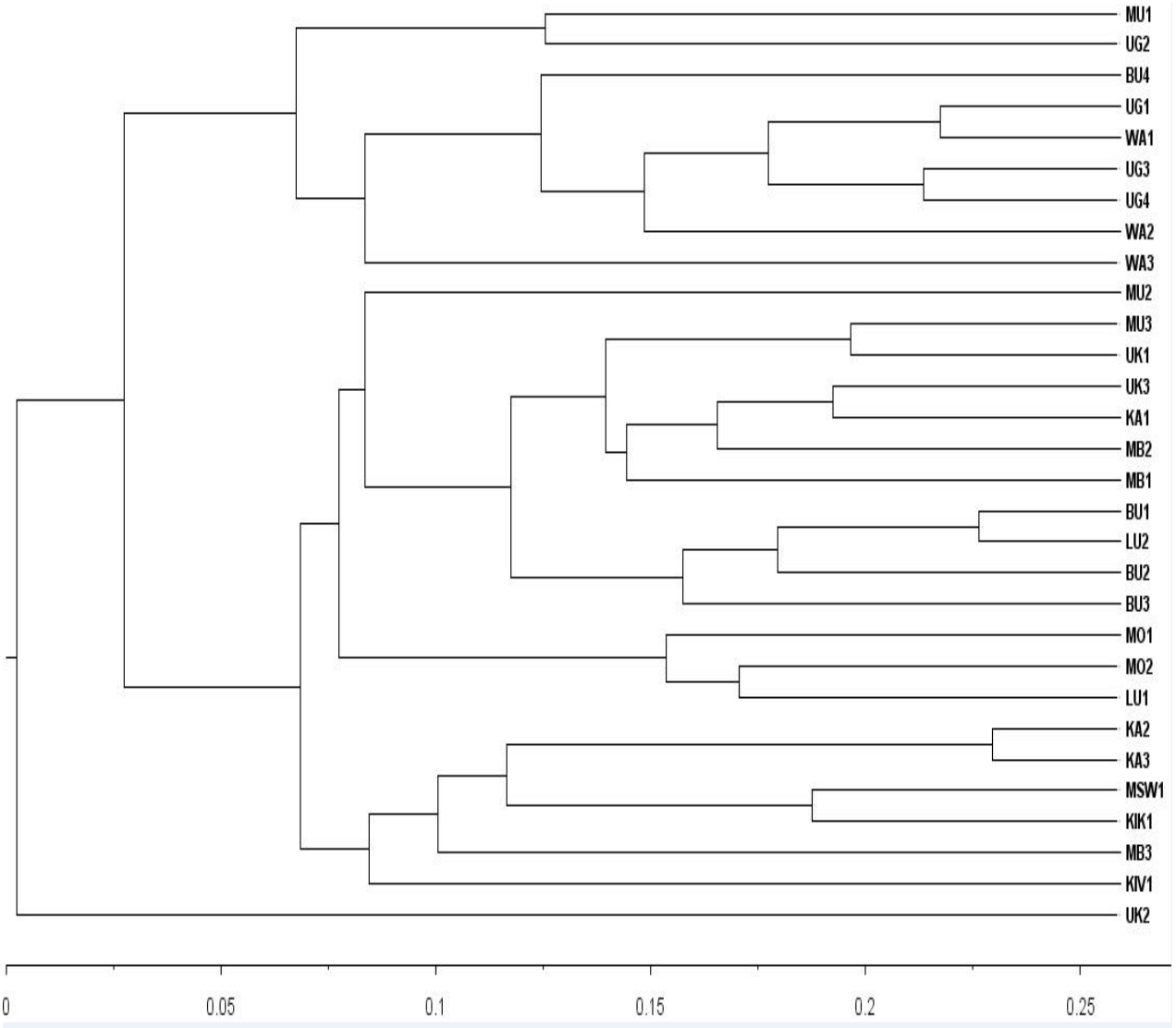


Figure 6.2. Dendrogram of genetic relationships of Jackfruits from different regions using SRAP markers, where Mu – Muranga, Ug-Ugenya, Mb-Mbale, Wa-Wakiso, Lu – Lunga Lunga, Bu –Busia, Mo- Mombasa, Kik-Kikoneni, Msw-Msambweni, Kiv-Kivulini and Uk-Ukunda.

6.3.6 Correlation between SSR and SRAP markers genetic diversity Parameters

A Pearson's correlation between the mean of polymorphic SSR markers parameters and those of the SRAP markers showed a high correlation of 0.78 as shown in Table 6.7.

Table 6.7. Correlation of genetic diversity parameters between SSR and SRAP markers

		SSR markers	SRAP markers
SSR Markers	Pearson Correlation	1	.781
SRAP Markers	Pearson Correlation	.781	1

6.3.7 Principal coordinate analysis (PCoA) of combined SSR and SRAP markers

The PCoA analysis of combined SSR and SRAP primers revealed that Mombasa 1, Mombasa 2, Busia 1, Busia 2 and Lunga Lunga 2 samples have higher genetic relationship. Wakiso 1, Wakiso 2, Ugenya 1, Ugenya 2 and Ugenya 3 were also found to have higher genetic relationship. Kampala 1 and Kampala 2 were closely related, while Muranga 2 and 3 were moderately similar. Mbale 1, Mbale 3, Ukunda 1, Ukunda 3 and Kikoneni 1were also moderately similar compared to the rest. Generally, Muranga and Wakiso samples, were highly dissimilar and this was also the case in Ugenya and Kampala samples (Figure 6.3).

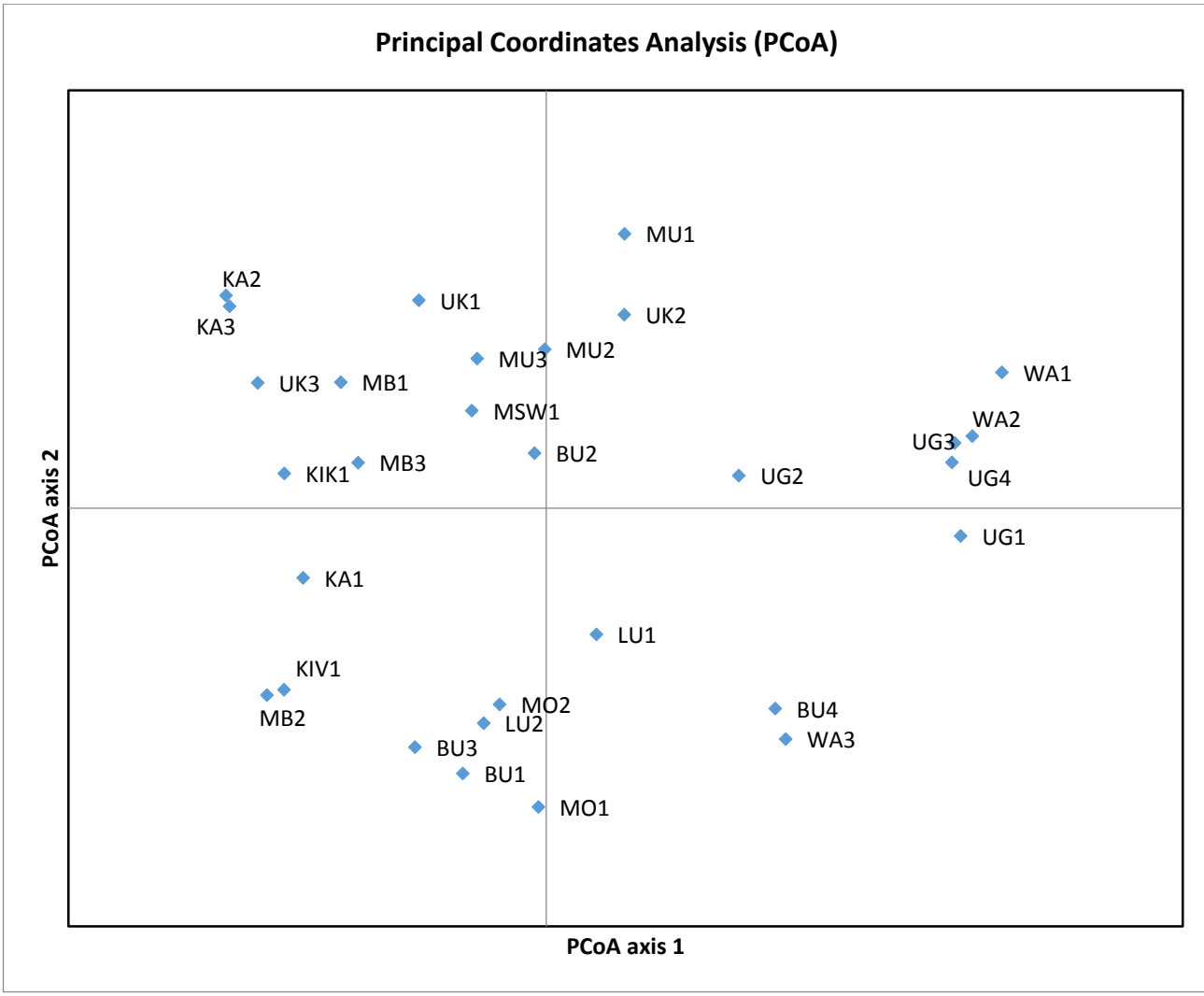


Figure 6.3. PCoA analysis of combined SSR and SRAP primers**,** where Mu – Muranga, Uk-Ukunda, Mb-Mbale, Wa-Wakiso, Lu – Lunga Lunga, Bu –Busia, Mo-Mombasa, Kik-Kikoneni, Msw-Msambweni, Kiv-Kivulini and Ug-Ugenya.

6.4 Discussion

The gene diversity value measures the difference in genetic information of samples and can be used to determine the genetic variation of samples from the same species. The gene diversity values for the polymorphic markers ranged from 0.47 to 0.62 using SSR markers. The average gene diversity value for all the polymorphic markers using SSR markers was found to be 0.55. The jackfruit genetic diversity using SSR markers,

indicate a moderate level of genetic diversity. This means that some of the varieties might have superior traits compared to others (Govindaraj et al., 2015).

The SRAP markers on the other hand, had more varied gene diversity values that ranged from 0.39 to 0.81. This means that the SRAP markers showed moderately higher, genetic diversity values compared to the SSR markers. This can be attributed to the fact that the SSR markers are specific and target specific genes in the organism as opposed to SRAP markers, which are non-specific and target coding regions of the organism (Li and Quiros, 2001). The SRAP markers are therefore able to amplify many regions in the genome compared to the SSR and this explains why the gene variation was higher in SRAP markers. However, the average gene diversity value for the polymorphic markers had a small variation, as the values was 0.61 for SRAP and 0.55 for SSR markers.

Both SSR and SRAP markers showed moderate genetic diversity, the findings were consistent with those of Shyamalamma et al. (2008), who found that jackfruit from selected regions in India, had moderate genetic diversity using AFLP markers and Gopalsamy et al. (2012), who also found moderate genetic diversity in jackfruit samples from India using RAPD markers. Plants with high genetic diversity may sometimes have more varieties that have the higher ability to withstand harsh conditions such as drought amongst others, compared to those that have similar genetic makeup. The identification of the superior species, would be therefore be important towards ensuring food and nutrition security.

The PIC value indicates the polymorphism of a marker and the value usually ranges from 0 to 1. If the value is higher than 5, it means the marker is highly polymorphic but if it is less then it means the marker has moderate or low polymorphism. The PIC value of the polymorphic SSR primers ranged from 0.37 to 0.56 indicating moderate polymorphism. On average, the PIC value was 0.48, which implies that the polymorphism was moderate.

The SRAP markers on the other hand, had PIC values that ranged from 0.78 to 0.36, which are slightly higher than those of the SSR markers. On average, the PIC value 0.54 in SRAP markers, which is also slightly higher than that of SSR. However, the value also implies that the polymorphism was moderate. The values of the two markers are however close and they both indicate moderate level of polymorphism. The findings are consistent with that of Schnell et al. (2001), who also found moderate polymorphism in jackfruit collected from different countries. The PIC values in SSR was consistent with those obtained in other plants since very few studies on genetic characterization have been done on jackfruit. A study by Kumar et al. (2015), revealed that the PIC value of mango cultivars in India ranged from 0.32 to 0.77, which was close to the values obtained in this study. A different study by Mathithumilan et al. (2013), found that the average PIC value in mulberry, which belongs to the same *Moraceae* family as Jack fruit had an average polymorphic information content (PIC) of 0.559.

Simpson's Diversity Index is mostly used to measure genetic diversity and it takes into account the species richness. The more the number of species that are genetically different, the higher the species richness. The value also takes into account the relative abundance of each of the genotype in the species and the evenness of the abundance. A population that has species richness and even relative abundance of the genotypes is likely to have a higher Simpson’s index. The diversity index values ranged from 0.43 to 0.52 in SSR and the average value was 0.48, which indicates moderate species richness and genetic diversity. The values were relatively lower compared to SRAP values and they ranged from 0.87 to 0.47. The average value was also a bit different as it was 0.61 in SRAP markers. In this study the Simpson’s index average value of polymorphic SRAP markers was also moderate. The findings imply that the jackfruit samples that were analyzed, had moderate richness and genetic diversity. However, the SRAP markers show that the richness is higher compared to the SSR markers. The diversity index using SSR was comparable to that obtained in a different medicinal

plant *Rhodiola rosea* L found in Russia that was also found to be 0.48 (Gyorgy et al., 2012).

The term Heterozygosity, is derived from the word heterozygous, which refers to organisms carrying two different alleles of the same gene. Heterozygosity is the measure of the relative abundance of heterozygotes. If the heterozygosity value is high, then the genetic diversity of the given species is also high. The observed heterozygosity values of polymorphic markers were found to range from 0.3 to 0.5 for SSR markers and the average value was found to be 0.42, which implies that the heterozygosity was moderate. The values for SRAP markers on the other hand, ranged from 0.23 to 0.78 and the average value was 0.46. The primers also show that jackfruit has moderate heterozygosity. This implies that there was moderate abundance of heterozygotes in the samples analyzed. There are no heterozygosity values that have been reported for jackfruit samples so far but the values obtained were slightly different from those obtained in a different medicinal plant called root plant. The heterozygosity in this plant was found to range from 0.47 to 0.84, implying that the plant is more heterogeneous compared to jackfruit (Gyorgy et al., 2012).

The Jaccard's similarity coefficient using the SSR markers ranged from 0.55 to 1.0. Most samples from the same region were generally found to show a high similarity coefficient compared to samples from the different regions. The trend in similarity and dissimilarity was such that most samples from the same region had high similarity values, while those from the different regions had low similarity values. The similarity values in SSR markers showed that there is moderate genetic diversity. Some of the samples from the same region that showed high similarity are: Kampala 2 and Kampala 3, Busia 1 and Busia 3, Ukunda 1 and Ukunda 3 amongst others.

The SRAP markers on the other hand, had similarity values that ranged from 0.38 to 0.81. The similarity values showed a wider variation compared to the SSR markers. This may be attributed to the fact that most SRAP markers do not generally target a

particular region of the DNA and hence they are likely to amplify many regions compared to SSR markers, which target a specific gene. However, for SRAP markers, the similarity values ranged from low to high and most of the values were moderate. The trend was similar to that of SSR coefficient values, as most samples from the same region had higher similarity than those from the different regions.

There are some regions that consistently showed high similarity coefficient values in both SSR and SRAP markers. Some of the samples from the same region that were found to show high similarity values by both SRAP and SSR markers include: Kampala 2 and Kampala 3, Busia 1 and Busia 3, Ukunda 1 and Ukunda 3, Lunga Lunga 1 and Lunga Lunga 3 amongst others. The high similarity coefficient of values from the same region implies that samples from most regions had low genetic diversity. This may be attributed to the fact that most regions share the same climatic conditions and the plants are therefore unlikely to undergo major genetic changes, so as to adapt to the environments. This may also be attributed to the fact that the seeds of the plants from the same region are likely to be used to propagate new plants in the same region. Cultivation of the trees through grafting was also found to be a popular mode of propagation by the farmers in some regions and this would also explain the low genetic diversity in some samples from the same region.

The markers also both showed high similarity values for samples from different regions and they include: Mombasa and Lunga Lunga samples, Ukunda and Kampala samples, Kivulini and Lunga Lunga, Lunga Lunga and Kikoneni, Msambweni and Kikokeni, Lunga Lunga and Msambweni, Wakiso and Busia, Muranga and Ukunda amongst others. The similarity of samples in the different regions may be attributed to the proximity of regions. For instance, Kikoneni Msambweni and Lunga Lunga regions are all located in the Southern Coast region of Kenya. Muranga samples were also found to have high similarity with the Ukunda samples, while some of the Ukunda samples were also found to be similar to those of the Kampala samples. The regions

are far from each other as some of them are located in two different countries. The probable reason may be that the plants were from the same origin.

The dissimilarity values on the other hand, were mostly found to be lower in samples from the same region but high in samples from different regions. The regions that had high dissimilarity were similar to the ones that had low similarity values. The regions that were found to have high dissimilar coefficient values by both SSR and SRAP markers were: Muranga and Mombasa, Lunga Lunga and Muranga, Msambweni and Muranga, Kikoneni and Muranga, Wakiso and Mombasa, Lunga Lunga and Wakiso, Msambweni and Wakiso, Mombasa and Mbale, Mbale and Wakiso. The high dissimilarity of these samples may be attributed to the fact that they do not share a common ancestry**.** The findings of the study were consistent with that of Schell et al. (2001), who studied jackfruit from different countries using AFLP markers and found that the similarity coefficient ranged from 0.567 to 0.950. The study was also consistent with that of Li et al. (2010), who found that the average similarity coefficient of jackfruit from China was 0.5000 using AFLP markers. The study also found that the plant had moderate genetic diversity.

The dendrogram of genetic diversity analysis of the jackfruits using SSRs revealed three major clusters. Most samples from the same geographical region were grouped under the same cluster. This implies that most samples from the same region had high genetic relatedness. A few samples from the same regions were grouped in different clusters, implying that they had high genetic variation with other samples from the same region. This may be as a result of mutation or difference in species varieties. Most samples from Busia, and Ugenya were grouped under the same cluster. Most samples from Muranga, Ukunda and Kampala were also grouped under the same cluster. Kampala 2 and Kampala 3 samples were found to have close genetic relationship, while. Muranga 1, 2 and Ukunda 3 samples were also found to have close genetic relationship. Busia, 1, 3, 4, Ugenya 1, 2, Mombasa 1 and Kikoneni 1 were also found to have a close genetic relationship.

In SRAP markers most samples from the same geographical region were clustered together. Most samples from Kivulini, Msambweni, Kikoneni and Kampala were grouped together. The Kampala 2 and 3 samples had high genetic relatedness, while Msambweni and Kikoneni, also had close genetic relationship. The samples from Lunga Lunga, Mombasa, Busia, Mbale, Muranga and Ukunda were also clustered together. Busia 1, 2 and 3 samples were also found to be related to Lunga Lunga 2, while Mombasa 1 and Mombasa 2 related to Lunga Lunga 1. Muranga 3 and Ukunda 1 were closely related, while Mbale 2 sample was found to be more related to Kampala 1 and Ukunda 3 compared to the rest. Mostly samples from Ugenya and Wakiso were also clustered together. Ugenya 1, 2 and 3 samples were found to be more related to Wakiso 1 and Wakiso 2 compared to the rest. Muranga 1 sample on the other hand, was closely related Ugenya 2 compared to the rest.

A correlation of the SSR and SRAP markers genetic diversity parameters showed a high positive correlation of 0.78. This implies that the results from both primers were consistent and they both showed that jackfruit has moderate genetic diversity. The findings of the study were similar to those of Shyamalamma et al. (2008), who clustered 50 jackfruits from different countries into three major clusters using AFLP markers and they were all found to have moderate genetic diversity. A different study by Gopalsamy et al. (2012), also grouped jackfruits obtained from different parts of India into three major clusters using RAPD markers.

The PCoA analysis of combined SSR and SRAP primers revealed that most samples from the same region had a closer relationship compared to samples from different regions. For instance, Mombasa 1, Mombasa 2, Busia 1 and Busia 2 samples were found to have close genetic relationship. This was also the case with Wakiso 1, Wakiso 2, Ugenya 1, Ugenya 2 and Ugenya 3, as well as Kampala 1 and Kampala 2 and Muranga 2 and Muranga 3. Muranga and Wakiso samples had a distant genetic relationship as well as Ugenya and Kampala samples. This may be because Muranga

and Wakiso regions and Ugenya and Kampala regions are relatively far apart and the different environmental and weather conditions may have caused genetic variations to occur in the plants so that that they can adapt to their environments. The findings of the study were consistent with those of (Shyamalamma et al. (2008), who found that plants from distant geographical locations had a higher dissimilarity in genetic relationship. The study also revealed that factors such as rainfall pattern also played a big role in affecting the genetic diversity of Jackfruits.

The SSR and SRAP markers were effective in genetic characterization of jackfruits from different regions. The genetic diversity parameters of both markers showed that jackfruit plants found in Kenya and Uganda have moderate genetic diversity. Most samples from the same region were found to have a higher genetic similarity compared to samples from different regions. The correlation of the genetic parameters determined using the SRAP and SSR was also relatively high (0.78). The jackfruits found in selected regions of Kenya and Uganda therefore have moderate genetic diversity and can therefore be grouped into three major clusters based on their genetic relatedness. The studies are consistent with Nakintu et al. (2019), who found Jackfruit from different geographical locations had moderate genetic diversity and PCoA analysis grouped the plants into three major clusters. However, the study had higher gene diversity and heterozygosity values compared to the ones obtained in this study. Notably, heterozygosity values ranged from 0.59- 0.79, while gene diversity values were from I = 1.71-1.08. The difference may be attributed to the difference in primers used. Additionally, the study corroborated with Kavya et al. (2019), who analysed genetic diversity of Indian jackfruit using UPGMA and obtained three major clusters.

CHAPTER SEVEN

7.0 GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

7.1 Nutritional Profile of jackfruit

The nutritional profile of jackfruit seeds and fruit revealed they are rich in nutrients. The seeds had a higher composition of nutrients compared to the pulp region. This implies that the jackfruit seeds are a better source of nutrients compared to the pulps. The value of the ash content in seeds was more than twice that of the pulp and this implies that the seeds are rich in minerals compared to the pulp. The lipid content was also found to be higher in the seeds compared to the pulp region. Therefore, the seeds are better sources of jackfruit lipid compared to the pulp region.

The pulp region was found to have a higher moisture content compared to the seeds, which means it has a shorter shelf life. This is because high moisture content makes the plant more prone to microbial infection. However, both the seeds and the pulp regions have relatively high moisture content 44-63% and they would therefore require further processing, in order to increase their shelf life. Both the seeds and pulp, were found to have protein and carbohydrates, with seeds having a relatively higher content compared to the pulp. The levels were however relatively high in both of them and hence the fruit can be targeted as a source of carbohydrate and proteins.

The jackfruit seeds and pulps were also found to be rich in essential minerals. The seed generally had higher mineral composition compared to the pulps, though in overall, they were found to be good sources of essential minerals that are required in the body such as potassium, calcium and sodium, which are important in the formation of strong bones and teeth. Additionally, the fruit was moderately rich in magnesium, zinc, iron and copper, which are important in a number of metabolic processes in the body. Therefore, the fruit is a potential source of essential minerals.

7.2 Phytochemical composition and antioxidant activity

The phytochemical composition had high variation in different tissues of the plant. The root had the highest phenolic, flavonoid and tannin content, while the pulp and seeds had the least. The phenolics, flavonoids and tannin composition was found to be relatively higher in the roots, barks and leaves compared to the edible regions. The different parts of jackfruit tree were also found to have high antioxidant activity. The roots, leaves and bark had significantly higher phytochemical content compared to the edible regions. Phytochemicals exhibit antioxidant activities, which is critical for proper functioning of the body. They eliminate reactive oxygen species and other oxidants, which may be harmful to the body. Notably, high levels of the oxidants increase the risks of cancer, premature aging, hypertension and diabetes amongst others (Beer et al., 2002). Moreover, a mixture of leaves and barks are useful in treatment of diabetes (Omar et al., 2011). This is attributable to the high composition of phytochemicals were obtained in this study.

7.3. Comparison of seed germination and DNA extraction protocols

The seed pre-treatment using 3% hydrogen peroxide is efficient in the germination of Jackfruit seeds. The modified protocol for DNA extraction provides an alternative method that can be used by laboratories that have low funding to extract DNA from jackfruit. The extraction of DNA from plants high in phytochemicals presents a serious challenge to most researchers.

7.4 Genetic characterization

The gene diversity parameters revealed that jackfruit from selected regions in Kenya and Uganda is rich in genetic diversity. The average gene diversity values for all the polymorphic markers was found to be 0.55 using SSR markers and 0.61 for SRAP markers. Both markers showed that jackfruit has moderate genetic diversity, however the level of diversity is higher in SRAP markers compared to the SSRs. The gene

diversity parameters of both primers indicated that the samples had moderate richness. There was moderate abundance of heterozygotes in the samples analyzed. Most samples from the same region showed high genetic relatedness compared to samples from different regions. This implies that there is a relationship between genetic relatedness and the regions. The dendrograms generated revealed three major clusters with numerous sub-clusters showing that jackfruit is rich in diversity.

7.5 Significance of the findings

The findings of the study, imply that both jackfruit seeds and pulp region are rich in important nutrients and can be potential alternative sources of nutrients such as proteins and carbohydrates. The seeds of jackfruits were found to be highly nutritious and, in most cases,, they are normally discarded. According to USAID (2018), the number of those affected by nutrition insecurity in Kenya is 3.5 million. With those highly affected being children who are less than 5 years. Jackfruit presents a potentially alternative good source of nutrients that can be propagated in this region, to help in reducing nutrition insecurity. The information obtained in this study can be used in popularizing the cultivation of jackfruit in Kenya, where it is scarce and has the potential of improving food and nutrition security.

Jackfruit seeds and pulps, therefore presents an alternative source of micronutrients that will minimize the micronutrient related diseases. Minerals are micronutrients since they are only required in small quantities by the body. The problem of micronutrient deficiency is a worldwide phenomenon. The CDC puts at 2 billion the number of individuals suffering from micronutrient deficiency in the world, where most of the affected individuals are from the developing countries (CDC, 2018). The use of jackfruit as a potential alternative source of essential minerals can contribute in reducing the micronutrient deficiency cases.

The different parts of the plant were found to be high in phytochemicals and therefore the tree can be potentially used for medicinal purposes. Tyangi et al. (2015), suggests that different parts of jackfruit are used for different medicinal purposes especially in India. The root extract is used for treating fever, asthma and skin conditions. The leaves are used as sedative in wounds and the leaves ashes are used in healing of abscesses and the mature bark is used in treating dysentery. This means that these parts have high medicinal value and the presence of high composition of phytochemicals found in these regions, may explain the high medicinal value of these parts of the tree.

The findings imply that the propagation of jackfruit seedlings in areas, where it can be grown can be achieved through pretreatment of fresh seeds in 3% hydrogen peroxide. One jackfruit can produce 100 and 500 seeds; hence, the seeds can be potentially germinated and distributed over a wide area. The fruit is the biggest in the world (up to 50 kgs), although the variety found in Kenya mostly ranges from 2 to 20 kgs. Notably, the tree takes four years to grow and once the tree is fully grown it can survive up to 100 years, while producing the fruits yearly. Therefore, the tree can provide food for many families because of its large size. The mass propagation of the jackfruit seedlings utilizing the protocol described in the study may encourage the cultivation of the tree in mutliple regions in Kenya. The planting of the seedlings will also be consistent with the current government initiatives of improving the forest cover in the country by planting more trees.

The study for optimization of DNA extraction in jackfruit using the different protocols has not been reported yet. The use of liquid nitrogen for DNA extraction is a limiting factor in most laboratories in limited resource settings as it is highly volatile and requires extreme freezing conditions, which may not be available in these laboratories. The alternative protocol enables researchers with limited funding to conduct genetic studies in plants with high secondary metabolites.

The findings of the study indicate that jackfruit is moderately rich in diversity. This means that there might be some varieties that have superior traits compared to others. The identification of the varieties with superior traits such as better adaptive capacity may facilitate the propagation of these trees through breeding programs.

7.6 Conclusion

The study reveals that the entire jackfruit tree is beneficial. Firstly, the edible region (seeds and pulps) can be consumed to provide nutrients in the body. Moreover, the other parts of the plant can be used for medicinal purposes. The entire tree provides a renewable alternative source of the phytochemicals and natural antioxidants, which can be harvested and used for therapeutic interventions of chronic illnesses. Notably, pulps and seeds have a rich nutritional profile; however, the seeds are more nutritious compared to the pulp region. The consumption of the seeds should be encouraged to minimize food and nutrition insecurity. Furthermore, jackfruit pulp and seeds are good sources of essential minerals such as potassium, calcium, sodium, iron, zinc, copper and magnesium. Hydrogen peroxide solution is efficient in germination of jackfruit seeds. Hence, the technique can be used for seed germination and propagation of jackfruit in Kenya. The modified CTAB-SDS based protocol is effective in extraction of DNA from young leaves. The technique does not employ the use of liquid nitrogen and beta mercaptoethanol; thus, it can be used in laboratories with limited freezing facilities. Jackfruit found in selected regions in Kenya and Uganda have moderate genetic diversity. This means that there may be varieties that have superior traits such as drought resistant or pest resistant amongst others that can be identified and propagated to alleviate food and nutrition insecurity in Kenya.

7.7 Recommendations

The jackfruit seeds are mostly discarded in Kenya and Uganda, and they were found to be highly nutritious and better source of carbohydrate and protein compared to the pulps. Therefore, the seeds can be ground and used for enhance the nutrition of maize, wheat and millet flour to reduce nutrition insecurity in these countries. Moreover, the

challenge of frequent drought in Kenya necessitates the propagation of varieties that have better adaptive ability in harsh conditions. Future studies can focus on identification of jackfruit varieties that have better adaptive traits such as drought and disease resistance. Additionally, the examination of the effect of different processing and preservation methods on the nutritional and phytochemical composition of jackfruits is crucial since the pulps and seeds of jackfruit require preservation due to the high moisture content. The major limitation of the study was obtaining mature fruits in Kenya, as the trees are quite rare and the ripening seasons were also different. The nutritional profile obtained in the study can be used in popularization of the fruit to increase its cultivation in Kenya, whereas the germination protocol can be used for large scale propagation of the trees in most parts of the country.

REFERENCES

Abedin, M. S., Nuruddin, M. M., Ahmed, K. U., and Hossain, A. (2012). Nutritive compositions of locally available jackfruit seeds (*Artocarpus heterophyllus*) in Bangladesh. *International Journal of Biosciences*, *2*(8), 1-7.

Abong, G. O., Okoth, M. W., Karuri, E. G., Kabira, J. N., and Mathooko, F. M. (2009). Nutrient contents of raw and processed products from Kenyan potato cultivars. *Journal of Applied Biosciences*, *16*, 877-886.

Abu bakar, Z., and Maimuna, A. (2013). Effect of hydrochloric acid, mechanical scarification, wet heat treatment on germination of seed of *Parkia biglobosa* African locust bean (Daurawa) case study of Gombe Local Government Area. *Journal of Applied Sciences and Environmental Management*, *17*(1), 119-123.

Abu Bakar, M. F., Karim, F. A., and Perisamy, E. (2015). Comparison of phytochemicals and antioxidant properties of different fruit parts of selected *Artocarpus* species from Sabah, Malaysia. *Sains Malaysiana*, *44*(3), 355-363.

Ajayi, I. (2008). Comparative study of the chemical composition and mineral element content of *Artocarpus heterophyllus* and *Treculia africana* seeds and seed oils. *Bioresource. Technology*, 99 (11), 5125–5129.

Allen, G. C., Flores-Vergara, M. A., Krasynanski, S., Kumar, S., and Thompson, W. F. (2006). A modified protocol for rapid DNA isolation from plant tissues using cetyltrimethylammonium bromide. *Nature protocols*, *1*(5), 2320 -2323.

Amos, William, and John Harwood. (1998). Factors affecting levels of genetic diversity in natural populations. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *353* (1366), 177-186.

Andrew, R. L., Wallis, I. R., Harwood, C. E., and Foley, W. J. (2010). Genetic and environmental contributions to variation and population divergence in a broad-spectrum foliar defence of *Eucalyptus tricarpa*. *Annals of Botany*, *105*(5), 707-717.

Andarwulan, N., Fardiaz, D., Wattimena, G. A., and Shetty, K. (1999). Antioxidant

activity associated with lipid and phenolic mobilization during seed germination of *Pangium edule Reinw*. *Journal of Agricultural and food chemistry*, *47*(8), 3158-3163.

AOAC (1995). *Official Methods of Analysis of AOAC International, 16th edition. Methods* 950.46, 928.08, 991.36, 920.153. Washington (D.C.)

Arina, A. N., and Azrina, A. (2016). Comparison of phenolic content and antioxidant activity of fresh and fried local fruits. *International Food Research Journal*, *23*(4), 1717.

Ashrafuzzaman, A, Sukarna, K., Dilafroza, K and Haque P. (2012). In Vitro Regeneration and Multiplication of Jackfruit (*Artocarpus Heterophyllus* L.), *Research Journal of Biology,* 2.2, 59-65.

Arruda, S. R., Pereira, D. G., Silva-Castro, M. M., Brito, M. G., and Waldschmidt, A. M. (2017). An optimized protocol for DNA extraction in plants with a high content of secondary metabolites, based on leaves of Mimosa tenuiflora (Willd.) Poir.(Leguminosae). *Genetics and molecular research: GMR*, *16*(3), 1-3

Arung, E. T., Shimizu, K., and Kondo, R. (2006). Inhibitory effect of artocarpanone from *Artocarpus heterophyllus* on melanin biosynthesis. *Biological and Pharmaceutical Bulletin*, *29*(9), 1966-1969.

Awal, H. M. A., and Gheyasuddin, S. (1991). Biochemical parameters of jackfruit seed-meal [Bangladesh]. *Bangladesh Journal of Agricultural Research (Bangladesh)*. 16(1): 17-21.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, *181*(4617), 1199.

Berquin, I. M., Min, Y., Wu, R., Wu, J., Perry, D., Cline, J. M. and Edwards, I. J. (2007). Modulation of prostate cancer genetic risk by omega-3 and omega-6 fatty acids. *The Journal of Clinical Investigation*, *117*(7), 1866-1875.

Burci, L. I. M., da Silva, C. B., de Oliveira, M., Dalarmi, L., Zanin, S. M. W.,

Miguel, O. U.G. and Miguel, M. D. (2015). Determination of antioxidant, radical scavenging activity and total phenolic compounds of Artocarpus heterophyllus (Jackfuit) seeds extracts. *Journal of Medicinal Plants Research*, *9*(40), 1013-1020.

Centre for Disease Control and Prevention. (CDC). (2018). Micronutrients fact. Retrieved from: https://www.cdc.gov/nutrition/micronutrient-malnutrition/micronutrients/index.html (Accessed on April 6 2019)

Collard, B. C., and Mackill, D. J. (2009). Start codon targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. *Plant Molecular Biology Reporter*, *27*(1), 86-89.

De Beer D, Joubert E, Gelderblom WC, Manley M. (2002). Phenolic compounds: A review of their possible role as *in vivo* antioxidants of wine. *South African Journal of Enology and Viticulture*. 2002;23(2): 48–58.

De Groote, Hugo, Timothy Nyanamba, and Raphael Wahome. (2010). Quality protein maize for the feed industry in Kenya. *Outlook on Agriculture*, *39*(4): 291-298.

Devos, K. M., Millan, T., and Gale, M. D. (1993). Comparative RFLP maps of the homoeologous group-2 chromosomes of wheat, rye and barley. *Theoretical and Applied Genetics*, *85*(6-7), 784-792.

Dellaporta, S. L., Wood, J., and Ticks, J. B. (1983). A plant molecular DNA minipreparation version 2. *Plant Molecular Biology Reporter*, *1*, 19-21.

Doosty, B., Drikvand, R., Salahvarzi, E., Amiri, H., and Hadian, J. (2012). Comparative analysis and optimization of different DNA extraction protocols in *Satureja khuzistanica*. *International Journal of Biology*, *4*(4), 112-116.

Doyle, J. J., and Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. *Focus*, *12*, 13-15.

Eke-Ejiofor, J., and Owuno, F. (2013). The physico-chemical and sensory properties of jackfruit (*Artocarpus heterophilus*) jam. *International Journal of Nutrition and Food Sciences*, *2*(3), 149-152.

Elevitch, C. R., and Manner, H. I. (2006). *Artocarpus heterophyllus* (jackfruit). *Species Profiles for Pacific Island Agroforestry*, *10*, 1-25.

El-Zaher, M. A. (2008). Using the grafting for propagation of the jackfruit and producing the rootstocks for the grafting. *American-Eurasian Journal of Agricultural and Environmental Sciences*, *3*(3), 459-473.

Eneji, E. G., Atangwho, I. J., Iwara, I. A., and Eyong, E. U. (2011). Micronutrient and phyto-chemical composition of root bark and twig extracts of *Gongronema latifolium*. *Journal of Medicine and Medical Sciences*, *2*(11), 1185-1188.

Evenson, R. E., and Gollin, D. (2003). Assessing the impact of the Green Revolution, 1960 to 2000. *Science*, *300*(5620), 758-762.

FAO (2009). *The State of Food Insecurity in the World*. . Rome. http://www.fao.org/news/story/en/item/161819/icode/

FAO. (2005). FAO's activities in nutrition and biodiversity. *Third Session of the Intergovernmental Technical Working Group on Plant Genetic Resources for Food and Agriculture*, Rome.

FAO and UNEP. 2020. The State of the World's Forests 2020. *Forests, Biodiversity and People*. Rome. https://doi.org/10.4060/ca8642en

FAO, WFP and IFAD. (2012). *The State of Food Insecurity in the World 2012: Economic growth is necessary but not sufficient to accelerate reduction of hunger and malnutrition*. Rome, FAO. Retrieved from: http://www.kenyafoodsecurity.org/images/files/shortrains/sra2013/2012_2013_SRA_Report.pdf>

FAO. (2014). *State of Food Insecurity in the World*, FAO, Rome, Italy.

FAO, IFAD, UNICEF, WFP, and WHO. (2017). *The State of Food Security and Nutrition in the World 2017*. *Building resilience for peace and food security*. Rome, FAO.

Fanzo, J. (2014). Strengthening the engagement of food and health systems to improve nutrition security: Synthesis and overview of approaches to address malnutrition. *Global Food Security*, *3*(3-4), 183-192.

Ferriol, M., Pico, B., and Nuez, F. (2003). Genetic diversity of a germplasm

collection of *Cucurbita pepo* using SRAP and AFLP markers. *Theoretical and Applied Genetics*, *107*(2), 271-282.

Frison, E. A., Cherfas, J., and Hodgkin, T. (2011). Agricultural biodiversity is essential for a sustainable improvement in food and nutrition security. *Sustainability*, *3*(1), 238-253.

Gajanana, T. M., Gowda,D., and Reddy, C. (2010). Exploring Market Potential and Developing Linkages – *A Case of Underutilized Fruit Products in India*, 23, 437–443.

Garcia, A. A., Benchimol, L. L., Barbosa, A. M., Geraldi, I. O., Souza Jr, C. L., and Souza, A. (2004). Comparison of RAPD, RFLP, AFLP and SSR markers for diversity studies in tropical maize inbred lines. *Genetics and Molecular Biology*, *27*(4), 579-588.

Garcia-Vallvé, S., Palau, J., and Romeu, A. (1999). Horizontal gene transfer in glycosyl hydrolases inferred from codon usage in Escherichia coli and *Bacillus subtilis*. *Molecular Biology and Evolution*, *16*(9), 1125-1134.

Gopalsamy, A, Sundaravadivelan K, Kumar, S. and Mariselvan, M. (2012). Molecular Marker (Rapd) Based Fingerprinting on Jackfruit to Estimate the Genetic Diversity of Jackfruit in Bangladesh. *International Journal of Biosciences (IJB*), 4, 3-7.

Goswami, C., Hossain, M. A., Kader, H. A., and Islam, R. (2011). Assessment of physicochemical properties of jackfruits'(*Artocarpus heterophyllus Lam*) pulps. *Journal of Horticulture, Forestry and Biotechnology*, *15*(3), 26-31.

Govindaraj, M., Vetriventhan, M., and Srinivasan, M. (2015). Importance of genetic diversity assessment in crop plants and its recent advances: an overview of its analytical perspectives. *Genetics Research International*, *2015*, 1-5.

Gulsen, O., Uzun, A., Canan, I., Seday, U., and Canihos, E. (2010). A new citrus linkage map based on SRAP, SSR, ISSR, POGP, RGA and RAPD markers. *Euphytica*, *173*(2), 265-277.

Gupta, D., Mann, S., Sood, A., and Gupta, R. K. (2011). Phytochemical, nutritional

and antioxidant activity evaluation of seeds of jackfruit (*Artocarpus heterophyllus* Lam.). *International Journal of Pharma and Bio Sciences*, *2*(4), 336-345.

Guo, Z. H., Fu, K. X., Zhang, X. Q., Bai, S. Q., Fan, Y., Peng, Y., and Ma, X. (2014). Molecular insights into the genetic diversity of *Hemarthria compressa* germplasm collections native to southwest China. *Molecules*, *19*(12), 21541-21559.

Gyorgy, Z., Szabó, M., Bacharov, D., and Pedryc, A. (2012). Genetic diversity within and among populations of roseroot (*Rhodiola rosea* L.) based on molecular markers. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 40(2), 266-273.

Hakim, E. H., Juliawaty, L. D., Syah, Y. M., and Achmad, S. A. (2005). Molecular diversity of *Artocarpus champeden* (*Moraceae*): A species endemic to Indonesia. *Molecular Diversity*, *9*(1-3), 149-158.

Hossain, M. D., Sarwar, M. S., Dewan, S. M. R., Hossain, M. S., Shahid-Ud-Daula, A. F. M., and Islam, M. S. (2014). Investigation of total phenolic content and antioxidant activities of *Azadirachta indica* roots. *Avicenna Journal of Phytomedicine*, *4*(2), 97.

Hunter, D. and Fanzo, J. (2013). Agricultural biodiversity, diverse diets and improving nutrition. In, Diversifying Food and Diets: Using Agricultural Biodiversity to Improve Nutrition and Health (Fanzo, J. and Hunter, D. et al eds), *Issues in Agricultural Biodiversity*, Earthscan, UK, 1-13.

Hwang, J. H., Ahn, S. G., Oh, J. Y., Choi, Y. W., Kang, J. S., and Park, Y. H. (2011). Functional characterization of watermelon (*Citrullus lanatus* L.) EST–SSR by gel electrophoresis and high resolution melting analysis. *Scientia Horticulturae*, *130*(4), 715-724.

Imani A, Rasouli M, Tavakoli R, Zarifi E, Fatahi R, Barba-Espín G, Martínez-Gómez P (2011). Optimization of seed germination in *Prunus* species combining hydrogen peroxide or gibberellic acid pre-treatments with stratification. *Seed Science and Technology,* 39(1):204-207.

IPGRI (2006). *The International Plant Genetics Resources Institute*: Retrieved 2019-01-09: https://www.bioversityinternational.org/

Jagtap, U. B., and Bapat, V. (2010). *Artocarpus*: a review of its traditional uses, phytochemistry and pharmacology. *Journal of Ethnopharmacology* 129,142–66.

Jobin-Décor, M. P., Graham, G. C., Henry, R. J., and Drew, R. A. (1997). RAPD and isozyme analysis of genetic relationships between *Carica papaya* and wild relatives. *Genetic Resources and Crop Evolution*, *44*(5), 471-477.

Kang, T. J., and Yang, M. S. (2004). Rapid and reliable extraction of genomic DNA from various wild-type and transgenic. *BMC Biotechnology, 4,* (20), 1-12.

Kavya, K., Shyamalamma, S., and Gayatri, S. (2019). Morphological and molecular Genetic diversity analysis using SSR markers in Jackfruit (*Artocarpus heterophyllus* Lam.) genotypes for pulp colour. *Indian Journal of Agricultural Research*, *53*(1), 8-16.

Khan, R., Zerega, N., Hossain, S., and Zuberi, M. I. (2010). Jackfruit (*Artocarpus heterophyllus* Lam.) diversity in Bangladesh: land use and artificial selection. *Economic Botany*, *64*(2), 124-136.

Kumar, M., Ponnuswami, V., Nagarajan, P., Jeyakumar, P., and Senthil, N. (2013). Molecular characterization of ten mango cultivars using simple sequences repeat (SSR) markers. *African Journal of Biotechnology*, *12*(47), 6568-6573.

Kumaran, A., and Karunakaran, R. J. (2007). In vitro antioxidant activities of methanol extracts of five *Phyllanthus* species from India. *LWT-Food Science and Technology*, *40*(2), 344-352.

Li, G., and Quiros, C. F. (2001). Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. *Theoretical and Applied Genetics*, *103*(2-3), 455-461.

Liu, H., Tan, W., Sun, H., Liu, Y., Meng, K., and Liao, W. (2016). Development and characterization of EST- SSR markers for *Artocarpus hypargyreus* (Moraceae). *Applications in plant sciences*, *4*(12), 1-4.

Lobo V, Patil A, Phatak A, Chandra N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews*. 4(8):118.

Luna, T., Wilkinson, K., and Dumroese, R. K. (2009). Seed germination and sowing options. *Nursery manual for native plants: A guide for tribal nurseries*, *1*, 133-151.

Madegwa, Y. M. (2015). Modelling nutrient dynamics and yield of finger millet (*Eleusine Coracana*) in semi-arid eastern Kenya, using the agricultural production systems simulator model (APSIM). *Department of Land Resource Management and Agricultural Technology, Faculty of Agriculture, University of Nairobi, Nairobi*.

Madrigal- Aldana, D. L., Tovar- Gómez, B., de Oca, M. M. M., Sáyago- Ayerdi, S. G., Gutierrez- Meraz, F., and Bello- Pérez, L. A. (2011). Isolation and characterization of Mexican jackfruit (Artocarpus heterophyllus L) seeds starch in two mature stages. *Starch- Stärke*, *63*(6), 364-372.

Mathithumilan, B., Kadam, N. N., Biradar, J., Reddy, S. H., Ankaiah, M., Narayanan, M. J., and Sreeman, S. M. (2013). Development and characterization of microsatellite markers for *Morus* spp. and assessment of their transferability to other closely related species. *BMC Plant Biology*, *13*(1), 194.

McBride, D. (2003). The hazards of liquid nitrogen. *The New Zealand Medical Journal*, *116*(1168), U305-U305.

Meng, H., Chen, S., Cheng, Z., Chai, D. Li, Y and Pak, J. (2012). SRAP markers for fruit shape in cucumber, *Pakistan Journal of Botany*, *44*(4), 1381-1384.

Mirajkar, S. J., Rai, A. N., Vaidya, E. R., Moharil, M. P., Dudhare, M. S., and ` Suprasanna, P. (2017). TRAP and SRAP molecular marker based profiling of radiation induced mutants of sugarcane (Saccharum officinarum L.). *Plant Gene*, *9*, 64-70.

Morton, J. F. (1987). Fruits of warm climates. Florida Flair Books, Miami.

Mukprasirt, A. and Sajjanaantakul, K. (2004) Physico-chemical properties of flour

and starch from jackfruit seeds (Artocarpus heterophyllus Lam.) compared with modified starches. *International Journal of Food Science and Technology, 39*, 271–276.

Mussane, C. R. B., Biljon, A. V., Herselman, L., Books, R. and Scarda, R. (2010). Morphological and genetic characterization of mango varieties in Mozambique. In *Second RUFORUM Biennial Meeting, Uganda* (pp. 991-995).

Mui, N.T., Ledin, I., Udén, P. and Binh, D,V. (2001). Effect of replacing a rice bran-soya bean concentrate with Jackfruit (Artocarpus heterophyllus) or Flemingia (*Flemingia macrophylla*) foliage on the performance of growing goats. *Livestock Production Science*. 72, 253-262.

Murray, M. G., and Thompson, W. F. (1980). Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research, 8*(19), 4321-4326.

Nahak, G., and Sahu, R. K. (2010). In vitro antioxidative acitivity of *Azadirachta indica* and *Melia azedarach* Leaves by DPPH scavenging assay. *Natural Science*, *8*(4), 22-28.

Nakintu, J., Albrecht, C., Müller, C. M., Kagoro-Rugunda, G., Andama, M., Olet, E. A., ... and Gemeinholzer, B. (2019). Exploring the genetic diversity of jackfruit (*Artocarpus heterophyllus* Lam.) grown in Uganda based on SSR markers. *Genetic Resources and Crop Evolution*, *67*(3), 605-619.

Nakintu, J., Olet, E., Andama, M. A., and Lejju, J. (2019). Ethno-varieties and Distribution of jackfruit tree (*Artocarpus heterophyllus* Lam.) in Uganda: implications for trade, food security and germplasm conservation. *East African Journal of Science, Technology and Innovation*, *1*(1). 27-51.

Narain, P. (2000). Genetic diversity-conservation and assessment. *Current Science-Bangalore*, *79*(2), 170-175.

Nemli, S., Kianoosh, T., and Tanyolac, M. B. (2015). Genetic diversity and population

structure of common bean (*Phaseolus vulgaris* L.) accessions through retrotransposon-based interprimer binding sites (iPBSs) markers. *Turkish Journal of Agriculture and Forestry*, *39*(6), 940-948.

Ocloo, F. C. K., Bansa, D., Boatin, R., Adom, T., and Agbemavor, W. S. (2010). Physico-chemical, functional and pasting characteristics of flour produced from Jackfruits (*Artocarpus heterophyllus*) seeds. *Agriculture and Biology Journal of North America*, *1*(5), 903-908.

Odoemelam, S, A. (2005). Functional properties of raw and heat processed jackfruit (*Artocarpus heterophyllus*) flour. *Pakistan Journal of Nutrition*, 4, 366-370.

Oliveira, E. J. D., Costa, J. L., Santos, L. F. D., Carvalho, F. M. D., Silva, A. D. S., and Dantas, J. (2011). Molecular characterization of papaya genotypes using AFLP
markers. *Revista Brasileira de Fruticultura*, *33*(3), 849-858.

Ojwang, R. A., Runo, S. M., Ben, M., and Ogoyi, D. O. (2015). Lipid profile and levels of omega-3 polyunsaturated fatty acids present in jackfruit (*Artocarpus heterophyllus*) Lam.(Moraceae) seeds and variation in different treatments. *African Journal of Biotechnology*, *14*(16), 1409-1417.

Ojwang, R. A., Muge, E. K., Mbatia, B., Mwanza, B., and Ogoyi, D. O. (2017). Comparative Analysis of Phytochemical Composition and Antioxidant Activities of Methanolic Extracts of Leaves, Roots and Bark of Jackfruit (*Artocapus heterophyllus*) from Selected Regions in Kenya and Uganda. *Journal of Advances in Biology and Biotechnology*, *16*(1), 1-13.

Ojwang, R, A. (2012). Lipids and levels of Omega-3 and Omega-6 PUFAs in Jackfruit (*Artocarpus Heterophyllus*) found in Kenya. *MSc Thesis*, Kenyatta University. Dept of Biochemistry and Biotechnology.

Omar, H. S., El-Beshbishy, H. A., Moussa, Z., Taha, K. F., and Singab, A. N. B. (2011). Antioxidant activity of *Artocarpus heterophyllus* Lam.(Jack Fruit) leaf extracts: remarkable attenuations of hyperglycemia and hyperlipidemia in streptozotocin-diabetic rats. *The Scientific World Journal*, *11*, 788-800.

Owuor, P. O., Obaga, S. O., and Othieno, C. O. (1990). The effects of altitude on the chemical composition of black tea. *Journal of the Science of Food and Agriculture*, *50*(1), 9-17.

Prashanth, S, R., Parani, M., Mohanty, B, P., Talame, V., et al. (2002). Genetic diversity in cultivars and landraces of *Oryza sativa* subsp. *indica* as revealed by AFLP markers. *Genome,* 45, 451-459.

Phaneendranath, B. R. (1980). Influence of amount of water in the paper towel on standard germination tests. *Journal of Seed Technology*, 82-87.

Qian, C. T., Chen, J. F., and Luo, X. D. (2006). Identification and characterization of cucumber alien translocation plant AT-04 with resistance to fusarium wilt. *Journal-Nanjing Agricultural University*, *29*(20), 1-3.

Rocha, E. A., Paiva, L. V., Carvalho, H. H. D., and Guimarães, C. T. (2010). Molecular characterization and genetic diversity of potato cultivars using SSR and RAPD markers. *Crop Breeding and Applied Biotechnology*, *10*(3), 204-210.

Schnell, R, J., Olano, C,T., Campbell, R, J and Brown, J, S. (2001). AFLP analysis of genetic diversity within a jackfruit germplasm collection. *Science of Horticuture.* 91: 261-274.

SCUC. (2006). Jackfruit *Artocarpus heterophyllus*. Field manual for extension workers and farmers. *SCUC,* Southampton, UK.

Sensoy S, Büyükalaca S and Abak K (2007). Evaluation of genetic diversity in Turkish melons (*Cucumis melo* L.) based on phenotypic characters and RAPD markers. *Genetic Resources of Crop Evolution.* 54, 1351-1365.

Shanmugapriya K, Saravana S, Payal H, Mohamed P, Binnie W. (2011). Antioxidant activity, total phenolic and flavonoid contents of *Artocarpus heterophyllas* and *Manilkara zapota* seeds and its reduction potential. *International Journal of Pharmacy and Pharmaceutical Sciences,* 3(5):256–260.

Shafiq, M., Mehmood, S., Yasmin, A., Khan, S. J., Khan, N. H., and Ali, S. (2017). Evaluation of phytochemical, nutritional and antioxidant activity of indigenously grown jackfruit (*Artocarpus heterophyllus Lam*). *Journal of Scientific Research*, *9*(1),
135-143.

Singh, R., & Tiwari, J. P. (1998). In-vitro clonal propagation of jackfruit (*Artocarpus heterophyllus Lamk*.). *Indian Journal of Horticulture*, *55*(3), 213-217.

Sika, K. C., Kefela, T., Adoukonou-Sagbadja, H., Ahoton, L., Saidou, A., Baba-Moussa, L., and Gachomo, E. W. (2015). A simple and efficient genomic DNA extraction protocol for large scale genetic analyses of plant biological systems. *Plant Gene*, *1*, 43-45.

Sirisha, N., Rao,V., Rao B, D., and Rao, R. T. (2014). Evaluation of antioxidant activities, phytochemical constituents and protein profiling of five varieties of jackfruit (*Artocarpus* species) seeds. *International Journal of Pharmaceutical Sciences*. 4(4), 626–631.

Shyamalamma, S. S., Chandra, S., Hegde, M., and Naryanswamy, P. (2008). Evaluation of genetic diversity in jackfruit (*Artocarpus heterophyllus* Lam.) based on amplified fragment length polymorphism markers. *Genetics and Molecular Research*, *7*(3), 645-656.

Soetan, K. O., Olaiya, C. O., and Oyewole, O. E. (2010). The importance of mineral elements for humans, domestic animals and plants-A review. *African Journal of Food Science*, *4*(5), 200-222.

Sudan, J., Raina, M., Singh, R., Mustafiz, A., and Kumari, S. (2017). A modified protocol for high-quality DNA extraction from seeds rich in secondary compounds. *Journal of Crop Improvement*, *31*(5), 637-647.

Sudha, G., Priya, M. S., Shree, R. I., and Vadivukkarasi, S. (2011). In vitro free radical scavenging activity of raw pepino fruit (*Solanum muricatum* aiton). *International Journal of Current Pharmaceutical Research*, *3*(2), 137-140.

Sundarraj, A. A., and Vasudevan, T. (2018). Jackfruit Taxonomy and Waste Utilization. *Vegetos: An International Journal of Plant Research and Biotechnology*, *31*(1), 67-73.

Swami, S. B., N. J. Thakor, P. M. Haldankar, and Kalse, S,B. (2012). Jackfruit and Its Many Functional Components as Related to Human Health: A Review. *Comprehensive Reviews in Food Science and Food Safety* 11, 565–576.

Sylvie, D. D., Anatole, P. C., Cabral, B. P., and Veronique, P. B. (2014). Comparison of in vitro antioxidant properties of extracts from three plants used for medical purpose in Cameroon: *Acalypha racemosa*, *Garcinia lucida* and *Hymenocardia lyrata*. *Asian Pacific Journal of Tropical Biomedicine*, *4*, S625-S632.

Szopińska, D. (2014). Effects of hydrogen peroxide treatment on the germination, vigour and health of Zinnia elegans seeds. *Folia Horticulturae*, *26*(1), 19-29.

Tambe, V. D., and Bhambar, R. S. (2014). Estimation of total phenol, tannin, alkaloid and flavonoid in *Hibiscus tiliaceus* Linn. wood extracts. *Journal of Pharmacognosy and Phytochemistry*, *2*(4), 41-47.

Takaya, J., Higashino, H., and Kobayashi, Y. (2004). Intracellular magnesium and insulin resistance. *Magnesium research*, *17*(2), 126-136.

Travlos, I. S., and Economou, G. (2006). Optimization of seed germination and seedling emergence of *Medicago arborea* L. *International Journal of Botany*, *2*(4), 415-420.

Tulyathan, V., Tananuwong, K., Songjinda, P., and Jaiboon, N. (2002). Some physicochemical properties of jackfruit (*Artocarpus heterophyllus* Lam) seed flour and starch. *Science Asia*, *28*(1), 37-41.

United Nations (UN). (2017). *World population projected to reach 9.8 billion in 2050, and 11.2 billion in 2100*. Retrieved from https://www.un.org/development/desa/en/news/population/world-population-prospects-2017.html

USAID. (2018). Kenya Nutritional Profile. Retrieved from www.usaid.gov/sites/default/files/documents/1864/Kenya-Nutrition-Profile-Mar2018-508.pdf (Accessed January 12, 2019)

Vinha, A. F., Moreira, J., and Barreira, S. V. (2013). Physicochemical parameters, phytochemical composition and antioxidant activity of the algarvian avocado (*Persea americana* Mill.). *Journal of Agricultural Science*, *5*(12), 100.

Walter, M., Kilian, J., and Kudla, J. (2002). PNPase activity determines the

efficiency of mRNA 3′- end processing, the degradation of tRNA and the extent of polyadenylation in chloroplasts. *The EMBO journal*, *21*(24), 6905-6914.

WHO. (2017). Mortality and global health estimates. *World Health Organization*. Retrieved from: http://1.http://www.who.int/gho/mortality_burden_disease/en (Accessed October 12, 2017).

Williams, J., and Haq, N. (2002). Global Research on Underutilized Crops - An assessment of current activities and proposals for enhanced cooperation. *International Centre for Underutilised Crops*, 1-50.

Winter, P., and Kahl, G. (1995). Molecular marker technologies for plant improvement. *World Journal of Microbiology and Biotechnology*, *11*(4), 438-448.

Wojtyla, Ł., Lechowska, K., Kubala, S., and Garnczarska, M. (2016). Different modes of hydrogen peroxide action during seed germination. *Frontiers in Plant Science*, *7*, 66, 1-16.

Appendices

Appendix 1

Table 9.1: SSR primer sequences

Primers	**Sequences**
AH 14 F	GCTTGTGGGTTCTGGGATCTAT
AH 14R	R: CAGACACTAGTTTGGATGTACT
AH 31 F	TCCTCTAACGTGCGCCCCTAAG
AH 31 R	AAACCCAGCGTGCCACCATTG
AH 46 F	GGAGAGGGCGGTGCAGTAGAA
AH 46 R	GCAGAGCAGACACTACAGTAGC
AH 59F	TCTCCTCCACCTCCTCCATTGT
AH 59R	GACCTTGGGACCCGCACTTCTT
AH 77F	CGAGAAGGTTCCGAGCCAGATT
AH 77R	CCCGACCAAGACCCGGAGTATA
AH 76F	GAACGGCAGATTTCACCATTTT
AH 76R	AGGATCAACTTAGCCCACTATA

Appendix 2

Table 9.2: SRAP marker combinations

Primers	**Sequences**
ME 5-EM 7	ME 5: 5'-TGAGTCCAAACCGGAAG-3' EM 7: 5'-GACTGCGTACGAATTCAA-3'
ME 5-EM 9	ME 5: 5'-TGAGTCCAAACCGGAAG-3' EM 9: 5'-GACTGCGTACGAATTCGA-3'
ME 5 –EM 10	ME 5: 5'-TGAGTCCAAACCGGAAG-3' EM 10: 5'-GACTGCGTACGAATTCAG-3'
ME8-EM 7	ME 8: 5'-TGAGTCCAAACCGGTGC-3' EM 7: 5'-GACTGCGTACGAATTCAA-3'
ME 8-EM 9	ME 8: 5'-TGAGTCCAAACCGGTGC-3' EM 9: 5'-GACTGCGTACGAATTCGA-3'
ME 8-EM 10	ME 8: 5'-TGAGTCCAAACCGGTGC-3' EM 10: 5'-GACTGCGTACGAATTCAG-3'
ME 11-EM 7	ME11: TGAGTCCAAACCGGAAC EM 7: 5'-GACTGCGTACGAATTCAA-3'
ME 11-EM 9	ME11: TGAGTCCAAACCGGAAC EM 9: 5'-GACTGCGTACGAATTCGA-3’
ME 11-EM 10	ME11: TGAGTCCAAACCGGAAC EM 10: 5'-GACTGCGTACGAATTCAG-3'