NUTRITIONAL VALUE OF BAMBARA GROUNDNUT (*Vigna subterranea* (L.) Verdc.): A HUMAN AND ANIMAL PERSPECTIVE

by

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PREFACE

The research contained in this thesis was completed by the candidate while based in the Discipline of Crop Science, School of Agricultural, Earth and Environmental Sciences, in the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg Campus, South Africa. The research was financially supported by the South African Department of Agriculture, Fisheries and Forestry (DAFF) 'Zero hunger' project.

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

_____ _____

Supervisor (A.T. Modi) Co-supervisor (K. Pillay)

Date: 03 November 2014

DECLARATION

I, Pumlani Gqaleni, declare that:

(i) the research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work;

(ii) this dissertation has not been submitted in full or in part for any degree or examination to any other university;

(iii) this dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;

(iv) this dissertation does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

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b) where their exact words have been used, their writing has been placed inside quotation marks, and referenced;

(v) where I have used material for which publications followed, I have indicated in detail my role in the work;

(vi) this dissertation is primarily a collection of material, prepared by myself, published as journal articles or presented as a poster and oral presentations at conferences. In some cases, additional material has been included;

(vii) this dissertation does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the References sections.

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ABSTRACT

Bambara groundnut (*Vigna subterranea* (L) Verdc.) is an indigenous African legume that is reported to have wide adaptation to a range of environments. It is popular among subsistence farmers in sub-Saharan African. However, research on the crop still lags behind that of other established legumes and in most places the crop is still cultivated from landraces, with no locally improved varieties available. The objective of the study was to evaluate the nutritional and agronomic potential of bambara groundnut. Three separate experiments were undertaken, (i) seed quality determination during germination, (ii) controlled environment study to determine yield and nutritional quality under water stress and (iii) field trials to determine the effect of seasons and location on nutrient composition. The results showed that the darker coloured seeds had a faster germination rate. Black speckled seeds had the highest (crude protein) CP after 8 (20.67%), 16 (22.11%), 24 (20.68 %), and 48 hours (20.77%), on the other hand cream seeds had the lowest CP after 16 (19.30%), 24 (18.71%), and 72 hours (19.16 %). The results showed that nutrient composition varied during early imbibition and the variations could be associated with seed colour and duration of imbibition. Under controlled environments, statistically significant differences were observed for plants under 100% ETc when compared with plants under 30% ETc with regards to stomatal conductance. Bambara groundnut landrace selections were able to adapt to the limited water under 30% ETc by closing their stomata. The lower stomatal conductance at 30% ETc relative to 100% ETc demonstrated a regulation of transpirational losses, through effective stomatal control. Under field conditions, the interactions between seasons, location, irrigation systems, sequential harvesting and crop varieties is one that needs sufficient planning so as to maximise nutrient quality and overall crop production. The nutritive value and mineral contents of bambara groundnut landrace selections varied considerably in response to water regimes, sequential harvesting, locations and seasons. These findings suggested that bambara groundnut is a drought resistant crop and can aid as an affordable all year round forage supplement for ruminants during the dry seasons.

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DEDICATION

I dedicate this thesis to my unborn son aka “Moses”. When the news came that you are on the way I picked up my pen again and started writing this theses, you are the reason I worked harder so as to make sure you are provided with all you could ever need.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

Agriculture is mainly carried out to produce food for human and animal consumption as well as raw materials for industry. In most African countries, farming is practiced by smallholder farmers for their own subsistence using traditional methods. These current levels of production are evidently not enough to meet the increasing demands for food due to the ever increasing population (Food and Agriculture Organisation (FAO), 2006). In 1900, the world population size was 1.5 billion. Today it is just over 7 billion and is expected to increase to 9 billion by 2050 (Michael, 2001).The highest rate of population growth will be in the zones which already suffer from poverty and food shortage; most of these zones are in Africa (FAO, 2006). South Africa's current population has recently surpassed the 50 million people mark (FAO, 2012). This suggests that South African agriculture will have to increase output in order to meet growing demand from an increasing population.

However, there are limitations to achieving this, chiefly, land and water are limited resources, with water set to become even more scarce in the near future (Hassan *et al*., 2005; Schulze, 2011). This suggests that there is a need for innovative and sustainable agricultural interventions in order to broaden the food basket and ensure future food security. One approach that has recently emerged is to evaluate and characterize underutilised indigenous and traditional food crops with a view to re-introducing them in rural areas (Mabhaudhi, 2009). One such crop of interest is bambara groundnut (*Vigna subterranea* L. Verdc), an indigenous African legume (Mabhaudhi *et al*., 2013).

Legumes have received much attention for utilization in a variety of food systems due to their wide distribution throughout the world and potentially high protein content (Mahala and Mohamed, 2010).They have been the basic source of food security in a number of developing

countries (Borget, 1992). In addition, legumes can be exploited for both human and animal use. However, very little efforts have been made to exploit less popular legumes in the fight against poverty and malnutrition. With the general scare that current subsistence farming systems are inadequate, current and expected population growths are and will continue to put pressure on these systems. Legumes that have not been thoroughly studied. For example, bambara groundnut has a great potential in addressing the problem of protein malnutrition in sub-Saharan Africa. Furthermore, with animal protein prices increasing daily, such legumes could prove to be a cheaper source of dietary protein.

Bambara groundnut, a self-pollinating annual legume, is one of the most favoured legumes by resource-limited farmers living in rural areas (Azam-Ali *et al.*, 2001). It is the third most important legume after peanuts (*Arachis hypogaea)* and cowpea (*Vigna unguiculata*) (Mkandawire, 2007). The crop is said to be tolerant to drought (Berchie *et al*., 2012), pests (Tweneboah, 2000) and produces a reasonable yield when grown under poor soil conditions (Messiaen, 1992). Azam-Ali *et al.* (2001) demonstrated that bambara groundnut is resilient to adverse environmental conditions as it tolerates low fertility soils and low rainfall Ocran *et al*. (1998) also reported that bambara groundnut could successfully grow in areas with less than 500 mm of annual rainfall. Local research on bambara groundnut (Mabhaudhi and Modi, 2011; Mabhaudhi *et al*., 2013) has also confirmed that local landraces of bambara groundnut are drought tolerant. The authors went on to suggest that bambara groundnut may be a suitable crop for cultivation in marginal areas with low rainfall.

In addition to its reported drought tolerance, bambara groundnut seed also makes a complete feed for both humans and animals. The above ground material and by-products of bambara groundnut can be used as feed ingredients and incorporated in the formulation of animal feeds. Bambara groundnut can be easily converted to 'meat', which may meet human needs for animal-protein. Nutritional analyses undertaken by various researchers revealed that on average, the seeds contain 63% carbohydrates, 19% protein and 6.5 % fat in the form of oil (Azam-Ali *et al*., 2001; Ijarotimi and Esho, 2009). From a human nutrition perspective, the protein is of high quality, having a good balance of essential amino acids and relatively high lysine (6.8%) and methionine

(1.3%) contents (Ellah and Singh, 2008). The gross energy content has been reported to be higher than that of other more popular legumes such as cowpea, lentils and pigeon pea (Poulter, 1981). The nutritional value of bambara groundnut provides a cheap source of good quality protein to poorly-resourced farmers in semi-arid areas (Amarteifio *et al.*, 2006) making it a good supplement for both human and animal diets. The dual purpose of bambara groundnut makes it an important future crop.

As previously alluded, there is growth in the human population. This growth has also led to a parallel increase in animal production in order to feed the growing human population. Despite the increase in animal production systems, numerous challenges threaten the sustainability of the animal industry (Aarnink and Verstegen, 2007). One of these challenges involves increases in animal feed costs (Aarnink and Verstegen, 2007). The continued increase in feed costs is largely due to the competition for raw materials between humans and livestock, and increased cost of agronomic inputs such as labour, fertilizers, herbicides and insecticides. Consequently, economically feasible raw materials are now being sought after for animal feeds. Soybean meal has so far been used as an animal feed supplement and meets the required energy and amino acid levels required in animal diets; however, soybean has a number of environmental constraints (Hartman *et al*., 2011). Poor crop establishment was reported on soybean planted on dry soils at sowing (Egli, 1998). Ball *et al*. (2000) also reported that soybean has a short vegetative period when planted in areas with high temperature or during summer. There is also the fact that soybean is widely consumed by humans, either directly or indirectly. This, once again, puts human consumption in direct competition with animal feed. Therefore, identification of new/alternative low-cost feed resources is essential to meet growing demand from both human and animal consumption. It is in this context that this study aims to evaluate the use of bambara groundnut, an underutilized legume crop, as an alternative dual purpose crop with potential to feed humans as well as animals.

Several studies have reported on the nutritional composition of bambara groundnut seeds, particularly as a protein source (Belewu *et al*., 2008; Ijarotimi and Esho, 2009; Oyeleke, 2012). Less effort has, however, been dedicated to the potential of bambara groundnut seeds and other

parts of the bambara plant, as a source of nutrition for both humans and animals. Utilization of the whole plant as a green fodder can be practised. The crop's functional properties (bulk density, water holding capacity, emulsifying capacity and stability, and foaming capacity and stability) would also make it useful in fortifying food materials that are low in protein (Onimawo *et al*., 1998). Studying the effects of sequential harvesting on the nutritional composition of bambara groundnuts (above ground material), can prove useful in bridging the knowledge gap on the crop's nutrient profile during growth and development. A number of nutritional properties (proteins, minerals, fibre content) are likely to change during crop growth; however, such information is currently lacking and poorly understood. Such information would be useful in determining the most suitable time for harvesting the above ground matter of bambara groundnut for optimum nutrient utilization in animal diets.

1.2 Justification

Given the fact that bambara groundnut is drought tolerant and highly nutritious, it has a great potential to address the protein-energy malnutrition problem in developing countries. Previous work on the nutritional content of bambara groundnut has not focused on the crop as a dual purpose crop used for both humans and animals in the context of crop management and landrace comparisons. Oyeleke *et al*. (2012) reported on the nutritional content of bambara groundnut from a narrow perspective of one variety. These gaps provide a premise for the current study to provide the nutritional profile of bambara groundnut landraces differing in seed colour and also to interrogate the potential use of bambara as a dual purpose crop. This information would help farmers to reduce feed costs and increase allow for the utilisation of bambara groundnut as a legume.

1.3 Aims and Objectives

The broad objective of the study was to determine the effects of sequential harvesting on three bambara groundnut landraces differing in seed colour under controlled environment as well as field conditions in response to water availability. The specific objectives were:

- to determine the effect of seed colour on the nutritional value of bambara groundnut seeds during germination and the association of germination time on the accumulation of various nutrients;

- to determine the effect of water stress and sequential harvesting on plant growth, development, yield and nutritional content of bambara groundnut selections; and

- to compare the effect of seasons, environmental conditions and irrigation on nutritional content and quality of bambara groundnut varieties.

CHAPTER 2

LITERATURE REVIEW

2.1 Origin and Taxonomy of Bambara Groundnut

Early researchers interested in the origin of bambara groundnut (*Vigna subterranea* L. Verdc) believed that the crop originated from Africa. Bambara groundnut belongs to the family *Leguminosae*, sub–family *Papilionoideae* (Goli, 1997). The genus *Vigna* also comprises a wild species type (*V. subterranea* var. *spontanea*) while *V. subterranea* var. *Subterranea* is the cultivated species. Bambara groundnut was derived from the name of a tribe from the Bambara people, central Mali near Timbuktu (Goli, 1997). The crop spread throughout Africa by means of migration of indigenous people. This crop is also found in other continents, for example Asia and North America. However, despite it being an indigenous African legume, its popularity has now been overshadowed by groundnuts (*Arachis hypogaea*).

In South Africa, bambara groundnut production occurs in KwaZulu-Natal, Eastern Cape, Mpumalanga, Limpopo and the Northern Province (Swanevelder, 1998). The Venda and the Bolebedu people claim to have brought bambara groundnut to South Africa (Bamshaiye *et al.*, 2011). The Venda people's contestation on the claim is sounder as the name 'Ndluhu-mvenda' meaning groundnut of Vendaland, is commonly used around the Venda regions. Farmers in Mpumalanga province claim that the introduction of the crop came about during dry winter seasons when major crops such as maize were not produced to their potential yields. Around Mpumalanga it was therefore called the poor man's crop, as it was an alternative source of food protein for the small scale farmers. It also provided a means of survival during times of drought-induced famine. Bambara groundnut is also known as ntoyoci (Bemba, Republic of Zambia), jugo beans (South Africa), izindlubu (Zulu, South Africa), indlubu (Xhosa, South Africa), Kwaruru (Hausa, Nigeria), Okpa (Ibo, Nigeria), Epa-Roro (Yoruba, Nigeria) and Nyimo (Shona, Zimbabwe) (Bamshaiye *et al*., 2011).

2.2 Uses of Bambara Groundnut

Bambara groundnut is considered to be an underutilized crop. The low yields associated with bambara groundnut production may be attributed to the fact that its production and crop

improvement have been neglected over the past years by researchers. This neglect has occurred despite the fact that bambara groundnut is important for small scale farmers due to its drought tolerance and commercial potential. The waning popularity of bambara groundnut in traditional African communities can be attributed to the fact that it takes a long time to cook, it has poor milling characteristics and contains anti-nutritional factors such as tannins and trypsin inhibitors (Barimalaa and Anoghalu, 1997). However, bambara groundnut still plays an important role and is widely utilized in traditional dishes in several African countries such as Côte d'Ivoire (Yao *et al*., 2005), Zimbabwe, Nigeria (Uvere *et al*., 1999) and Cameroon (Goli, 1997).

Bambara groundnut is primarily used for human consumption. The seeds are consumed at different developmental stages, either immature or fully ripe. The immature seeds can be consumed fresh, boiled, grilled, as a meal or mixed with immature groundnuts or green maize (Bamshaiye *et al.,* 2011). Mature bambara groundnut seeds are very hard, hence boiling becomes a prerequisite before any further preparation. Ripe seeds are milled to produce flour which can be used to make biscuits and/or otherwise mixed with cereals and boiled to make porridge (Bamshaiye *et al.,* 2011). Ripe dry seeds are also roasted, broken into pieces, boiled, crushed and eaten as a relish. In Zimbabwe, a peanut like snack is also produced through roasting of bambara groundnut and can also be dried and stored for later use (de Kock, undated).

Commercial canning of bambara groundnuts has been practiced in Ghana, the nuts were canned in gravy by a government factory and over 40,000 cans were produced annually (Begemann, 1986). In Zimbabwe canned bambara beans were commercially produced for the market as 'Tulimara Nyimo Beans' and recommended for addition to soups, stews and salads. However, the successful commercialization of bambara groundnut in Zimbabwe was hampered by problems such as transport difficulties as the roads were not suitable for truck deliveries, distances to the farms, the fuel crisis and food shortages which often resulted in the beans not always being available for sale by local farmers. Other rival factors included storage facilities (the beans needed to be fumigated and stored in cold rooms), marketing strategies (lack of awareness of bambara groundnuts commercial products both locally and internationally as well as limited funds for marketing activities), relatively expensive compared with other legumes, distribution (not widely distributed to local supermarkets with large populations and also not available in villages (de Kock, undated).

Despite Zimbabwe's production constraints, it has been successful in exporting more than 3000 tonnes of bambara groundnut to South Africa and Swaziland (Hampson *et al.,* 2001).To date there has been no commercialization of bambara groundnut in South Africa. This means that South Africa is lagging behind. Lack of research efforts towards commercial utilization of bambara groundnut in South Africa is delaying the legume's inception into a commercial scale. South Africa boasts better infrastructure and capacity than Zimbabwe, therefore it can be in a better position to successfully commercialize bambara groundnut. The current study aims to contribute to the development of some scientific knowledge that may be needed in order to successfully commercialize bambara groundnut in South Africa.

2.3 Agronomic Practices

In most African countries, bambara groundnut is intercropped with maize, cowpea and various other major commodities. It is also grown in rotation as it improves the nitrogen status of the soil (Berchie *et al.,* 2012; Jørgensen *et al,* 2011). Bambara groundnut can be planted from late October, through November to early December, after good rains. Sinefu (2011) evaluated planting dates as a tool for managing water stress in bambara groundnut in the KwaZulu-Natal area of South Africa. Conclusions from that study showed that bambara planted at the optimum planting dates (November) had the best yields compared with late planting dates (January). Bambara groundnut has been reported to take 7 to 15 days to emerge (Swanevelder, 1998). However, recent studies using local South African landraces have reported slow emergence of up to 35 days after planting (Mabhaudhi *et al*., 2011; Mabhaudhi and Modi, 2013; Mabhaudhi *et al.*, 2013). Seeds stored for about 12 months germinate well, but longer storage results in a loss of viability (Ayamdoo *et al.*, 2013). Vegetative growth takes place in spring and early summer and pods form only in late summer and autumn (Sinefu, 2011).

Bambara groundnut is a typical short-day plant. Flowering starts 30 to 35 days after sowing and may continue until the end of the plant's life. Mabhaudhi and Modi (2013) reported that it took 86 to 88 days after planting for 50% flowering to occur in bambara groundnut under irrigated field conditions while it took 64 to 66 days after planting under a rain shelter (Mabhaudhi *et al*., 2013). However, they concurred that flowering continued up until the end of plant life. However, flowering may occur much earlier when water is limiting. Pod and seed development take place

approximately 30 to 40 days after fertilization. The fruit of bambara groundnut develops above or below the soil surface, although in practice few varieties are surface bearers. The bambara groundnut pod is small, about 1–5 cm long, round or slightly oval shaped and wrinkled with mostly one or sometimes two seeds. The seed develops during a further 10 days. Seeds are mature when the parenchymatous layer surrounding the embryo has disappeared. The seeds are round, 1 to 5 cm in diameter, smooth and very hard when dried. The crop has a growth period of about 130 to 174 days (Mabhaudhi and Modi, 2013; Mabhaudhi *et al.*, 2013).

Bambara needs bright sunshine, high temperatures and evenly distributed rainfall during the season to achieve best growth potential. Average temperatures between 20°C to 28°C are most suitable. It requires a frost-free period of at least 3 to 5 months. It has also been reported that very low yields and crop failure occurred for bambara groundnut planted during May in KwaZulu-Natal; this is typically the onset of winter in the province and the crop's growth cycle may have coincided with frost occurrence (Mabhaudhi *et al.*, 2013). The plant is highly adaptable and tolerates harsh conditions better than most legume crops. Bambara groundnut requires moderate rainfall from sowing until flowering. An annual rainfall of 500 to 600 mm is required. The plant tolerates heavy rainfall, except at maturity.

Bambara groundnut grows well in well-drained soil. It requires a soil pH of 5.0 to 6.5. Bambara groundnut gives the best yields in a deeply ploughed field with a fine seedbed. The established planting density ranges from 6 to 29 plants m^2 (Zulu, 1989). Bambara seed varies in size and therefore planting densities can vary from 25 to 75 kg/ha. The recommended spacing is 10 to 15 cm in single rows of 45 to 90 cm apart. Planting density is usually low especially when crops are not in rows. In conditions of high moisture levels and in heavy soils (not recommended) seed can be planted 2.5 to 3.0 cm deep and 5.0 to 7.5 cm deep in sandy soil (Zulu, 1989).

2.4 Nutritional Value of Bambara Groundnut

2.4.1 Animal nutrition perspective

With the increase in feed costs in the animal industry, the use of plant protein sources has become more necessary. Legumes such as soybean meal, groundnut cake and cowpea have been well utilized as plant protein sources and therefore extensively experimented and researched on.

Bambara groundnut has long been used as an animal feed (Linnemann, 1991). Its seeds have been successfully used in poultry feeding (Oluyemi *et al.*, 1976). The leaves are also suitable for animal grazing because they are rich in nitrogen and phosphorus (Rassel, 1960). In weaner pig diets, up to 10% bambara groundnut inclusion level was found economical for producing affordable and cheaper pork (Onyimonyi and Okeke, 2007). Bambara groundnut landraces that are resistant to foliar diseases would have a dual role of providing seeds for human use and above ground material for livestock feed. Maize is an unbalanced ration and is normally prepared with different inclusion levels of various available legumes. Bambara groundnut has high crude protein content (17-25%) (Belewu *et al.*, 2008) and can be a good protein supplement for maize diets prepared for animal consumption.

Bambara groundnut varieties provide up to 25% protein when compared to other legumes. These sought after protein levels can be valuable in improving animal feed diets with low protein contents. Bambara groundnut by-products such as bambara groundnut sievate, which is a result of processing bambara groundnut into flour for human use, has undergone adequate research and it was suggested that it can be used in poultry diets (Ekenyem and Odo, 2011; Ugwu and Onyimonyi, 2008). Research findings from studies on the use of bambara groundnut seeds and bambara groundnut by-products have given premise on further studying the leaves of the plant, identifying their potential use as an animal food source. The current study aims to characterize bambara groundnut as an alternative animal feed source by assessing its nutritional composition and value.

2.4.2 A human nutrition perspective

Bambara groundnut possesses sufficient quantities of nutrients such as proteins, vitamins and minerals. Bambara groundnut seeds provide an important source of crude protein (up to 24%), carbohydrates (up to 63%) and fats (up to 6.5%). The crop also has a good balance of essential amino acids (Table 2.1), and is rich in essential amino acids compared with the exotic *Arachis hypogaea* (Belewu *et al.*, 2008). Table 2.2 shows the proximate composition of one variety of bambara groundnut. A study by Bamishiaye *et al.* (2011) showed that there was not much difference in proximate composition between different varieties of bambara groundnut seeds. The crop has poor phosphorus and magnesium content and fair calcium content. A recent study on the

evaluation of the nutritional quality of complementary foods from popcorn, African locust bean and bambara groundnut concluded that germinated popcorn-bambara groundnut blends are the most suitable for infant diets (Ijarotimi and Keshinro, 2012).

2.5.1 Anti-nutritional factors

Anti–nutritional factors (ANF's) are substances that are generated in natural feed ingredients by the normal metabolism of plant species and interaction of different mechanisms. These factors hinder the optimal utilisation of the food (Apata and Ologhobo, 1997) by inhibiting protein digestibility, thus forming irreversible complexes with proteins, which reduces the bioavailability of amino acids (Tibe *et al*., 2007). Anti-nutritional factors are not intrinsic properties of a compound and their activity depends upon the digestive process of the animal which is fed an ANF-rich feed (Aganga and Tshwenyane, 2003). The utilisation of leaves, pods, and edible twigs of shrubs and trees as animal feed is limited by the presence of ANFs. Despite the nutritional benefits of bambara groundnut, there are nutritional constraints such as ANF's.

Several studies on bambara groundnut have identified ANF's such as trypsin inhibitor (Tibe *et al*., 2007), phytate (Nwanna *et al.,* 2005), and tannins (Borget,, 1992; Tibe *et al*., 2007). Borget, (1992) identified low levels of trypsin inhibitor in bambara groundnut seeds although these levels have been reported to be higher than those of pigeon pea (Fasoyiro *et al*., 2005) and chickpea (Apata and Ologhobo, 1997). In a comparative study between bambara groundnut and soybean, bambara groundnut seeds were reported to contain a higher anti–trypsin activity and the activity depended largely on the landrace (Tibe *et al*., 2007). High levels of phytate have been reported in bambara groundnut and are associated with reducing Ca availability (Nwanna *et al*., 2005). Poulter (1981) found a correlation between seed colour and the level of tannins present in bambara groundnut seeds. Cream coloured seeds had the lowest tannin level while brown and red contained higher levels, respectively (Nwokolo, 1996; Amarteifio *et al*., 2006; Tibe *et al*., 2007). This pattern was also observed in sorghum varieties where brown varieties contained more condensed tannins than white varieties (Amarteifio *et al*., 2006). Bambara groundnut landraces have lower tannin concentrations compared with cowpea (Asante *et al*., 2004) and pigeon pea (Fasoyiro *et al*., 2005). However, in a separate study by Akindahunsi and Salawu (2005), it was concluded that low levels of tannins had beneficial effects on human and animal nutrition. This suggests that bambara groundnut may be beneficial to both human and animal diets.

Table 2.1: Amino acid composition of bambara groundnut seeds. Source: (Belewu *et al*., 2008).

Amino acids	Average (% protein)
Alanine	4.4
Arginine	6.8
Aspartic acid	11.0
Cystine	1.5
Glutamic acid	16.9
Glycine	3.7
Histidine	3.1
Isoleucine	4.1
Leucine	7.6
Lysine	6.7
Methionine	1.3
Phenylalanine	5.5
Serine	4.7
Threonine	3.5
Tryptophan	1.2
Tyrosine	3.4
Valine	4.9

2.5.2 Processing methods of overcoming ANF's detrimental effects

Heat treatment has been reported to eliminate some or most of the ANF activities in legumes (Apata and Ologhobo, 1997). Heat treatments such as boiling or roasting are usually effective in destroying trypsin inhibitors. Heat-treating bambara groundnut could improve the performance of bambara groundnuts on growing broiler chicks. Trypsin inhibitor is also inactivated by autoclaving. Other effective forms of processing for reducing and/or eliminating ANF's include cooking, soaking, milling, hulling, germination and fermentation (Frunji *et al*., 2003). Traditional methods of cooking bambara groundnut normally involve soaking them overnight or for a few hours before boiling them. This suggests that indigenous knowledge may have evolved to develop ways of overcoming some of these ANF's. Onwuka (2006) observed 37 to 79% ANF reduction after boiling both pigeon pea and cowpea for 80 minutes. However, some of these processes do not always increase the feeding value of the feed, making it a less attractive feed to the animal (Nwanna *et al*., 2005).

Table 2.2: Proximate composition and fibre fractions of bambara groundnut seeds. Source: (Belewu *et al*., 2008).

Main analysis	Avg	SD	Min	Max
Dry matter	% as fed	86.8	3.4	83
Crude protein	% DM	19.8	3.1	16.7
Crude fibre	% DM	10.8	5.6	3.4
NDF	% DM	24.2	-	-
ADF	% DM	16	-	14
Lignin	% DM	3.2	-	-
Ether extract	% DM	5.6	0.9	4.6
Ash	% DM	4.4	3.8	5.1
Starch (polarimetry)	% DM	41.2	-	-
Gross energy	MJ/kg DM	19.4		

2.6 Potential of Bambara Groundnut as a Food Security Crop

Bambara groundnut is important for farmers because it is a legume capable of fixing atmospheric nitrogen, thus contributing to soil fertility. Bambara groundnut can produce reasonable yields with low input and is an ideal crop for resource-limited smallholder farmers. Nigeria and Zambia have been categorized as being the major bambara groundnut producing countries (Purseglove, 1992; Enwere, 1998). However, even in these countries, the crop is mainly grown for subsistence with the surplus being sold on the local markets. Thus the crop does not enter world trade (Enwere, 1998).

Plant protein has a worldwide food security role and provides about 65% of the world's supply of proteins for humans with up to 15% coming from legumes (OECD and Food and Agriculture Organization of the United Nations, 2010). Protein-energy malnutrition is a major health problem in developing countries (OECD and Food and Agriculture Organization of the United Nations, 2010). Amongst the legumes, bambara groundnut landraces can provide up to 25% of proteins. Inclusion of bambara groundnuts in human rations could replace expensive animal protein sources, and therefore prove economical to disadvantaged rural communities. The crop has a

potential to boost food security in rural areas. Some African tribes have been reported to extract oil from bambara groundnut seeds through roasting and pounding of the seeds.

In Swaziland, about 98% of farmers regard bambara groundnut as a profitable crop (Begemann *et al.,* 2002). In Zimbabwe the same company that produced "Nyimo beans" also has potential new products: Chilli Nyimo Beans, Baked Nyimo Beans (in a tomato sauce), Mixed 3 Bean Salad, Dried and Salted Nyimo Beans (like salted peanuts), Nyimo Bean Cereal, Biscuits and Snack Bars, Nyimo Bean Flour, Nyimo Bean Milk (similar to Soya Milk). In restaurants in Angola and Mozambique, boiled salted seeds are often served as appetizers. Bambara groundnut has the potential to contribute to food security with respect to its drought tolerance potential. This becomes more important, especially in sub-Saharan Africa where cultivation of other legumes is risky due to unfavourable rainfall conditions.

2.7 Conclusions

Bambara groundnut's importance as a food source for both humans and animals is undoubted. It has been documented that bambara groundnut landraces have the ability to produce high yields both under field and controlled environments (Mwale *et al*., 2007; Wang *et al*, 2003). The crop also boasts good nutritional values, which provide a cheap source of good quality protein to poorly-resourced farmers in semi-arid areas (Amarteifio *et al.,* 2006). Currently, less effort has been dedicated to the potential of bambara groundnut seeds and the above ground parts of the bambara groundnut plant, as a source of nutrition for both humans and animals. Bamgbose *et al.* (2006) concluded that bambara groundnut seeds can be used as a source of animal protein for man and livestock, giving it a great potential to address the protein-energy malnutrition problem in developing African countries. Given the rate at which agriculture production is to growth in world population, the production and use of bambara groundnut should be improved.

CHAPTER 3

MATERIALS AND METHODS

3.1 Plant Material

Seeds of a bambara groundnut landrace were obtained from Capstone Seeds located in Howick, Pietermaritzburg, South Africa. Typical of most landraces, the seed comprised of several seed coat colour variations. Previous studies by Sinefu (2011) and Mabhaudhi (2012) have both pointed to the fact that seed coat colour can be used as a selection criterion for improved establishment in bambara groundnut. Therefore, in this study, three distinct colours were selected: cream (C1), brown speckled (B1) and black speckled (B2) (Figure 3.1).

Figure 3.1: Bambara groundnut colour selections C1 = Cream, C2 = Brown speckled and C3 = Black speckled.

3.2 Seed Quality

3.2.1 Standard germination test (SG)

Seed viability was tested using the standard germination test. Each treatment (seed colour) was represented by 25 seeds which were replicated four times. The seeds were germinated between moistened double-layered brown paper sheets rolled and tied with elastic bands on both ends. Prepared rolled papers were then placed in sealed plastic bags and germinated in a growth chamber at 25°C for eight days (ISTA, 1999). Daily germination observations were recorded for the eight days they were kept in the growth chamber and final germination was recorded on the ninth day. Measurements taken included seedling root, shoot lengths, root: shoot ratio and dry matter.

Germination speed, as defined by the germination velocity index (GVI) was calculated according to the formula by Maguire (1962);

$$\mathbf{GVI = G1/N1 + G2/N2 +\ldots + Gn/Nn} \qquad \textbf{Equation 3.1}$$

where:

GVI = germination velocity index,

G1, G2…Gn = number of germinated seeds in first, second… last count, and

N1, N2…Nn = number of sowing days at the first, second… last count.

Mean time to germination (MGT) was calculated according to the formulae by Ellis and Roberts (1981):

$$\boldsymbol{MGT = \frac{\sum Dn}{\sum n}} \qquad \textbf{Equation 3.2}$$

where:

MGT = mean germination time, and

n = the number of seed which were germinated on day D.

3.2.2 Seed electrolyte conductivity (EC)

Electrolyte leakage or conductivity (EC) measures the amount of solute leakage in seeds. The R&A CM100 Model Single Cell Analyzer (state manufacturer and place of manufacture) was

used to analyse electrolyte leakage. Seeds (50 seeds) from each colour selection replicated four times were individually weighed and put into CM 100 cells, each filled with 2 ml distilled water. EC was measured over 0 h, 1 h, 3 h, 6 h, 12 h and 24 h periods. Seed mass (g), water activity (aw) and electrolyte conductivity were measured at each interval.

3.2.3 Proximate analyses of germinated seeds

The seeds were taken after each germination time interval and were freeze dried at -60ºC for 72 hrs. After freeze–drying they were ground through a 1-mm screen of a mill hummer. Chemical analysis was done following the Association of Official Analytical Chemists standard procedures (AOAC, 1990). The detailed methods adapted were as follows: detergent fibres (NDF & ADF) were analysed according to the method described by Van Soest *et al*. (1991). Dry matter (DM) was determined by drying samples in a fanned oven at 100ºC for 24 hours. Nitrogen was determined by the micro- Kjeldahl method and crude protein (CP) was calculated as N × 6.25. Ether extract was determined according to the soxhlett procedure (AOAC 920.39). Ash was determined by igniting fibre samples in a furnace at 550 ºC overnight (AOAC 942.05). The carbohydrate content was determined by difference, addition of all the percentages of moisture, fat crude protein, and ash, crude fibre were subtracted from 100% .This gave the amount of nitrogen free extract otherwise known as carbohydrate.

3.3 Controlled Environment: Crop Response to Water Stress and Proximate Analyses

Seed bed trials were conducted under controlled environment conditions (27/15°C day/night; 65% Relative Humidity and natural day length) at the University of Kwazulu-Natal's Controlled Environment Research Unit (CERU). The experiments were conducted under simulated drought conditions where temperature and relative humidity were monitored electronically using a HOBO 2K logger (Onset Computer Corporation, Bourne, USA).

3.3.1 Experimental design and trial management

The experimental design was a split-plot arranged in a randomised complete block design. There were two irrigation treatments (main factor) (100% vs. 30% Crop water requirement (ETc)) and three sequential harvesting treatments; H1: 25% of the leaves were removed sequentially (bi-

weekly untill final harvest) H2: 25% of leaves were removed sequentially (monthly until final harvest) H0: No sequential harvesting untill final harvest. Leaf removal occurred randomly from plant parts. There were three seed colours: Cream (C1), Brown speckled (C2) and Black speckled (C3). The experiment was replicated three times.

Seeds were planted in seed beds with soil whose field capacity had previously been determined *in situ*. Each main plot had plots of 12 rows; the rows were 1.5 m in length and spacing between rows was 0.5 m. Plant spacing was 0.15 m within rows. Plant population was 11 plants per row with nine plants being experimental plants.

Both seed beds were initially watered to field capacity and there after irrigated at 100% ETc to allow maximum crop stand. After maximum crop stand was reached, the above mentioned water treatments were imposed. Seed beds were routinely hand weeded to ensure no competition for water and solar radiation took place. Fertilizer was not applied on all treatments so as to simulate conditions under which local subsistence farmers cultivate the crop. However, soil analysis results showed that fertility was adequate to recommend crop production without taking into account N-fixation.

3.3.2 Field capacity test

Three drained pots were filled up with the experimental soil with each representing a replicate. Water was added to the pots until saturation was reached. The pots were then left to drain for 5 hrs, allowing them to reach field capacity. Soil was then removed from the pots and transferred to labelled brown paper bags which were weighed before being oven dried. The samples were dried at 80˚C for 72 hours, after which dry mass of the soil was measured. The formula used to determine the field capacity of the soil was as follows;

$$\boldsymbol{SWC(\theta m) = \left(\frac{\theta w - \theta d}{\theta d}\right) x\ 100\%} \qquad \textbf{Equation 3. 3}$$

where; Θm =gravimetric field water capacity

Θw = wet mass of soil, and

Θd = dry mass of soil.

3.3.3 Data collection

Data collection commenced four weeks after planting. Data collection included: emergence up to 35 days after planting, plant height, leaf number, chlorophyll content index, soil water content and stomatal conductance measured on a weekly basis. Plant height was measured from the soil surface to the base of the tallest leaf. Leaf number was counted for leaves with at least 50% green leaf area (Mabhaudhi and Modi, 2013) and each trifoliate leaf was counted as one leaf. Stomatal conductance was measured using a steady state leaf porometer (Model SC- 1, Decagon Devices USA) on the abaxial leaf surface. While chlorophyll content index (CCI) was measured using a chlorophyll content meter (CCM- 200 PLUS, Opti–Sciences, USA) on the adaxial leaf surface. Measurements of stomatal conductance and chlorophyll content index were taken from fully exposed and expanded leaves between 1200–1400 hrs during the periods in between irrigation events when the soil was drying (Mabhaudhi and Modi, 2013). Soil water content was monitored using an ML2x theta probe connected to a HH2 handheld moisture meter (Delta-T, UK). Yield and yield components were measured at harvest. Nutritional analyses data was taken at 8 weeks after planting (leaf material) and at final harvest (both seeds and leaf material); All the seeds and leaf material that was sequentially harvested was freeze dried for three days, soon after leaves were removed from freeze drier and ground under liquid nitrogen using a mortar and pestle and stored at -12°C.

3.4 Field Experiment

The field experiment was planted at the University of KwaZulu-Natal's Ukulinga Research Farm in Pietermaritzburg (29°37'S; 30°16'E; 845 m a.s.l) during the 2013/14 season under irrigated and rainfed conditions. Ukulinga has a warm subtropical climate with an average annual rainfall of about 694 mm received mainly during the summer months. Ukulinga farm has a semi-arid environment characterised by clay loamy soil. An automatic weather station situated within a 50 m radius was used to monitor weather parameters from the trial.

3.4.1 Experimental design

The experimental design was a split-plot design arranged in a randomized complete block design (RCBD), with three replications. The main plots comprised two irrigation treatments (irrigated and rainfed), while the sub-plot treatment was made up of four bambara groundnut seed colours

(C1 = cream, C2 = Brown speckled, C3 = Black speckled). The sub-sub-plot comprised three sequential harvesting treatments (H1 and H2) and a control (H0). The experiment was replicated three times. The trial was planted on an area of 404.4 m^2. Each main plot was 142.2 m^2 with a spacing of 10 m between the plots. The sub plot size was 4.7 m^2, spacing between plants was 0.15 m * 0.50 m translating to 62 plants per plot of which 35 were experimental plants. Sequential harvesting commenced two weeks from the date of the first open flower, this period allowed uniformity in leaf growth and therefore reduced bias: Refer to section 3.3.1 for detailed description on the sequential harvesting.

3.4.2 Agronomic practices

Soil samples were taken before the start of the experiments for soil fertility and textural analyses. Land preparations were done by disking and rotovating, providing fine seed beds. Weeding was done by hoeing.

3.4.3 Data collection

Plant emergence was measured weekly, up to when 90% of plants had emerged. Stomatal conductance, chlorophyll content index, plant height and leaf number were determined weekly. Refer to section 3.3.4 for detailed descriptions of data collection.

3.4.3.1 Proximate analyses

Leaf material was sequentially harvested and stored for nutritional analysis. At final harvest the seeds and leaf material were collected. See section 3.3.4 for detailed descriptions on nutritrional data collection.

3.4.3.2 Weather data

Weather (rainfall, Tmin and Tmax) data for the duration of the study was obtained from measurements collected by an automatic weather station (AWS) located about 100 m from the study site.

3.5 Statistical Analyses

All data were analysed using ANOVA from GenStat® Version 16 (VSN International, UK). Means were separated using Duncan's Multiple Range Test in GenStat® at the 5% level of significance.

CHAPTER 4

SEED QUALITY AND NUTRITIONAL COMPONENTS OF SELECTED BAMBARA GROUNDNUT COLOUR SELECTIONS

4.1 Introduction

Research has shown that seeds of Bambara groundnuts are useful for food and beverage consumption for humans while the leaves can be used for both human and animal consumption (Black and Halmer, 2006). Nutrient and mineral availability in seeds varies depending on the environment the crop is grown. In a study using bambara groundnut sown at four different locations, it was found that the total yield was different in the locations using the same bambara groundnut landrace (Masindeni, 2006).

Seeds are very diverse in colour and size. Varieties that produce smaller seeds can generate many more seeds per plant, while those with larger seeds invest more resources into those seeds and normally produce fewer and big seeds (Igor, 2007). Plants with darker seed colours have been associated with good seed development, hence, better yields, while lighter colours have lower yields (Mabhaudhi, 2012). This was confirmed in a study on cowpea (Odindo, 2007), who showed that seed coat colour has an influence on seed germination and quality.

Seed quality is defined as the overall value of seed lot for its intended use, particularly looking at the physiological quality (viability, germination and vigour) (McDonald and Copeland, 1997). It affects the ability of the seed to overcome the variable conditions experienced by seed during crop establishment. High seed quality is essential for good crop establishment and how the crop will perform under field conditions. In general, poor quality seed may result in reduced germination and emergence rates, poor tolerance to sub-optimal conditions and low seedling growth rates (Powell and Mathews, 1984). A study by Odindo (2007) reported that seed coat colour has an influence on seed germination and quality on cowpea.

This study aimed to compare and contrast seed quality components (germination, viability, and vigour) from bambara groundnut landraces differing in seed colours and to critically identify

knowledge gaps in terms of chemical and mineral composition that exist in bambara groundnut seed colours during imbibition. Materials and methods are explained in 3.2.1 above.

4.2 Results

4.2.1 Standard germination tests

Results of germination parameters are shown in Table 4.1. A comparison of seed colours showed highly significant ($P<0.001$) differences due to black specked seeds having a very high germination capacity, while the other two seed colours did not differ significantly (Table 4.1). Black speckled bambara groundnut seeds had the highest dry mass (9.229g) followed by brown speckled and cream respectively. There were significant differences ($P<0.05$) among bambara groundnut selections with respect to root and shoot lengths; black speckled seeds produced longer shoot and roots than brown speckle and cream, respectively (Table 4.1). Significant differences ($P<0.05$) were also observed for germination velocity index (GVI) where the plain cream seeds gave the highest GVI (4.421) and black speckled seeds had the lowest GVI (2.529) (Table 4.1). Daily germination showed highly significant differences ($P<0.001$) among bambara groundnut seed colour selections (Figure 4.1). Germination started between days 3 and 4. Black speckled seeds were fast to germinate followed by brown speckled then cream seeds with the slowest germination.

Table 4.1: Performance of bambara groundnut seed colour selections (cream, brown– and black–speckle) during a standard germination test. Note: Brown sp. = Brown speckled and black sp. = Black speckled); GVI = Germination velocity index; MGT = Mean germination time; EC = Electrical Conductivity.

Variety	GVI	MGT (days)	EC (µS/g)	Root length (mm)	Shoot length (mm)	Root: Shoot ratio	Dry mass (g)
Cream	4.421	7.237	27.5	7.022	2.00	3.633	7.559
Brown sp.	2.940	7.115	616.2	10.12	3.056	3.361	8.581
Black sp.	2.529	7.237	338.6	10.45	3.167	3.633	9.229
$LSD_{(P=0.05)}$	0.770	0.264	90.9	2.492	0.869	1.434	0.567

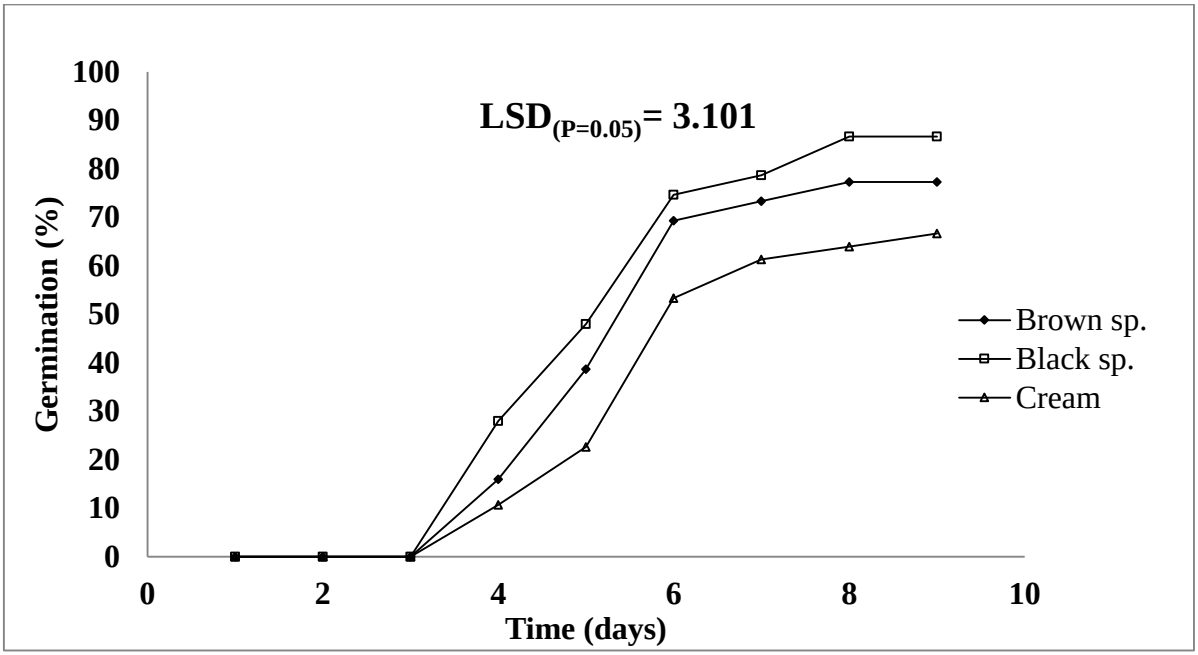

Figure 4.1: Daily germination of different bambara groundnut colour selections (Brown speckle, Black speckle and light Cream) during the first nine days in the germination chamber.

4.2.2 Electrolyte leakage

Electrolyte leakage differed significantly ($P<0.001$) amongst the bambara groundnut seed colour selections (Table 4.1). Brown speckled seeds had the highest leakage followed by black speckled and cream seeds, respectively (Figure, 4.2).

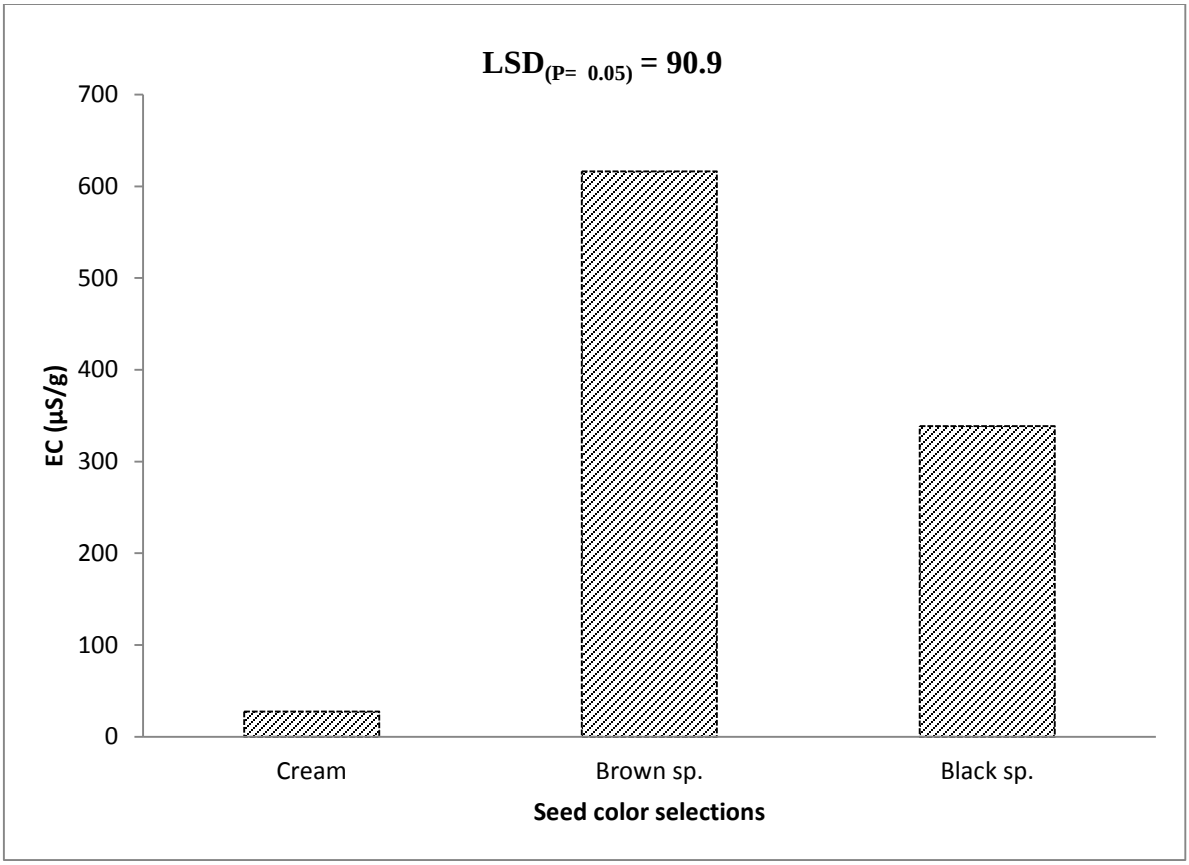

Figure 4.2: Electrolyte leakage as a measure of seed vigour in three bambara groundnut seed colours.

4.2.3 Nutritional quality

4.2.3.1 Chemical composition of bambara groundnut seeds during early germination

During germination there was a marked increase in moisture content, as expected. The values determined for moisture content for black speckled (3.33%), brown speckled (4.87%) and cream (4.19%) seeds were significantly different ($P<0.001$) (Figure 4.3). Brown speckled seeds had the highest moisture content values (Figure 4.3). The values determined for ash content in black speckled (3.921 %) and cream (3.945 %) seeds were significantly different ($P<0.001$) from those of brown speckled (4.164 %) seeds (Figure 4.4). Brown speckled seeds had the highest ash values at 8 (4.10%), 16 (4.04%), 24 (4.11%), 48 (4.27%), 72 (4.29%), and 24 (4.17%) hours, on the other hand black had the lowest ash content at 8 (3.60%) and 120 hours (4%) with cream having the lowest values at 16 (3.888%), 24 (3.78%), 48 (3.78%), and 72 hours (3.99%) (Figure 4.4). No significant differences were observed for fat content during early germination amongst the three bambara groundnut seed colour landrace selections (Figure 4.5). However, it was noted that the

darker seed colours (Black and brown speckled) had more fat content than cream seeds, were after 16, 24, 48, 72 and 120 hours darker seed colours dominated (Figure 4.5).

Significant differences (P<0.001) in ADF content were observed during early germination for the three colour selections (Figure 4.6). After 120 hours of germination, black speckled seeds had the highest ADF content (13.70%) and brown speckled had the lowest (10.98%) (Figure 4.6). The highest ADF content (13.70% = black speckled) was observed after 120 hours of germination and the lowest (8.61% = black speckled) was observed 24 hours of germination. The values determined for NDF in black (13.56%) and brown speckle seeds (13.67%) were significantly different (P<0.001) from that of cream seeds (15.23%) (Figure 4.7). After 72 and 120 hours of early germination cream seeds had the highest NDF levels of 14.04% and 23.22%, with black speckled seeds having the lowest at 72 hours and brown speckled also had the lowest after 120 hours of germination (Figure 4.7). Differences (P<0.05) were observed for crude protein (CP) over early germination time amongst the seeds landrace selections. Black speckled seeds had the highest CP after 8 (20.67%), 16 (22.11%), 24 (20.68%), and 48 hours (20.77%), on the other hand cream seeds had the lowest CP after 16 (19.30%), 24 (18.71%), and 72 hours (19.16%) (Figure 4.8). After 120 hours of germination brown speckled seeds had the highest CP content (21.45%) followed by cream (20.64%) and black speckled seeds (20.55%) (Figure 4.8).

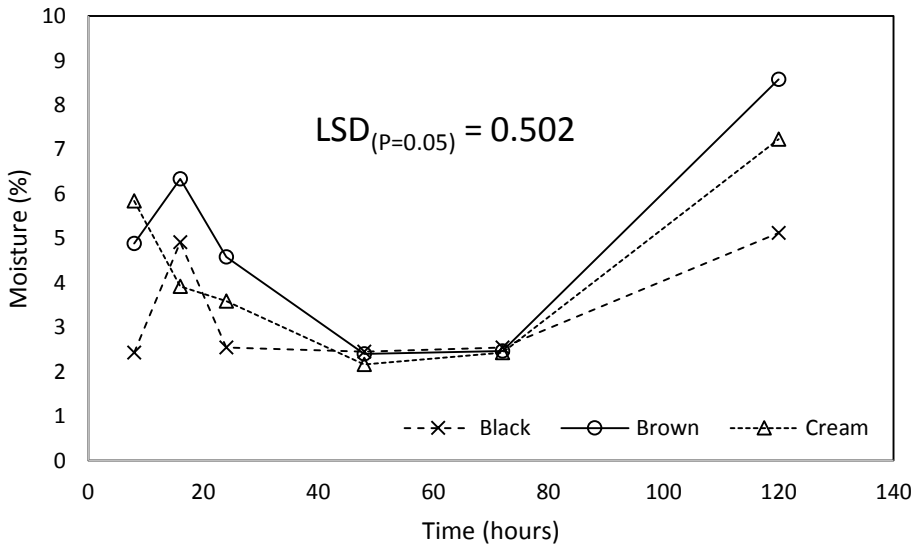

Figure 4.3: Moisture content of three bambara groundnut colour selections during early germination.

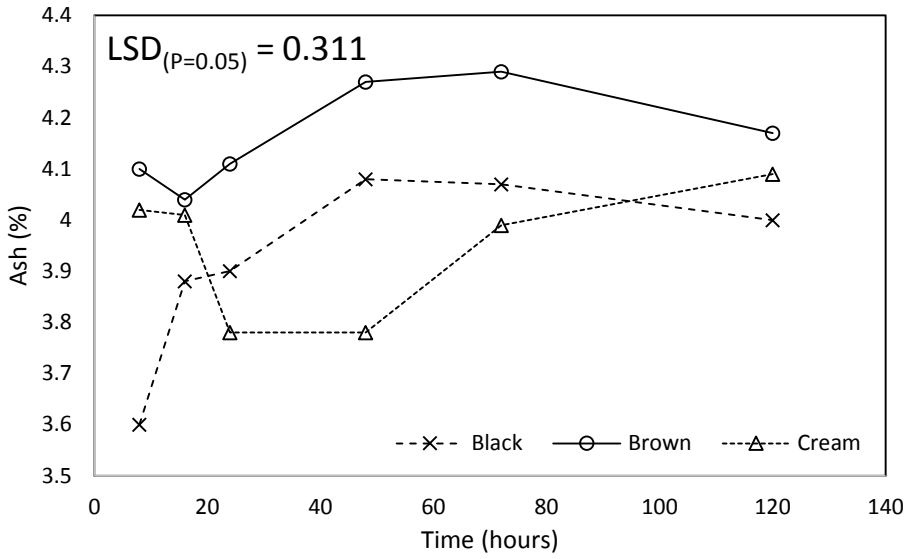

Figure 4.4: Ash content of three Bambara groundnut colour selections during early germination.

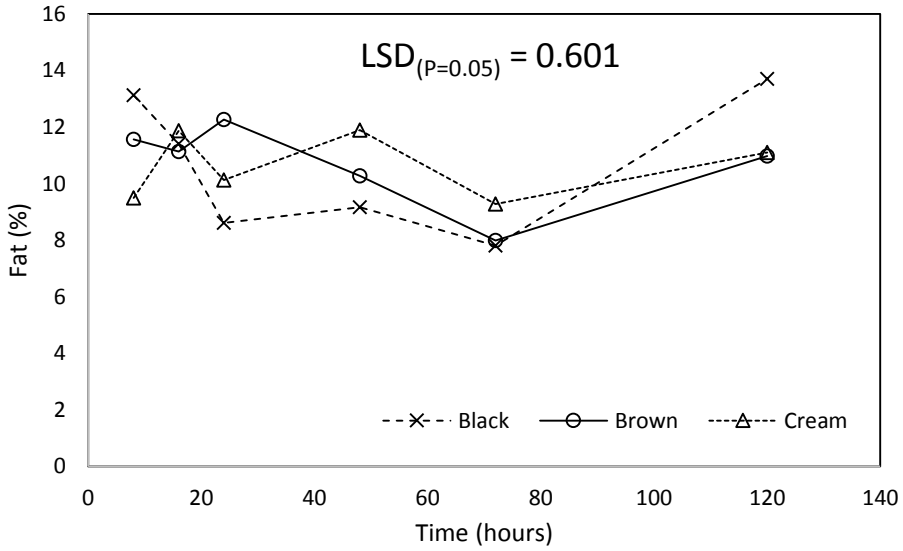

Figure 4.5: Fat (F) content of three bambara groundnut colour selections during early germination.

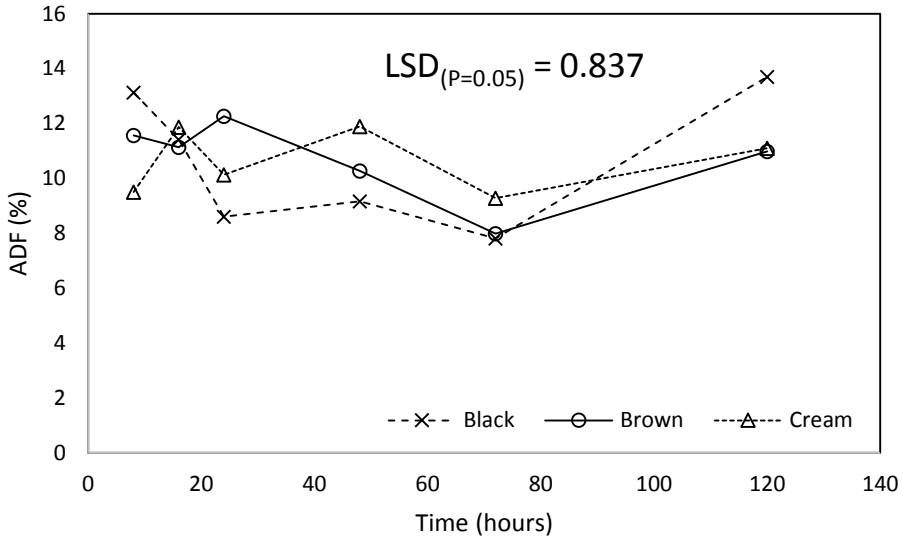

Figure 4.6: Acid Detergent Fibre (ADF) content of three bambara groundnut colour selections during early germination.

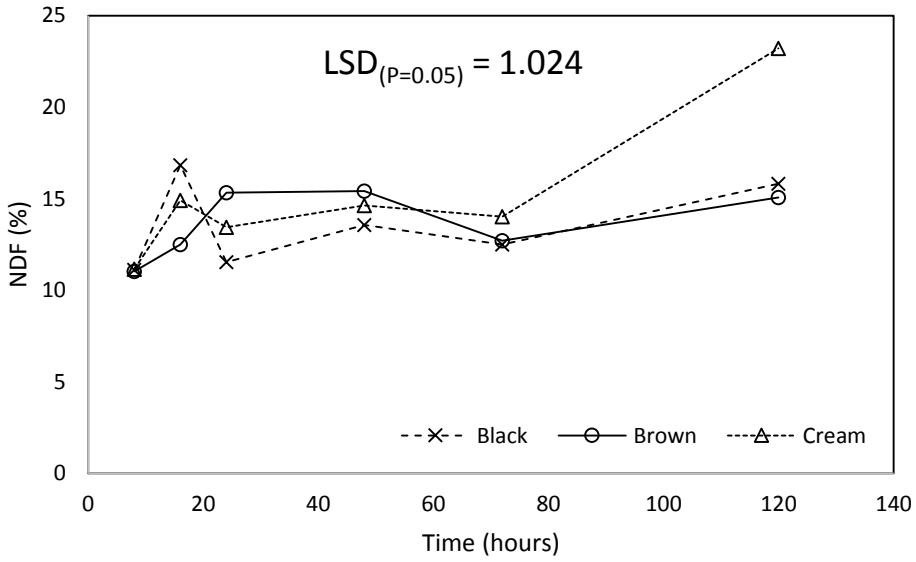

Figure 4.7: Neutral Detergent Fibre (NDF) content of three bambara groundnut colour selections during early germination.

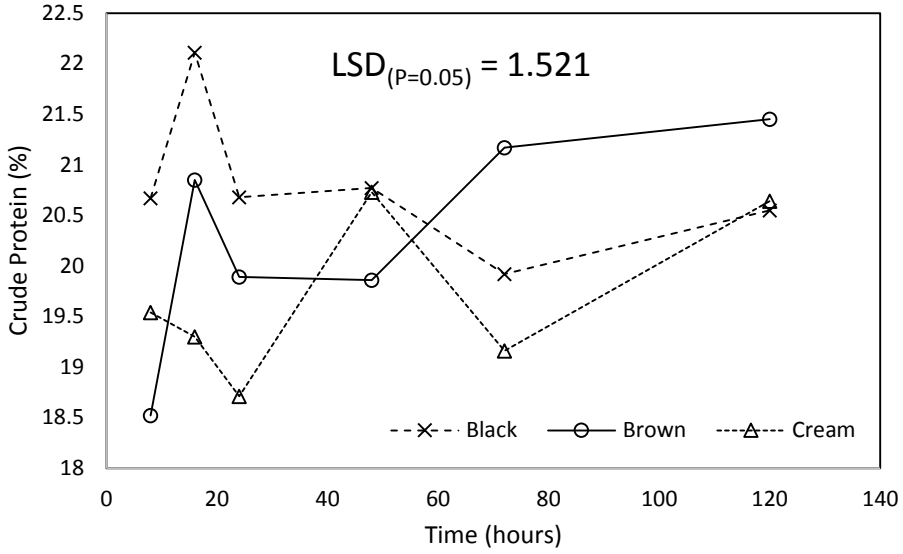

Figure 4.8: Crude protein (CP) content of three bambara groundnut colour selections during early germination.

4.2.3.2 Mineral composition of bambara groundnut seeds during early germination

Changes in selected mineral elements during germination are shown in Figures 4.9 to 4.17. While statistical analysis showed significant differences between seed colours (Appendix 1), the general the changes in mineral content did not show a consistent trend (Figures 4.9 to 4.17).

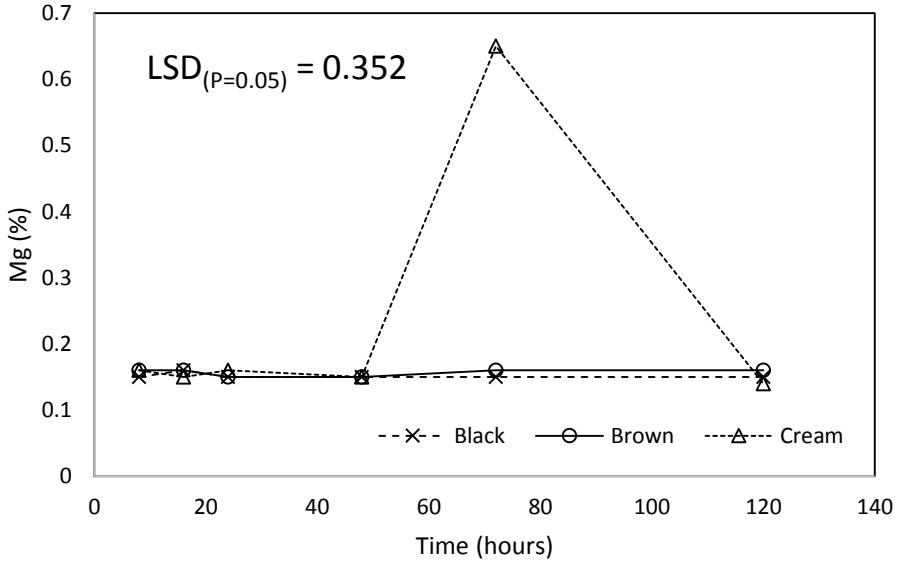

Figure 4.9: Magnesium (Mg) content of three bambara groundnut colour selections during early germination.

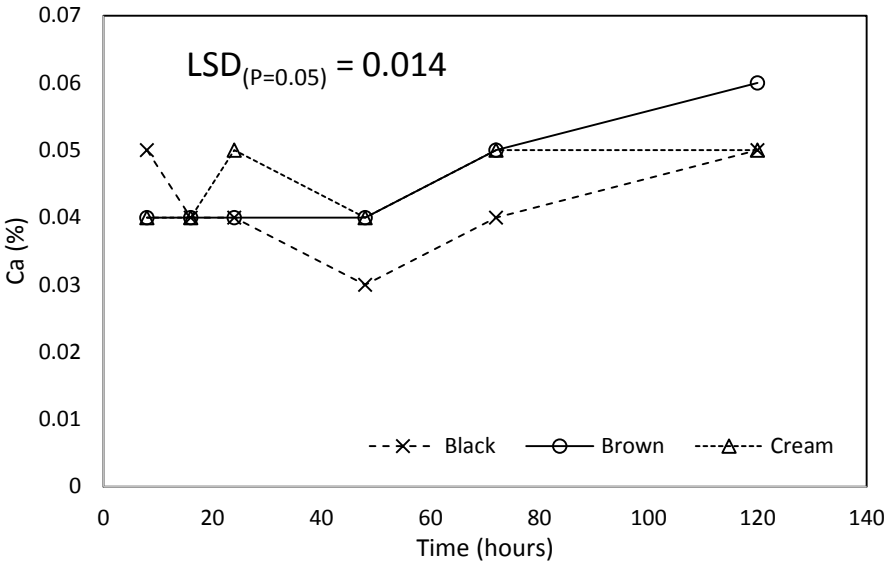

Figure 4.10: Calcium (Ca) content of three bambara groundnut colour selections during early germination.

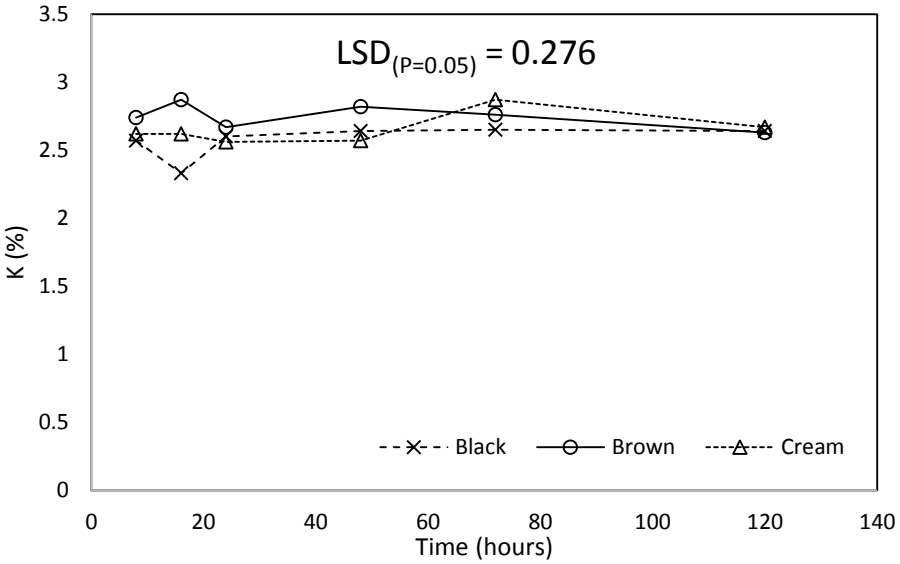

Figure 4.11: Potassium (K) content of three bambara groundnut colour selections during early germination.

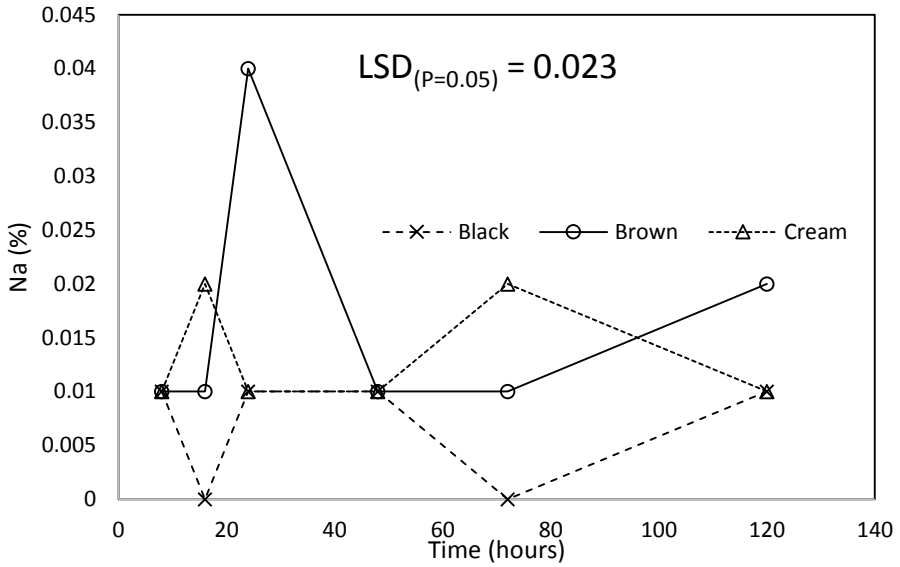

Figure 4.12: Sodium (Na) content of three bambara groundnut colour selections during early germination.

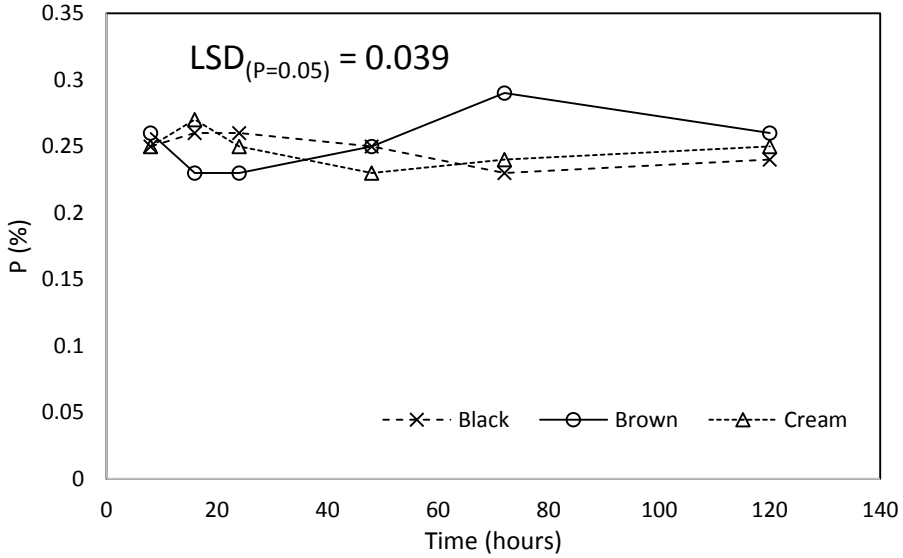

Figure 4.13: Phosphorus (P) content of three bambara groundnut colour selections during early germination.

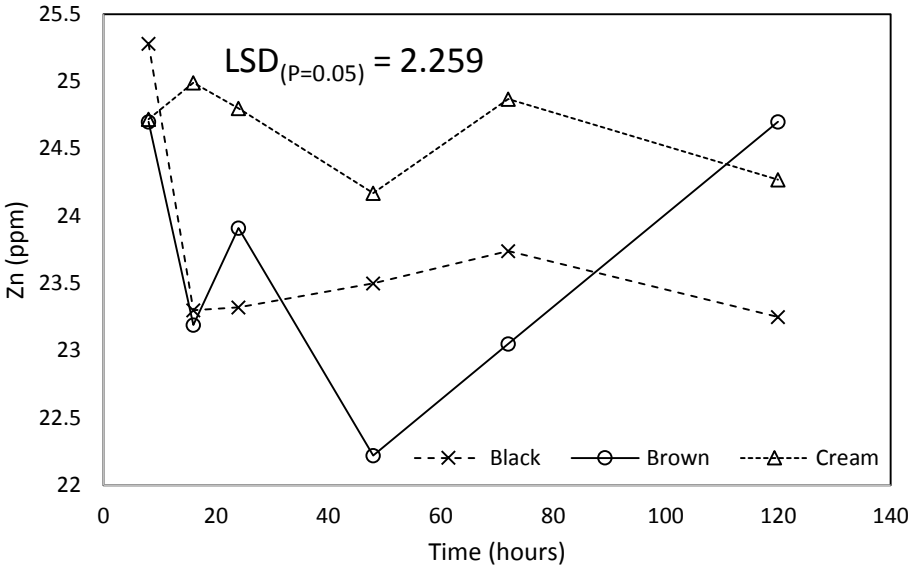

Figure 4.14: Zinc (Zn) content of three bambara groundnut colour selections during early germination.

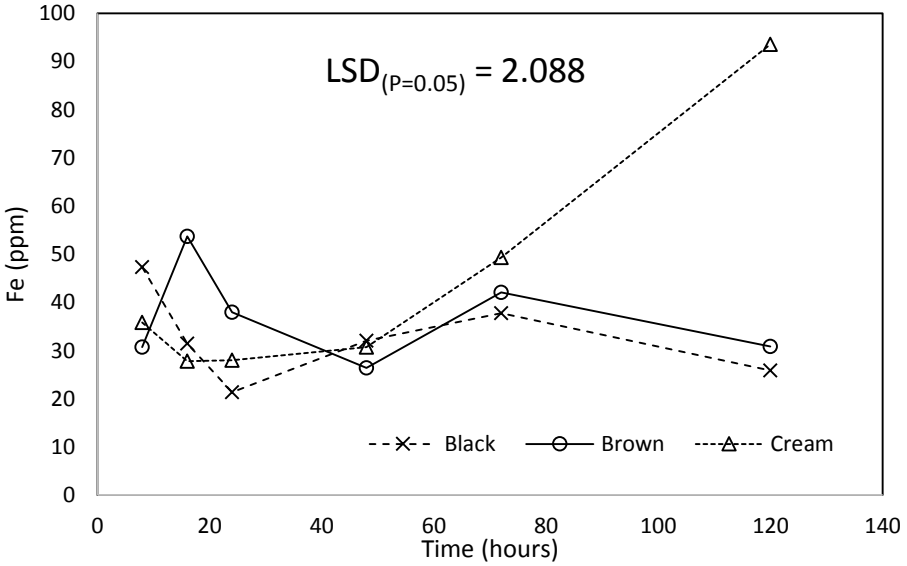

Figure 4.15: Iron (Fe) content of three bambara groundnut colour selections during early germination.

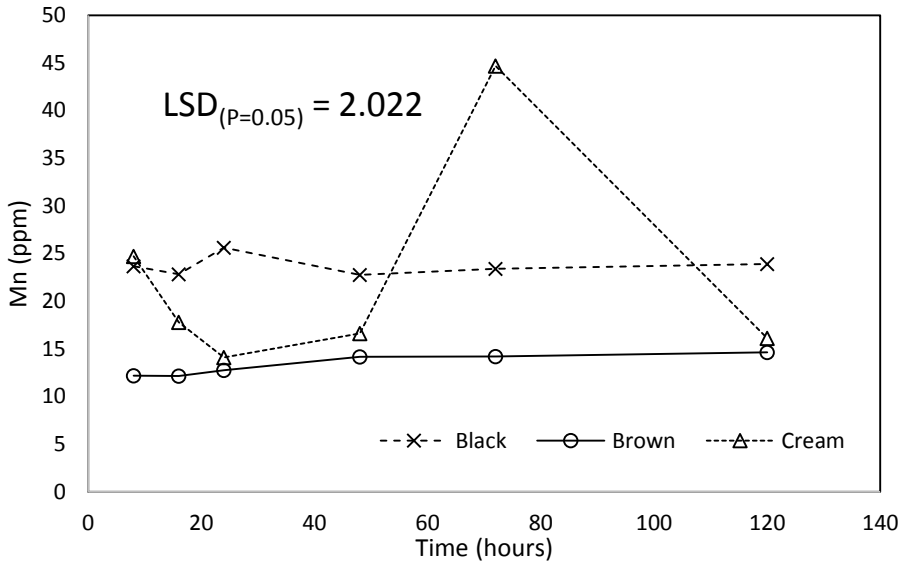

Figure 4.16: Manganese (Mn) content of three bambara groundnut colour selections during early germination.

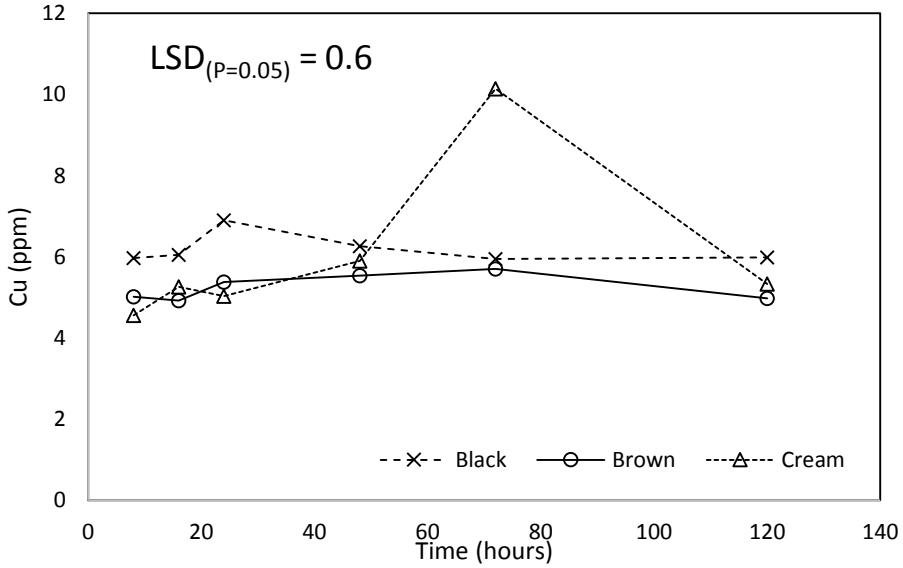

Figure 4.17: Copper (Cu) content of three bambara groundnut colour selections during early germination.

4.3 Discussion

The standard germination test is used as a measure of viability (ISTA, 1999; Peñaloza, 2005) with the ultimate objective of gaining information with respect to field planting value of the seed. Seed viability is defined as the property of the seed that enables it to germinate under favourable conditions, provided that any dormancy is removed prior to the germination test (Basu, 1995).

Findings from different studies have shown that seed colour has an influence on seed quality (Powell, 1989; Zulu and Modi, 2010; Sinefu, 2011). In this study, black speckled seeds had the highest germination and the lighter colours (brown and cream) had lower germination rates. These results were similar to that of Sinefu (2011), where dark coloured seeds performed better than the light coloured seed selection by about 5%. Similar observations were also noted with green pea (Atak *et al.*, 2006) and clover (Dalianis, 1980). Seed colour can also be linked to different physiological processes that the seed undergoes before the process of germination (Odindo, 2007). In the current study, significant differences were observed in viability between seed colours. Correlations were reported between seed colour and seed performance during germination in two separate studies (Asiedu and Powell, 1998; Pimpini *et al*. 2002).

The results of the proximate composition of the three bambara groundnut landrace selections during imbibition confirmed the general theory of seed germination changes, which affect the mass and the length of the seedling (Bewley and Black, 1994). However, these findings are somewhat in contrast with those of Obizoba and Atti (1994) on millet.

Protein levels have been reported to increase over the imbibition period in seeds (Bewley and Black, 1994). This was consistent with findings from the current study which also showed increase in crude protein. Crude protein contains more non-protein N, which is known to reduce digestion absorption and utilization (Echendu *et al*., 2009). Imbibition increased protein levels from 18.52% in 24 hours to 21.45% in 120 hours of germination for Brown speckled seeds; 18.71 to 20.64% for cream seeds and no significant increase was observed for the black speckled seeds. The increase in the crude protein might be as a result of enzyme hydrolysis of insoluble protein to soluble protein, which is more available (Echendu *et al*., 2009). Other legumes have been observed to increase their protein content as well during germination, e.g., ground beans (Echendu *et al*., 2009) and cowpea (Chikwendu, 2008).

The slight increase of ash content for the three bambara groundnut landrace selections is in agreement with Echendu *et al*. (2009), who observed an increase in ash content from 24-96 hours of imbibition. The work of Chikwendu (2008) showed that the increase in ash content during imbibition was due to endogenous enzyme hydrolysis of complex organic compounds which release more nutrients allowing anti-nutrients to leach into the ground.

The fat content of the bambara groundnut landrace selections was higher than the fat content observed for groundnut (Echendu, 2009), even though no significant differences were found between the bambara groundnut landrace selections. The high fat values might be due to non-conversion of free fatty acids to carbohydrates which may result in an increase in fat composition during the imbibition period (Afam-Anene and Onouha, 2006).

For NDF and ADF, no significant differences were observed among bambara groundnut selections between 0-75 hrs. After 100 hours of imbibition, a significant difference ($P<0.001$) was observed where cream had more NDF traces as compared to the darker colours. A similar pattern was observed from 0-100 hours with ADF but with black emerged to having more ADF traces after 100 hours of imbibition. It can be concluded that light coloured bambara groundnut seeds have more NDF traces after 100 hours of imbibition as compared to the lower traces found on the darker seed colours.

The mineral content changes observed in this study were not conclusive in that there were no clear trends. The expectation was that as imbibition proceeded, mineral elements would clearly increase, especially phosphorus (El-Mahdy, 2003). Previous studies have shown that after > 90 hours of germination, the dry weight of seeds decreased while total ash content increased (Borade *et al*, 1984; Ching, 1966; Dawood *et al*, 2013). It has been observed that during germination the phytic acid values diminish and the water soluble inorganic phosphorus values increase. Phytase activity, which is absent in the ungerminated seeds originates after germination and the phosphatase activity is increased in the germinated seeds (Ching, 1966; Dawood *et al*, 2013). Changes in calcium, magnesium, iron, manganese, copper and zinc are found to be dependent on the loss of dry weight which occurs more during processing than just germination or imbibition of seeds Dawood *et al*, 2013. The limitation of the current study was that it relied on changes

during germination and there was no determination of phytase/phosphatase activity for correlation with changes in mineral content during imbibition.

CHAPTER 5

EFFECTS OF WATER STRESS, SEED COLOUR AND SEQUENTIAL HARVESTING ON NUTRIENT QUALITY AND OVERALL GROWTH OF BAMBARA GROUNDNUTS LANDRACES UNDER CONTROLLED ENVIRONMENT CONDITIONS

5.1 Introduction

Traditional and indigenous crops are still being cultivated under poor farming conditions by smallholder farmers with little or no knowledge on how to improve their farming practices. The need to improve farming practices requires a multidisciplinary research on these crops so as to preserve their germplasm, improve their agronomic potential and also appreciate and assess nutritional value of the crops (Doku *et al.*, 1971; Swanevelder, 1998). Like most underutilized crops, bambara groundnut is cultivated by smallholder farmers, on arid, semi-arid and other marginal conditions across Africa (Linnenann and Azam-Ali, 1993). It has been identified as a drought tolerant crop that can produce yield where other crops, such as groundnut, fail completely (Harris and Azam-Ali, 1993). According to Collinson *et al*. (1997), bambara groundnut is considered drought tolerant because of its ability to close its stomata to reduce water loss and adjust its osmotic potential. Water stress in plants affects the plant's metabolic processes such as growth, photosynthesis and enzyme activity (Turner and Stewart, 1986). Boyer (1968) studied the effect of water stress on maize (*Zea mays* L.) and observed that when leaf water potential decreased, leaf area also decreased while photosynthesis was affected later. Yoshiyuki *et al*. (2014), on the other hand, observed that growth of some legumes reduced under water stress resulting in smaller leaf area which transpired less water, and this was considered as a first line of defence mechanism against drought, which in overall altered with plant yield (Rachie and Silvester, 1977. There is a need to identify suitable environmental conditions for bambara groundnut to determine the agronomic of landraces. Information generated from such an investigation will not only aid in quantifying the nutritional content of different bambara groundnut landraces but also to assess the potential of this crop as a food security crop. Hence, the objective of this study was to investigate the effects of sequential harvesting on nutritional content of bambara groundnut landraces grown under different water stress conditions. The materials and methods are presented in chapter 3 above.

5.2. Results

5.2.1 Soil water content

Highly significant differences (P<0.001) were observed in soil water content with respect to different water regimes (Figure 5.1). The highest water content was observed after 12 weeks after planting under 100% ETc. Between 4 and 8 weeks, 30% ETc was below permanent wilting point (PWP). As from 7 weeks after planting 100% ETc showed consistent water content above PWP (Figure 5.1).

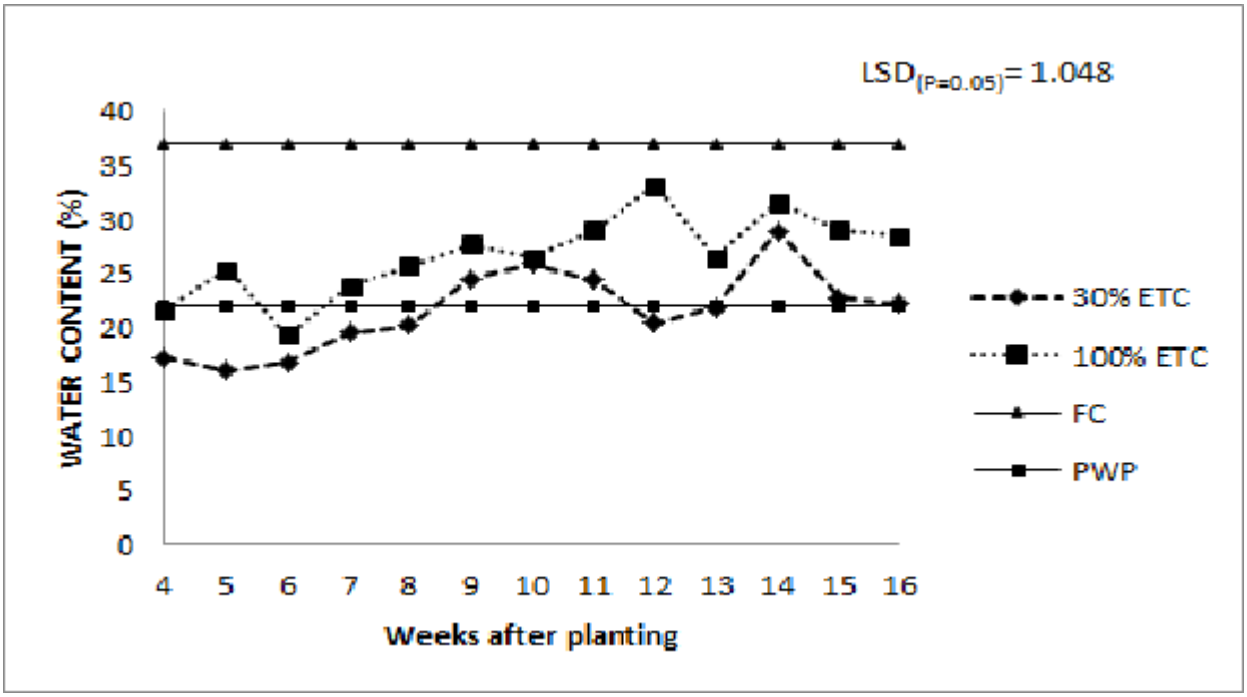

Figure 5.1*:* Soil water content at different water regimes (30% & 100% ETc) over a period of 16 weeks

5.2.2 Crop physiology

5.2.2.1 Stomatal Conductance and Chlorophyll Content Index

An analysis of the stomatal conductance (SC), comparing the water regimes (30% and 100% ETc) and seed colours over the season showed that there were significant differences ($P < 0.05$) between water regimes (Table 5.1). Providing 100% ETc increased stomatal conductivity by 28% compared with 30% ETc (Table 5.1). There were no significant differences between seed colours. Neither was there a significant interaction between water regime and seed colour. With respect to chlorophyll content, there were no significant differences between water regimes, nor seed colours. There was also no significant interaction between water regime and seed colour (Table 5.1).

Table 5.1: Stomatal conductance (SC) and chlorophyll content index (CCI) of three bambara groundnut landraces sorted by seed coat colour in response to soil water regime conditions during controlled environment conditions.

Water Regime	**Landrace**	**SC (mmol m^{-2} s^{-1})**	**CCI**
	Cream	34.39	11.29
30% ETc	Brown Speckled	33.36	9.15
	Black Speckled	32.82	10.15
Mean		**33.52**	**10.19**
	Cream	46.97	9.82
100% ETc	Brown Speckled	49.64	12.30
	Black Speckled	43.24	9.71
Mean		**46.62**	**10.61**
$LSD_{(P=0.05)}$ Water		7.463	2.119
$LSD_{(P=0.05)}$ Landrace		5.219	3.940
$LSD_{(P=0.05)}$ Water*Landrace		7.056	4.609

5.2.3 Crop growth

5.2.3.1 Emergence, plant height and leaf number

With regards to emergence, significant differences (P<0.001) were observed between seed colours at both 30% ETc and 100% ETc (Figure 5.2). Significant differences (P<0.001) were also observed between the water regimes. There were also significant interactions (P<0.05) between seed colour and water regimes. Black speckled seeds had the highest emergence from day 7 to day 35 at 100% ETc, and under 30% ETc black speckled seeds again had the highest emergence from 7 to 28 days after which brown seeds showed the best performance (Figure 5.2). Cream seeds had the lowest emergence throughout. Thus, the darker speckled seeds had a better emergence, overall (Figure 5.2).

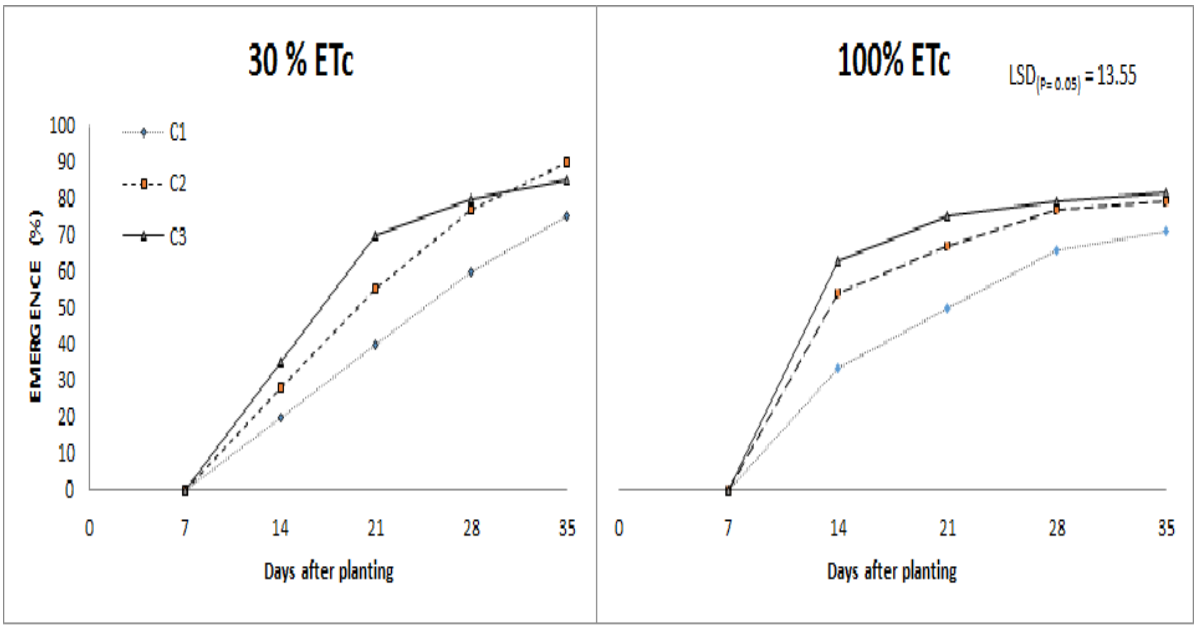

Figure 5. 2*:* Emergence of three bambara landraces (Cream-C1, Brown-C2, & Black-C3) planted under two water regimes (30% & 100% ETc).

Plant growth and development was determined in terms of plant height and leaf number. Results showed that over the growing period, there were no significant differences between water regimes with respect to plant height (Table 5.2). This finding was true for landrace seed colour (Table 5.2). However, there were significant differences between water regimes, with respect to leaf number, with 100% ETc giving 27% more leaf number than 30% ETc (Table 5.2).

Table 5.2: Plant height and leaf number of Bambara groundnut landraces, sorted by seed coat colour, in response to soil water regime during plant growth under controlled environment conditions.

Water Regime	Landrace	Plant Height (cm)	Leaf Number
	Cream	18.77	5.57
30% ETc	Brown Speckled	18.95	5.35
	Black Speckled	18.14	5.86
Mean		**18.62**	**5.60**
	Cream	19.27	7.79
100% ETc	Brown Speckled	18.68	7.16
	Black Speckled	19.27	7.92
Mean		**19.07**	**7.63**
$LSD_{(P=0.05)}$ Water		1.237	0.913
$LSD_{(P=0.05)}$ Landrace		1.045	0.567
$LSD_{(P=0.05)}$ Water*Landrace		1.331	0.807

5.2.4. Yield parameters

The key indicators of yield performance that were determined in this study were harvest index, grain yield, pod number, pod mass, and grains per pod (Figures 5.3 – 5.7). Results showed that there was a significant grain yield difference between water regimes and landrace colours due to cream landrace (Figure 5.3). The high water regime caused (23%) better grain yield than the lower water regime (Figure 5.3). With respect to harvest index, there were significant differences between water regimes and landraces (Figure 5.4). The differences were shown with respect to the speckled landraces (Figure 5.4). The high water regime was not significantly different across landraces. For the cream landrace, there were no significant differences between water regimes. For the speckled landraces, the higher water regime produced a significantly higher (~30%) harvest index than the lower water regime (Figure 5.4).

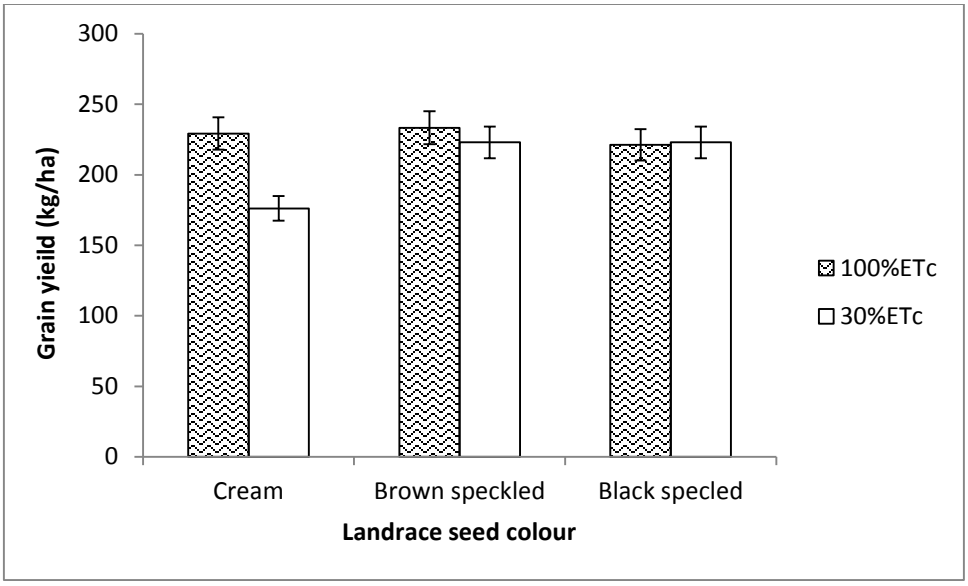

Figure 5.3: Comparison of bambara groundnut landrace response to soil water regime during growth under controlled environment conditions with respect to grain yield.

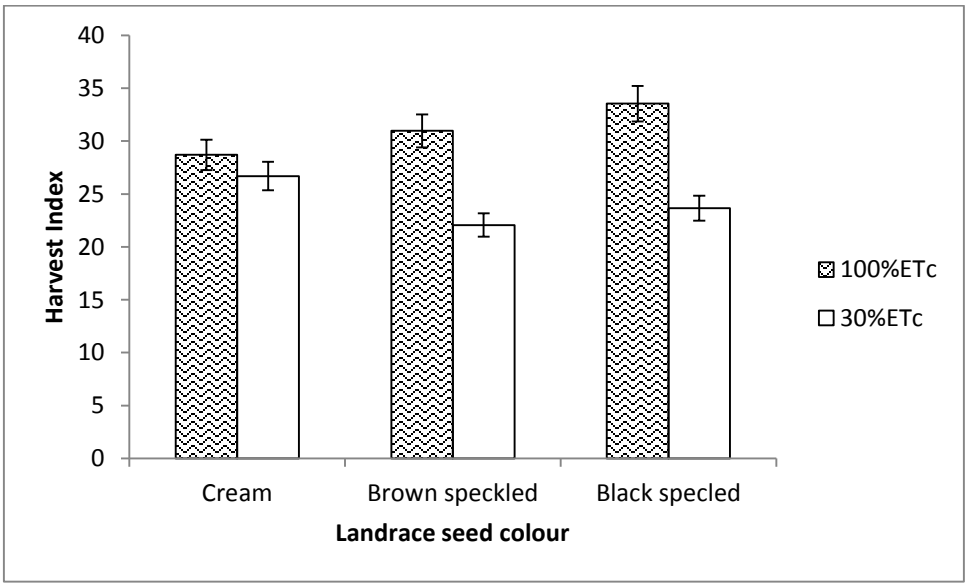

Figure 5.4: Comparison of bambara groundnut landrace response to soil water regime during growth under controlled environment conditions with respect to harvest index.

Pod number per plant showed a significant differences between both water regimes and landraces (Figure 5.5). These differences were found in the brown speckled landrace (Figure 5.5). However, there was a general trend of high water regime giving a higher pod number per plant by 1 to 6% compared with the lower water regime (Figure 5.5). Pod mass followed a similar trend as pod number per plant (Figure 5.6). It was interesting to note that there were no significant differences between water regimes and landraces with respect to the number of grains per pod (Figure 5.7).

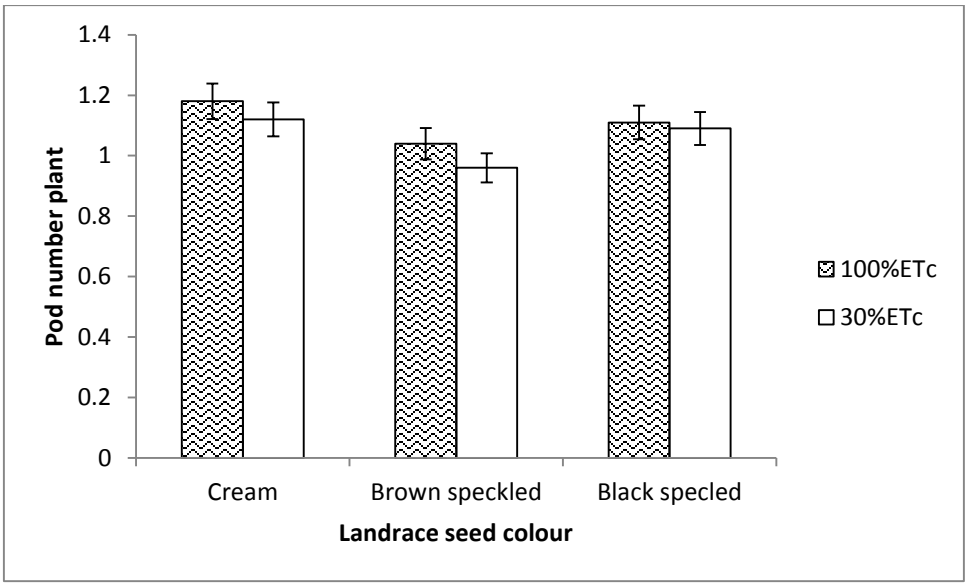

Figure 5.5: Comparison of bambara groundnut landrace response to soil water regime during growth under controlled environment conditions with respect to pod number per plant.

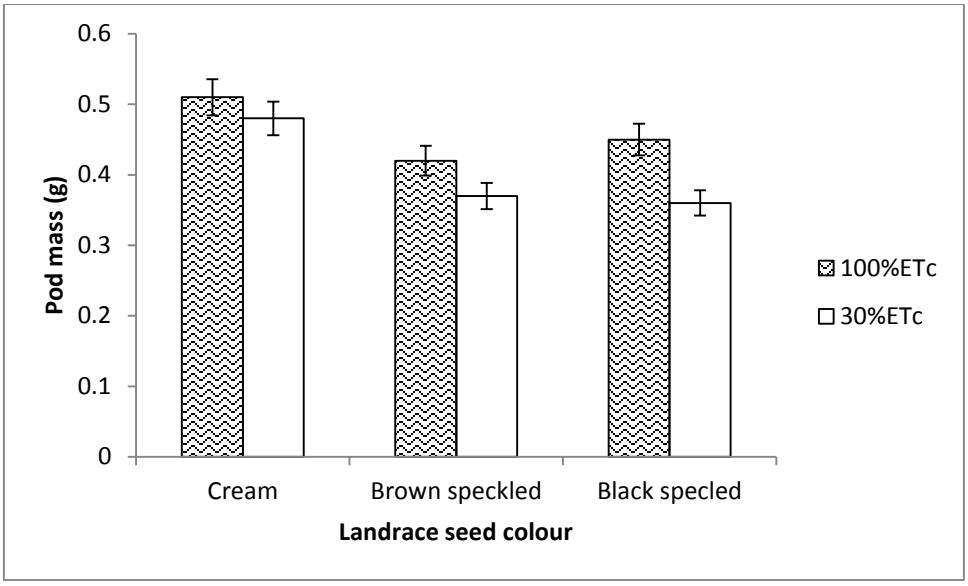

Figure 5.6: Comparison of bambara groundnut landrace response to soil water regime during growth under controlled environment conditions with respect to pod mass.

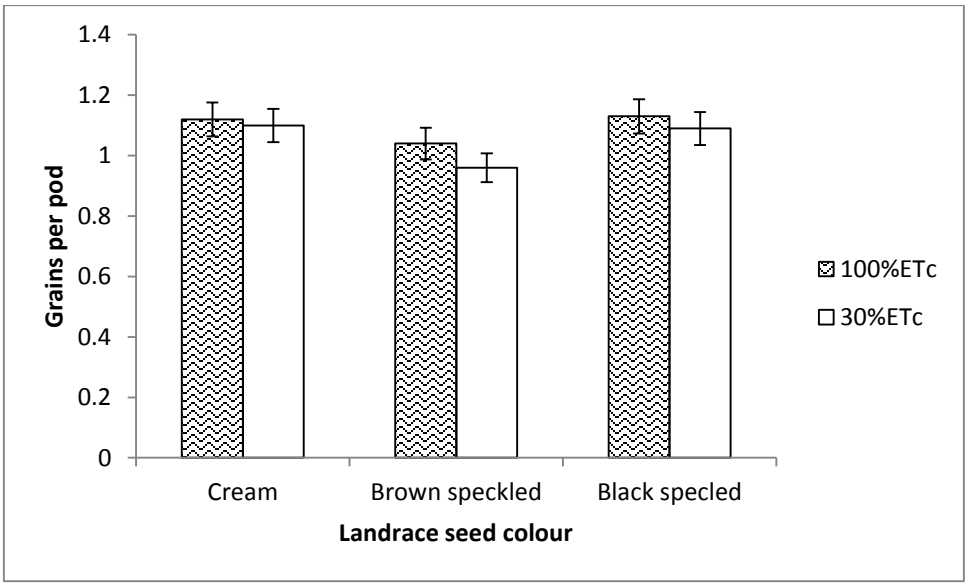

Figure 5.7: Comparison of bambara groundnut landrace response to soil water regime during growth under controlled environment conditions with grains per pod.

5.2.5 Proximate composition

The results of the proximate composition of bambara groundnut leaf material grown under two water regimes are shown in Table 5.3. Significant differences ($P<0.001$) were observed for water regime, harvest time, landrace (Table 5.3). For all proximate plant components, plants grown at 100% ETc had higher moisture content compared with those grown at 30% ETc, regardless of harvest time. Mature plants, harvested 16 weeks after planting, were generally characterised by increased proximate components that those harvested 8 weeks earlier (Table 5.3). This trend was shown for all landraces, although the landraces were significantly different. Fat, was the only nutritional component that did not show a significant change in response to harvest time and water regime (Figure 5.3). Analysis of seed material at harvest maturity showed significant ($P<0.05$) differences between water regimes and landraces (Table 5.4). On average, there was a higher moisture content in seeds grown at 100%ETc compared to 30%ETc (Figure 5.4). Neutral detergent fibre (NDF) content was not significantly affected by water regime. Crude protein (CP) and fat content increased in response to higher water content during growth, whereas both ash and acid detergent fibre (ADF) declined on average. The differences among landraces showed no consistent trend with respect to a particular seed component.

Table 5.3: Proximate composition of the above ground material (leaves and branches) of bambara groundnut at 8 weeks after planting and at harvest (16 weeks). NDF = Neutral detergent fibre; CP = Crude protein; ADF = Acid detergent fibre.

Water regime	Time(weeks)	Landrace	Moisture (%)	NDF (%)	CP (%)	FAT (%)	Ash (%)	ADF (%)
	8	Black speckled	4.135	24.22	11.96	3.28	4.14	20.63
		Brown speckled	4.505	32.45	15.62	2.53	4.22	20.75
		Cream	7.425	26.23	12.26	3.45	6.33	19.74
30% ETc	16	Black speckled	5.02	44.78	8.45	3.00	16.61	34.85
		Brown speckled	4.02	38.35	11.57	2.33	15.40	25.22
		Cream	5.705	45.22	9.2	2.8	15.22	33.16
	8	Black speckled	6.235	38.285	10.73	3.03	6.36	26.32
		Brown speckled	8.015	37.285	12.92	2.54	6.68	25.65
		Cream	7.985	35.28	12.64	2.44	5.78	25.75
100%ETc	16	Black speckled	6.95	34.96	10.88	3.27	11.55	25.19
		Brown speckled	4.85	44.75	11.46	3.25	20.96	35.17
		Cream	5.415	44.3	10.15	2.84	16.34	31.01
	$LSD_{(P=0.05)\ (Water\ regime)}$		0.31	0.33	0.93	0.12	1.67	0.68
	$LSD_{(P=0.05)\ (Time)}$		0.27	0.34	0.87	0.13	1.71	0.71
	$LSD_{(P=0.05)(Landrace)}$		0.23	0.289	0.891	0.12	1.69	0.68

Table 5.4*:* Proximate composition of bambara groundnut seeds at final harvest. NDF = Neutral detergent fibre; CP = Crude protein; ADF = Acid detergent fibre.

Water regime	Landrace	Moisture (%)	NDF (%)	CP (%)	Fat (%)	Ash (%)	ADF (%)
30% FC	Black	4.87	44.87	8.48	3.00	14.47	33.47
	Brown	3.9	39.72	10.92	2.23	14.14	28.09
	Cream	5.40	44.73	8.78	2.88	13.84	33.54
100% FC	Black	5.36	36.96	10.10	3.29	11.73	26.63
	Brown	4.52	45.98	9.74	3.30	22.82	32.20
	Cream	5.44	44.1	10.76	2.98	14.71	30.89
$LSD_{(P= 0.05)}$ (Water regime)		0.077	0.36	0.13	0.04	0.762	0.35
$LSD_{(P=0.05)}$ (Landrace)		0.061	0.31	0.10	0.042	0.66	0.30

5.2.6 Mineral composition

The results of analysis of selected minerals in bambara groundnut leaves and seeds at harvest maturity are shown in Tables 5.5 and 5.6. There were significant differences between water regimes, time of harvesting and landraces, with respect to mineral composition of leaf material (Table 5.5). Plants grown under 100%ETc produced 12% more Ca, 2% more Mg, 12% more K, 18% more Na and 15% more P, but there was no difference in the amount of Zn (at 6% on average) compared with plants grown at 30%ETc (Table 5.5). Harvesting time had an effect of increasing Ca by 22% at harvest maturity (16 weeks) compared with 8 weeks after planting, within 30ETc (Table 5.5). Within 100%ETc, the Ca improvement was 34% at 16 weeks after planting (Table 5.5). A similar trend was observed for the other mineral elements.

There was an average 21% increase in all mineral nutrients due to growing plants at 100% ETc compared with 30%ETc (Table 5.6). The individual nutrients showed the following increases in response to higher water regime: Ca (6%), Mg (13%), K 3%), P (18%) and Zn (22%), but there was not a significant effect on Na content (Table 5.6).

Table 5.4: Mineral composition of bambara groundnut leaves at 8 weeks after planting and at final harvest (16 weeks).

Water regime	Time(weeks)	Landrace	Ca (%)	Mg (%)	K (%)	Na (%)	P (%)	Zn (ppm)
	8	Black speckled	0.24	2.36	0.03	0.4	4.61	64.5
		Brown speckled	0.24	1.94	0.03	0.35	4.49	63.2
		Cream	0.21	2.18	0.03	0.35	3.47	50.5
30% ETc	**16**	Black speckled	0.3	2.03	0.07	0.39	5.56	70.8
		Brown speckled	0.26	1.77	0.08	0.40	4.61	61.3
		Cream	0.33	2.08	0.08	0.45	5.31	59.3
	8	Black speckled	0.23	2.04	0.03	0.34	4.23	30.6
		Brown speckled	0.25	2.33	0.06	0.46	4.59	44.4
		Cream	0.23	1.95	0.04	0.34	3.74	69.9
100% ETc	**16**	Black speckled	0.37	1.79	0.08	0.49	6.23	46.7
		Brown speckled	0.34	2.28	0.07	0.68	7.37	81.0
		Cream	0.36	2.08	0.08	0.57	6.86	75.8
$LSD_{(P=0.05)\ (Water\ regime)}$			0.02	0.01	0.02	0.01	0.02	0.3
$LSD_{(P=0.05)\ (Time)}$			0.02	0.01	0.02	0.01	0.02	0.3
$LSD_{(P=0.05)\ (Landrace)}$			0.06	0.04	0.06	0.02	0.06	1.05

Table 5.5: Mineral composition of bambara groundnut seeds at final harvest in a controlled environment.

Water regime	Variety	Ca (%)	Mg (%)	K (%)	Na (%)	P (%)	*Zn (ppm)*
30%ETc	Black speckled	1.11	0.30	2.01	0.07	0.48	53.49
	Brown speckled	1.14	0.26	1.74	0.07	0.45	46.55
	Cream	1.13	0.31	2.08	0.10	0.55	53.1
100%ETc	Black speckled	1.32	0.35	1.99	0.08	0.52	56.51
	Brown speckled	1.35	0.31	2.09	0.06	0.60	73.78
	Cream	1.39	0.33	1.93	0.07	0.52	66.16
$LSD_{(P=0.05)}$ (Water regime)		0.034	0.016	0.048	0.008	0.019	3.41
$LSD_{(P=0.05)}$ (Landrace)		0.028	0.013	0.039	0.007	0.015	2.78

5.3 Discussion

To be able to grow plants need to take up water from the soil and CO_2 from the atmosphere and use it in photosynthesis (Arve *et al*., 2011). This is done by CO_2 uptake through the stomatal pore, where water is simultaneously transpired. Water transpiration drives the water uptake by the roots and transport through the xylem. When the stomata are open CO_2 is taken up while water is transpired. When the stomata are closed little CO_2 is taken up and the transpiration is lowered. By opening and closing the stomata plants can regulate the amount of water lost, by sacrificing CO_2 uptake, when the environmental conditions are unfavourable. Since stomatal closure has negative effects on CO_2 uptake, photosynthesis, transpirational cooling as well as water and nutrient uptake it is important to close the stomata only when the benefit of water retention outweighs the negative effects. In this study the positive effect of adequate water availability on stomatal conductance (SC) was confirmed in that was a 28% improvement in SC when plants were grown under 100%ETc compared with 30%ETc. However, chlorophyll content index (CCI) was not affected by water regime during growth. This was a surprising finding, which needs further investigation.

Water stress can be defined as reduced water availability; either by water scarcity (drought) or osmotic stress (high salt concentrations) or water logging; too much water. Water stress may reduce photosynthesis, respiration and ion uptake, change the metabolic and growth patterns in the plant and in severe cases result in plant death (Teulat *et al.*, 1997). In nature water stress is common either for long or short periods of time, depending on the local climate. Most plants therefore have some adaptation or response to enhance the growth and survival rate during water stress and subsequent recovery. Although the current study did not investigate the adaptation patterns of bambara groundnut to water stress, evidence of crop adaptation to low water content during growth was indicated by no significant difference between 100%ETc and 30%ETc, with respect to plant height, and only 27% increase in leaf number under 100%ETc growth conditions. Also, there were only a few case of statistically significant differences between water regimes with respect to yield parameters. However, the trend in these differences in association with landraces requires further investigation.

Normally, proximate analysis of plant material used for feed or food is a quantitative method to determine different macronutrients. Basically it is the partition of plant material compounds into six categories by means of common chemical properties (Agomuo, 2011; Lien, 2014). The categories are moisture (crude water), crude ash (CA), crude protein (CP), ether extracts

(fats or lipids; EE), crude fibre (CF) and nitrogen free extractives (NFE) (Lien, 2014). In recent years the over 100 year old proximate system has been advanced and improved. Especially the imprecision of CA, CF and NFE as well as CP had been criticized (Agomuo, 2011; Lien, 2014). Modern methods to determine the exact composition of the CA fraction via atomic absorption spectroscopy and the CP fraction via amino acid analysers, near infrared spectroscopy (NIRS), etc. have been established. Improving the information gained from analysis of feedstuffs and diets also involves the determination of sugars and starch (polarimetric methods) contained in the NFE fraction of the proximate analysis (Agomuo, 2011; Lien, 2014). In this study, plant material analysis also include mineral elements in order to broaden the scope of nutritional value that is found in response to soil water regime and stage of plant development when the material is harvested.

CHAPTER 6

PROXIMATE NUTRIENT COMPOSITION OF SEEDS AND LEAVES OF BAMBARA GROUNDNUT LANDRACES PLANTED UNDER RAINFED AND IRRIGATED FIELD CONDITIONS OVER TWO SEASONS IN TWO LOCATIONS

6.1 Introduction

Legumes have been part of the human diet since the beginning of agriculture. A number of legume species are still an irreplaceable dietary protein source for both animals and humans (Wang *et al*., 2003). Legumes can be consumed as seedlings, young leaves, fresh immature pods and dry seeds. The leaves are known to add taste and flavour, as well as substantial amounts of protein, fibre, minerals and vitamins to the diet (Mitchell *et al*., 2009). However, research has only been devoted to dry seeds only. Bambara groundnut (*Vigna subterranea*) forms part of some of the most underutilized legumes on earth. This crop is widely distributed across Africa. Bambara groundnut was once said to be the third most important grain legume in sub-Saharan Africa. Due to the increasing demand for protein food sources, the United Nations Protein Advisory Group has identified the improvement of underutilized legumes as a vital area of research.

There is paucity of literature on the proximate and nutrient compositions of bambara groundnut seeds and leaves. Apart from the quantitative determination of specific nutrients in bambara groundnut, it has also been shown that the amount of a particular nutrient is influenced by climate, age at harvest, irrigation and plant genotype which is mostly characterised by the seed colour (Kane *et al*., 1997). Bambara groundnut leaves and seeds feature prominently in various dishes around Africa. However, published information is scant on its nutrient and proximate composition, correlations between nutrients, seasons, time of harvest, response to water availability, landrace selections and locations. Also knowledge of bambara groundnut proximate composition would enable people to know the better type of seeds and leaves to eat or feed to animals at any given point in time. The present study aims at drawing attention to the proximate and nutritive value of bambara groundnut with a view to providing the necessary information towards effective utilisation of this legume in various food applications.

6.2 Results

6.2.1 Meteorological data

The winter trial was planted on 3 May, 2013 and harvested on 1 August, 2013 at both locations (Figure 6.1 and 6.2). The summer trial was planted between December, 2013 and March, 2014 at Ukulinga research farm. At Ukulinga, rain fell more frequently during the winter trial which had the highest rainfall compared to Swayimani. There were very low rainfall observations at Swayimani, going down to as low as 3 mm during the June month. In summer, the rainfall was highest in February and March, 2014. At Ukulinga, the rainfall was significantly higher in summer than winter. Monthly average temperatures during winter were significantly low. A comparison of the two sites showed that Swayimani had the lowest winter temperatures. During the summer trial the average temperatures at Ukulinga was 29°C.

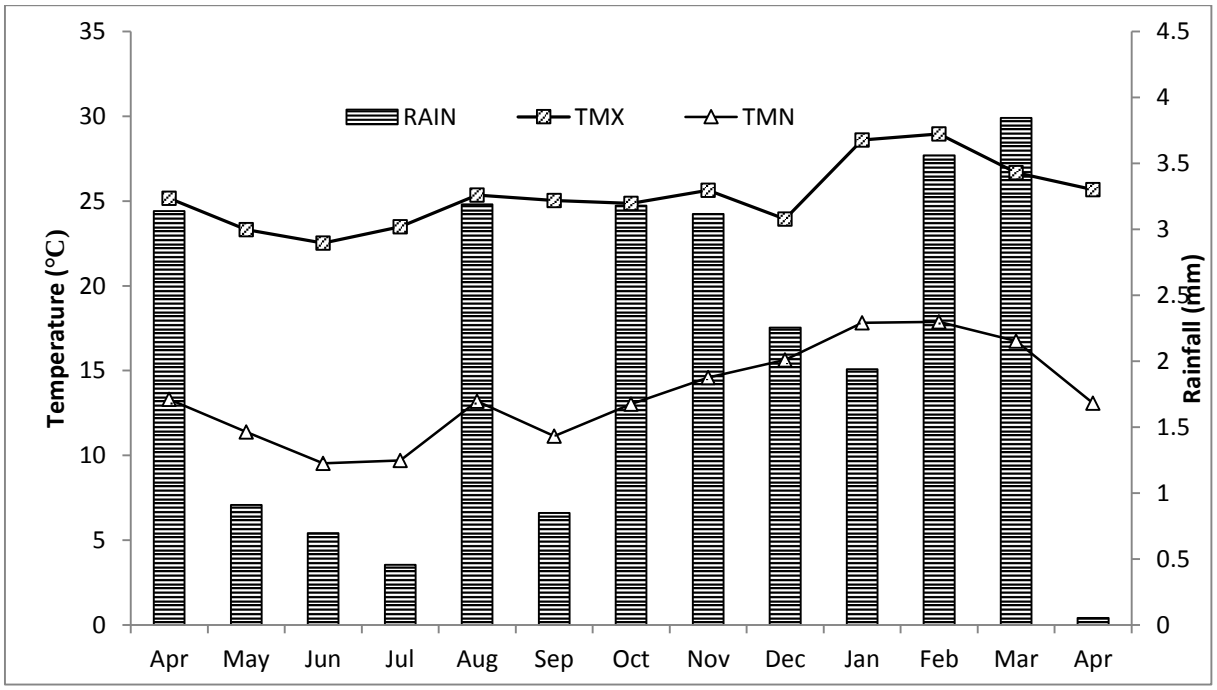

Figure 6.1: Monthly average temperatures (maximum and minimum) and rainfall recorded at Ukulinga Farm from April, 2013 to April, 2014.

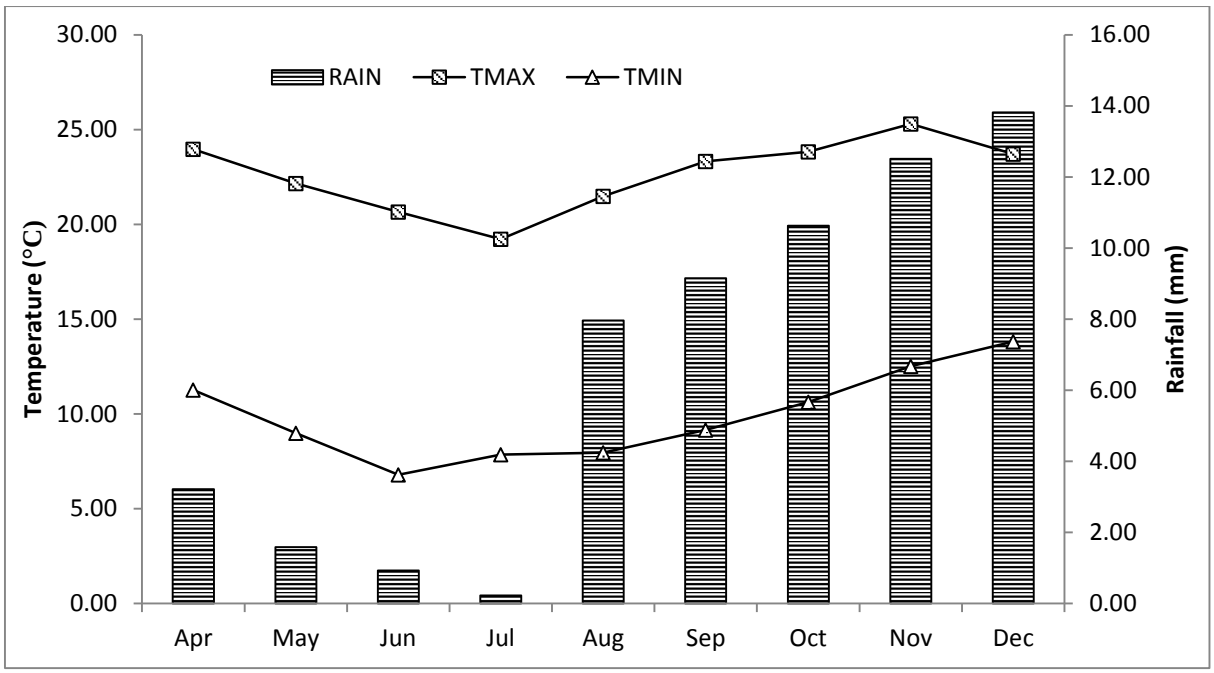

Figure 6.2: Monthly average temperatures (maximum and minimum) and rainfall recorded at Swayimani from April to December, 2013.

6.2.2 Proximate and Nutritional analyses

Table 6.1 presents the proximate composition leaves harvested at Swayimani and Ukulinga from two harvest periods during winter under rain-fed conditions. The moisture content at Swayimani ranged from1.24- 2.83%. No significant differences ($P>0.05$) were observed on both time and between landrace selections. However, based on mean values harvesting at 12 weeks after planting (2.17%) secured more moisture content than at 6 weeks after planting (1.69). Generally, cream seeds had more moisture content (2.24%) than brown (1.95%) and black (1.60%) respectively. With regards to moisture content at Ukulinga, significant differences ($P<0.05$) were observed, landrace selections, time and between all interactions from the treatments under consideration. Moisture content increased by 22% over time. The brown sp. variety had the most moisture content of 6.72% followed by cream (5.96%) and black sp. (5.37%) respectively. There were significant differences ($P<0.001$) between locations with regards to moisture. More moisture was observed at Ukulinga (5.37%) than at Swayimani (1.93%). However there were no significant differences between sites with regards to variety and time.

There were significant differences ($P<0.05$) between landrace selections and time with regards to protein content at Swayimani. The darker seeds (Black sp. and brown sp.) had

higher protein content when compared to cream seeds. Protein content was observed to decrease overtime, as 20.75% of protein was observed at 6 weeks after planting and 18.91% was observed at 12 weeks after planting, the two harvest times had a 9% protein content difference between them. There was a significant interaction ($P<0.05$) between time and landrace selections. Protein content at 6 weeks after planting ranged between 18.86-24.02% and at 12 weeks after planting it ranged between 16.67-20.39%. Highly significant differences ($P<0.001$) were observed time, variety and interactions between them with regards to protein content at Ukulinga. More protein content was observed after 12 weeks of planting (12.55%) as compared to 11.32% observed after 6 weeks of planting, however the opposite trend was observed at Swayimani. The black sp. variety had 3% and 8% more protein when compared with brown sp. and cream landrace selections respectively. A similar trend on the protein contents of the landrace selections under investigation was also observed at Swayimani, however landrace selections from Swayimani had 40% more protein than landrace selections from Ukulinga research farm. These findings display a huge difference on planting sites with regards to protein. Significant differences ($P<0.05$) were also observed between the two locations on protein content with regards to variety and time, landrace selections from Swayimani had more protein in both time periods.

There were no statistical differences ($P>0.05$) between landrace selections, time and their interactions with respect to Swayimani. However, mean differences showed that about 9% of fat decreased on the leaves between 6 and 12 weeks harvests. Black sp. leaves had 14% and 25% more fat when compared with brown sp. and cream leaves respectively. At 6 weeks after planting fat ranged between 2.82-3.31% and at 12 weeks after planting it ranged between 2.36-3.71%.No differences ($P>0.05$) were observed between landrace selections, time and variety time × variety. The fat content ranged between 3.32- 1.92%. Swayimani leaf landrace selections had 25% more fat content when compared with leaf landrace selections from Ukulinga research farm. Even though no statistical significance was observed, black sp. leaf landrace selections had more fat content of 2.60% followed by brown sp. (2.54%) and cream (2.53%) respectively. The same trend was also observed at Swayimani with regards to landrace selections. However, no statistical differences were observed between locations when it comes to ash in all treatments.

There were no significant differences ($P>0.05$) between landrace selections, time and their interactions with respect to ash. Leaf material from cream seeds had the most ash of 24.53% followed by black sp. (20.85%) and brown sp. (16.19%). At Ukulinga, no differences

(P>0.05) were observed between landrace selections, interactions between time and variety and variety, variety and time with respect to ash content. There was a 30% decrease in ash content over time at Ukulinga research farm. However, this was not the case at Swayimani but the times did not show statistical differences. Cream leaf variety had more ash (20.60%) followed by brown sp. (19.06%) and black sp. (18.07%), the same observations were observed at Swayimani with the same trend. No differences were observed between the two locations with regard to fat under all the treatments.

With regards to the fibre content (ADF and NDF), no significant differences (P>0.05) were observed between landrace selections and time. More NDF was found on black sp. leaves (45.41%) compared with cream (40.22%) and brown sp. (38.58%). Leaf material harvested from cream seed landraces were observed to have about 9 and 21% more ADF compared with leaf material harvested from black sp. and brown sp. Seeds, respectively. A decrease over time of about 3% ADF was observed between 6 and 12 weeks harvests. With regards to ADF and NDF at Ukulinga, no significant differences (P>0.05) were observed between landrace selections and their interactions. Time showed significant differences (P<0.05) for both ADF and NDF. Cream seeds were observed to contain more ADF and NDF at both locations. Leaf landrace selections from Swayimani contained less ADF and NDF when compared with leaf landrace selections harvested from Ukulinga research farm. Table 6.2 represents the mineral composition of leaf material harvested from three Bambara groundnut seed landrace selections during winter at Swayimani and Ukulinga. At Swayimani, all minerals except sodium showed no significant differences (P>0.05) between landrace selections and time. However trends were observed in all mineral there mineral content showed a decreasing trend with time, were less minerals were observed at 12 weeks after planting. There minerals decreased by 12% (Ca), 8% (Cu), 9% (K), 50% (Mg), 18% (Mn), 71% (Na), 7% (P) and 6% (Zn). Cream seeds had higher percentages of potassium, magnesium, sodium, phosphorus zinc and iron content when compared with seeds from both brown sp. and black sp. At Ukulinga, manganese had the dominating percentage amongst the minerals, making Bambara groundnut a good source of manganese. All nutrients showed a decreasing trend with time in all leaf landrace selections. Calcium content ranged between 0.76-0.91%. Leaf landrace selections under irrigated crops showed a better accumulation of calcium (0.86%) when compared with rain-fed leaf landrace selections (0.78%). Brown sp. seeds had more calcium (0.86%) when compared with cream (0.82%) and black sp. (0.79%). Significant differences (P<0.05) were observed between landrace selections with regards to copper, manganese, phosphorus and zinc.

Table 6.1: Proximate analysis (on 100% dry matter basis) of leaves harvested at two different locations (Ukulinga and Swayimani) and two different harvest periods

Location	Time(weeks)	Variety	Moisture (%)	Ash (%)	Fat (%)	ADF (%)	NDF (%)	CP (%)
Swayimani	**6**	Black sp.	1.24	17.96	3.22	29.64	45.25	24.02
		Brown sp.	2.19	19.79	3.31	26.55	38.97	18.86
		Cream	1.64	23.68	2.82	35.95	40.05	19.38
	12	Black sp.	1.96	23.74	3.71	31.65	45.56	19.65
		Brown sp.	1.72	12.60	2.65	26.52	38.19	20.39
		Cream	2.83	25.38	2.36	31.41	40.39	16.67
Ukulinga	**6**	Black sp.	3.58	25.60	2.06	31.19	41.64	11.33
		Brown sp.	7.70	14.84	2.55	35.09	45.28	11.59
		Cream	4.17	20.87	1.92	35.40	44.27	11.03
	12	Black sp.	5.00	22.27	2.45	33.30	43.62	11.22
		Brown sp.	4.35	30.21	2.17	41.12	45.83	11.56
		Cream	7.42	18.49	2.49	34.51	43.17	11.24
$LSD_{(P=0.05)Variety(A)}$			1.03	11.02	0.77	5.78	4.16	1.01
$LSD_{(P=0.05)Location(B)}$			0.84	8.99	0.63	4.72	3.39	0.82
$LSD_{(P=0.05)A*B}$			1.46	15.58	1.09	8.17	5.88	1.42

Table 6.2: Chemical analysis of leaves harvested at two different locations (Ukulinga and Swayimani) and two different harvest periods

Location	Time(weeks)	Variety	K (%)	P (%)	Ca (%)	Na (%)	Mg (%)	Zn (ppm)	Cu (ppm)	Mn (ppm)
Swayimani	**6**	Black sp.	1.80	0.29	0.44	0.51	0.32	57.82	5.81	101.27
		Brown sp.	1.79	0.23	0.46	0.92	0.36	52.88	8.69	73.77
		Cream	1.85a	0.29	0.41	0.21	0.57	62.22	4.58	61.29
	12	Black sp.	1.67a	0.24	0.33	0.14	0.20	52.04	5.80	59.98
		Brown sp.	1.74a	0.28	0.43	0.19	0.23	58.39	5.63	65.56
		Cream	1.58a	0.23	0.36	0.15	0.20	52.17	6.04	68.53
Ukulinga	**6**	Black sp.	1.53	0.38	0.81	0.13	0.32	44.91	16.13	312.28
		Brown sp.	1.35	0.32	0.87	0.17	0.36	41.02	11.69	178.41
		Cream	1.79	0.37	0.75	0.12	0.34	44.91	15.31	281.67
	12	Black sp.	1.49	0.26	0.72	0.12	0.33	38.58	11.87	173.64
		Brown sp.	4.35	1.64	0.41	0.81	0.14	0.39	47.05	15.75
		Cream	1.59	0.31	0.75	0.14	0.33	37.81	10.93	258.49
$LSD_{(P=0.05)Variety(A)}$			0.24	0.06	0.11	0.15	0.15	7.26	2.23	58.30
$LSD_{(P=0.05)Location(B)}$			0.19	0.05	0.09	0.12	0.12	5.93	1.82	47.60
$LSD_{(P=0.05)A*B}$			0.47	0.13	0.22	0.30	0.29	14.52	4.46	116.59

Table 6.3 presents the proximate composition of leaves harvested after 12 weeks after planting at Ukulinga research farm during summer and winter, under irrigated and rain-fed conditions. Significant differences ($P<0.001$) were observed between irrigation systems and between landrace selections during winter. Leaf landrace selections under irrigated irrigation system had 13% more protein when compared with rain-fed landrace selections. Black speckled seeds had the most protein content (14.31%) followed by brown speckled (12.52%) and lastly cream (11.70%). Protein content during winter under irrigated and rain-fed conditions was 6% and 4% less when compared to the summer trial respectively. There were no significant differences ($P>0.05$) amongst the two seasons with regards to protein. However, according to mean values all landrace selections performed better during summer when compared to winter with regards to the mean protein. Across seasons significant differences ($P<0.05$) were observed between irrigation systems and landrace selections.

With regards to ash, irrigation systems and an interaction between irrigation systems and landrace selections showed highly significant differences ($P<0.001$) during summer. No statistical differences ($P>0.05$) were observed between landrace selections during summer. According to the mean values black speckled seeds had the least ash content (21.81%) followed by brown speckled (27.39%) and cream (31.81%) with the most ash content. A similar trend was observed during the winter trial but the overall ash content was 29% lower. Significant differences ($P<0.05$) were observed between seasons with regards to ash, with summer having 28.26% and winter with15.91%. Irrigation systems also showed significant differences ($P<0.05$) between seasons, with irrigated leaves having 25% more ash when compared to rain-fed.

There were no significant differences ($P>0.05$) between irrigations and landrace selections with regards to fat content in summer. However mean values showed that irrigated landrace selections had more fat compared to landrace selections under rain-fed conditions, this was also the case during the winter trials. Landrace selections under the winter trial had an overall 44% more fat content when compared with the summer landrace selections. Differences were observed on an interaction between landrace selections and irrigation systems with regards to fat content during summer. Fat content differed ($P<0.001$) across seasons with winter having 2.27% and summer having 1.33% on average.

Moisture content showed significant differences ($P<0.01$) amongst irrigation systems and landrace selections during summer season. Cream landrace selections under irrigated

conditions had the most moisture content (8.86%) followed by black speckled (7.17%) and brown speckle (5.67%), the same trend was observed under irrigated conditions. Again the same trend was observed on the landrace selections with regards to moisture during the winter trial. However, moisture content showed no significant differences ($P>0.05$) across seasons. Landrace selections and irrigation systems showed significant differences across seasons with regard to moisture. Cream (7.91%) had the most moisture content across seasons followed by black speckle (6.45%) and brown speckle (5.93%). Rain fed irrigation systems had the lowest moisture contents under both winter and summer when compared with irrigated leaves.

No significant differences ($P>0.05$) were observed between landrace selections and irrigation systems with regards to NDF and ADF. However, significant differences were observed on an interaction between landrace selections and irrigation systems with regards to ADF. Landrace selections under rain-fed conditions had 2% more ADF than landrace selections under irrigated conditions. Significant differences ($P<0.01$) were observed between seasons with regards to NDF and ADF with summer landrace selections having about 20% more NDF and ADF than winter landrace selections.

Table 6.4 represents the mineral composition of leaf material harvested from three Bambara groundnut seed landrace selections during summer and winter respectively at Ukulinga research farm. Significant differences ($P<0.001$) between irrigation systems were observed with regards to calcium, copper and manganese. There were no significant differences ($P<0.001$) between landrace selections in all minerals. According to mean values, black speckled landrace selections had the most calcium (1.11%) followed by brown speckle (1.08%) and cream (0.90%). Landrace selections under rain-fed conditions had more calcium (1.12%) relative to landrace selections under irrigated conditions (0.94%). All minerals were more concentrated on the darker landrace selections (brown speckle and black speckle) relative to cream landrace selections. Significant differences ($P<0.001$) favouring summer were observed in all minerals excluding potassium, sodium and phosphorus which were greater during the winter season. This showed that most minerals are more concentrated on the Bambara groundnut landrace selections during summer.

Table 6.3: Proximate analysis (on 100% dry matter basis) of leaves harvested at Ukulinga locations under two irrigation treatments in two seasons (winter and summer).

Season	Treatment	Variety	Moisture (%)	NDF (%)	CP (%)	Fat (%)	Ash (%)	ADF (%)
Winter	**Rain-fed**	Black sp.	5.40	43.62	11.22	2.45	22.27	33.3
		Brown sp.	4.35	45.83	11.56	2.17	30.21	41.12
		Cream	7.42	43.17	11.24	2.49	18.49	34.51
	Irrigated	Black sp.	8.34	33.74	14.25	3.18	7.35	24.21
		Brown sp.	7.70	39.7	13.68	2.97	8.24	25.56
		Cream	7.70	35.32	13.37	3.32	8.93	26.22
Summer	**Rain-fed**	Black sp.	5.32	52.26	13.45	1.74	23.88	45.76
		Brown sp.	4.94	56.83	12.16	1.07	24.82	51.45
		Cream	7.45	50.91	9.94	1.45	19.49	41.73
	Irrigated	Black sp.	7.17	51.60	15.62	1.69	19.73	42.64
		Brown sp.	5.67	52.8	12.88	1.71	29.96	44.56
		Cream	8.86	54.13	11.74	0.94	44.13	48.98
$LSD_{(P=0.05)Variety\ (A)}$			1.41	4.83	0.99	0.60	9.57	7.636
$LSD_{(P=0.05)Irrigation(B)}$			1.62	5.57	1.14	0.69	11.05	8.82
$LSD_{(P=0.05)Season(C)}$			1.15	3.94	0.81	0.49	7.81	6.24
$LSD_{(P=0.05)A*C}$			1.99	6.83	1.39	0.849	13.53	10.79

Table 6.4: Proximate analysis of leaves harvested at Ukulinga locations under two irrigation treatments in two seasons (winter and summer).

Season	Treatment	Variety	K (%)	P (%)	Ca (%)	Na (%)	Mg (%)	Zn (ppm)	Cu (ppm)	Mn (ppm)
Winter	**Rain-fed**	Black sp.	1.49	0.26	0.72	0.12	0.33	38.58	11.87	173.64
		Brown sp.	1.64	0.41	0.81	0.14	0.39	47.05	15.75	540.04
		Cream	1.59	0.31	0.75	0.14	0.33	37.81	10.93	258.49
	Irrigated	Black sp.	1.52	0.23	0.79	0.16	0.32	32.56	6.52	113.93
		Brown sp.	1.73	0.31	0.8	0.14	0.33	34.46	8.1	103.34
		Cream	1.65	0.4	0.96	0.11	0.4	36.72	10.61	116.27
Summer	**Rain-fed**	Black sp.	1.15	0.14	1.18	0.07	0.39	34.69	16.01	217.73
		Brown sp.	0.75	0.14	1.10	0.04	0.38	45.20	16.83	415.34
		Cream	1.32	0.16	1.08	0.05	0.39	38.86	13.03	563.79
	Irrigated	Black sp.	1.21	0.13	1.04	0.06	0.43	32.73	15.17	1462.81
		Brown sp.	1.39	0.15	1.06	0.06	0.33	44.82	15.34	1034.21
		Cream	1.19	0.15	0.73	0.06	0.31	40.41	23.66	3176.65
$LSD_{(P=0.05)Variety\ (A)}$			0.252	0.06	0.21	0.05	0.03	9.48	3.13	774.21
$LSD_{(P=0.05)Irrigation(B)}$			0.291	0.07	0.24	0.06	0.04	10.95	3.62	893.98
$LSD_{(P=0.05)Season(C)}$			0.21	0.05	0.17	0.04	0.03	7.74	2.56	632.14
$LSD_{(P=0.05)A*C}$			0.36	0.08	0.30	0.07	0.05	13.40	4.43	1094.89

Table 6.5presents the proximate composition of seeds harvested after 12 weeks after planting at Ukulinga research farm during summer, under irrigated and rain-fed conditions. Seed landrace selections and irrigation systems showed differences at $P<0.05$ with respect to protein. Differences ($P<0.001$) were further observed on an interaction between the two treatments. Black speckled seeds had 11% and 5% more protein when compared with brown speckled and cream seeds respectively. Seeds harvested under irrigated conditions had 6% more protein content relative to seeds harvested under rain-fed conditions. Relatively seeds contained 31% more protein than the leaf material. Irrigation had a positive influence towards overall protein content in all seed landrace selections studied. There were no significant differences ($P>0.05$) between landrace selections with regards to fat content. However differences ($P<0.05$) were observed between irrigation systems. More fat was concentrated on seeds under irrigated conditions (6.5%) relative to rain-fed conditions (5.6%). According to mean values, seeds had 76% more fat than leaves.

Ash content differed significantly ($P<0.05$) between landrace selections and between irrigation systems. The overall ash content on the seeds was 81% lower when compared with the leaves. Irrigated seed landrace selections had more ash content (5.3%) than rain-fed landrace selections (5%), the same trend was observed when studying the leaf material. Moisture content also differed significantly ($P<0.05$) with regards to landrace selections and irrigation systems. Cream seeds had 22% and 32% more moisture content when compared with black speckled and brown speckled seeds respectively under irrigation system, the same pattern was also observed under rain-fed conditions. Moisture was more concentrated on the seeds than on the leaves. No significant differences ($P>0.05$) were observed between both treatments and their interactions with respect to NDF and ADF, this was the case with the leaf material at both winter and summer seasons. The results show that fibre content is the same under all the Bambara groundnut landrace selections under study.

Table 6.6 presents the mineral composition of seeds harvested after 12 weeks after planting at Ukulinga research farm during summer, under irrigated and rain-fed conditions. There were significant differences ($P<0.05$) between irrigation systems with regards to potassium, magnesium and phosphorus. Significant differences were also observed on an interaction between seed landrace selections and irrigation systems with respect to manganese and sodium. When comparing the mineral composition of the leaves and seeds, the seeds had lower concentrations of calcium, copper, magnesium, manganese, sodium and zinc, higher concentrations of potassium and phosphorus were also observed on the seeds.

Table 6.5: Proximate analysis (on 100% dry matter basis) of seeds harvested at Ukulinga during summer.

Treatment	Variety	Moisture (%)	NDF (%)	CP (%)	Fat (%)	Ash (%)	ADF (%)
Rain-fed	**Black sp.**	8.32	18.28	17.18	6.42	5.11	11.50
	Brown sp.	7.81	14.21	17.70	6.66	4.97	9.833
	Cream	11.59	16.87	18.25	6.33	4.97	12.25
Irrigated	**Black sp.**	9.74	15.85	21.49	5.79	5.38	12.35
	Brown sp.	8.21	16.54	16.74	5.32	5.25	10.99
	Cream	12.09	18.89	18.58	5.82	5.31	11.33
$LSD_{(P=0.05)}$ Variety(A)		1.43	2.91	1.12	0.72	0.10	1.97
$LSD_{(P=0.05)Treatment(B)}$		1.17	2.38	0.92	0.58	0.08	1.61
$LSD_{(P=0.05)\ A*B}$		2.02	4.12	1.59	1.01	0.15	2.79

Table 6.6: Mineral analysis of seeds harvested at Ukulinga during summer.

Treatment	Variety	K (%)	P (%)	Ca (%)	Na (%)	Mg	Zn (ppm)	Cu (ppm)	Mn (ppm)
Rain-fed	Black sp.	1.659	0.340	0.050	0.010	0.168	27.361	5.977	18.714
	Brown sp.	1.657	0.341	0.046	0.022	0.172	27.431	6.787	16.607
	Cream	1.641	0.345	0.051	0.009	0.170	25.911	5.830	15.115
Irrigated	Black sp.	1.746	0.378	0.053	0.013	0.173	24.712	6.032	15.991
	Brown sp.	1.705	0.364	0.053	0.012	0.174	23.966	5.883	15.984
	Cream	1.721	0.376	0.056	0.019	0.177	25.225	6.129	18.755
$LSD_{(P=0.05)}$ Variety(A)		0.052	0.013	0.009	0.006	0.006	1.271	1.175	1.821
$LSD_{(P=0.05)}$Treatment(B)		0.042	0.011	0.008	0.006	0.005	1.038	0.960	1.487
$LSD_{(P=0.05)}$ A*B		0.073	0.018	0.013	0.010	0.008	1.798	1.662	2.576

6.3 Discussion

The proximate and mineral results presented clearly indicate the potential of Bambara groundnut as being a possible source of scarce nutrients such as protein. Under the right management, location and planting season Bambara groundnut protein content can go as high as 24. 02%, which compares favourably with that reported for the more conventional legumes such as faba beans (Duc *et al*., 1999; Musalam *et al*., 2004), but higher than the records of Nworgu (2004), (18.3%), Aletor and Omodara (1994) (10.4%).

Seasonal variations and environments have been proven to influence the overall nutritional and chemical quality of legumes (George *et al*., 2005). Hence, the current study critically investigated the variations in the proximate analysis of Bambara groundnut seed and leaf landrace selections at different harvesting seasons (winter and summer) and environments. The nutritive value and mineral contents of the three Bambara groundnut landrace selections varied considerably among landrace selections, irrigation systems and seasons (Fi.gs 6.3 and 6.4). However, protein content was not significantly different across seasons, but it was significant amongst irrigation systems and landrace selections. These findings were similar to those reported by Abdalla Saleem *et al*. (2012) when studying the nutritive value of the leaves and fruits of three *Grewia* species.

The protein content of the leaves of Bambara groundnut in both seasons were higher than the minimum crude protein level of 7% required for optimum rumen function (van Soest, 1994). Bambara groundnut leaves can be a good source of protein throughout the year, providing basic animal diets especially during the dry season were animals are normally limited to poor nutritional herbaceous feeds. The good protein content on Bambara groundnut leaf landrace selections can also contribute in fodder making, and can therefore allow ease of transportation to environments with short grazing seasons with regards to ruminants. Legume fibre tends to digest faster than grasses, allowing ruminants to ingest more legumes (Buckmaster, 1990). Therefore, inclusion of Bambara ground nut leaf landrace selections which have high protein contents on animal diets is of high importance.

The seeds harvested during summer also showed superior amounts if protein and moisture as compared to the leaf material at final harvest. Similar observations were observed by Fasakin (2004). The high ash content during summer as observed on the current study may be due to high

concentrations of minerals which are precursors to the proximate formation in summer than in winter. For example, minerals such as potassium calcium and magnesium are components of ash. In both seasons the ash content range exceeded 8-10% as reported by Gohl (1981) who concluded that ash content exceeding these values is an indication of contamination of fat. Abdalla Saleem (2012) also added by suggesting that high ash contents are also an indication of low organic matter. Neutral detergent fibre is a good indicator for crop quality, it is related to the intake potential and energy value.

The NDF of the three landrace selections during summer ranged between 50.91-56.83% and during winter ranged between 33.74-45.83% which indicated a high digestibility as concluded by Kingamkono *et al*. (2004). On the current study it was observed that there was more NDF during summer compared to winter, these findings are in agreement with that of Abdalla Saleem *et al*. (2012). The ADF content of the three bambara groundnut leaf landrace selections was lower than (60.4-60.7%) as reported by Anele *et al.* (2008), as the amount required for proper utilisation by ruminants without decreasing their feed intake. High ADF content is known for resulting in slow digestion and also limits dry matter intake. The fat content in winter ranged between 2.17-3.32% and in summer ranged between 0.94-1.74% with no significant differences between landrace selections. The winter fat content is in harmony with the average fat content for *Ceratotheca sesamoids* Endl, reported by Fasakin (2004).

Most minerals except potassium, sodium and phosphorus showed to be favoured by summer conditions. An appreciable amount of these minerals was observed during summer relative to winter. These results coincide with the high rainfall ranges observed during summer at Ukulinga. More chemical reactions occur under moist conditions (George *et al.,* 2005) which result to accumulation of such minerals as observed in the current study. The same findings were observed under irrigated material whether seeds or leaves. These observations are similar to those of Alghamdi (2009) and Musallam *et al*. (2004). The seeds also were found to have more minerals than the leaves.

CHAPTER 7

GENERAL DISCUSSION

7.1 Introduction

Drought strongly affects grain yield in several regions of the world. Plant growth and plant water status in response to soil water deficit play an important role in tolerance to drought and in yield stability. Protein-energy malnutrition is a major health challenge in developing countries (FAO, 2012). This is ascribed to low nutritional quality of traditional food stuff and high cost of quality protein foods (FAO, 2012). Malnutrition often results in death, disability, stunted mental and physical growth. The interaction of poverty, poor health and poor feed sources has a multiplier effect on the general welfare of nations. It is well known that high cost of fortified nutritious foods in many developing countries is always beyond the reach of most families (Ijarotimi and Keshinro, 2012) hence many families depend on inadequately processed and low quality traditional foods as their basic meals. As the shortage of food continues in developing countries, legumes are being investigated and promoted more than before to help address malnutrition (Karikari *et al*., 1995). It is therefore necessary that their levels of consumption, which are already too low in several developing countries, should be increased (Borget, 1992).

Legumes are rich, not only in proteins, but in other nutrients such as oils. Legumes are also used as supplementary feeds for livestock (Aletor and Adeogun, 1995). Legumes' leaf material can also be harvested at all stages of growth and used to feed livestock (fresh) or used to form part of forage. In many cases, the mineral contents are not considered to be absolute and may vary depending on variety, climatic condition, location, irrigation conditions, and season during which they are grown (Abdalla Saleem *et al*., 2012; Dansi, *et al*, 2012; Onyeonagu and Eze, 2013).

7.2 Future Teaching, Learning and Research Possibilities

The following recommendations may be made, based on observations made during this study;

- Anti-nutritional factors are known to reduce the nutritional quality of crops. Therefore, there is a need for genetic improvement of the edible parts of crops such as the leaves and seeds to reduce anti-nutritional factors.
- It is advisable to consider research on biological evaluation of nutrient content of bambara groundnut so as to determine bioavailability of nutrients.
- Processing methods of legumes have lately shown significant differences on the overall nutrient quality of crops. Therefore, the effect of processing methods on the nutritive and chemical value of bambara groundnut landraces is necessary.
- This study provides an example of a multidisciplinary study between agronomy, animal and human nutrition, therefore continuous research of this nature is highly recommended.

7.3 Summary Conclusions

The results of seed quality from three bambara groundnut landrace selections showed variability in seed quality and emergence traits. The darker seeds were observed to have better seed quality, with respect to germination, electrolyte leakage and proximate analyses. The differences observed were attributed to seed coat colour. Bambara groundnut seed germination was associated with seed colour. Seed colour can be used for germplasm selection to grow the crop under various conditions. Imbibing seeds have proven to have major contributions on the seeds' nutritional composition. Such techniques can be used to maximise nutrient contents of crops at harvest.

In this study, physiological responses (stomatal conductance and chlorophyll content) and plant growth responses (plant height and leaf number) were evaluated. Stomatal conductance was observed to decrease with increase in water stress. Low stomatal conductance which results in low transpiration also reduces CO_2 flow on leaves. Low levels of CO_2 in leaves down–regulate photosynthesis, which simultaneously decreases chlorophyll availability in the plant (Makakheri *et al.*, 2010). The current results agree with the above statement, were plants with low stomatal conductance had low CCI. Bambara groundnut landrace selections grown under 30% ETc were

able to adapt to limited water availability by closing their stomata; this suggests that under normal conditions these varieties would perform better. Due to the low leaf population on H1 plants there was less water loss via transpiration, which means stomatal activity was very limited resulting in lower stomatal values. Minimising leaf number can be used as a drought mechanism in water stressed environments.

Both the seed and leaf material of bambara groundnut harvested from both the greenhouse and field experiments contained the required protein levels. The decline in crude protein levels from leaf material after 8 weeks can be attributed to assimilate remobilisation from the leaves to the reproductive organs during flowering and fruiting (Hewitt and Marrush, 1986). Based on the results from the current study, sequential harvest of leaves at different stages of growth indicated that most nutrients analysed were found during early stages of crop development. Based on these preliminary results, it is recommended to harvest the leaves between 8-12 weeks after planting. These leaves can be used for human consumption or as a supplement in animal feeds. The results of leaf nutrient analysis showed that bambara groundnut landrace selections contain most of the nutrients required for good health. It was shown to be an excellent source of calcium, magnesium, copper, zinc, manganese and sodium.

The interaction between seasons, location, irrigation systems, sequential harvesting and crop varieties is one that needs sufficient planning so as to maximise nutrient quality and overall crop production. It was concluded that all of these factors have a huge impact on the crop's nutrient profile. Summer results showed better overall nutrient quality than winter crops. Providing supplementary irrigation was shown to be beneficial to both yield and nutritional value of bambara groundnut. Even though nutritional value was lower in winter and under rainfed conditions, it was observed that bambara groundnut still produces reasonably good quality nutrients under these conditions. These findings suggest that bambara groundnut is a drought tolerant crop and can aid as a cheap all year round forage supplement for ruminants during the dry seasons.

Bambara groundnut is an indigenous African legume, where it is the third most important legume in terms of consumption and socioeconomic impact in semi-arid Africa behind peanut (*Arachis*

hypogaea) and cowpea (*Vigna unguiculata*). The crop makes few demands on the soil, and is known to be drought tolerant and relatively disease free. This study attempted to confirm the drought tolerance and nutritional value attributes of the crop. The approach that was used in this study was to determine whether it has potential nutritional values for human and animal feed. The approach of analysing leaf material midway through growth (8 weeks after planting) and at harvest maturity (16 weeks after planting) showed that the crop has a wider food potential. Farmers can use the leaves to as a green vegetable and/or hay and the effect on the final seed yield will not be total crop failure.

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APPENDICES

Appendix 1: Analysis of variance tables for chapter 4

Variate: GVI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.9540	0.4770	4.13	
Rep.*Units* stratum					
Variety	2	5.9441	2.9721	25.73	0.005
Residual	4	0.4620	0.1155		
Total	8	7.3602			

Variate: MGT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.06569	0.03284	2.41	
Rep.*Units* stratum					
Variety	2	0.13964	0.06982	5.13	0.079
Residual	4	0.05440	0.01360		
Total	8	0.25973			

Variate: Root_Shoot_ratio

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.7117	0.3559	0.89	
Rep.*Units* stratum					
Variety	2	0.1868	0.0934	0.23	0.802
Residual	4	1.6002	0.4000		
Total	8	2.4987			

Variate: Root_length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.

Rep stratum	2	0.169	0.084	0.07	
Rep.*Units* stratum					
Variety	2	21.509	10.754	8.90	0.034
Residual	4	4.836	1.209		
Total	8	26.513			

Variate: Shoot_length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.2269	0.1135	0.77	
Rep.*Units* stratum					
Variety	2	2.4877	1.2438	8.47	0.036
Residual	4	0.5872	0.1468		
Total	8	3.3017			

Variate: Dry_mass

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.34120	0.17060	2.73	
Rep.*Units* stratum					
Variety	2	4.25304	2.12652	33.97	0.003
Residual	4	0.25042	0.06260		
Total	8	4.84466			

Variate: Fresh_mass

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.6380	0.8190	2.32	
Rep.*Units* stratum					
Variety	2	51.5908	25.7954	73.05	<.001
Residual	4	1.4125	0.3531		
Total	8	54.6413			

Variate: ec_hr_weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	9	28305224.	3145025.	12.24	
Rep.*Units* stratum					
Seed_colour	2	41639487.	20819743.	81.00	<.001
Residual	708	181987943.	257045.		
Total	719	251932653.			

Variate: Germination

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	616.69	308.35	9.56	
Rep.*Units* stratum					
Seed_colour	2	2591.21	1295.60	40.18	<.001
day	8	85942.91	10742.86	333.18	<.001
Seed_colour.day	16	1458.57	91.16	2.83	0.002
Residual	52	1676.64	32.24		
Total	80	92286.02			

Variate: ADF

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.9889	0.9889	6.28	
Rep.*Units* stratum					
Variety	2	0.0385	0.0193	0.12	0.886
Time	5	49.4602	9.8920	62.80	<.001
Variety.Time	10	46.8865	4.6886	29.77	<.001
Residual	17	2.6778	0.1575		
Total	35	100.0519			

Variate: Ash

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.11378	0.11378	5.23	
Rep.*Units* stratum					
Variety	2	0.42813	0.21407	9.83	0.001
Time	5	0.22239	0.04448	2.04	0.124
Variety.Time	10	0.37009	0.03701	1.70	0.161
Residual	17	0.37005	0.02177		
Total	35	1.50445			

Variate: Crude

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	5.6328	5.6328	10.83	
Rep.*Units* stratum					
Variety	2	7.3329	3.6665	7.05	0.006
Time	5	8.4743	1.6949	3.26	0.030
Variety.Time	10	15.2961	1.5296	2.94	0.024
Residual	17	8.8386	0.5199		
Total	35	45.5746			

Variate: Fat

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.42871	0.42871	5.28	
Rep.*Units* stratum					
Variety	2	0.50368	0.25184	3.10	0.071
Time	5	3.92419	0.78484	9.66	<.001
Variety.Time	10	1.85290	0.18529	2.28	0.065
Residual	17	1.38089	0.08123		

Total	35	8.09037			

Variate: Moist

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.20330	0.20330	3.60	
Rep.*Units* stratum					
Variety	2	14.34514	7.17257	126.94	<.001
Time	5	91.81844	18.36369	325.00	<.001
Variety.Time	10	20.41460	2.04146	36.13	<.001
Residual	17	0.96056	0.05650		
Total	35	127.74203			

Variate: NDF

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.0204	0.0204	0.09	
Rep.*Units* stratum					
Variety	2	20.9006	10.4503	44.36	<.001
Time	5	159.5809	31.9162	135.46	<.001
Variety.Time	10	99.9473	9.9947	42.42	<.001
Residual	17	4.0053	0.2356		
Total	35	284.4546			

Variate: Mg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.02056	0.02056	0.74	
Rep.*Units* stratum					
Variety	2	0.05320	0.02660	0.96	0.404
Time	5	0.14314	0.02863	1.03	0.432
Variety.Time	10	0.27858	0.02786	1.00	0.480
Residual	17	0.47319	0.02783		
Total	35	0.96866			

Variate: Ca

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.00070714	0.00070714	16.16	
Rep.*Units* stratum					
Variety	2	0.00009905	0.00004953	1.13	0.346
Time	5	0.00124689	0.00024938	5.70	0.003

Variety.Time	10	0.00058956	0.00005896	1.35	0.283
Residual	17	0.00074407	0.00004377		
Total	35	0.00338671			

Variate: K_Ca_Mg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.08290	0.08290	4.86	
Rep.*Units* stratum					
Variety	2	0.18560	0.09280	5.44	0.015
Time	5	0.09390	0.01878	1.10	0.396
Variety.Time	10	0.26872	0.02687	1.57	0.197
Residual	17	0.29007	0.01706		
Total	35	0.92119			

Variate: Na

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.0008464	0.0008464	7.13	
Rep.*Units* stratum					
Variety	2	0.0005416	0.0002708	2.28	0.133
Time	5	0.0004369	0.0000874	0.74	0.607
Variety.Time	10	0.0010374	0.0001037	0.87	0.573
Residual	17	0.0020182	0.0001187		
Total	35	0.0048804			

Variate: P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.0030037	0.0030037	8.68	
Rep.*Units* stratum					
Variety	2	0.0001864	0.0000932	0.27	0.767
Time	5	0.0008117	0.0001623	0.47	0.794
Variety.Time	10	0.0066982	0.0006698	1.94	0.111
Residual	17	0.0058842	0.0003461		
Total	35	0.0165842			

Variate: Zn

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	1.602	1.602	1.40	
Rep.*Units* stratum					

Variety	2	7.343	3.672	3.20	0.066
Time	5	8.132	1.626	1.42	0.267
Variety.Time	10	8.883	0.888	0.78	0.651
Residual	17	19.482	1.146		
Total	35	45.443			

Variate: Fe

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	1		0.6330	0.6330	0.66	
Rep.*Units* stratum						
Variety	2		821.0258	410.5129	427.58	<.001
Time	5		1921.1802	384.2360	400.21	<.001
Variety.Time	8	(2)	6409.5472	801.1934	834.50	<.001
Residual	15	(2)	14.4013	0.9601		
Total	31	(4)	8841.1063			

Variate: Mn

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.8105	0.8105	0.88	
Rep.*Units* stratum					
Variety	2	758.8612	379.4306	413.03	<.001
Time	5	449.3154	89.8631	97.82	<.001
Variety.Time	10	904.2839	90.4284	98.44	<.001
Residual	17	15.6172	0.9187		
Total	35	2128.8882			

Variate: Cu

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.55548	0.55548	6.86	
Rep.*Units* stratum					
Variety	2	5.97944	2.98972	36.91	<.001
Time	5	16.90296	3.38059	41.74	<.001
Variety.Time	10	27.82931	2.78293	34.36	<.001
Residual	17	1.37696	0.08100		
Total	35	52.64416			

Appendix 2: Analysis of variance tables for chapter 5

Variate: H20_CONTENT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.00	0.00	0.00	
Reps.*Units* stratum					
FC	1	582.20	582.20	35.15	<.001
Residual	230	3809.51	16.56		
Total	233	4391.71			

Variate: avg_Stomatal

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	284.2	142.1	0.81	
Reps.FC stratum					
FC	1	10034.6	10034.6	57.02	0.017
Residual	2	352.0	176.0	0.88	
Reps.FC.seed_colour stratum					
seed_colour	2	513.5	256.8	1.29	0.328
FC.seed_colour	2	342.5	171.3	0.86	0.460
Residual	8	1598.4	199.8	0.61	
Reps.FC.seed_colour.*Units* stratum					
	216	71087.1	329.1		
Total	233	84212.4			

Variate: avg_cci

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	159.3	79.7	5.61	
Reps.FC stratum					
FC	1	10.3	10.3	0.72	0.484
Residual	2	28.4	14.2	0.12	
Reps.FC.seed_colour stratum					
seed_colour	2	27.3	13.7	0.12	0.888
FC.seed_colour	2	229.5	114.8	1.01	0.407
Residual	8	911.0	113.9	0.77	
Reps.FC.seed_colour.*Units* stratum					
	216	32043.3	148.3		
Total	233	33409.1			

Variate: avg_plant_height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	114.871	57.435	11.89	
Reps.FC stratum					
FC	1	12.144	12.144	2.51	0.254
Residual	2	9.664	4.832	0.60	
Reps.FC.seed_colour stratum					
seed_colour	2	3.965	1.982	0.25	0.786
FC.seed_colour	2	19.263	9.631	1.20	0.349
Residual	8	64.064	8.008	1.35	
Reps.FC.seed_colour.*Units* stratum					
	216	1276.831	5.911		
Total	233	1500.800			

Variate: avg_Leaf_no

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	8.217	4.108	1.56	
Reps.FC stratum					
FC	1	241.391	241.391	91.63	0.011
Residual	2	5.269	2.634	1.12	
Reps.FC.seed_colour stratum					
seed_colour	2	16.430	8.215	3.48	0.082
FC.seed_colour	2	1.665	0.832	0.35	0.713
Residual	8	18.879	2.360	0.47	
Reps.FC.seed_colour.*Units* stratum					
	216	1073.316	4.969		
Total	233	1365.167			

Variate: Grain_No_Pod

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.16030	0.08015	236.51	
Reps.FC stratum					
FC	1	0.00036	0.00036	1.05	0.413
Residual	2	0.00068	0.00034	0.03	
Reps.FC.seed_colour stratum					
seed_colour	2	0.04840	0.02420	1.89	0.213
FC.seed_colour	2	0.00964	0.00482	0.38	0.698

Residual	8	0.10242	0.01280		
Total	17	0.32180			

Variate: Harvest_Index_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	170.10	85.05	1.13	
Reps.FC stratum					
FC	1	209.10	209.10	2.79	0.237
Residual	2	150.04	75.02	3.85	
Reps.FC.seed_colour stratum					
seed_colour	2	13.32	6.66	0.34	0.720
FC.seed_colour	2	60.32	30.16	1.55	0.270
Residual	8	155.88	19.49		
Total	17	758.76			

Variate: Pod_No_Plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.4038	0.2019	11.97	
Reps.FC stratum					
FC	1	0.1663	0.1663	9.85	0.088
Residual	2	0.0337	0.0169	0.02	
Reps.FC.seed_colour stratum					
seed_colour	2	1.9657	0.9829	1.31	0.322
FC.seed_colour	2	6.9195	3.4598	4.61	0.047
Residual	8	6.0074	0.7509		
Total	17	15.4965			

Variate: Pod_mass_g

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.16840	0.08420	25.09	
Reps.FC stratum					
FC	1	0.02801	0.02801	8.35	0.102
Residual	2	0.00671	0.00336	0.18	
Reps.FC.seed_colour stratum					
seed_colour	2	0.02893	0.01447	0.78	0.490
FC.seed_colour	2	0.01151	0.00576	0.31	0.741
Residual	8	0.14809	0.01851		
Total	17	0.39165			

Variate: Total_Biomass_g

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.6469	0.3234	3.26	
Reps.FC stratum					
FC	1	0.3556	0.3556	3.58	0.199
Residual	2	0.1985	0.0992	0.96	
Reps.FC.seed_colour stratum					
seed_colour	2	0.2285	0.1142	1.11	0.376
FC.seed_colour	2	0.0081	0.0041	0.04	0.961
Residual	8	0.8248	0.1031		
Total	17	2.2624			

Variate: Total_grain_mass_plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.11363	0.05682	3.55	
Reps.FC stratum					
FC	1	0.01502	0.01502	0.94	0.435
Residual	2	0.03201	0.01601	1.19	
Reps.FC.seed_colour stratum					
seed_colour	2	0.01843	0.00922	0.68	0.532
FC.seed_colour	2	0.06941	0.03471	2.57	0.137
Residual	8	0.10789	0.01349		
Total	17	0.35640			

Variate: Grain_No_Pod

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.16030	0.08015	236.51	
Reps.FC stratum					
FC	1	0.00036	0.00036	1.05	0.413
Residual	2	0.00068	0.00034	0.03	
Reps.FC.seed_colour stratum					
seed_colour	2	0.04840	0.02420	1.89	0.213
FC.seed_colour	2	0.00964	0.00482	0.38	0.698
Residual	8	0.10242	0.01280		
Total	17	0.32180			

Variate: Yield_kg_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.

Reps stratum	2	6394.3	3197.1	7.68	
Reps.FC stratum					
FC	1	679.8	679.8	1.63	0.330
Residual	2	833.0	416.5	1.11	
Reps.FC.seed_colour stratum					
seed_colour	2	356.5	178.2	0.47	0.639
FC.seed_colour	2	178.6	89.3	0.24	0.794
Residual	8	3014.3	376.8		
Total	17	11456.5			

Variate: ADF

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.1358	0.1358	7.41	
Rep.FC stratum					
FC	1	42.2292	42.2292	2305.03	0.013
Residual	1	0.0183	0.0183	0.07	
Rep.FC.Variety stratum					
Variety	2	6.1931	3.0965	12.39	0.019
FC.Variety	2	115.9065	57.9532	231.90	<.001
Residual	4	0.9996	0.2499	2.11	
Rep.FC.Variety.*Units* stratum					
Time	1	594.6940	594.6940	5026.89	<.001
Harvest	1	7.5580	7.5580	63.89	<.001
FC.Time	1	73.7923	73.7923	623.76	<.001
Variety.Time	2	34.6590	17.3295	146.48	<.001
FC.Harvest	1	29.3826	29.3826	248.37	<.001
Variety.Harvest	2	32.2018	16.1009	136.10	<.001
Time.Harvest	1	30.3410	30.3410	256.47	<.001
FC.Variety.Time	2	301.0110	150.5055	1272.21	<.001
FC.Variety.Harvest	2	168.2194	84.1097	710.97	<.001
FC.Time.Harvest	1	110.4412	110.4412	933.55	<.001
Variety.Time.Harvest	2	40.7128	20.3564	172.07	<.001
FC.Variety.Time.Harvest	2	265.8575	132.9287	1123.63	<.001
Residual	18	2.1294	0.1183		
Total	47	1856.4825			

Variate: Ash

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.9750	0.9750	2.45	
Rep.FC stratum					
FC	1	7.6430	7.6430	19.24	0.143
Residual	1	0.3972	0.3972	0.51	
Rep.FC.Variety stratum					

Variety	2	45.6563	22.8282	29.04	0.004
FC.Variety	2	50.6426	25.3213	32.21	0.003
Residual	4	3.1449	0.7862	1.13	
Rep.FC.Variety.*Units* stratum					
Time	1	1266.4110	1266.4110	1824.30	<.001
Harvest	1	10.2441	10.2441	14.76	0.001
FC.Time	1	0.8071	0.8071	1.16	0.295
Variety.Time	2	25.2525	12.6263	18.19	<.001
FC.Harvest	1	3.2202	3.2202	4.64	0.045
Variety.Harvest	2	32.0708	16.0354	23.10	<.001
Time.Harvest	1	5.5342	5.5342	7.97	0.011
FC.Variety.Time	2	71.2392	35.6196	51.31	<.001
FC.Variety.Harvest	2	23.2733	11.6366	16.76	<.001
FC.Time.Harvest	1	40.1487	40.1487	57.84	<.001
Variety.Time.Harvest	2	108.2125	54.1062	77.94	<.001
FC.Variety.Time.Harvest	2	156.4610	78.2305	112.69	<.001
Residual	18	12.4954	0.6942		
Total	47	1863.8291			

Variate: Fat

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.007078	0.007078	4.94	
Rep.FC stratum					
FC	1	0.001660	0.001660	1.16	0.477
Residual	1	0.001433	0.001433	0.65	
Rep.FC.Variety stratum					
Variety	2	2.032600	1.016300	458.60	<.001
FC.Variety	2	1.988179	0.994090	448.58	<.001
Residual	4	0.008864	0.002216	0.48	
Rep.FC.Variety.*Units* stratum					
Time	1	0.025353	0.025353	5.49	0.031
Harvest	1	0.091846	0.091846	19.89	<.001
FC.Time	1	1.934260	1.934260	418.93	<.001
Variety.Time	2	0.393622	0.196811	42.63	<.001
FC.Harvest	1	0.244049	0.244049	52.86	<.001
Variety.Harvest	2	0.159978	0.079989	17.32	<.001
Time.Harvest	1	1.494986	1.494986	323.79	<.001
FC.Variety.Time	2	0.155133	0.077567	16.80	<.001
FC.Variety.Harvest	2	1.140540	0.570270	123.51	<.001
FC.Time.Harvest	1	1.250835	1.250835	270.91	<.001
Variety.Time.Harvest	2	2.860411	1.430206	309.76	<.001
FC.Variety.Time.Harvest	2	0.715770	0.357885	77.51	<.001
Residual	18	0.083108	0.004617		
Total	47	14.589707			

Variate: Crude

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.

Rep stratum	1	0.30476	0.30476	1.98	
Rep.FC stratum					
FC	1	0.28815	0.28815	1.87	0.402
Residual	1	0.15424	0.15424	0.98	
Rep.FC.Variety stratum					
Variety	2	34.56246	17.28123	109.99	<.001
FC.Variety	2	29.66097	14.83049	94.39	<.001
Residual	4	0.62848	0.15712	1.93	
Rep.FC.Variety.*Units* stratum					
Time	1	74.75911	74.75911	917.22	<.001
Harvest	1	0.84331	0.84331	10.35	0.005
FC.Time	1	18.82080	18.82080	230.91	<.001
Variety.Time	2	6.15274	3.07637	37.74	<.001
FC.Harvest	1	0.15226	0.15226	1.87	0.189
Variety.Harvest	2	1.64786	0.82393	10.11	0.001
Time.Harvest	1	2.12455	2.12455	26.07	<.001
FC.Variety.Time	2	0.47211	0.23606	2.90	0.081
FC.Variety.Harvest	2	17.55361	8.77681	107.68	<.001
FC.Time.Harvest	1	29.60870	29.60870	363.27	<.001
Variety.Time.Harvest	2	10.69320	5.34660	65.60	<.001
FC.Variety.Time.Harvest	2	72.63952	36.31976	445.61	<.001
Residual	18	1.46711	0.08151		
Total	47	302.53394			

Variate: NDF

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.0301	0.0301	0.05	
Rep.FC stratum					
FC	1	91.4793	91.4793	164.62	0.050
Residual	1	0.5557	0.5557	1.62	
Rep.FC.Variety stratum					
Variety	2	155.4414	77.7207	226.98	<.001
FC.Variety	2	10.2275	5.1137	14.93	0.014
Residual	4	1.3697	0.3424	1.55	
Rep.FC.Variety.*Units* stratum					
Time	1	884.2309	884.2309	4004.21	<.001
Harvest	1	40.8543	40.8543	185.01	<.001
FC.Time	1	211.1875	211.1875	956.36	<.001
Variety.Time	2	236.5769	118.2884	535.67	<.001
FC.Harvest	1	2.1921	2.1921	9.93	0.006
Variety.Harvest	2	14.9803	7.4902	33.92	<.001
Time.Harvest	1	132.9137	132.9137	601.90	<.001
FC.Variety.Time	2	534.4817	267.2408	1210.19	<.001
FC.Variety.Harvest	2	159.8634	79.9317	361.97	<.001
FC.Time.Harvest	1	17.7144	17.7144	80.22	<.001
Variety.Time.Harvest	2	72.0898	36.0449	163.23	<.001
FC.Variety.Time.Harvest	2	108.0783	54.0391	244.71	<.001

Residual	18	3.9749	0.2208		
Total	47	2678.2417			

Variate: Moist

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.02105	0.02105	0.31	
Rep.FC stratum					
FC	1	15.48202	15.48202	229.23	0.042
Residual	1	0.06754	0.06754	30.97	
Rep.FC.Variety stratum					
Variety	2	9.74700	4.87350	2234.65	<.001
FC.Variety	2	7.16110	3.58055	1641.79	<.001
Residual	4	0.00872	0.00218	0.08	
Rep.FC.Variety.*Units* stratum					
Time	1	22.24866	22.24866	829.18	<.001
Harvest	1	1.43466	1.43466	53.47	<.001
FC.Time	1	1.15647	1.15647	43.10	<.001
Variety.Time	2	28.73653	14.36827	535.49	<.001
FC.Harvest	1	1.37498	1.37498	51.24	<.001
Variety.Harvest	2	7.35756	3.67878	137.10	<.001
Time.Harvest	1	0.37748	0.37748	14.07	0.001
FC.Variety.Time	2	0.30633	0.15317	5.71	0.012
FC.Variety.Harvest	2	0.17286	0.08643	3.22	0.064
FC.Time.Harvest	1	10.02925	10.02925	373.78	<.001
Variety.Time.Harvest	2	10.04439	5.02219	187.17	<.001
FC.Variety.Time.Harvest	2	4.27280	2.13640	79.62	<.001
Residual	18	0.48298	0.02683		
Total	47	120.48238			

Appendix 3: Analysis of variance tables for chapter 6

Locations (Ukulinga*kwaswayimani)
Variate: Crude protein

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.2203	0.2203	0.26	
Rep.*Units* stratum					
Variety	2	15.6190	7.8095	9.34	0.004
Time	1	4.9829	4.9829	5.96	0.033
Location	1	433.2529	433.2529	518.26	<.001
Variety.Time	2	9.2830	4.6415	5.55	0.022
Variety.Location	2	14.0113	7.0057	8.38	0.006

Time.Location	1	5.2548	5.2548	6.29	0.029
Variety.Time.Location	2	9.2833	4.6417	5.55	0.022
Residual	11	9.1957	0.8360		
Total	23	501.1034			

Variate: Ash

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	122.4	122.4	1.22	
Rep.*Units* stratum					
Variety	2	44.9	22.4	0.22	0.803
Time	1	16.5	16.5	0.16	0.693
Location	1	13.9	13.9	0.14	0.717
Variety.Time	2	20.2	10.1	0.10	0.905
Variety.Location	2	132.4	66.2	0.66	0.536
Time.Location	1	14.6	14.6	0.15	0.710
Variety.Time.Location	2	289.5	144.7	1.44	0.277
Residual	11	1101.9	100.2		
Total	23	1756.3			

Variate: Fat

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	1.3333	1.3333	2.71	
Rep.*Units* stratum					
Variety	2	0.8538	0.4269	0.87	0.447
Time	1	0.0005	0.0005	0.00	0.974
Location	1	3.2735	3.2735	6.65	0.026
Variety.Time	2	0.9288	0.4644	0.94	0.419
Variety.Location	2	0.7277	0.3639	0.74	0.500
Time.Location	1	0.2503	0.2503	0.51	0.491
Variety.Time.Location	2	0.3267	0.1633	0.33	0.724
Residual	11	5.4127	0.4921		
Total	23	13.1074			

Variate: Moist

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.

Rep stratum	1	0.2231	0.2231	0.25	
Rep.*Units* stratum					
Variety	2	5.9832	2.9916	3.39	0.071
Time	1	1.2732	1.2732	1.44	0.255
Location	1	70.9507	70.9507	80.46	<.001
Variety.Time	2	18.0787	9.0394	10.25	0.003
Variety.Location	2	1.9565	0.9783	1.11	0.364
Time.Location	1	0.0021	0.0021	0.00	0.962
Variety.Time.Location	2	6.5065	3.2532	3.69	0.059
Residual	11	9.7002	0.8818		
Total	23	114.6742			

Variate: ADF

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	70.90	70.90	2.57	
Rep.*Units* stratum					
Variety	2	34.60	17.30	0.63	0.552
Time	1	3.65	3.65	0.13	0.723
Location	1	139.00	139.00	5.04	0.046
Variety.Time	2	37.51	18.76	0.68	0.527
Variety.Location	2	137.11	68.56	2.49	0.129
Time.Location	1	16.03	16.03	0.58	0.462
Variety.Time.Location	2	9.00	4.50	0.16	0.851
Residual	11	303.30	27.57		
Total	23	751.12			

Variate: NDF

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	30.63	30.63	2.15	
Rep.*Units* stratum					
Variety	2	21.38	10.69	0.75	0.495
Time	1	0.27	0.27	0.02	0.892
Location	1	39.58	39.58	2.78	0.124
Variety.Time	2	2.65	1.33	0.09	0.912
Variety.Location	2	97.60	48.80	3.42	0.070
Time.Location	1	0.41	0.41	0.03	0.869
Variety.Time.Location	2	2.91	1.46	0.10	0.904
Residual	11	156.83	14.26		
Total	23	352.27			

Variate: Ca

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.000037	0.000037	0.00	
Rep.*Units* stratum					
Location	1	0.954894	0.954894	98.98	<.001
Time	1	0.033986	0.033986	3.52	0.087
Variety	2	0.013022	0.006511	0.67	0.529
Location.Time	1	0.001815	0.001815	0.19	0.673
Location.Variety	2	0.013888	0.006944	0.72	0.508
Time.Variety	2	0.013562	0.006781	0.70	0.516
Location.Time.Variety	2	0.012892	0.006446	0.67	0.532
Residual	11	0.106117	0.009647		
Total	23	1.150213			

Variate: Cu

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	1.573	1.573	0.38	
Rep.*Units* stratum					
Location	1	123.556	123.556	30.09	<.001
Time	1	22.881	22.881	5.57	0.038
Variety	2	17.996	8.998	2.19	0.158
Location.Time	1	37.144	37.144	9.05	0.012
Location.Variety	2	4.454	2.227	0.54	0.596
Time.Variety	2	3.524	1.762	0.43	0.661
Location.Time.Variety	2	35.227	17.613	4.29	0.042
Residual	11	45.164	4.106		
Total	23	291.518			

Variate: K

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.00264	0.00264	0.06	

Rep.*Units* stratum					
Location	1	0.11545	0.11545	2.51	0.141
Time	1	0.07023	0.07023	1.53	0.242
Variety	2	0.04535	0.02267	0.49	0.623
Location.Time	1	0.01171	0.01171	0.26	0.623
Location.Variety	2	0.02815	0.01407	0.31	0.742
Time.Variety	2	0.01096	0.00548	0.12	0.889
Location.Time.Variety	2	0.01771	0.00886	0.19	0.827
Residual	11	0.50496	0.04591		
Total	23	0.80714			

Variate: Mg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.03711	0.03711	2.09	
Rep.*Units* stratum					
Location	1	0.00670	0.00670	0.38	0.552
Time	1	0.06552	0.06552	3.68	0.081
Variety	2	0.02746	0.01373	0.77	0.486
Location.Time	1	0.06584	0.06584	3.70	0.081
Location.Variety	2	0.00980	0.00490	0.28	0.764
Time.Variety	2	0.03968	0.01984	1.12	0.362
Location.Time.Variety	2	0.00686	0.00343	0.19	0.827
Residual	11	0.19569	0.01779		
Total	23	0.45467			

Variate: Mn

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	3934.	3934.	1.40	
Rep.*Units* stratum					
Location	1	127696.	127696.	45.51	<.001
Time	1	59276.	59276.	21.12	<.001
Variety	2	31989.	15995.	5.70	0.020
Location.Time	1	77266.	77266.	27.53	<.001
Location.Variety	2	37314.	18657.	6.65	0.013
Time.Variety	2	43864.	21932.	7.82	0.008

Location.Time.Variety	2	35913.	17956.	6.40	0.014
Residual	11	30867.	2806.		
Total	23	448120.			

Variate: Na

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.00520	0.00520	0.29	
Rep.*Units* stratum					
Location	1	0.29779	0.29779	16.63	0.002
Time	1	0.22868	0.22868	12.77	0.004
Variety	2	0.15443	0.07722	4.31	0.041
Location.Time	1	0.22633	0.22633	12.64	0.005
Location.Variety	2	0.13396	0.06698	3.74	0.058
Time.Variety	2	0.12452	0.06226	3.48	0.068
Location.Time.Variety	2	0.10495	0.05248	2.93	0.095
Residual	11	0.19701	0.01791		
Total	23	1.47287			

Variate: P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.002168	0.002168	0.66	
Rep.*Units* stratum					
Location	1	0.021921	0.021921	6.70	0.025
Time	1	0.000088	0.000088	0.03	0.873
Variety	2	0.014612	0.007306	2.23	0.154
Location.Time	1	0.000958	0.000958	0.29	0.599
Location.Variety	2	0.018464	0.009232	2.82	0.103
Time.Variety	2	0.022722	0.011361	3.47	0.068
Location.Time.Variety	2	0.003812	0.001906	0.58	0.575
Residual	11	0.036011	0.003274		
Total	23	0.120757			

Variate: Zn

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.

Rep stratum	1	186.89	186.89	4.30	
Rep.*Units* stratum					
Location	1	1956.59	1956.59	44.97	<.001
Time	1	14.64	14.64	0.34	0.574
Variety	2	36.11	18.05	0.41	0.670
Location.Time	1	150.22	150.22	3.45	0.090
Location.Variety	2	30.62	15.31	0.35	0.711
Time.Variety	2	189.30	94.65	2.18	0.160
Location.Time.Variety	2	6.54	3.27	0.08	0.928
Residual	11	478.61	43.51		
Total	23	3049.51			

Seasons (summer*winter)
Variate: Crude protein

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	2.2019	2.2019	3.01	
Rep.*Units* stratum					
Variety	2	8.4999	4.2500	5.82	0.028
Seasons	1	0.0009	0.0009	0.00	0.974
Irrigation	3	23.6701	7.8900	10.80	0.003
Variety.Seasons	2	4.1937	2.0968	2.87	0.115
Variety.Irrigation	6	3.5145	0.5858	0.80	0.595
Residual	8	5.8466	0.7308		
Total	23	47.9275			

Variate: Fat

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.0352	0.0352	0.13	
Rep.*Units* stratum					
Variety	2	0.2210	0.1105	0.41	0.678
Seasons	1	12.3261	12.3261	45.51	<.001
Irrigation	3	1.8964	0.6321	2.33	0.150
Variety.Seasons	2	0.1613	0.0807	0.30	0.750
Variety.Irrigation	6	0.6101	0.1017	0.38	0.875
Residual	8	2.1670	0.2709		

Total	23	17.4171			

Variate: ADF

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	11.22	11.22	0.26	
Rep.*Units* stratum					
Variety	2	69.26	34.63	0.79	0.486
Seasons	1	1278.11	1278.11	29.14	<.001
Irrigation	3	362.27	120.76	2.75	0.112
Variety.Seasons	2	1.48	0.74	0.02	0.983
Variety.Irrigation	6	123.63	20.60	0.47	0.814
Residual	8	350.86	43.86		
Total	23	2196.83			

Variate: NDF

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	38.01	38.01	2.17	
Rep.*Units* stratum					
Variety	2	68.94	34.47	1.97	0.202
Seasons	1	887.10	887.10	50.60	<.001
Irrigation	3	193.72	64.57	3.68	0.062
Variety.Seasons	2	3.68	1.84	0.10	0.902
Variety.Irrigation	6	27.16	4.53	0.26	0.942
Residual	8	140.27	17.53		
Total	23	1358.87			

Variate: Moisture

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	5.502	5.502	3.70	
Rep.*Units* stratum					
Variety	2	16.776	8.388	5.64	0.030

Seasons	1	0.004	0.004	0.00	0.960
Irrigation	3	20.652	6.884	4.63	0.037
Variety.Seasons	2	1.423	0.712	0.48	0.636
Variety.Irrigation	6	8.648	1.441	0.97	0.501
Residual	8	11.896	1.487		
Total	23	64.901			

Variate: Ca

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.05439	0.05439	1.64	
Rep.*Units* stratum					
Variety	2	0.00053	0.00026	0.01	0.992
Seasons	1	0.24989	0.24989	7.55	0.025
Irrigation	3	0.21442	0.07147	2.16	0.171
Variety.Seasons	2	0.03008	0.01504	0.45	0.650
Variety.Irrigation	6	0.07054	0.01176	0.36	0.888
Residual	8	0.26487	0.03311		
Total	23	0.88474			

Variate: Cu

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.001	0.001	0.00	
Rep.*Units* stratum					
Variety	2	17.103	8.552	1.16	0.361
Seasons	1	274.477	274.477	37.21	<.001
Irrigation	3	133.157	44.386	6.02	0.019
Variety.Seasons	2	11.839	5.920	0.80	0.481
Variety.Irrigation	6	158.532	26.422	3.58	0.050
Residual	8	59.015	7.377		
Total	23	654.125			

Variate: K

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.01869	0.01869	0.39	

Rep.*Units* stratum					
Variety	2	0.04264	0.02132	0.45	0.654
Seasons	1	1.26042	1.26042	26.44	<.001
Irrigation	3	0.03144	0.01048	0.22	0.880
Variety.Seasons	2	0.02770	0.01385	0.29	0.755
Variety.Irrigation	6	0.58105	0.09684	2.03	0.174
Residual	8	0.38137	0.04767		
Total	23	2.34333			

Variate: Mg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.0009584	0.0009584	1.08	
Rep.*Units* stratum					
Variety	2	0.0015253	0.0007626	0.86	0.458
Seasons	1	0.0021589	0.0021589	2.44	0.157
Irrigation	3	0.0081505	0.0027168	3.08	0.091
Variety.Seasons	2	0.0134417	0.0067208	7.61	0.014
Variety.Irrigation	6	0.0273751	0.0045625	5.16	0.019
Residual	8	0.0070674	0.0008834		
Total	23	0.0606773			

Variate: Mn

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	486982.	486982.	1.08	
Rep.*Units* stratum					
Variety	2	1517237.	758619.	1.68	0.246
Seasons	1	13125973.	13125973.	29.11	<.001
Irrigation	3	10605679.	3535226.	7.84	0.009
Variety.Seasons	2	2072630.	1036315.	2.30	0.163
Variety.Irrigation	6	5532962.	922160.	2.05	0.172
Residual	8	3606965.	450871.		
Total	23	36948428.			

Variate: Na

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.000368	0.000368	0.20	
Rep.*Units* stratum					
Variety	2	0.001500	0.000750	0.42	0.673
Seasons	1	0.039141	0.039141	21.70	0.002
Irrigation	3	0.000196	0.000065	0.04	0.990
Variety.Seasons	2	0.000019	0.000010	0.01	0.995
Variety.Irrigation	6	0.002925	0.000487	0.27	0.936
Residual	8	0.014429	0.001804		
Total	23	0.058577			

Variate: P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.004821	0.004821	1.90	
Rep.*Units* stratum					
Variety	2	0.020114	0.010057	3.96	0.064
Seasons	1	0.190439	0.190439	75.01	<.001
Irrigation	3	0.000768	0.000256	0.10	0.957
Variety.Seasons	2	0.013345	0.006673	2.63	0.133
Variety.Irrigation	6	0.019691	0.003282	1.29	0.358
Residual	8	0.020310	0.002539		
Total	23	0.269488			

Variate: Zn

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	73.05	73.05	1.08	
Rep.*Units* stratum					
Variety	2	549.47	274.74	4.06	0.061
Seasons	1	44.95	44.95	0.66	0.438
Irrigation	3	130.72	43.57	0.64	0.608

Variety.Seasons	2	169.31	84.65	1.25	0.336
Variety.Irrigation	6	81.35	13.56	0.20	0.967
Residual	8	540.71	67.59		
Total	23	1589.57			

Seeds ukulinga
Variate: Cruden Protein

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.0105	0.5053	0.67	
Rep.*Units* stratum					
Irrigation	1	6.7360	6.7360	8.87	0.014
Variety	2	13.4810	6.7405	8.88	0.006
Irrigation.Variety	2	22.6080	11.3040	14.89	0.001
Residual	10	7.5922	0.7592		
Total	17	51.4278			

Variate: ADF

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	5.108	2.554	1.08	
Rep.*Units* stratum					
Irrigation	1	0.580	0.580	0.25	0.631
Variety	2	8.446	4.223	1.79	0.216
Irrigation.Variety	2	3.770	1.885	0.80	0.477
Residual	10	23.590	2.359		
Total	17	41.494			

Variate: Ash

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.016327	0.008163	1.24	
Rep.*Units* stratum					
Irrigation	1	0.397535	0.397535	60.40	<.001
Variety	2	0.061787	0.030894	4.69	0.036

Irrigation.Variety	2	0.005000	0.002500	0.38	0.693
Residual	10	0.065812	0.006581		
Total	17	0.546462			

Variate: Fat

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	2.7164	1.3582	4.39	
Rep.*Units* stratum					
Irrigation	1	3.0944	3.0944	10.01	0.010
Variety	2	0.0452	0.0226	0.07	0.930
Irrigation.Variety	2	0.6014	0.3007	0.97	0.411
Residual	10	3.0912	0.3091		
Total	17	9.5486			

Variate: NDF

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	23.987	11.993	2.34	
Rep.*Units* stratum					
Irrigation	1	1.854	1.854	0.36	0.561
Variety	2	19.579	9.790	1.91	0.199
Irrigation.Variety	2	21.219	10.609	2.07	0.177
Residual	10	51.286	5.129		
Total	17	117.924			

Variate: Ca

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.00008934	0.00004467	0.88	
Rep.*Units* stratum					

Irrigation	1	0.00010697	0.00010697	2.10	0.178
Variety	2	0.00005062	0.00002531	0.50	0.622
Irrigation.Variety	2	0.00001291	0.00000646	0.13	0.882
Residual	10	0.00050861	0.00005086		
Total	17	0.00076845			

Variate: Cu

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.9918	0.4959	0.59	
Rep.*Units* stratum					
Irrigation	1	0.1510	0.1510	0.18	0.680
Variety	2	0.4732	0.2366	0.28	0.759
Irrigation.Variety	2	1.2135	0.6068	0.73	0.507
Residual	10	8.3475	0.8348		
Total	17	11.1771			

Variate: K

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.018804	0.009402	5.80	
Rep.*Units* stratum					
Irrigation	1	0.023265	0.023265	14.35	0.004
Variety	2	0.001874	0.000937	0.58	0.579
Irrigation.Variety	2	0.001333	0.000667	0.41	0.674
Residual	10	0.016217	0.001622		
Total	17	0.061493			

Variate: Mg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.00003627	0.00001813	0.91	
Rep.*Units* stratum					

Irrigation	1	0.00008499	0.00008499	4.24	0.056
Variety	2	0.00002778	0.00001389	0.69	0.522
Irrigation.Variety	2	0.00003069	0.00001535	0.77	0.490
Residual	10	0.00020026	0.00002003		
Total	17	0.00037998			

Variate: Mn

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	8.765	4.382	2.19	
Rep.*Units* stratum					
Irrigation	1	0.044	0.044	0.02	0.886
Variety	2	3.403	1.701	0.85	0.457
Irrigation.Variety	2	31.543	15.772	7.87	0.009
Residual	10	20.046	2.005		
Total	17	63.799			

Variate: Na

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.00003664	0.00001832	0.56	
Rep.*Units* stratum					
Irrigation	1	0.00001021	0.00001021	0.31	0.590
Variety	2	0.00008796	0.00004398	1.34	0.306
Irrigation.Variety	2	0.00030785	0.00015392	4.68	0.037
Residual	10	0.00032918	0.00003292		
Total	17	0.00077184			

Variate: P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.0001308	0.0000654	0.65	
Rep.*Units* stratum					
Irrigation	1	0.0041801	0.0041801	41.69	<.001
Variety	2	0.0001972	0.0000986	0.98	0.407

Irrigation.Variety	2	0.0001810	0.0000905	0.90	0.436
Residual	10	0.0010026	0.0001003		
Total	17	0.0056916			

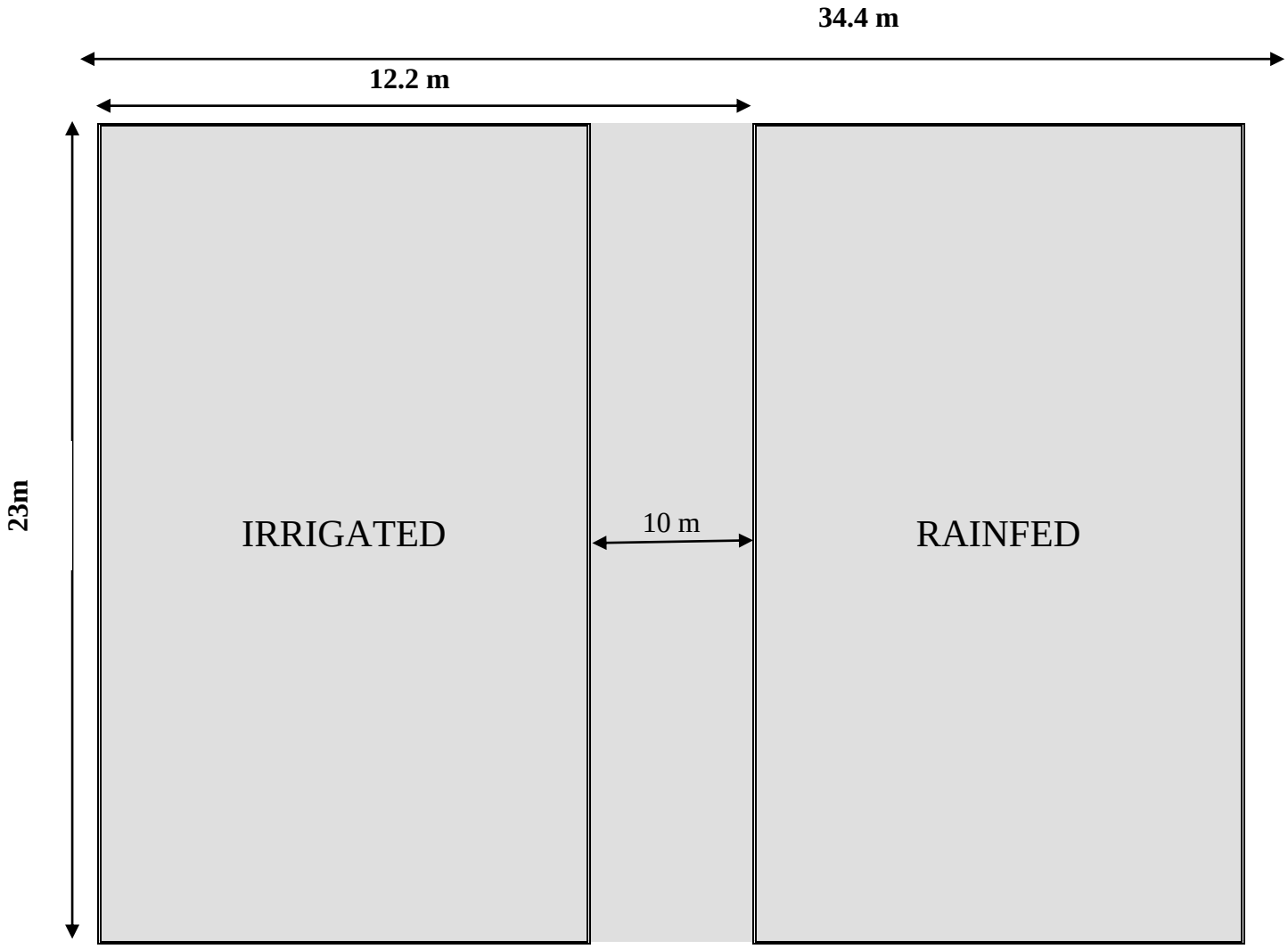
Variate: Zn

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	5.8207	2.9103	2.98	
Rep.*Units* stratum					
Irrigation	1	23.1152	23.1152	23.67	<.001
Variety	2	0.7005	0.3503	0.36	0.707
Irrigation.Variety	2	6.1247	3.0624	3.14	0.088
Residual	10	9.7640	0.9764		
Total	17	45.5251			

- 0.15m *0.50 m translating to 48 plants per plot of which 24 will be the experimental plants

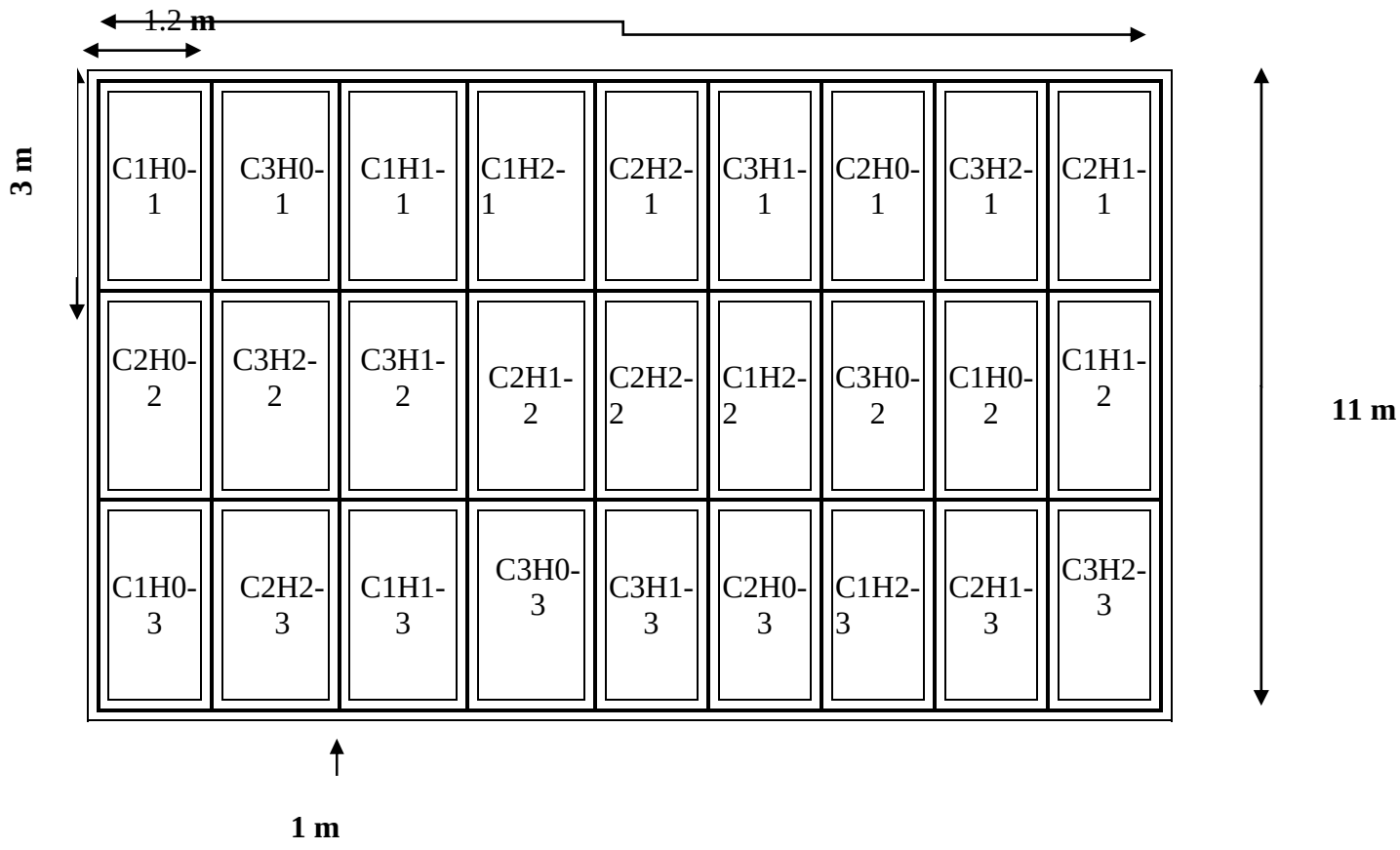
Appendix 4: Field experiment trial overview

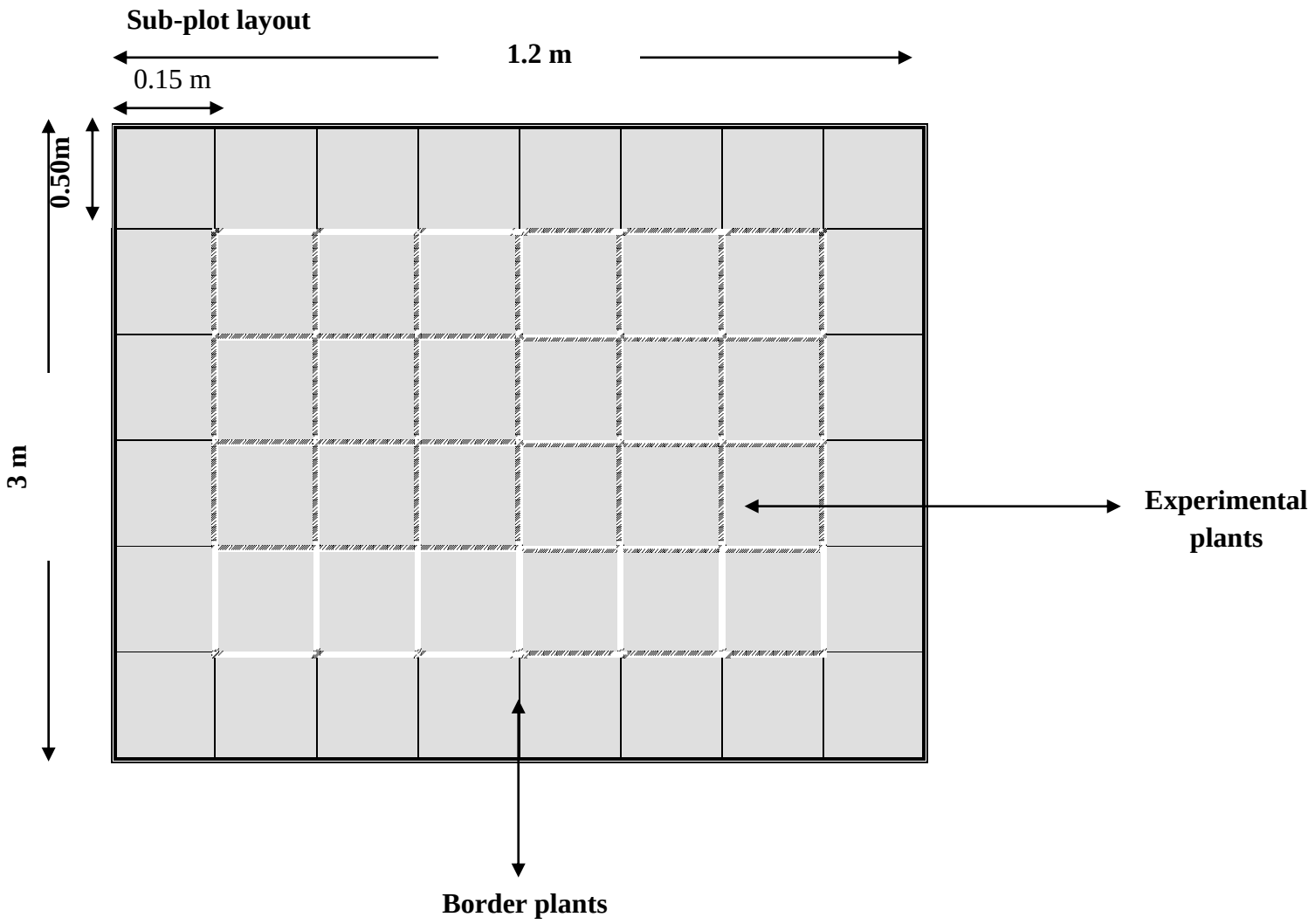
Trial overview (Main plots)



Field trial layout for Bambara groundnut main plot

18.8 m


1.2 m
3 m
C1H0-1
C3H0-1
C1H1-1
C1H2-1
C2H2-1
C3H1-1
C2H0-1
C3H2-1
C2H1-1
C2H0-2
C3H2-2
C3H1-2
C2H1-2
C2H2-2
C1H2-2
C3H0-2
C1H0-2
C1H1-2
C1H0-3
C2H2-3
C1H1-3
C3H0-3
C3H1-3
C2H0-3
C1H2-3
C2H1-3
C3H2-3
11 m
1 m


Sub-plot layout
1.2 m
0.15 m
0.50m
3 m
Experimental plants
Border plants