AGRO-MORPHOLOGICAL, NUTRITIONAL AND GENETIC DIVERSITY ANALYSES OF BAMBARA GROUNDNUT (*Vigna subterranea* (L.) Verdc)

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Abstract

Bambara groundnut (*Vigna subterranea* (L.) Verdc) is a legume crop with potential to address food insecurity in sub-Sahara Africa. However, a lack of agronomic, genetic and nutritional information on the crop hinders its full potential utilization.

Nineteen Bambara groundnut lines were evaluated in the field in 2017 and 2018 at two sites. The lines showed significant differences (P<0.05) for all the measured traits. Lines such as IITA686, Cream and Uniswa Red-R were found to have superior performance for multiple traits such as number of seeds per plant, seed mass per plant, plant height and mid-leaf width.

The genetic variation among the Bambara groundnut lines was assessed using 20 polymorphic SSR markers The markers exhibited an average polymorphic information content (PIC) of 0.57 and the observed heterozygosity was 0.58, showing that the lines exhibited a considerable level of outcrossing. The lines were clustered into three groups based on the principal coordinate analysis. The highest genetic distance was 0.60 between Tiganecuru and S19. Lines such as IITA686, Cream and Uniswa Red-R that had good performance for multiple agronomic traits occurred within a genetic distance of 0.40 making them genetically divergent enough for generating crosses for Bambara groundnut improvement. The ash, fat, proteins, starch, calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), phosphorous (P), sodium (Na), copper (Cu), and zinc (Zn), acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents were determined in the Bambara groundnut lines using combustion and chemical digestion procedures. The nutritional content varied significantly (p<0.05) among the lines with lines S19, Gresik, Pong-Br-UNK, Pong-Cr exhibiting high means for starch and protein content ranging from 11.05 to 11.94%. Genotypes Mix, Pong-Br-UNK, 42-1, Gresik, Uniswa Red-R, and Brown were clustered together based on their high starch, Na, Ca, fat, and Mn contents. The negative correlations among some of the nutritional content would be a challenge for simultaneous selection to breed nutritious Bambara groundnut lines. The lines with high content for multiple nutritional elements such as 211-57, Pong-Br-EN and Uniswa Red-G were recommended for production.

It was imperative to determine interrelations among agronomic traits and nutritional content with seed mass for indirect selection. Among the agronomic traits, number of seeds (NS $r=0.58$, $p<0.01$), number of healthy seeds (NHS, $r=0.51$, $p<0.05$) and plant height (PH, $r=0.45$, $p<0.05$) exhibited the strongest associations with seed mass. These traits had NS, NHS and PH high direct effects on seed mass of 2.04, 1.72 and 0.60, respectively. These findings provide a means to facilitate indirect selection of genotypes with high seed mass productivity via proxy.

Overall, the study found significant agro-morphological and genetic variation among the Bambara groundnut lines, which would be a prerequisite for Bambara groundnut improvement. The superior lines identified for multiple traits and genetic divergence were IITA686, Cream and Uniswa Red-R.

Key words: Agro-morphology; Bambara groundnut; Genetic variation; Seed quality

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 National Research Foundation (NRF) through Freestanding Doctoral Scholarship.

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. As accepted by the University of KwaZulu-Natal, this thesis is in the form of published, accepted and submitted journal articles, which are indicated in each chapter.

Declaration

I, Nokuthula Cherry Hlanga, 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:

a) their words have been re-written but the general information attributed to them has been referenced;

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.

Student signature: [redacted]

Supervisor signature: [redacted]

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Dedication

This thesis is dedicated to both my parents Khathazile Zulu and Madimetja Hlanga.

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Chapter 1 Introduction

Background

Many countries in the sub-Saharan Africa region face critical shortages of food at national and household levels. South Africa is food secure at the national level, although there is widespread food insecurity at the household level (Department of Statistics, 2017). Food insecurity threatens 54% of the rural population that depend on subsistence agriculture (Kruger *et al*., 2012). Food insecurity is a significant concern for the government as it contributes to neo-natal deaths among childbearing women, malnutrition and cognitive under development in children and starvation among the general populace. There is a need to improve crop production and productivity to combat food insecurity in sub-Sahara Africa.

Low crop production and productivity in sub-Saharan Africa is attributed to several factors including climate change, lack of technical support for the agriculture sector, inherent poor soils to support crop production, poorly developed seed systems to supply improved cultivars and a lack of adaptive strategies (Kruger *et al*., 2012). Climate change is a major challenge in subsistence farming as smallholder farmers lack adaptive and coping strategies compared to commercial farmers. They depend on seasonal rainfall, which has become increasingly erratic recently (Thornton *et al*., 2009). As a result, subsistence farmers incur high yield losses leading to the perpetuation of food insecurity among rural households. In addition, climate change exacerbates the challenges posed by poor soil fertility. There is a widespread distribution of soils with inherently low fertility statuses in sub-Saharan Africa and frequent nutrient mining without inadequate nutrient replenishment perpetuate poor soil fertility (Mapfumo *et al*., 2017). These abiotic stresses often occur in combination with biotic stresses such as pests and diseases and high weed pressure. The occurrence of multiple stresses in sub-Saharan Africa has caused numerous failures of commodity crops such as maize, wheat and rice.

The recurrent production constraints such as drought, heat and pests and disease stresses affecting commodity crops necessitate the need to include orphan crops in mainstream agro-systems. Orphan crops include cereals such as sorghum (*Sorghum bicolor* (L.) Moench) and millet (*Panicum glaucum* L.), legumes such as pigeon pea (*Cajanus cajan* (L.) Millspaugh), Bambara groundnut, vegetables and root crops such as cassava (*Manihot esculenta* Crantz) and yam (*Dioscorea alata* L).. These crops are known to be significantly more adaptable to extreme and erratic environmental conditions compared to commodity crops such as rice (*Oryza sativa*), maize (*Zea mays*), and wheat (*Triticum aestivum*) (Zenabou *et al*., 2014). The orphan crops play an important role in providing a source of food and income among farmers in marginal areas. These crops have relatively higher tolerance to drought, heat and ion toxicity due to their extensive rooting and physiological abilities, which enable them to produce reasonable yield under harsh environmental challenges (DAFF, 2010).

Bambara groundnut (*Vigna subterranea* (L.) Verdc) is one of the neglected legume crops in Africa despite its huge potential as a source of nutrition among the rural communities. The crop can potentially be utilized in diversified forms such as fresh or dried food. The seeds can be used fresh, dry or for extraction of milk (DAFF, 2010). The crop contains carbohydrate (63%), protein (19%), oil (6.5%) and appreciable amounts of essential minerals such as calcium, iron, potassium and sodium (DAFF, 2010). The nutritional quality of Bambara groundnut makes it a suitable candidate for diversifying sources of nutrition among rural households that depend heavily on staple crops such as maize that are carbohydrate-rich but lack essential minerals and proteins to meet dietary requirements.

The inclusion of Bambara groundnut under rural agriculture production systems would be suitable as it is known to be a low input crop. In addition, it has relatively high tolerance to edaphic constraints such as drought and soil acidity and salinity. Taffouo et al. (2010) reported that white seed coat (WSC) landraces were relatively more tolerant to salinity. They suggested that the WSC landraces could be cultivated in the coastal and semi-arid saline soils and also provide a source of genes for salt tolerance in breeding programs. Its drought and heat stress tolerance render it more suitable for production under erratic rainfall and high temperature conditions experienced in many parts of the sub-Sahara region (Tadele and Assefa, 2012).

The rationale of the study

There is a need to improve the productivity of Bambara to validate its inclusion in diverse cropping systems. Improvement of Bambara is hinged on a thorough understanding of its morphological and physiological characters as a preliminary step towards efficiently exploiting its genetic diversity. This approach will also help breeders and farmers by generating vital baseline information on the currently known landraces. Evaluating diverse landraces for their traits will also help to characterize useful germplasm for breeding programmes and increase selection efficiency for specific environmental conditions. Thus, there is a need to characterise different Bambara groundnut landraces using morphological, physiological and genetic differences influencing their growth, development and yield under field and tunnel conditions and nutritional content.

Problem Statement

Climate change, poor soils and non-availability of inputs that are commonly encountered in sub-Sahara Africa curtail the production of commodity crops such as maize, wheat and rice that have high management and input requirements. As such, there are increasingly more proposals to include orphan crops for production under low input subsistence agro-systems to increase crop production and enhance food security. Bambara groundnut is known to be significantly adaptable to extreme and erratic environmental conditions and its production will boost food security in marginal areas. This will increase the resilience in the ever-changing farming environment. However, there is a lack of research and development efforts aimed at imporving production and productivity of Bambara groundnut in comparisons to the commodity crops.

Aim of the study

The study aimed to assess diversity and polymorphisms among Bambara germplasm that potentially contribute to identifying suitable lines for production and breeding.

Specific Objectives

The specific objectives were:

i. To characterise 19 different Bambara groundnut landraces using morphological and physiological differences influencing their growth, development and yield under field and greenhouse conditions to select superior landraces for production.

ii. To assess genetic variation and relatedness among Bambara groundnut lines using SSR markers as a preliminary step towards Bambara improvement in South Africa.

iii. To assess nutritional content among the 19 Bambara groundnut lines using standard combustion protocol and scanning electron microscopy to identify lines with high content of multiple nutrients.

iv. To deduce multivariate relationship among agro-morphological and physiological traits and nutritional content among 19 Bambara landraces to facilitate indirect selection for high yield.

Hypotheses

i. The Bambara were diverse in agronomic performance and physiological traits
ii. The Bambara landraces consisted of genetically divergent individuals
iii. The nutritional content will vary with regard to the place of origin of the Bambara landraces
iv. The multivariate relationships among the traits and yield would allow indirect selection

Thesis structure

The thesis is written in paper format and consists of six experimental chapters that distinct but linked to each other by the objectives. `Where a chapter has been published or submitted to a journal for publication, a foot note is presented providing the details. The thesis is preceded by an introduction section providing a background, problem statement, aims and objective and hypotheses of the study.

Chapter 2 is a literature review that provides background information on Bambara groundnut, status of Bambara groundnut production in sub-Sahara Africa and its potential for utilization and production in mainstream agriculture. The literature review chapter advocates for the inclusion of Bambara groundnut in mainstream agriculture as an intervention to food insecurity and low crop productivity due to abiotic constraints.

Chapter 3 is based on the evaluation of Bambara groundnut in the field for agro-morphological diversity. The chapter identifies superior lines for varietal selection and for breeding purposes. The data was subjected to different statistical analyses to identify superior lines.

Chapter 4 reports on genetic diversity using simple sequence repeats (SSR) markers. The analysis of genetic markers provided a basis for understanding the agro-morphological variations observed in Chapter 2.

Chapter 5 reports on nutritional content of the Bambara groundnut lines. A number of nutrients were analysed, and the data was subjected to multivariate analysis to identify lines that had content of multiple nutrients. Superior lines were identified as important for food and nutrition intervention.

Chapter 6 provided an alternative method for assessing nutritional content in Bambara groundnut lines. The electron microscopy method provided a means for quantitative and qualkitative assessment of mineral element content in Bambara and provided complementary observations to chapter 5.

Chapter 7 is a report on the assessment of the interrelationships among agronomic traits and nutrient content with seed mass accumulation in Bambara groundnut lines. It provides opportunities to identify potential direct and indirect selection pathways for simultaneous improvement of seed mass productivity and nutritional content and agronomic performance in Bambara groundnut.

Lastly, the thesis provides a general discussion of research findings and provides the implications of the study on Bambara groundnut production and its potential improvement.

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Chapter 2 Literature Review

2.1 Introduction

Many developing countries are facing food shortages due to several factors including climate change, soil nutrient deficiencies, inefficient farming methods and lack of access to improved varieties of crops. Climate change, manifesting as drought stress, is the single most important challenge faced by many farmers in sub-Sahara Africa (SSA) (Barrios *et al.*, 2006). The majority of farmers lack strategies to cope against drought stress. The impact of drought is exacerbated by soil nutrient deficiencies endemic in the SSA region (Shiferaw *et al.*, 2014). Most of the soils in SSA are degraded and are inherently deficient in organic matter and major nutrients such as nitrogen, which negatively affects their crop production potential. These factors affect the choice of crops cultivated by the farmers. However, the majority of farmers in SSA also frequently attain low yields because they lack access to improved varieties or adapted crops with high yield potential. The cultivation of underutilised and neglected staple crops, which are highly adapted to low input agro-systems has been proposed as an intervention strategy to improve their status of orphan crops to significant contributors to food security in this region (Tadele, 2009).

2.2 Orphan crops

Orphan crops are difficult to define precisely but terms such as underutilized crops, lost crops of Africa, minor crops, neglected crops, and crops for the future have been used to refer to orphan crops (Tadele, 2009). They are often associated with the cultural heritage of their places of origin, poorly documented as to their cultivation and utilization and are characterized by a lack of formal seed supply systems. However, orphan crops play an important role in food chain systems of many developing countries due to their high adaptation to specific agro-ecological niches and marginal areas (Table 1). They also have relatively higher tolerance to biotic and abiotic constraints than highly managed cereals such as rice (*Oryza sativa*), maize (*Zea mays*), and wheat (*Triticum aestivum*) (Tadele, 2009). Orphan legume crops include groundnut (*Arachis hypogaea*), grass pea (*Lathyrus sativus*) and Bambara groundnut (*V. subterranea*).

The utilization of orphan crops is usually at subsistence level for human consumption. Cereals such as maize, wheat and rice provide the largest proportion of calories for poor communities and the inclusion of orphan crops has been promoted to diversify their diets and improve human nutrition (Pingali, 2012). Orphan crops can also be used as animal feed and to generate income for resource-poor farmers (Pingali, 2012).

Owing to their low economic importance compared to commodity crops such as rice, maize, and wheat, orphan crops have been neglected by both the international scientific community and industry (Foyer *et al.*, 2016). As a result, there is a lack of crop improvement programs focusing on the orphan crops. Subsequently, the production of orphan crops is predominantly based on the cultivation of landraces, which lack uniformity and have low yield potential (Odeigah and Osanyinpeju, 1998).

Table2.1: Major orphan crops of Africa and their important traits

Common Name	Botanical name	Type of crop	Region importance	Important trait
African eggplant	*Solanum aethiopicum*	Leafy vegetable	All regions	High yielding
African rice	*Oryza glaberrima*	Cereal	Western	Pests and diseases resistance
African yam bean	*Sphenostylis stenocarpa*	Root crop	All regions	High protein content
Amaranth	*Amaranthus* s	Leafy vegetable	All regions	Fast growing
Bambara groundnut	(*Vigna subterranea* (L.) Verdc)	Legume	All regions	Rich in protein, drought tolerant
Baobab	*Adansonia digitate*	Leafy vegetable/fruit	All regions	Drought tolerant
Barbados cherry	*Malpighia glabra*	Fruit	All regions	Rich in vitamin
Cassava	*Manihot esculentum*	Root crop	All regions	Drought tolerant

Adapted from (Tadele, 2009)

Among the orphan crops, the cultivation and improvement of the orphan legumes has been promoted widely due to a number of reasons including their ability to fix nitrogen, drought resilience and high protein content to improve diets of the rural poor (Tadele, 2009). Legume crops can alleviate protein and micronutrient deficiencies associated with the predominance of cereal-based diets in developing countries (Cullis and Kunert, 2017). Orphan crops are generally more adapted to extreme soil, climatic conditions and with high tolerance to abiotic environmental stresses such as drought that exist in many parts of the world. Bambara groundnut is one of the underutilized legumes that has gained attention over the recent years due to its high potential to overcome production constraints frequently encountered under small-scale agriculture systems and alleviate dietary nutritional deficiencies and poverty among the rural communities (Unigwe *et al.*, 2016).

2.3 Bambara groundnut and food security

Bambara groundnut is the second most important food legume and the third food crop, after maize and groundnut, grown by small-scale farmers in many African countries (Dri Yao, 2015). The crop fits well in traditional agriculture systems where it is often cultivated as an intercrop with maize and cowpeas or as a sole crop (DAFF, 2016). The utilization of Bambara groundnut is well documented and is reported to occur in diversified forms such as fresh or dried food or for extraction of chemicals with nutritional or pharmaceutical values (Murevanhema and Jideani, 2013). In some African countries, fresh seeds can be prepared by boiling and toasting or used to produce flour for different purposes such as bread or porridge making (Mubaiwa *et al.*, 2018). The grains contain 63% carbohydrate, 19% protein, 6.5% oil, and essential minerals such as calcium, iron, potassium, sodium and essential fats (Oyeyinka *et al.*, 2017; Yao *et al.*, 2015). The reported nutritional quality of Bambara groundnut makes it a suitable candidate for diversifying nutritional sources among poor rural households that depend heavily on staple crops such as maize that are carbohydrate-rich but lack essential minerals and proteins to meet dietary requirements (Alhamdi et al., 2020). The crop has also been reported to possess some medicinal qualities (Oyeyinka *et al.*, 2017). In addition, the crop can also be used to feed livestock. However, despite the great potential, its contribution to rural economies, national agriculture production and nutritional requirements for rural populations is still lagging far behind that of the commodity cereal crops due to a lack of improved seed systems and crop improvement programs (Abu and Buah, 2011).

2.4 Environmental condition effects on Bambara groundnut

Bambara groundnut, like any other crops, suffers from fluctuations in environmental conditions such a precipitation, temperature, soil properties and prevalence of pests and diseases (DAFF, 2016). The occurrence of drought in many parts of the world due to climate change has decimated yield potential of many crops (Rosenblatt and Schmitz, 2016). The majority of farming systems in SSA incur significant crop losses due to drought stress because about 90% of the agriculture systems are dependent on seasonal rainfall (Amjath-Babu *et al.*, 2016). The inclusion of Bambara groundnut in mainstream agriculture systems could help to reduce the impact of drought since Bambara groundnut is well known to be relatively more drought-tolerant than many grain and cereal crops such as maize, wheat and common beans, which dominate the agricultural landscape of SSA. Bambara groundnut uses a combination of mechanisms to tolerate drought stress, including stomatal regulation of gas exchange, reduction of leaf area and maintenance of a relatively high leaf water status and high levels of photosynthesis (Chai *et al.*, 2016). Several characteristics such as reducing leaf number, plant height, reduced canopy size and early maturity have been reported to confer drought tolerance to Bambara groundnut, making it a strong candidate for cultivation under drought prone environments (Karikari and Tabona, 2004; Mabhaudhi *et al*., 2013). According to Karikari and Tabona. (2004), canopy spread, root:shoot ratio, 1000-seed weight and number of seeds per pod are among parameters that could be used for indirect selection for drought tolerance in Bambara groundnut in Botswana and other areas where drought is a common occurrence. Chai *et al.* (2016) showed that drought stress reduced stomatal conductance significantly among Bambara groundnut lines but those that had high stomatal density and reduced leaf area managed to escaped drought.

The ability to tolerate harsh environmental conditions enables Bambara groundnut to produce reasonable yield (Jørgensen *et al.*, 2010). Reduced plant canopy size and maturity are often associated with increased water use efficiency and better drought tolerance in plants although literature has shown that there may be yield penalties (Mabhaudhi *et al.*, 2013). Drought and heat stress tolerance render crops more suitable for production under erratic rainfall and high temperature conditions experienced in many parts of the sub-Sahara Africa region (Chai *et al.*, 2016).

2.4.1 Heat stress

Bambara groundnut can be grown in the arid and semi-arid regions as it can tolerate warm temperatures. The optimum temperature range for seed germination for Bambara groundnut is between 30 and 35 °C, which is higher than that of common bean (DAFF, 2016). Bambara groundnut can tolerate warm temperatures but does not tolerate freezing temperatures during the growing season. Although the crop is relatively heat tolerant, extreme temperatures induce leaf senescence resulting in the reduction of biomass production potential (Jørgensen *et al.*, 2010). Under heat stress conditions, Bambara groundnut has been shown to respond by maintaining or reducing leaf area and leaf area index. The lower number of leaves under heat has been associated with higher partitioning of dry matter into pods (Berchie *et al.*, 2012).

Studies have reported that Bambara groundnut tolerates warm temperature and low water availability by closing its stomata and increasing its water use efficiency (Jørgensen *et al.*, 2010). Berchie, *et al.* (2012) showed that some heat tolerant lines maintained high leaf area and leaf area index for periods between 45 and 120 days after sowing under high temperature conditions.

2.4.2 Pests and diseases

Bambara groundnut is generally resistant to diseases and has fewer insect pest than cereal crops such as maize and wheat. The common insects that attack Bambara groundnut are groundnut jassid (*Empoasca facialis*), groundnut hopper (*Hilda patruelis*) and brown leaf beetles (*Ootheca mutabilis*). Important storage pests include *Callosobruchus maculate*, *C. subinnotatus* and *C. tenocampa hilda*, bruchid beetles and maize weevils. These pests can cause significant yield and biomass loss in Bambara groundnut if not controlled, although the yield loss is not often as high as in maize and wheat. *Alectra vogelii* (Mhlilwane) was found to be a serious root parasite of Bambara groundnut, especially in Mpumalanga and causes yield reduction of up to 49 %. Two local varieties, MPB51 and MPB31 have been developed by the Lowveld Research Unit for higher yield (2355 kg) and high tolerance to *A. vogelii*, respectively (DAFF, 2016).

Bambara also suffers from fungal diseases such as Cercospora leaf spot (*Cercospora* s), powdery mildew (*Erysiphe polygoni*) and fusarium wilt (*Fusarium oxypolygoni*). Symptoms of *Cercospora* leaf spot are reddish-brown circular spots on the leaves, as well as lesions on the stems, petioles and pods. In severe attacks, leaves fall and the plants may die. The disease can be controlled by practising crop rotation or burning of crop debris of the previous season as well as use of resistant cultivars. *Fusarium* wilt causes vascular discolouration, yellowing, necrosis and wilting. Affected plants become stunted and die. *Fusarium* wilt can be controlled by use of resistant cultivars and crop rotation. There are no chemicals registered for the control of diseases and pests of Bambara groundnut in South Africa (DAFF, 2016).

2.5 Nitrogen fixation

Many soils under smallholder farming systems in SSA are characterised by low nitrogen content. In addition, farmers lack financial resources to procure fertilizers and as a result they do not apply fertilizers or apply below optimal rates (Chianu *et al.,* 2011). The widely reported low productivity of cereals such as maize and wheat under smallholder farming systems can be partially attributed to low nitrogen use. Like most orphan crops, Bambara groundnut is cultivated by subsistence farmers under low nitrogen application (Mulongoy and Gueye, 1992). Reports show that Bambara groundnut nodulates with cowpea-type *Bradyrhizobia* bacteria and can nodulate with isolates from other tropical legumes, which indicates that the species is less selective in its bacterial requirements (Laurette *et al.*, 2015). Bambara groundnut is known to grow under soils with different fertility, moisture and temperatures levels that cause the crop to nodulate with different strains of bacteria from one site to another (Laurette *et al.*, 2015). *Bradyrhizobia* nodulation under dry, hot and low fertility soils in Nigeria were serologically and morphologically different compared to the ones under wet, humid and more fertile soils of Ibanda (Laurette *et al.*, 2015). The adaptability of *Bradyrhizobia* under soils of diverse moisture content in Africa shows how legumes such as Bambara groundnut, kersting bean and cowpea survive in soils with different moisture, temperature and pH levels. However, the differences in yield recorded for the different crops under different environments suggests that there exists considerable variation in symbiotic efficacy of native *Bradyrhizobia* species that nodulate with the different species.

Nitrogen fixation by grain legumes such as Bambara groundnut contributes to soil nitrogen (N) economy in the field providing rotational benefits to subsequent crops such as production of high N content organic residues that contribute to integrated soil fertility management (ISFM) (Laurette *et al.*, 2015). Sudano-Sahelian zone of Nigeria reported that Bambara groundnuts fixed up to 28.42 kgN/ha (Egbe *et al*., 2013). The diversification of cropping systems through the use of crop rotation including legumes, cereals, root and tuber crops can be perceived as key towards sustainable intensification of agriculture in SSA under nitrogen deficient soils.

Figure2.1: Root nodulation fungi on legume roots

Adapted from (Jennings, 2015)

2.6 Bambara groundnut diversity

Morphological, biochemical and genetic characteristics have been used to evaluate diversity of Bambara groundnut accessions. The choice of the method for characterisation is influenced by factors such as availability of expertise, time, financial and technical resources. Morphological characteristics are the most widely used indicators of genetic diversity due to requirement of relatively simple methods compared to the requirements for determination and analyses of biochemical or genetic markers.

2.6.1 Morphological diversity

Farmers grow local landraces from previous harvests, or buy from local markets, because there are no improved varieties readily available. This has been due to a lack of research and development dedicated towards the genetic improvement of Bambara groundnut.

Current production trends show that Bambara groundnut production is dominated by landraces or farmers' varieties (Touré *et al.*, 2012).

The production of Bambara groundnut is dominated by landraces in all the major growing regions particularly in SSA. Landraces are more phenotypically and genotypically diverse than pure lines, and are excellent sources of genetic variation for breeding (Gbaguidi *et al*., 2018) although they may have low yield potential. Initial collections and evaluations of Bambara groundnut landraces were carried out by the International Institute of Tropical Agriculture (IITA) (Table 1.2), which maintains a genebank of Bambara groundnut accessions (Mohhamed, 2014). Several countries in Africa maintain their own national genebanks with multiple accessions of Bambara groundnut landraces in their germplasm collections (Table 1.3). Some of these collections have been evaluated for diversity, multiplication or for research on agronomic traits such as seed yield and planting population (Mohhamed, 2014).

Table 2.2: Total and safely duplicated accessions under IITA

Crop	Total no in collection	Safely duplicated	Safely duplicated %
Cowpea	15,379	11,761	76%
Soybean	4,841	1,522	31%
Bambara groundnut	1,752	932	53%
Maize	1,565	713	46%
Wild *Vigna* s	1,543	1,517	98%
African yam bean	456	27	6%
Yam	1,619	1,386	86%
Cassava	2,794	2,257	81%
Grand total	29,949	20,115	67%

Adapted from (IITA, 2019)

Table 2.3: Countries or Institutions holding Bambara groundnut germplasm collections

Country/Institution	**Number of accessions**
Benin	3
Botswana	26
Burkina Faso	143
France, ORSTOM	1000
Ghana, University of Ghana	80
Ghana, Savanna Agricultural Research Institute (SARI)	90
Ghana, Plant Genetic Resources Centre (PGRC)	166
Guinea	43
Kenya, National Genebank	6
Kenya, Kenya Agricultural Research Institute (KARI)	2
Kenya, National Museums	2
Mali	70
Mozambique	12
Namibia	23
Nigeria, IITA	2035
Nigeria	Not available
Niger	79
South Africa, Grain Crops Institute	198
South Africa, Institute for Veld and Forage Utilization	117
South Africa, Department of Agriculture	20
Tanzania, The National Plant Genetic Resources Centre of	22
Zambia, University of Zambia	463
Zambia, The National Plant Genetic Resources Centre	124
Zimbabwe	129

Adapted from (Mohhamad, 2014)

Most Bambara groundnut accessions have been evaluated using agro-morphological descriptors, metabolic parameters, and nutritional and chemical composition (Ndiang *et al.*, 2014). The characterization is the key for crop improvement and to aid in food insecurity intervention. Many studies have used crop morphology as the first step towards differentiating and identifying important traits and the extent of their diversity. Previous studies have shown that there are 15 to 20 agronomic characteristics that are commonly used in morphological characterization of Bambara groundnut. The number of days to emergence, flowering and maturity have been reported extensively and used widely to differentiate variability in phenological development among landraces (Ndiang *et al.*, 2014). Rate of phenological development has been linked to the ability of the crop to escape drought and other unfavourable terminal stresses. Other traits such as leaf shape, size and colour are commonly reported and associated with drought stress (Qaseem *et al.*, 2018). Morphological characterization studies have identified three types of growth habits namely bunch, semi-bunch and spreading among Bambara groundnut landraces (Odongo 2018). Findings by International Institute for Tropical Agriculture (IITA) following characterization of 1384 Bambara groundnut accessions showed that 8, 45 and 47% of accessions were spreading, bunch and semi-bunchy types, respectively. Landraces that are spreading are used in intercropping to form a more rapid ground cover and aid in early weed suppression. Both the bunch and semi-bunch types grown under monoculture were shown to achieve optimum yield under high plant populations. However, Bambara groundnut germplasm held at IITA has not been adequately characterized for its use in breeding programmes, compared to cowpea and groundnut. Olukolu *et al.* (2012) proposed the integration of qualitative and quantitative traits with molecular characterization in germplasm studies for pre-breeding.

2.6.2 Genetic diversity

Evaluating genetic variation is fundamental for initiating breeding programs for successful crop improvement. Genetic diversity is described as the degree of differentiation between or within species (Molosiwa, 2012). Natural diversity that exists in landraces has been exploited in many breeding programs. However, natural variability is eroded due to deliberate breeding targeting only a few traits (like yield and its component traits), frequent use of few selected genotypes as parents and introduction of a limited number of elite lines. The existence of Bambara groundnut landraces, farmer conserved varieties and accessions maintained in gene banks such as IITA provides adequate genetic variation for exploitation, provided the genetic diversity is adequately characterized.

Genetic diversity among landraces of Bambara is important in the context of challenges of drought and low nitrogen stresses as they may serve as an important source of novel genes conferring tolerance to different biotic and abiotic stresses. In this context, knowledge of all aspects of genetic diversity, factors affecting genetic diversity, different methods of diversity analysis, their measurement and the software for carrying statistical analysis becomes imperative in order to maximize its exploitation.

Genetic characterization offers the capacity to detect genetic diversity that exceeds that of traditional (phenotypic) methods (Mohhamed 2014). Deoxyribonucleic acid (DNA) markers linked to agronomic traits can increase the efficiency of classical breeding by significantly reducing the number of backcross generations (Mohhamed 2014). Molecular markers are known to be stable, have high precision and are not affected by the environment or plant developmental stage. Molecular markers have been used in Bambara groundnut diversity studies (Minnaar-Ontong et al., 2021). However, simple sequence repeat (SSR) markers were shown to be markers of choice for diversity analysis, particularly for Bambara groundnut landraces (Alhamdi et al., 2020). They are short tandem repeats of DNA which are multi-allelic, co-dominant and evenly distributed throughout the genome of a species (Dettori*et al*., 2015). They are useful markers to use when investigating pure line selections such as those of Bambara groundnut landraces (Mayes *et al.*, 2013). Being PCR-based, SSR markers are technically simple to deploy and are amenable to high throughput assays. In recent years, SSR markers have become integral in marker-assisted selection (MAS) in early generation breeding populations (Mohhamed, 2014). Molecular markers should not be an alternative to morphological markers, but, rather, complementary to conventional breeding (Mohhamed, 2014).

2.7 Nutritional and biochemical of Bambara groundnut

Nutritionally, Bambara groundnut represents a cheap protein-rich source that can improve the food and nutrition security status of rural households. Biochemical analysis for carbohydrate, fat, starch, protein and mineral content revealed that Bambara groundnut produces an almost balanced diet (Kaptso *et al.*, 2015). Chemical composition and physicochemical variation have been observed among Bambara groundnut landraces.

Starch, protein and mineral elements are some of the important biochemical properties used to evaluate Bambara groundnut. The starch granule size and shape in Bambara groundnut were found to vary with botanical origin, landrace, growth stage of the plant, environmental conditions, extraction and purification methods of the grains (Kaptso *et al.*, 2015). In particular, the starch granules of the white variety were larger (size range 10–35 μm) and polygonal while those from the black variety were smaller (size range 6–15 μm) and spherical in shape. In addition, the peak of gelatinization temperature was higher for white variety (81.7 °C) than for black variety (77.5 °C). Oyeyinka *et al.* (2016) reported oval and irregularly shaped starch granules with size ranging between 24 and 29 μm. In a separate study, Chowdhury et al. (2015) reported significant variation in mineral content in five landraces of Bambara groundnut. The landraces grown in Swaziland had higher mineral contents than those grown in Botswana and Namibia. The variation in biochemical structure and physiology of Bambara has been investigated using scanning electron microscopy (SEM) imagery based on the morphology of starch, minerals and seed coat thickness (Table 1.4). Recently, there has been an increase in the use of light microscopy in determining variation in mineral element content among Bambara groundnut accessions (Oyeyinka and Oyeyinka, 2018).

The SEM is a technique that utilises a focused beam of electrons to produce an image of the sample surface. The electrons from the beam are focused to hit the sample surface and get deflected off the sample specimen. The microscope is connected to a detector that decodes the amount and nature of the deflected electrons and constructs a magnified image that can be viewed (Goldstein *et al*., 2017). The most common technique in SEM is the Energy Dispersive X-Ray Spectrotomy (EDX), which requires the initial cryo-fracturing of the samples in liquid nitrogen. The procedure is destructive as it involves the dissection of the Bambara groundnut into two halves longitudinally. The SEM is premised on the superficial coating of the sample surface by gold particles spluttered by a high precision coating instrument. The variation in the coating is viewed under the electron microscopy in a vacuum mode and captured by a scanner. The thickness of the coat will determine how much of the light will be allowed to pass through based on the X-ray phenomenon used widely. The combination of the electron microscope and scanner can determine the amount and wavelength of the light passing through the sample. The differences in the coating thickness are decoded and can be analysed statistically as indicators of variation in structural composition and mineral content among different samples. This technique has been successful to discern variation in nutrient content, seed coat thickness and non-adherence of the seed coat to the cotyledons among Bambara groundnut genotypes (Ogundele and Emmambux, 2018) as well as other legumes such as peas (Sharma *et al*., 2015, Lazarević *et al*., 2017), soybean (Otobe *et al*., 2015) and common bean (Sandhu *et al*., 2018).

Table 2.4: Morphology, X-ray diffraction pattern and degree of crystallinity of Bambara groundnut starch.

Seed coat colour	Granule size (µm)	Measuring method	Shape description	Diffraction pattern	Crystallinity (%)
Not specified	NR	NR	NR	C	38.75
Not specified	18–36	LM, SEM	Predominantly oval, Few granules were concave and round	NR	NR
Not specified	45.57	SEM	Oval, round	A	43.7
White	10–35	SEM	Irregular, polygonal	A	41.1
Black	6–15	SEM	Spherical	A	40.4
Not specified	26	SEM	Oval, few round and irregular	C	29.4–35.3
Maroon, Brown, Cream	24–29	SEM	Oval, few round and irregular	A	30.5–33.0

LM: Light microscopy, SEM: Scanning electron microscopy. Adapted from (Oyeyinka and Oyeyinka, 2018)

2.8 Correlation, Regression and Path Coefficient Analyses

Most production focuses on yield as the most important trait in crop production. Due to G X E interactions yield expression is variable under different environments (Biarnès-Dumoulin *et al*., 1996). Yield is a complex trait due to variability of heritable traits under different environmental conditions and selection pressures, which makes it difficult and ineffective for direct selection of yield. Thus, the improvement of Bambara groundnut yield can be achieved through exploitation of the relationship between yield and its related traits. The relationship has been used in several studies to overcome the complexity associated with grain yield heritability. Grain yield selection and evaluation should be carried out with relative precision using models that minimise environmental influence and which are able to differentiate and quantify the contributory factors.

Correlations estimate the nature of relationship that may exist between variables. Correlations are found where variables have a cause and effect relationship in which one variable is dependent on the other such that a change in the independent variable causes a change in the dependent variable (Bello *et al*., 2010). The relationship can either be positive or negative; strong or weak. Where variables are positively correlated both independent and dependent variables change in the same direction whereas in negatively correlated variables, the variables change in opposite directions. Information on correlations is important in crop improvement where selection of yield is indirect and achieved through selection of secondary traits (Makanda *et al*., 2009). However, correlations are inadequate in describing the importance of each trait in contributing to the final yield (Blanco *et al*., 2012). The inadequacy can be misleading where observed variations are due to more than one indirect cause (Blanco *et al*., 2012). Therefore, there is a need for a more in-depth analysis of the interactions to understand the importance of each trait and rank their importance for targeting during selection. One way to achieve this is by using the path coefficient analysis (Lule *et al*., 2012).

Path coefficient analysis is important in partitioning the observed change in the dependent variable into contributory effects by each independent variable (Lule and Mengistu, 2014) It is a useful way of examining direct and indirect relationships of complex traits (Lule and Mengistu, 2014). Understanding of the grain yield-secondary traits relationship will greatly improve selection methods as it helps to rank the traits in order of their importance in yield improvement. The breeder will then target traits with highest contributory effects for selection. Therefore, it was prudent to investigate the role of secondary traits in determining yield in the current study.

2.9 Conclusion

Bambara groundnut is one of the world's main staple crops and an important legume for direct human consumption. The crop has high nutritional value (protein source), drought tolerant characteristics and N-fixation properties. Therefore, it has potential to contribute to food and nutritional security in SSA, especially amongst smallholder farmers. However, smallholder farmers face challenges in achieving high yields for Bambara groundnut. The challenges include a lack of improved cultivars adaptable for production under rain-fed conditions. In order to improve productivity of Bambara groundnut under smallholder farming and for the crop to be lucrative for mainstream agriculture, there is a need genetic improvement of the crop and development of improved cultivars that will be easily accessible by farmers. Development of improved cultivars depends on genetic diversity and availability of germplasm. Hence, assessing agro-morphological, nutritional composition and genetic diversity in Bambara represents a preliminary step towards Bambara groundnut improvement.

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Chapter 3 Agro-Morphological diversity of Bambara groundnut lines evaluated under field conditions

Abstract

Many countries in sub-Saharan Africa are affected by multidimensional food insecurity due to a number of factors that include climate change induced drought stress. There is a need to identify and evaluate naturally resilient crops to improve food security. Bambara groundnut (*Vigna subterranea* (L.) Verdc) is a resilient legume with potential to improve food security in sub-Saharan Africa. Nineteen Bambara groundnut lines were evaluated for emergence, leaf number, plant height, leaf size, canopy diameter, chlorophyll content index, stomatal conductance, flowering and yield components in 2017 and 2018 under field conditions in two sites. A completely randomised block design, with three replications was used at each site. The lines showed significant differences ($P<0.05$) for all the measured traits. Differences in rate of emergence among the lines were attributed to differences in seed coat thickness and metabolic efficiency. The number of leaves per plant, plant height and canopy size varied significantly concomitant with differences in stomatal conductance and chlorophyll content. The first principal component (PC) accounted for 30.4% of the variation and was strongly correlated with NHS ($r=0.90$, $p<0.001$) and NS ($r=0.90$, $p<0.001$). Second PC explained 22.7% of the variation and was associated with LN ($r=0.86$, $p<0.001$), CD ($r=0.85$, $p<0.001$). The traits associated with PC1 and PC2 were identified as the most important traits for discriminating among the lines. The genetic variation exhibited among the lines provides opportunities for selection during Bambara groundnut improvement. However, the negative correlations among some of the traits would be a challenge for simultaneous selection during the breeding process.

KEYWORDS: agronomic traits, characterization, food security, genetic diversity, phenotypic selection

3.1 Introduction

Many countries in the sub-Saharan Africa region face critical shortages of food at national and household levels due to several factors that include, lack of technical support for the agriculture sectors, inherent poor soils to support crop production and lack of adaptive strategies in the changing climate (Baiphethi and Jacobs, 2009). These factors occur individually, sequentially or in combination, resulting in devastating effects on crop production. South Africa is food secure at the national level although there is widespread food insecurity at the household level. Food insecurity threatens 54% of the in rural population that depend on subsistence agriculture (Shisanya and Mafongoya, 2016). Food insecurity is a major concern for governments and civic organisation as it causes neo-natal deaths among childbearing women, leads to stunted growth and poor cognitive development in children and related to decimation of the labour force due to diet related deaths (Dewing *et al.*, 2013).

Climate change related stresses such as drought and high temperature have contributed to poor crop productivity in sub-Saharan Africa in both commercial and subsistence farming systems. However, the impact of climate change effects are more profound under subsistence farming as the farmers lack adaptive and coping strategies compared to commercial farmers (Ziervogel *et al.*, 2014). The majority of the subsistence farmers depend on seasonal rainfall, which has become increasingly erratic and below average due to climate change (Shrestha and Nepal, 2016). In addition, the farmers in sub-Sahara Africa rely on local knowledge and farming methods, which may not be adequate, efficient or suitable to increase resilience in the ever-changing farming environment. As a result, subsistence farmers incur high yield losses or total crop failure leading to perpetuation of food insecurity among rural households (Shrestha and Nepal, 2016).

Climate change induced stresses, poor soil fertility and the non-availability of inputs commonly encountered in sub-Sahara Africa farming systems adversely affect the production of crops such as maize, wheat or rice, which have high management and input requirements (Shrestha and Nepal, 2016). As such, there are increasingly more proposals to include resilient or orphan crops for production under low input subsistence farming systems. Resilient crops include cereals such as sorghum (*Sorghum bicolor* (L.) Moench) and millet (*Panicum glaucum* L.), legumes such as pigeon pea (*Cajanus cajan* (L.) Millspaugh), Bambara groundnut, vegetables and root crops such as cassava (*Manihot esculenta* Crantz) and yam (*Dioscorea alata* L). These crops are relatively more tolerant to drought, heat and ion toxicity stresses due to their extensive rooting ability (Ojiewo *et al.*, 2015) and are known to be sources of food and income for subsistence farmers in the marginal areas.

Bambara groundnut has been identified as a suitable crop for improving food production in sub-Saharan Africa due to a number of factors including its high drought tolerance, nitrogen fixing ability and high nutritional content. The crop can potentially be utilized as fresh or dried food. The seeds can be used fresh, dry or used for extraction of milk (Ijarotimi and Esho, 2009). The Bambara groundnut grain contains carbohydrate (65%), protein (18%), oil (6.5%) and essential minerals such as calcium, iron, potassium and sodium (Mazahib *et al*., 2013). The nutritional quality of Bambara makes it a suitable candidate for diversifying nutritional sources among rural households that depend heavily on staple crops such as maize that are carbohydrate-rich but lack essential minerals and proteins to meet dietary requirements. Taffouo *et al*. (2010) reported that white seed coat (WSC) Bambara groundnut lines were relatively more tolerant to salinity.

They suggested that the WSC lines could be cultivated in the coastal and semi-arid saline soils and provide a source of genes for salt tolerance. Its drought and heat stress tolerance render it more suitable for production under erratic rainfall and high temperature conditions experienced in many parts of the sub-Sahara African region (Tadele and Assefa, 2012). Mabhaudhi *et al*. (2013) reported that Bambara groundnut lines with brown and red seed coats had reduced canopy size, early flowering and maturity, and maintained high water use efficiency under water stress rendering them drought tolerant. Agricultural soils in sub-Saharan Africa are commonly depleted of soil C, an integral component for controlling soil biogeochemical processes resulting in low soil pH (Wood *et al*., 2015). As a result, the soil is of poor nutrient quality, lack structure and highly acidic as they contain low soil organic matter for buffering. Resulting in many crops failing to grow to full potential without soil amendments. Inputs such as fertilizer are not affordable or easily accessible to small holder farmers, leading to sub-Sahara having the lowest per capita fertilizer usage in the world (Ramaila *et al*., 2011). While Bambara groundnut crops can potentially contribute to food security, it has been neglected in breeding programs relative to the focus paid on the major crops (Dawson *et al.*, 2018). Consequently, there have been calls to consider its inclusion in breeding programs to improve its productivity and promote its inclusion in cropping systems to diversify sources of nutrition in subsistence farming systems (Dawson *et al.*, 2018).

The productivity of Bambara groundnut s still low among subsistence farmers due to a lack of improved cultivars for production. Most farmers cultivated landraces that are segregating leading to yield variability across seasons, that lack farmer preferred traits such as high palatability and reduced processing time (Mubaiwa *et al*., 2017). Thus, there is a need to develop new and improved cultivars to meet market demands and respond to environmental constraints. Therefore, there is need to improve productivity of Bambara to validate its inclusion in diverse cropping systems. Improvement of Bambara is hinged on thorough understanding of its morphological and physiological characters as a preliminary step towards efficiently exploiting its genetic diversity. This approach will also help breeders and farmers by generating vital baseline information on the currently known lines. Evaluating diverse lines for their traits will also help to characterize useful germplasm for breeding programmes and increase selection efficiency for specific environmental conditions. The objective of this study was to characterise 19 different Bambara groundnut lines using morphological and physiological differences influencing their growth, development and yield under field conditions to select superior genotypes for recommendation.

3.2 Materials and Methods

3.2.1 Planting material

Nineteen Bambara groundnut lines were used in this study (Table 3.1). The seeds were sourced from subsistence farmers of Tugela Ferry (28°45’ S; 30°27’E) and Deepdale (30°33' S; 29°54' E) in KwaZulu-Natal, and the University of Nottingham (UK). The lines form part of germplasm maintained at the University of KwaZulu-Natal for Bambara groundnut improvement program.

3.2.2 Site description

The field trials were conducted at two sites over a period of two years. The site was the field at the Controlled Environment Facility (CEF) of the University of KwaZulu–Natal at Pietermaritzburg campus (29°37'34.0"S 30°24'13.4"E). The second site was the field at the Ukulinga Research Farm of the University of KwaZulu-Natal in Pietermaritzburg (29° 37′ S; 30° 16′ E; 775 m a. s. l.). The average temperatures during the experiment in

2017 at CEF and Ukulinga were 26^{0}C and 28^{0}C, repectively. In 2018, the average temperatures were 24^{0}C and 27^{0}C for CEF and Ukulinga. The average rainfall received during the trial in 2017 at CEF and Ukulinga were 610mm and 694mm respectively. The CEF received 790mm of rainfall in 2018 while Ukulinga received 669mm.

Table 3.1: List of Bambara groundnut lines used in the study showing their origins and seed coat colour.

Line	Source	Country of Origin	Seed coat colour
IITA686	University of Nottingham	Tanzania	Light brown
DIP-C	University of Nottingham	Botswana	Cream
S19	University of Nottingham	Namibia	Black
Uniswa Red-G	University of Nottingham	Swaziland	Dark red
Uniswa Red-R	University of Nottingham	Swaziland	Red
Kenya Capstone	Capstone Seed company	South Africa	Cream
Gresik	University of Nottingham	Indonesia	Black
Brown	Local Farmers	South Africa	Brown
Light brown	Local Farmers	South Africa	Light, brown
Cream	Local Farmers	South Africa	Cream
M09-4	University of KwaZulu-Natal	Zimbabwe	Brown
211-57	Capstone Seed company	South Africa	Dark cream
Tiganecuru	University of Nottingham	Botswana/Mali	White
42-1	University of KwaZulu-Natal	Zambia	Black
Mix	Local Farmers	South Africa	Mixture of colours
KANO2	University of Nottingham	Nigeria	Light cream
Pong-Br-EN	Local farmers	Unknown	Light brown
Pong-Br-UNK	Local farmers	Unknown	Light brown with sparkle
Pong-Cr	Local farmers	Unknown	Cream

3.2.3 Experimental design and field experiment

The experiments were laid out using complete randomized block designs replicated three times at each site. The land was disced and rotovated to achieve a fine tilth. All the trials were planted in December and harvested in May of the subsequent year. Two seeds were planted per station at 3cm depth. The plots were single rows measuring 0.5 × 0.5m per genotype. Other agronomic practices were as per standard Bambara groundnut production practice in South Africa (DAFF 2010). The soil moisture was monitored by digital moisture sensors using the ML3 Theta Kit (Delta T Devices UK) and tensiometers sunk at 30 and 60 cm depths at several points in the field. Irrigation water was applied through irrigation pipe to maintain adequate soil moisture (average 80% of field capacity) three times per week.

3.2.4 Data Collection

3.2.4.1 Plant growth and physiology

The evaluation of plant growth and physiology parameters was initiated soon after the emergence (VE) stage. Plant height (PH), number of leaves (LN) were measured on three plants per plot and recorded as averages per plot. Plant height was measured from the ground level to the tip of the fully matured leaf using a tape measure (Stanley 3m Power lock steel). The number of leaves per plant were counted as the number of fully developed unifoliate to trifoliate leaf that were visible. Mid-leaf length (MLL) and mid-leaf width (MLW) were measured on the middle leaf of the trifoliate. Canopy diameter (CD) was measured on three plants per plot using a measuring tape. The widest part of the canopy was measured. Stomatal conductance (SC) was determined using the Model SC–1 steady state leaf porometer (Decagon Devices, Inc., USA). A portable chlorophyll meter, the

SPAD-502 Plus (Konica Minolta, Japan) was used to measure chlorophyll content index (CCI) on the fully expanded trifoliate and solar radiation exposed leaves.

3.2.4.2 Yield parameters

entire plot was harvested by digging the entire plant from the ground. After harvesting shoot, roots, pod (S, R, R) and whole plant mass from each experimental plot were weighed with a digital sensitive balance (Masskot, FX320, Switzerland) and average mass (g) per plot was recorded. After shelling the crop, seed mass (SM) per plant was weighed with a digital sensitive balance (Masskot, FX320, Switzerland). Thereafter, number of seeds were counted and categorised into damaged (D) and healthy seeds (NHS) from the pods.

3.2.5 Data Analyses

Means, standard deviations, minimums, maximums and coefficients of variation were computed for each growth and yield trait. Subsequently, the data were subjected to analyses of variance (ANOVA) using GenStat® Version 18 (VSN International, United Kingdom) after testing for normality and homogeneity of variance across seasons and sites. The means were separated by Fischer's Unprotected least significant difference (LSD) at 0.05 significance level. Subsequently, Pearson correlation, principal component and hierarchical analyses were conducted to deduce trait and line associations using R software (R Core Team, 2019).

3.3 Results

3.3.1 Physiological parameters

The Bambara groundnut lines exhibited significant ($p<0.001$) genetic variation for rate of emergence. The emergence rate among the lines ranged from 45 to 98%. The highest emergence rate was observed for the line MO9 (98%). The average emergence rate was 68% while Tiganecuru had the lowest emergence rate of 45% (Figure 3.1). The variation among the Bambara lines for chlorophyll content was narrow and did not show significant variation. However, the chlorophyll content indices exhibited variability with plant growth. chlorophyll content indices were observed to increase with plant growth (Figure 3.2). The highest chlorophyll content index was 61.5 observed at week 4 and then it started to decline towards maturity with the lowest index measured at week 9. Similarly, stomatal conductance occurred within a narrow range among the Bambara groundnut lines (Figure 3.3). The stomatal conductance increased with plant growth in general. The lowest stomatal conductance of 40.4 was measured at week 4 while at week 9, the highest conductance of 363.5 was recorded.

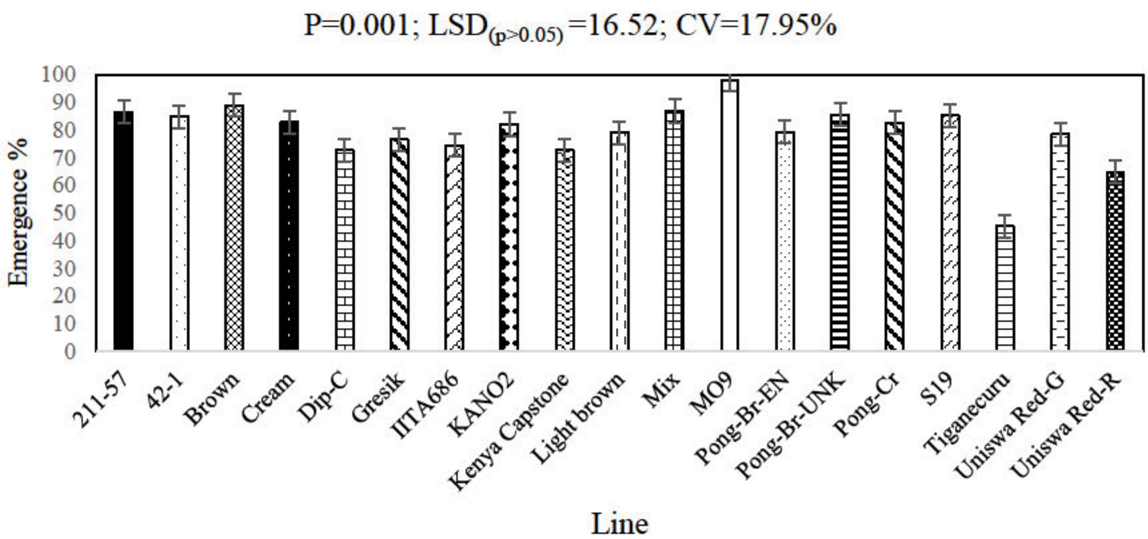

Figure 3.1: A comparison of final emergence for 19 Bambara groundnut lines in the field. The bars represent standard error of means (±4.14).

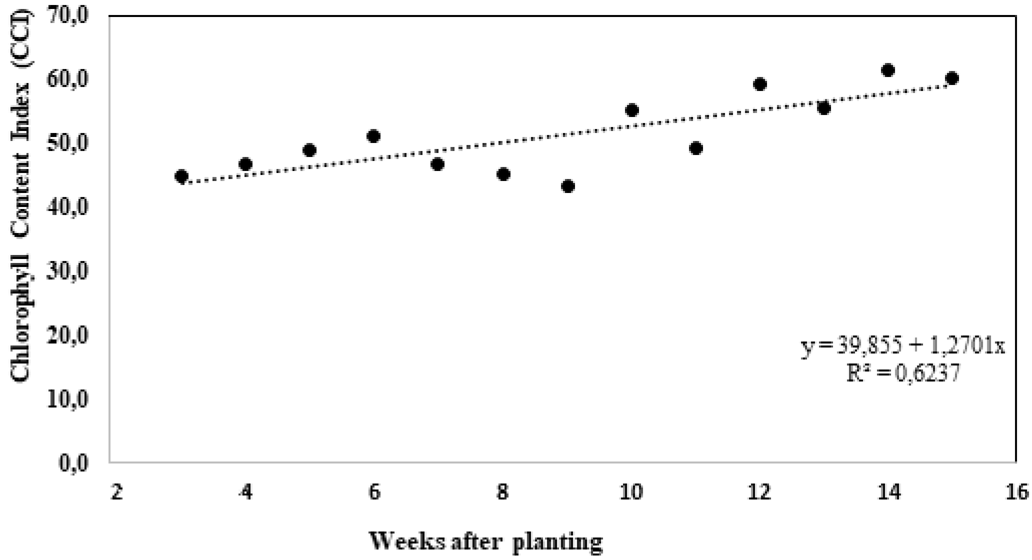

Figure3.2: A comparison of final chlorophyll content index (CCI) for 19 Bambara groundnut lines in the field.

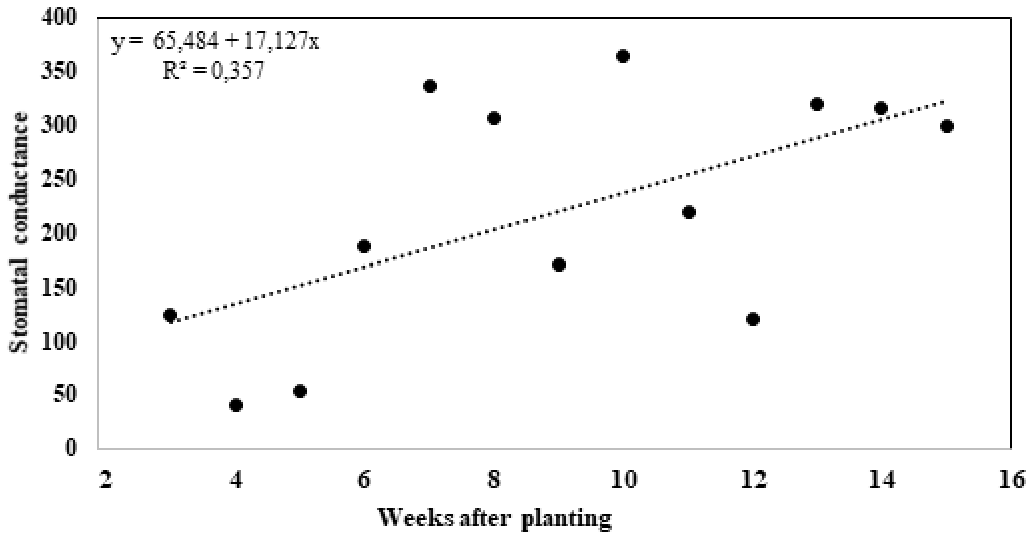

Figure 3.3: A comparison of final stomatal conductance for 19 Bambara groundnut lines in the field.

3.3.2 Growth parameters

Plant height differed widely and significantly ($p<0.001$) among the Bambara groundnut lines. The plant height ranged from 14.15 to 16.99 cm. The line Pong-Cr was shortest while Kenya Capstone was the tallest (Figure 3.4). There was significant (p=0.001) variation for number of leaves per plant among the Bambara groundnut lines (Figure 3.5). The leaf numbers ranged between 20.00 and 29.00 with lines 8- DIP C and 42-1 having the lowest and highest numbers of leaves per plant. The number of leaves per plant exhibited significant variability as the plant growth progressed over weeks after planting (Figure 3.6). The number of leaves per plant increased gradually to week 100. There was a sharp increase after week 11 to week 13. Thereafter, the number of leaves decreased gradually until physiological maturity after week 14 and 15. The Bambara groundnut lines exhibited significant (p=0.001) differences in mid-leaf length. The lines Mix, Dip-C and IITA686 had the shortest leaves (average of 6.2cm). The observed average mid-leaf length for most of the lines was between 6.2 and 6.8cm. The lines with the longest leaves (average 7.0cm) included MO9, Light brown and Pong-Br-UNK (Figure 3.7). The final mid-leaf width varied significantly (p=0.001) from 1.4 to 2.0cm among the Bambara groundnut lines. The line 211-57 was observed to have the narrowest leaves. On the other hand, lines such as Cream, SI9-3 and Uniswa Red-R were observed to have the widest mid-leaf width averaging 2.0cm each (Figure 3.8). There were significant differences (p=0.003) among the Bambara groundnut lines for canopy diameter. The widest canopy diameter was recorded for line 211-57 with 32 cm and least canopy diameter was observed for Dip-C with a mean of 28 cm (Figure 3.9). The range for canopy diameter was between 26 and 32 cm.

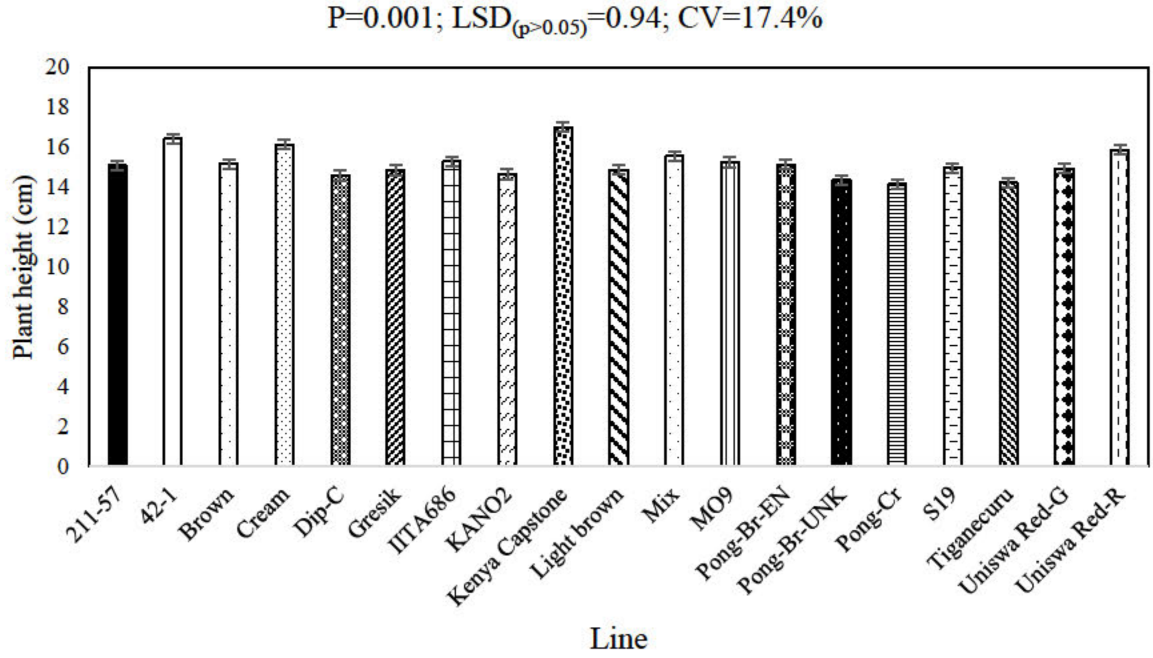

Figure 3.4: A comparison of final plant height for 19 Bambara groundnut lines in the field. The bars represent standard error of means (±0.24).

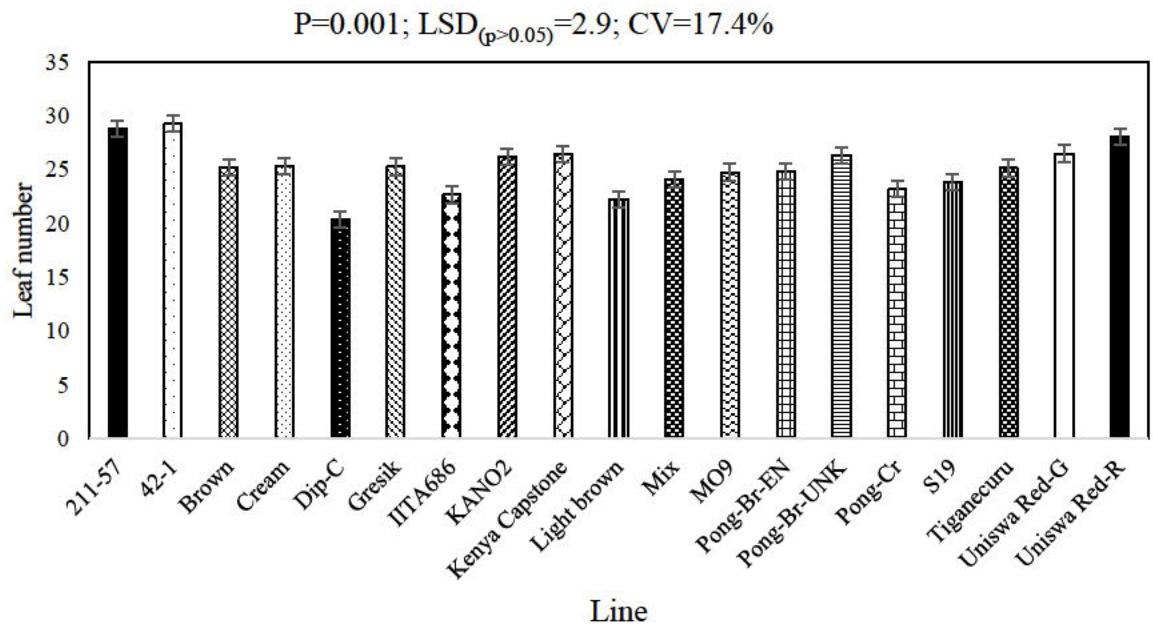

Figure 3.5: A comparison of final leaf number for 19 Bambara groundnut lines in the field. The bars represent standard error of means (±0.75).

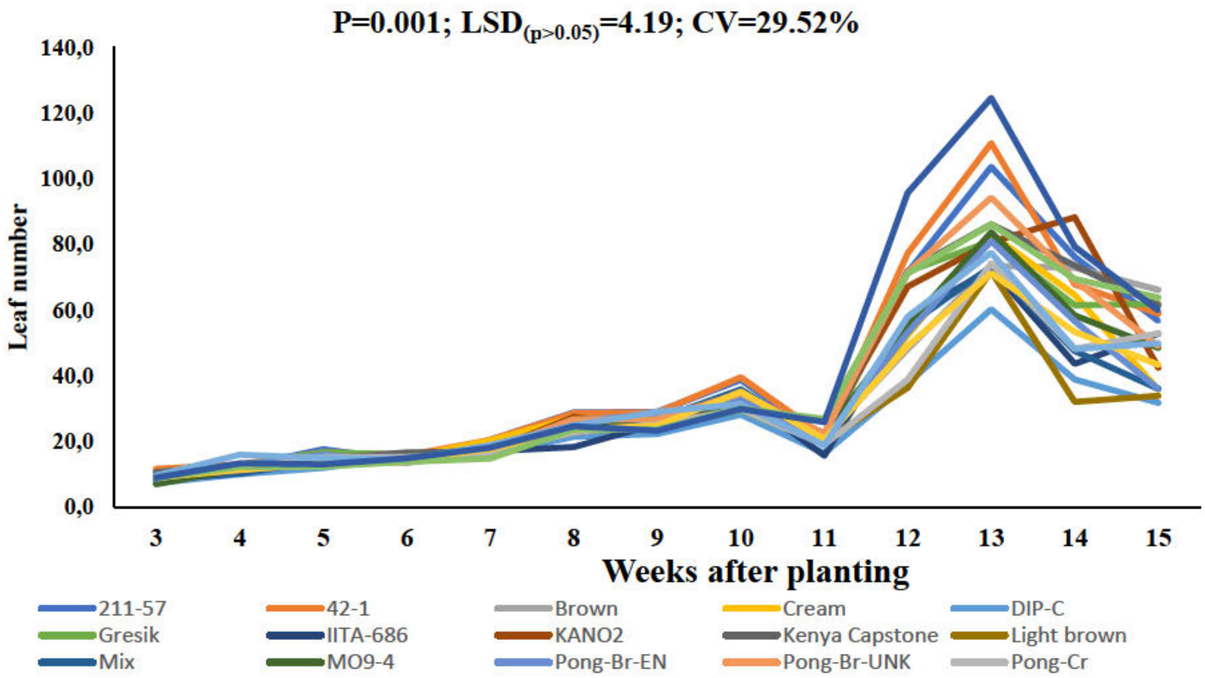

Figure 3.6: A comparison of final leaf number for 19 Bambara groundnut lines 15 weeks after planting.

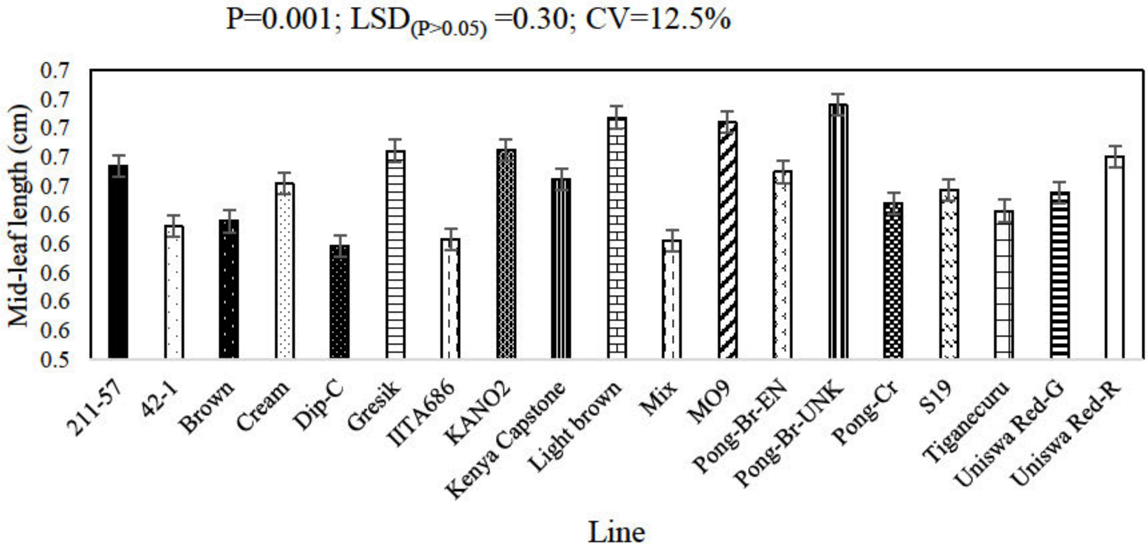

Figure 3.7: A comparison of final mid-leaf length for 19 Bambara groundnut lines. The bars represent standard error of means (±0.075).

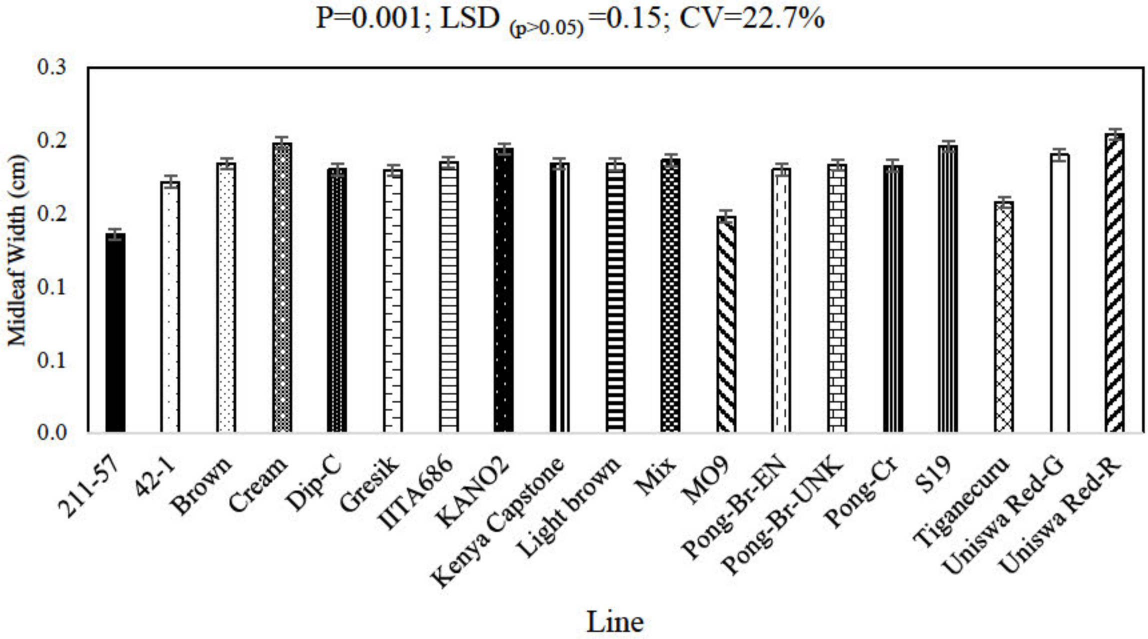

Figure 3.8: A comparison of final mid-leaf width for 19 Bambara groundnut lines in the field. The bars represent standard error of mean (±0.039).

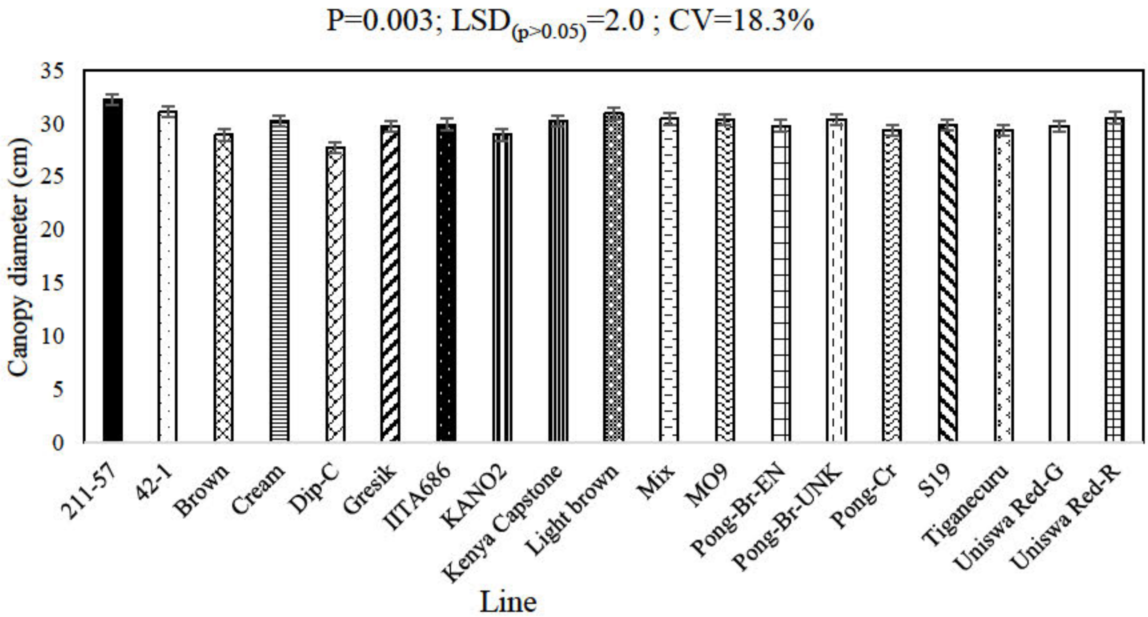

Figure 3.9: A comparison of final canopy diameter for 19 Bambara groundnut lines in the field. Standard error bar represents standard deviation (±0.52).

2.3.3 Biomass parameters

There were significant ($p<0.001$) differences among the Bambara groundnut lines in plant biomass accumulation. Tiganecuru was observed to have accumulated the lowest plant biomass (44.1 g plant ^{1}). The highest plant biomass was accumulated by the lines Kenya Capstone, 211-57, KANO2, Uniswa Red-G, 42-1, MO9 and Mix with respective plant biomass means of 129.9, 117.1, 117.0, 113.9, 107.7, 107.0 and 106.6 g plant^{-1} (Figure 3.10). Tiganecuru was observed to have the lowest shoot biomass of 26.8g while the lines including Kenya Capstone, Uniswa Red-G, Mix and KANO2 had high shoot biomass of 100.1, 85.1, 84.1 and 81.4 g plant^{-1}, respectively (Figure 3.11). The range of root biomass accumulated by the Bambara groundnut lines was between 17.1 g plant^{-1} recorded for the line Cream and 45.8 g plant^{-1} recorded for line MO9 (Figure 3.12). The genetic differences among the Bambara groundnut lines had significant effects ($p=0.001$) on seed mass productivity. The two lines Pong-Br-UNK (20.5 g plant^{-1}) and Pong-Cr (21.31 g plant^{-1}) were among the lines with the lowest seed mass productivity. The lines with the highest seed mass productivity included IITA686, mix and Brown with mean seed mass productivity of 104.03, 78.11 and 76.71 g plant ^{1}, respectively (Figure 3.13).

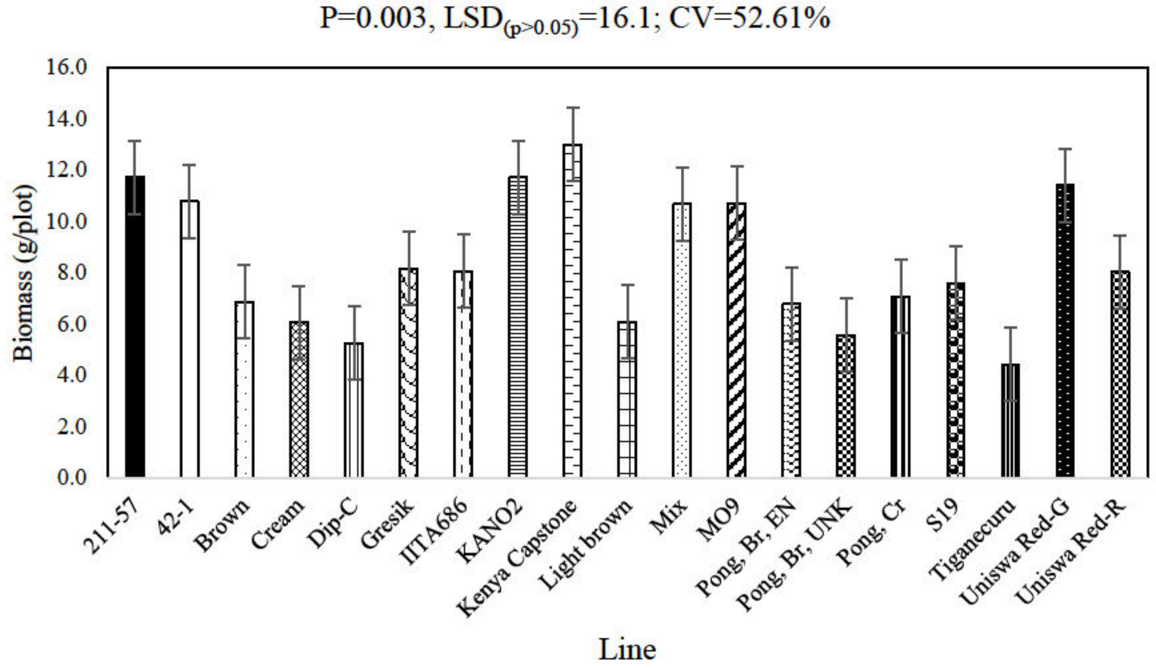

Figure 3.10: A comparison of final biomass for 19 Bambara groundnut lines in the field. The bars represent standard error of mean (±5).

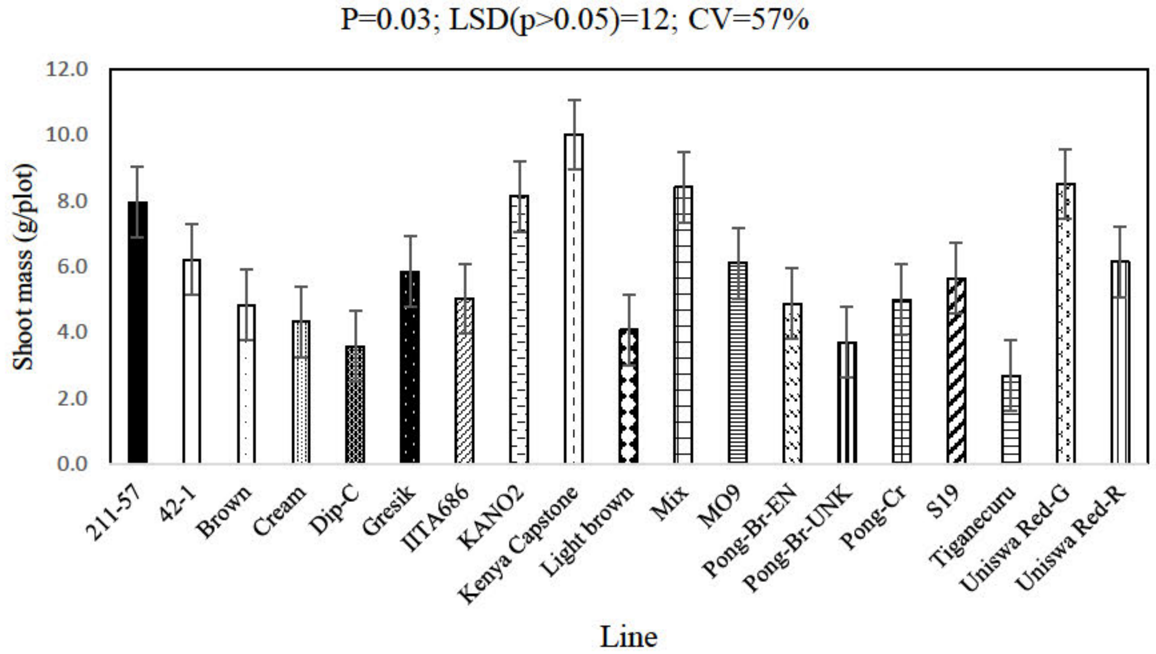

Figure 3.11: A comparison of final shoot mass for 19 Bambara groundnut lines in the field. Standard error bar represents standard deviation (±10.7).

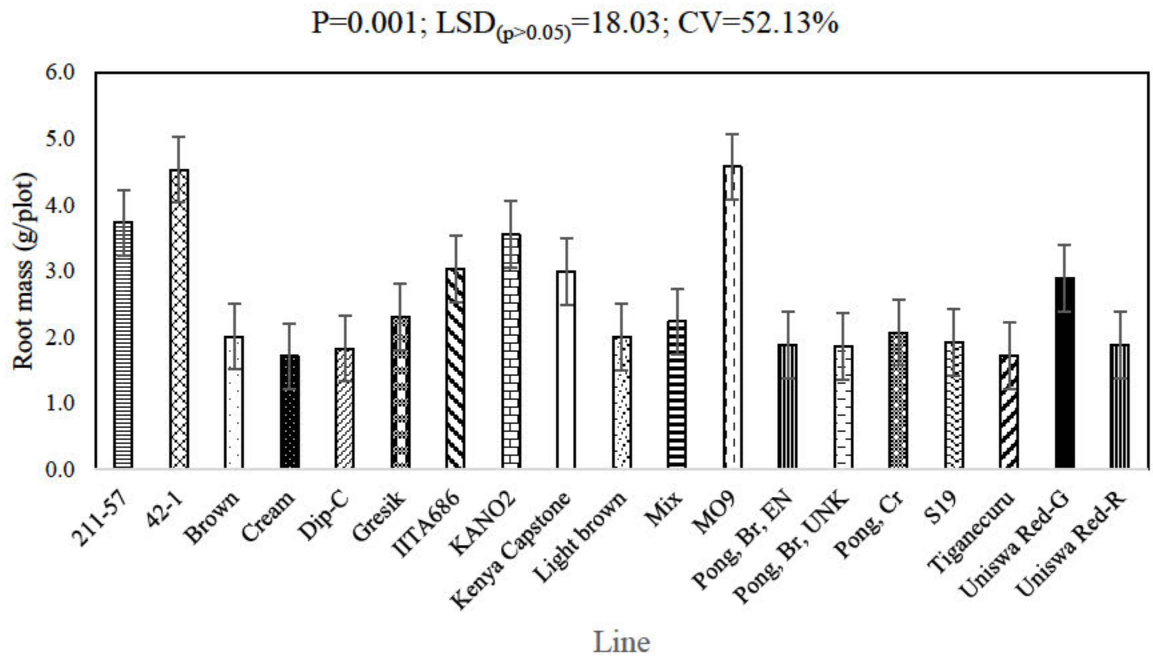

Figure 3.12: A comparison of final root mass for 19 Bambara groundnut lines in the field. The bars represent standard error of means (±14.3).

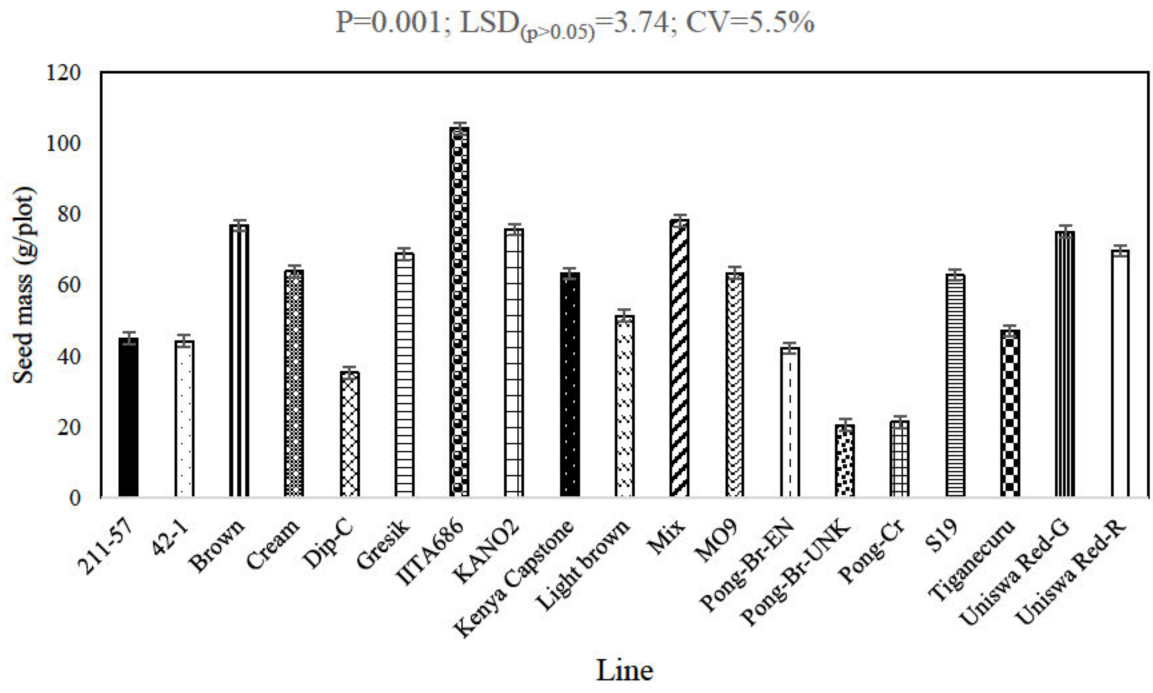

Figure 3.13: A comparison of final seed mass for 19 Bambara groundnut lines in the field. The bars represent standard error of mean (±1.6).

3.3.4 Multivariate analysis

3.3.4.1 Principal components

Three principal components (PC) with Eigen values ≥1 explained about 70% of the variation in the agro-morphological traits among the 19 Bambara lines (Table 3.2). The first and second PCs accounted for 30.4 and 22.7% of the variation. The traits that contributed the most to PC1 were NHS and NS, each contributing more than 25% to the variation explained by PC1. On the second PC, traits such as LN and CD accounted for more than 30% each of the variation on PC2 loadings (Figure 3.14). Three traits MLL, SC and CCI had no significant contribution on either PC1 or PC2, failing to contribute above the threshold of 10% each.

Table 3.2: Eigen values and variance for principal components for 19 Bambara groundnut lines evaluated in two locations

	PC1	**PC2**	**PC3**
Eigen value	3.04	2.27	1.69
% of var	30.37	22.66	16.91
Cumulative % of var	30.37	53.03	69.93
NS	0.90	-0.01	-0.22
NHS	0.90	-0.05	-0.27
SM	0.73	0.15	0.02
PH	0.54	0.61	0.06
LN	-0.01	0.86	0.12
MLL	-0.39	0.32	-0.06
MLW	0.59	-0.37	0.41
CD	-0.12	0.85	0.09
SC	-0.23	-0.24	-0.83
CCI	-0.07	-0.32	0.83

PH=plant height, EMG=final emergence rate, LN=leaf number per plant, MDL=mid-leaf diameter, MDW=mid-leaf diameter, CD=canopy diameter, RDM=root dry mass, PDM=pod dry mass, SDM=shoot dry mass, SNP=seed number per plant, HSP=healthy seed number per plot, SM=seed mass per plot

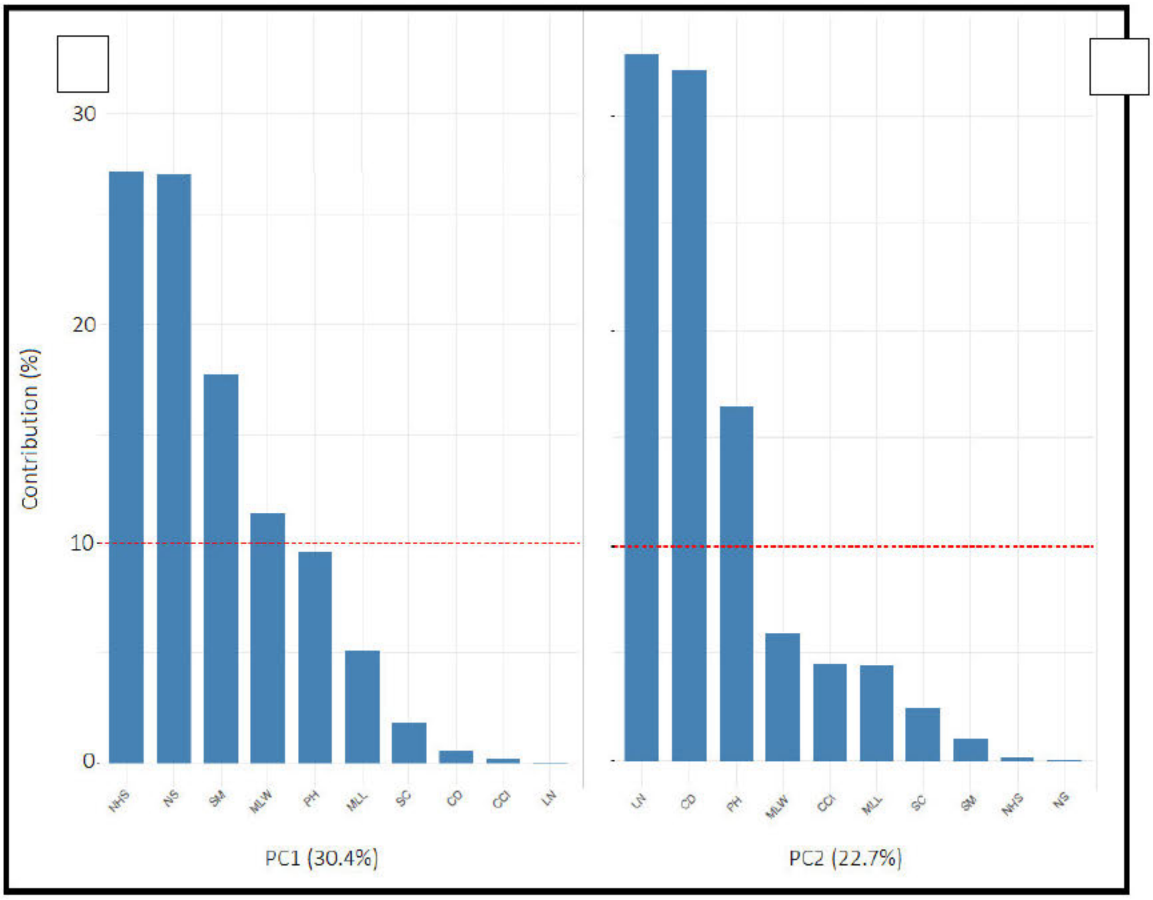

Figure 3.14: The contribution of each trait to the variation explained by A) first and B) second principal components

3.3.4.2 Interrelationship

The interrelationship among the traits showed that there were some significant correlations among the agro-morphological traits (Figure 3.15). Canopy diameter, LN and MLL were positively correlated as they were plotted in the same quadrant. Similarly, PH and SM were positively associated by the virtue of occurring in the same quadrant. There were other traits such as the yield parameters SM, NS and NHS that were positively correlated as shown by the acute angles between their vectors despite being plotted in two different quadrants. Negative correlations were observed between PH and SC, MLL and MLW, and LN and CC highlighted by the obtuse angles between their respective vectors. The traits exhibited variable associations with the two different PCS. The first PC was strongly correlated with NHS ($r=0.90$, $p<0.001$), NS ($r=0.90$, $p<0.001$), SM (r=0.73, p<0.001), MLW (r=0.59, p<0.01) while the second PC was associated with LN (r=0.86, p<0.001), CD (r=0.85, p<0.001) and PH (r=0.61, p<0.01) (data not shown).

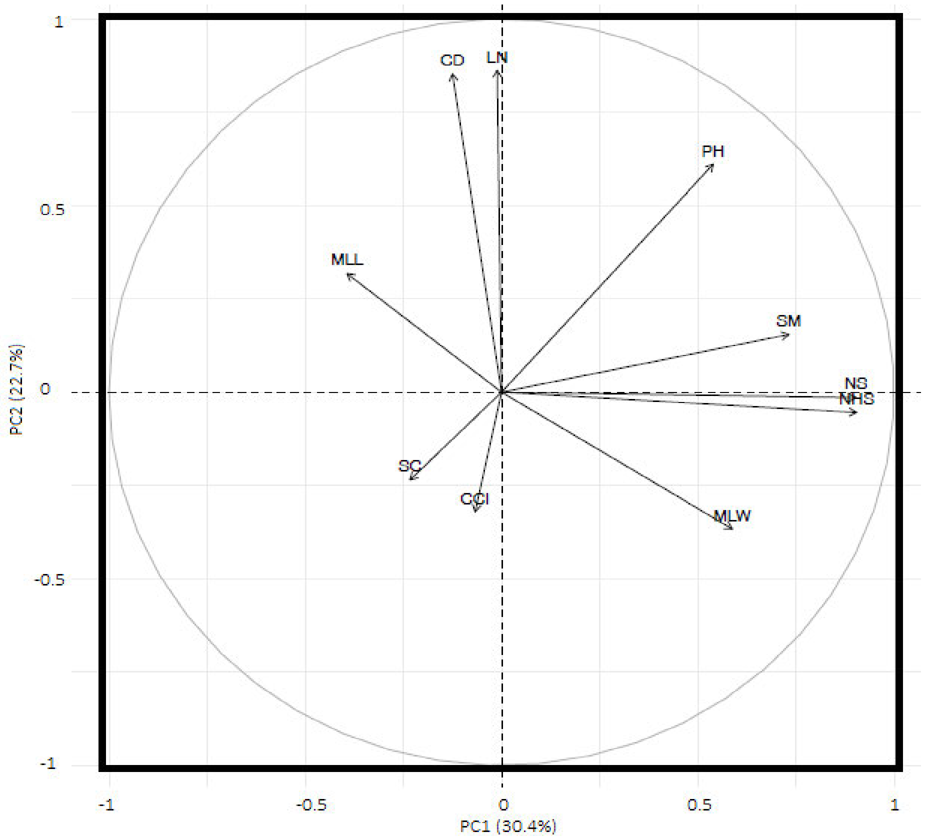

Figure 3.15: The interrelationship among trait variables and principal components for the Bambara groundnut genotypes

3.3.4.3 Line-trait associations

Figure 3.16 depicts the multi-variate relationships among line and traits. The strength of association between line and trait is determined by the proximity of the line to the trait vector while the distance of line from the origin represents the relative genotypic mean for the particular trait. Lines furthest from the origin and are in close proximity to a trait vector show that they excelled in that particular trait. Lines Cream and IITA were closely associated with SM while Uniswa Red-G was in close proximity to the vector for CCI, and KANO2, Brown, S19, Kenya Capstone and Uniswa Red-R excelled in MLW and PH. Lines that exhibited a tendency for high stomatal conductance were Pong-Br-EN, Gresik, DIP-C, MO9. Line Mix was not associated with any particular trait vector.

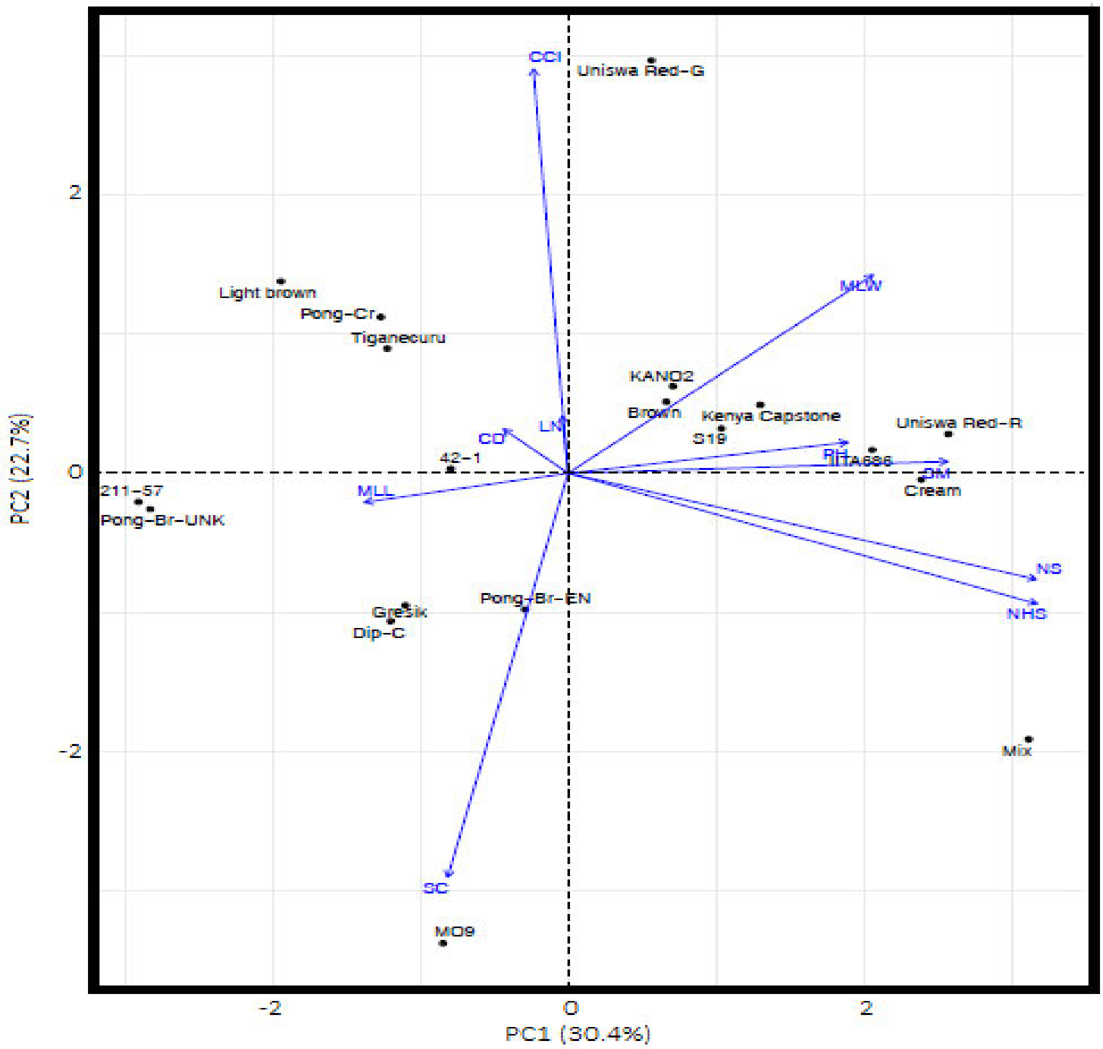

Figure 3.16: Multivariate associations depicting genotype-trait relationship among the growth and yield traits and 19 Bambara groundnut lines

3.3.4.4 Line hierarchical clustering

The unweighted pair group method with arithmetic mean (UPGMA) dendrogram revealed that the 19 lines could be grouped into three distinct clusters based on similarity in their agronomic performance (Figure 3.17). There were two clusters that were comprised of 8 lines each while the smallest cluster contained three lines only. The cluster that contained IITA866, Mix, Cream and Uniswa Red-R was characterised by high mean values for NS, NHS, SM, PH and MLW. The other cluster that contained Kenya Capstone, S19 and Pong-Br-EN exhibited high mean values for LN, MLL, CD and SC.

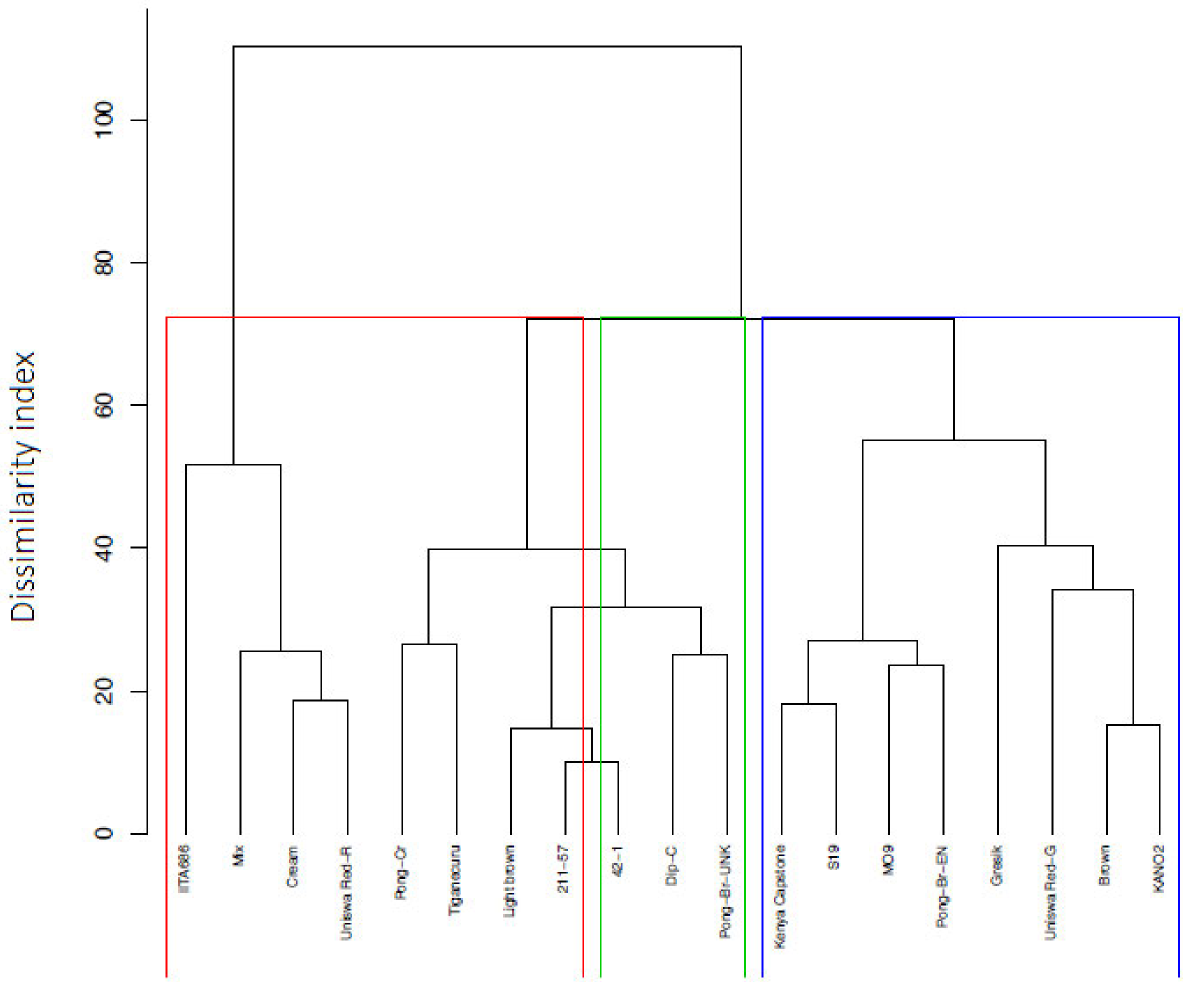

Figure 3.17: Dendrogram depicting the interrelatedness of 19 Bambara groundnut lines based on growth and yield traits across two locations

3.4 Discussion

3.4.1 Line and environmental impact on growth and yield traits

The significant differences observed among the Bambara groundnut lines for physiological, growth and biomass parameters indicate that there was useful variation among the lines to facilitate varietal selection or identification of superior genotypes for recommendation. Differences in agro-morphological characteristics are underpinned by differences in genetic constitution that results in different levels of adaptation to environmental conditions. The genotypes such as M09, Brown and Mix could be attributed to differences in seed coat thickness and metabolic efficiency. Hard seed coats prolonged germination resulting in low rates of germination as observed by Chibarabada *et al*. (2014). In contrast, Mandizvo *et al*. (2019) found no differences in seed coat thickness among landraces of Bambara and concluded that seed emergence and vigour could not be explained by differences in seed coat thickness. Touré *et al.* (2012) attributed the variation in seed germination among genotypes to seed health, physiological condition and size. However, lines that emerge early coupled with early seedling vigor should be recommended especially in sub-Sahara Africa to take advantage of availability of moisture early in the growth season. This would contribute to escaping terminal drought stress that is prevalent in sub-Sahara Africa. The narrow variation among the lines for chlorophyll content and stomatal conductance show that these traits would be difficult to include in germplasm characterisation or as selection criteria. Useful traits should be easily measured and exhibit wide variation for providing opportunities to identify superior genotypes. The progressive increase in chlorophyll content and stomatal conductance over the growth period is concomitant with development of photosynthetic capacity. This could be useful to target lines with possible early maturity or ability to accumulate photosynthetic capacity early in the season. Meena and Massawe. (2013) identified an early maturing landrace that coupled high chlorophyll content index, high stomatal conductance and high biomass accumulation, which showed the potential usefulness of these traits in selection of productive lines. Lines with higher chlorophyll content would have high photosynthetic potential leading to high plant growth rate (Oyeyinka and Oyeyinka, 2018).

Among the growth parameters, plant height, number of leaves, mid-leaf length, mid-leaf width, and canopy diameter exhibited significant variation among the lines showing that the lines had different photosynthetic capability and yield potential. Plant height, leaf size and canopy diameter are important determinants of solar radiation interception, photosynthesis, leaf area index and ultimately the amount of photosynthates produced by a line during the vegetative and reproduction stages. Uniswa Red was among the lines with high leaf number, whch corroborated findings by Al Shareef *et al*. (2014) who also reported the highest leaf number in the same genotype. Lines that have high foliage productivity would be useful for dual prupose in sub-Sahara Africa where the leaves are also used as vegetables or as livestock feed. A gtall genotypes with high number of leaves and wide canopy would likely have high capacity for establishment and grain yield production and should be candidate for selection after establishing its yield productivity. The lines Kenya capstone, 42-1 and 211-57 exhibited consistently favourable performance for leaf numbers, plant height, midleaf length, midleaf width, canopy diameter, and yield parameters compared to the other lines, which resulted in these lines having high biomass productivity.

3.4.2 Associations among lines and growth and yield parameters

About 70% of the variation in agro-morphological traits was explained by the first three PCs, showing that the traits were highly discriminatory as a large proportion of the variation could be explained by a relatively few numbers of PCs. The observed high contribution of NHS, NS, SM and MLW on PC1 and their strong association with PC1 show that these traits were highly discriminatory or exhibited the widest variation among the genotypes, which provide a basis for line evaluation and characterization. Although, these traits were potentially more useful in line characterization, it does not imply that they are more important than the other traits in terms of functionality. The lack of significant contribution by MLL, SC and CCI shows that they were least discriminatory among the growth and yield traits. Differences in trait contribution to PCs and importance in discriminating lines has also been reported previously. For instance, Zenabou *et al.* (2014) and Feri *et al.* (2019) found that terminal leaf width, plant height and petiole length were the most discriminatory traits in a population of Bambara evaluated in Cameroon. In South Africa, Mohammed *et al*. (2014) found that leaf colour at emergence, petiole colour, leaflet joint pigmentation and calyx colour were the most discriminatory traits in evaluating agro-morphological diversity among 49 Bambara genotypes. Variation in traits that are identified as important is influenced by test population and environmental effects.

3.4.3 Interrelationship

The variable associations exhibited by the traits can be favourable or undesirable for simultaneous selection. Traits that exhibit desirable correlation can be selected simultaneously. The positive correlations that were observed among CD, LN and MLL will allow for simultaneous and direct selection. Simultaneous selection for lines that exhibit high SC and CCI and MLL will be challenged by the negative association of these traits with SM. The negative correlation could be related to high vegetative growth at the expense of reproduction in lines that have long leaves and large canopy diameter similar to reports on that it is difficult to develop dual purpose cowpeas with high grain yield and more foliage by (Dube and Fanadzo, 2013). Conventional selection based on agro-morphological performance will be inefficient to improve Bambara groundnut where important traits exhibit unfavourable correlations. Phenotyping should be integrated with molecular characterization to increase efficiency in selection and speed up Bambara groundnut improvement. Zenabout *et al.* (2014) also found variable correlations among agro-morphological traits in Bambara, although the magnitude was different from those reported in this study. Differences in magnitude and nature of associations among traits vary from one study to another due to differences in germplasm used and environmental conditions under which the studies were carried out. Agro-morphological traits are quantitative traits that exhibit environmental plasticity, which affects their interrelationship in different environments (Gratani, 2014).

3.4.4 Line trait associations for multi-trait selection

Line performance in multiple traits was visualized in a genotype-trait biplot that is based on singular value decomposition using the principal components. Simultaneous visualization of lines and performance in multiple traits is a more comprehensive tool than the conventional methods of comparing means of individual traits at a time (Montesinos-López *et al*., 2018). Lines such as Cream and IITA686 were in close proximity to the SM vector showing that they had attained high seed mass production. These lines were also plotted in the positive direction of the vectors for PH, NS, NHS and MLW showing that they potentially have consistently good performance in these traits. The selection of Cream and IITA686 would fulfil dual purposes of high grain yield and vegetative growth for vegetables or livestock feed and likely improve these traits in future generations. Dual purpose legumes are important in food systems of sub-Sahara where the leaves are utilized during the season prior to grain harvest and breeding for dual purpose has been reported with relative success in cowpea (Dube and Fanadzo, 2013). Other lines exhibited associations and excellence in other traits. For instance, Uniswa Red-G had exceptionally high CCI but its performance in yield parameters of SM, NS and NHS maybe average. Lines that exhibit highly exceptional excellence in a single trait can be selected as useful donor lines for genes conferring that particular trait. Lines such as Light brown, Pong-Cr, Tiganecuru, 211-57 and Pong-Br-UNK that were not associated with a particular vector show that they performed below the average for most traits. Lines IITA686, Cream and Uniswa Red-R are recommended for selection for their exceptional performance in SM, PH, NS and NHS while Pong-Br-EN, Gresik, Dip-C and M09-4 can be selected to improve SC. The hierarchical clustering clustered IITA686, Cream and Uniswa Red-R together, indicating that the clustering was based on their excellence in NS, NHS, SM, PH and MLW. Clustering based on agro-morphological traits is common in diversity and evaluation studies on Bambara and other legumes (Bonny *et al.,* 2019; Zavinon *et al*., 2019; Aliyu *et al*., 2016; Zenabou *et al.,* 2014).

3.5 Conclusion

The variables used to distinguish the differences in character between Bambara groundnut lines were able to show significant differences. The investigation showed that there was significant diversity amongst the Bambara groundnut lines and that they were influenced by environmental conditions. The variability among the Bambara groundnut lines confirmed the initial hypothesis that line diversity can be observed with the use of morphological, physiological and yield parameters. The environment also played a critical role in conditioning the differences in the growth and development of the lines. The results contribute information necessary for future nutrition and Bambara groundnut improvement through development of adaptable cultivars suitable for different environments. Lines such as IITA686, Cream and Uniswa Red-R were found to have superior performance for multiple traits such as number of seed per plant, seed mass per plant, plant height and mid-leaf width.

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Chapter 4 Assessment of genetic diversity among Bambara groundnut lines using SSR markers

ABSTRACT

Evaluating genetic diversity in a set of germplasm forms a basis for crop improvement. This is especially important for crops such as Bambara, which have been neglected in national breeding programs. The objective of this study was to characterise genetic variation among 19 Bambara groundnut lines using 20 polymorphic simple sequence repeat (SSR) markers to identify lines that would be recommended for initiating Bambara groundnut improvement programs in South Africa. The genetic parameters were calculated using GenAlex software. The markers exhibited considerable polymorphism for discriminating the genotypes, with an average polymorphic information content (PIC) of 0.57. Similarly, the observed heterozygosity was 0.58, showing that the lines exhibited a considerable level of outcrossing within range for autogamous crops. The lines were clustered into three groups based on the principal coordinate analysis, explaining a cumulative 37% of the variation among the genotypes. The relatedness was confirmed by the dendrogram with the least genetic distance being zero between genotypes Brown and Light brown and the highest genetic distance being 0.60 between Tiganecuru and S19. Lines such as IITA686, Cream and Uniswa Red-R that had good performance for multiple agronomic traits occurred within 0.40 genetic distance making them genetically divergent enough for generating crosses for Bambara groundnut improvement. The most divergent genotypes with favourable agro-morphological characteristics will be recommended as genetic resources for Bambara improvement and variety development programs.

Key words: Bambara groundnut**,** genetic diversity, landraces, polymorphisms, SSR markers

4.1 Introduction

Bambara groundnut (*Vigna subterranea* (L.) Verdc) is an important legume crop in Africa and ranks third after ground nut (*Arachis hypogea*) and cowpea (*Vigna unguiculta*) in terms of production. The relatively high tolerance to nitrogen deficiency and drought stress reported for Bambara groundnut render it more suitable for production under small-holder agriculture systems of sub-Sahara Africa (SSA), where the majority of farmers have to contend with critical water shortages and degraded soils (Mapfumo *et al*., 2017). It is mainly grown for its highly nutritious seeds, which contain 18%–25% protein, 55%–72% carbohydrate and 6%–7% oil (Somta *et al*., 2011). Furthermore, Bambara groundnut fits seamlessly into mixed and rotation systems due to its short season cycle (110-150 days) and ability to fix atmospheric nitrogen, which contributes to soil fertility. Commercially, Bambara groundnut has potential for food and feed production and industrial applications. The seeds of Bambara groundnut are eaten fresh as snacks by boiling or roasting but can also be dried and processed into fine flour and milk. Bambara groundnut is also used as animal feed as it is rich in nitrogen and phosphorus. However, despite its importance and ability to tolerate marginal conditions, average Bambara groundnut yields obtained by smallholder farmers remain very low to make substantial contribution to mainstream agriculture. Principally, the low yields are a consequence of a combination of poorly developed agriculture systems, environmental factors and the lack of improved Bambara groundnut cultivars in contrast to other legumes such as groundnuts or mung bean. Developing improved cultivars of Bambara groundnut would increase its production and productivity in SSA and improve its status as an important agricultural commodity crop.

Bambara groundnut production in South Africa is carried out by small-holder farmers who cultivate locally adapted landraces that have low yield potential. There is a need to develop modern and improved varieties to increase productivity and penetrate the market value chain. Improving Bambara groundnut will benefit both smallholder and commercial farmers. Both the small scale and commercial farmers will benefit from improved yield potential while commercial farmers will likely adopt improved cultivars for traits such as uniformity, high yield and improved processing qualities. There is an evident lack of improved Bambara groundnut cultivars in many parts of Africa.

Like most neglected crops, most of the research on Bambara has centred on evaluation for local adaptation, with little or no emphasis on breeding or genetic improvement. Bambara is naturally adapted for self-pollination and hybridization may not be achieved easily, which can contribute to narrow genetic variation for important agro-morphological traits. Genetic variation is a critical limiting factor in crop improvement programs. Despite the difficulty associated with hybridizing Bambara, there is a need to evaluate genetic variation within available germplasm as a preliminary step to devise a crop improvement strategy. Genetic diversity in Bambara groundnut has been evaluated using various morphological descriptors, and biochemical and molecular markers. While morphological and biochemical traits provide useful baseline information on genotype performance, their importance is undermined by environmental interference, which may lead to inconsistencies and inaccuracies during genotype characterization (Aljumaili *et al*., 2018; Mululalem *et al*., 2018). The advent of DNA markers has helped to circumvent the challenges posed by environmental effects on genotype characterization.

Bambara germplasm has been evaluated for genetic diversity using several DNA markers including amplified fragment length polymorphism (AFLP) (Ntundu *et al*., 2004), restriction fragment length polymorphism (RFLP) (Puozaa *et al*., 2017), random amplified polymorphic DNA (RAPID) (Fatimah *et al*., 2018) and simple sequence repeats (SSRs) (Molosiwa *et al*., 2015; Somta *et al*., 2011) and more recently, single nucleotide polymorphisms (Ho *et al*., 2017). The choice of markers to use for genetic analysis is informed by the required extent of genome coverage, cost effectiveness, repeatability, amenability to automation and anticipated polymorphism among other factors. This study used SSRs because they exhibit a high degree of polymorphism, allelic variation and uniformity in coverage of the genome, are easy to detect when using the PCR platform and they have high discriminating power (Powell *et al*., 1996). The effectiveness and discriminatory power of SSR markers is unique for different populations. Therefore, there is a need to evaluate each panel of germplasm to determine the genetic variation at disposal for the intended agricultural use and possibly Bambara groundnut improvement programs. Odongo *et al*. (2018) successfully distinguished variation among 105 lines of Bambara groundnut in Kenya using SSR markers.

In this study, a total of 19 landraces, which form a part of a core collection at the University of KwaZulu Natal, were evaluated for genetic variation using 20 polymorphic SSR markers to identify the genetic basis of variation observed in the divergent genotypes. These lines were previously evaluated for agro-morphological variation. The phenotypic variation provided baseline information on the differences observed among the lines and this current study was necessary to assess the genetic basis for the observed phenotypic variation. The objective of this study was to assess genetic variation and relatedness among Bambara groundnut lines using SSR markers as a preliminary step towards Bambara improvement in South Africa.

4.2 Materials and methods

4.2.1 Plant materials

Nineteen genotypes of Bambara groundnut were used in this study. The genotypes exhibited diverse morphological and agronomic characteristics (Table 4.1).

Table 4.1: List of Bambara groundnut lines used in the study showing their origins and seed coat colour.

Line	Source	Country of Origin	Seed coat colour
IITA686	University of Nottingham	Tanzania	Light brown
DIP-C	University of Nottingham	Botswana	Cream
S19	University of Nottingham	Namibia	Black
Uniswa Red-G	University of Nottingham	Swaziland	Dark red
Uniswa Red-R	University of Nottingham	Swaziland	Red
Kenya Capstone	Capstone Seed company	South Africa	Cream
Gresik	University of Nottingham	Indonesia	Black
Brown	Local Farmers	South Africa	Brown
Light brown	Local Farmers	South Africa	Light, brown
Cream	Local Farmers	South Africa	Cream
M09-4	University of KwaZulu-Natal	Zimbabwe	Brown
211-57	Capstone Seed company	South Africa	Dark cream
Tiganecuru	University of Nottingham	Botswana/Mali	White
42-1	University of KwaZulu-Natal	Zambia	Black
Mix	Local Farmers	South Africa	Mixture of colours
KANO2	University of Nottingham	Nigeria	Light cream
Pong-Br-EN	Local farmers	Unknown	Light brown
Pong-Br-UNK	Local farmers	Unknown	Light brown with sparkle
Pong-Cr	Local farmers	Unknown	Cream

4.2.2 Deoxyribonucleic acid (DNA) extraction

The 19 Bambara genotypes were planted at the University of KwaZulu-Natal (29°37'34.0"S 30°24'13.4"E) in South Africa. Two seeds of each genotype were sown in a plastic pot in a greenhouse and raised to the three-leaf stage of growth. Three leaves of each genotype were harvested and collected fresh for DNA extraction. The DNA was extracted following the Cetyl-tetramethyl ammonium bromide (CTAB) method, where about 200 mg of the fresh leaf material were mixed with 500 µL of CTAB buffer. The mixture was incubated for one hour at 65°C and centrifuged at full speed. The resultant supernatant was decanted and gently mixed with 400-µl chloroform: iso-amyl alcohol (24:1). The mixture was centrifuged again and then DNA was precipitated by salt and ethanol. The supernatant aqueous phase containing DNA was transferred to a clean microfuge tube and suspended in a TE buffer.

The polymerase chain reaction (PCR) amplification used a total volume of 12 µl of PCR mix to run the reaction. The PCR mix was made up of 0.72 µl magnesium chloride ((50mM $MgCl_2$), 1.2 µl dNTPs (25 uM), 0.12 µl Taq (5U/uL), 0.06 µl foward primer (10 uM), 0.3 µl reverse primer (10 uM), 1.2 µl of 1x reaction buffer, 6.16 µl PCR grade water and 0.24 µl fluorscent dye. The first cycle of denaturation was set for 2 min at 55 ^{o}C followed by 33 cycles of denaturation for 1 min at 55 ^{o}C, annealing of 63 ^{o}C for 2 min, and an extension for 2 min at 72 ^{o}C. The amplified DNA samples were visually analysed via the fluorescent dye on Genetic Analyzer 3130xl labelled. The DNA samples were separated by capillary electrophoresis on an ABI 3013 automatic sequencer. The Gene Mapper 4.0 was used to decode the electrophrograms and the fragments were numerically scored.

4.2.3 Simple sequence repeat (SSR) marker analysis

Twenty simple sequence repeats (SSRs) selected based on previous published work on Bambara were used in this study. The forward and reverse primers for the markers are presented in Table 4.2.

Table 4.2: SSR markers and their forward and reverse primers

SSR marker	Forward primer	Reverse primer	Anealing Temperature ºC
P1	AACTTGCCATACGTGGAAGG 59	ACACGCTGCATAATTCACCA	59
P7	GTAGGCCCAACACCACAGTT	GGAGGTTGATCGATGGAAAA	55
P10	TCAGTGCTTCAACCATCAGC	GACCAAACCATTGCCAAACT	55
P15	AGGAGCAGAAGCTGAAGCAG	CCAATGCTTTTGAACCAACA	55
P16	CCGGAACAGAAAACAACAAC	CGTCGATGACAAAGAGCTTG	57
P19	AGGCAAAAACGTTTCAGTTC	TTCATGAAGGTTGAGTTTGTCA	55
P21	CAAACTCCACTCCACAAGCA	CCAACGACTTGTAAGCCTCA	57
P23	CAGTAGCCATAATTTGCTATGAACA	CGAATCACCATTCAATACGC	55
P30	AATGCAAGATTTTGGCTTGG	CCCACTCAAACCATACACCA	59
P31	GCTAAGGTGGAGTGGTGGAA	CAATCATCTTTTGCGCTTCA	57
P32	TTCACCTGAACCCCTTAACC	AGGCTTCACTCACGGGTATG	57
P33	ACGCTTCTTCCCTCATCAGA	TATGAATCCAGTGCGTGTGA	57
P37	CCGATGGACGGGTAGATATG	GCAACCCTCTTTTTCTGCAC	55
P44	TGTGGGCGAAAATACACAAA	TCGTCGAATACCTGACTCATTG	59
Pco	GAGTCCAATAACTGCTCCCGTTTG	ACGGCAAGCCCTAACTCTTCATTT	59
D8	GCATCTTTACAGCAAGAGTTTCAA	TGGATCTTCCTCATTGCAGTATAA	59
D11	GAGGAAATAACCAAACAAACC	CTTACGCTCATTTTAACCAGACCT	59
D14	GAACGAAGCCAGGATAATGATAGT	CGAAAGCGACAACTCACTACTAAA	59
D15	TGACGGAGGCTTAATAGATTTTTC	GACTAGACACTTCAACAGCCAATG	59
E7	CATGATTTGTTGTGATGATGAT	AACAACAAATGTACCAAAGAATCG	51

4.2.4 Data analysis

The fragment lengths derived from the Gene Mapper 4 were compiled in Excel and analysed in GenAlex version 6.5 following Peakall and Smouse, (2007). The total number of alleles per locus (Na), number of effective alleles per locus (Ne), Shannon's information index (I), observed heterozygosity (Ho) and gene diversity (He) were computed based on the formulae presented by Nei and Li (1979). For each microsatellite, the polymorphic information content (PIC) was calculated as $PIC = 1 - (\sum_1^n pi^2)$ where pi is the frequency of i^{th} allele. The genetic dissimilarity coefficients among the 19 genotypes were calculated and used to generate a dendrogram based on the unweighted pair group method with arithmetic mean (UPGMA) in DARwin 6.0 (Perrier and Jacquemoud-Collet, 2006).

4.3 Results

4.3.1 Polymorphisms of the SSR markers

There were 101 alleles (Na) identified in the markers (Table 4.3). The number of alleles per marker ranged from 1 to 12 with a mean of 5.05 alleles per polymorphic marker. Markers P32 and P15 had the highest number of alleles, with 12 and 10 alleles, respectively. The number of effective alleles (Ne) was 67.6 with a mean of 3.38 effective markers per locus. The markers revealed substantial heterozygosity in the lines with both expected and observed heterozygosity closer to 0.60. The markers exhibited considerable polymorphic information content (PIC) with an average of 0.57. The markers with the lowest PIC values were P22 and D8, concomitant with their low number of effective alleles and heterozygosity while markers P32 and P15 were the most informative with the highest PIC values of 0.88 and 0.87, respectively. In total, 80% of the markers had high PIC values above 0.5. Markers P31, P44, P21 and D8 were less informative as they had PIC values below 0.5.

Table 4.3: Genetic parameters as revealed by the 20 SSRs marker on the 19 genotypes

Marker	Na	Ne	He	uHe	I	PIC
P1	3.00	2.19	0.54	0.56	0.86	0.54
P7	4.00	2.7	0.63	0.65	1.12	0.63
P10	9.00	4.85	0.79	0.82	1.9	0.79
P15	10.00	7.76	0.87	0.89	2.17	0.87
P16	4.00	3.03	0.67	0.69	1.17	0.67
P19	9.00	5.69	0.82	0.85	1.94	0.82
P21	1.00	1.00	0.00	0.00	0.00	0.00
P23	2.00	2	0.5	0.51	0.69	0.5
P30	5.00	2.71	0.63	0.65	1.19	0.63
P31	2.00	1.11	0.1	0.1	0.21	0.1
P32	12.00	8.6	0.88	0.91	2.33	0.88
P33	4.00	2.67	0.63	0.64	1.11	0.63
P37	5.00	2.76	0.64	0.65	1.24	0.64
P44	2.00	1.23	0.19	0.19	0.34	0.19
Pco	7.00	5.6	0.82	0.84	1.83	0.82
D8	1.00	1.00	0.00	0.00	0.00	0.00
D11	3.00	2.31	0.57	0.58	0.94	0.57
D14	8.00	4.69	0.79	0.81	1.77	0.79
D15	6.00	3.62	0.72	0.74	1.51	0.72
E7	4.00	2.11	0.53	0.54	0.95	0.53
Mean	5.05	3.38	0.57	0.58	1.16	0.57
Standard Error	0.71	0.49	0.06	0.06	0.16	0.06

Na = number of different alleles, Ne = number of effective alleles, I = Shannon's diversity index, He =expected heterozygosity, uHe = unbiased expected heterozygosity, PIC=polymorphic information content

4.3.2 Cluster and principal coordinate (PCoA) analyses of SSR markers

The UPGMA cluster analysis based on genetic dissimilarity using the neighbour-joining method grouped the 19 genotypes as shown in Figure 4.1. The genotypes showed genetic variation, while genotypes Light brown and Brown, and Uniswa-Red R and Uniswa-Red G showed close relatedness.

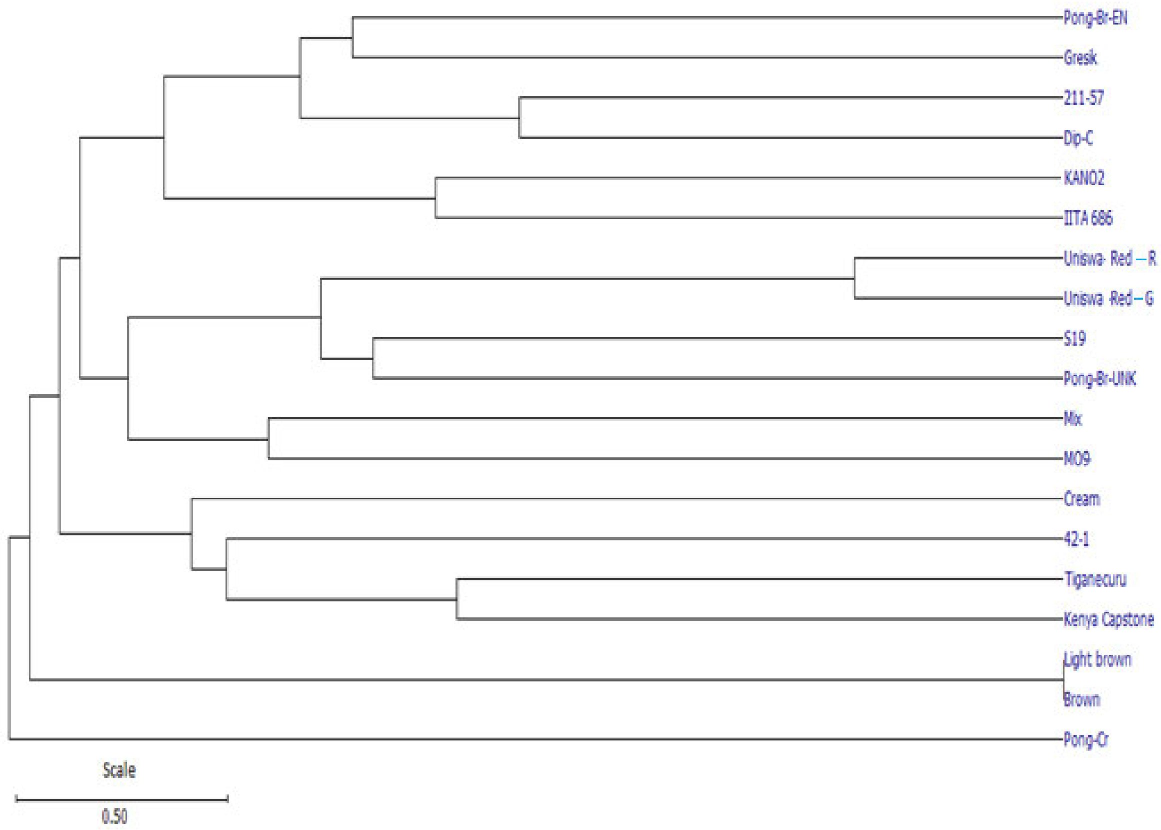

Figure 4.1: The neighbour joining UPGMA dendogram showing the relationship among the 19 Bambara lines

The resultant genetic distances showed that the longest genetic distances were 0.60 between Tiganecuru and S19 while the shortest was zero between Brown and Light brown lines (Table 4.4). The average genetic distance between all the lines was 0.38.

Table 4.4: Genetic distances among 19 Bambara groundnut genotypes assessed based on 20 SSR markers

	Pong-Br-EN	KANO2	211-57	Mix	Light brown	Cream	Uniswa-Red R	S19	Brown	Tiganecuru	Pong-Cr	42-1	Gresik	Dip-C	Uniswa Red-G	MO9	Pong-Br-UNK	Kenya Capstone	IITA
Pong-Br-EN	0																		
KANO2	0.49	0																	
211-57	0.28	0.53	0																
Mix	0.18	0.45	0.38	0															
Light brown	0.24	0.53	0.4	0.3	0														
Cream	0.28	0.47	0.36	0.3	0.38	0													
Uniswa Red-R	0.38	0.53	0.4	0.3	0.4	0.4	0												
S19	0.52	0.49	0.48	0.5	0.48	0.44	0.44	0											
Brown	0.24	0.53	0.4	0.3	0	0.38	0.4	0.5	0										
Tiganecuru	0.48	0.45	0.52	0.5	0.52	0.46	0.56	0.6	0.52	0									
Pong-Cr	0.42	0.53	0.44	0.4	0.48	0.54	0.48	0.5	0.48	0.48	0								
42-1	0.37	0.52	0.43	0.3	0.37	0.3	0.43	0.5	0.37	0.39	0.47	0							
Gresik	0.34	0.45	0.32	0.3	0.4	0.48	0.4	0.5	0.4	0.52	0.36	0.53	0						
Dip-C	0.24	0.49	0.28	0.3	0.4	0.38	0.48	0.5	0.4	0.52	0.48	0.41	0.36	0					
Uniswa Red-G	0.36	0.53	0.36	0.3	0.4	0.36	0.04	0.4	0.4	0.52	0.48	0.39	0.4	0.44	0				
MO9	0.38	0.57	0.34	0.3	0.5	0.34	0.48	0.5	0.5	0.5	0.44	0.41	0.46	0.48	0.46	0			
Pong-Br-UNK	0.36	0.49	0.4	0.3	0.44	0.48	0.32	0.4	0.44	0.52	0.4	0.47	0.36	0.52	0.32	0.4	0		
Kenya Capstone	0.44	0.53	0.48	0.4	0.48	0.4	0.4	0.5	0.48	0.4	0.44	0.43	0.48	0.48	0.36	0.5	0.48	0	
IITA686	0.35	0.44	0.33	0.3	0.53	0.43	0.45	0.5	0.53	0.49	0.37	0.48	0.25	0.37	0.41	0.47	0.41	0.45	0

The principal component analyses (PCoA) based on the Eigenvalues and the cumulative percentage of the principal component (PC) scores was conducted. The first two PCs of the PCoA accounted for a cumulative variation of 38.83% (Figure 4.2). The SSR markers separated the lines into three different quadrants, although one genotype (Uniswa Red-R) was placed in its own quadrant. The distribution of the genotypes into the three main clusters was not homogeneous. The Uniswa Red-R and Uniswa Red-G genotypes were clustered in different quadrants despite the short genetic distance between the genotypes. In contrast, Pong-Br-UNK, Pong-Br-EN and Pong-Cr were clustered in different quadrants concomitant with the large genetic distance separating them. The genotypes in the third quadrant exhibited the shortest distance while genotypes IITA686 and Brown exhibited the highest dissimilarity.

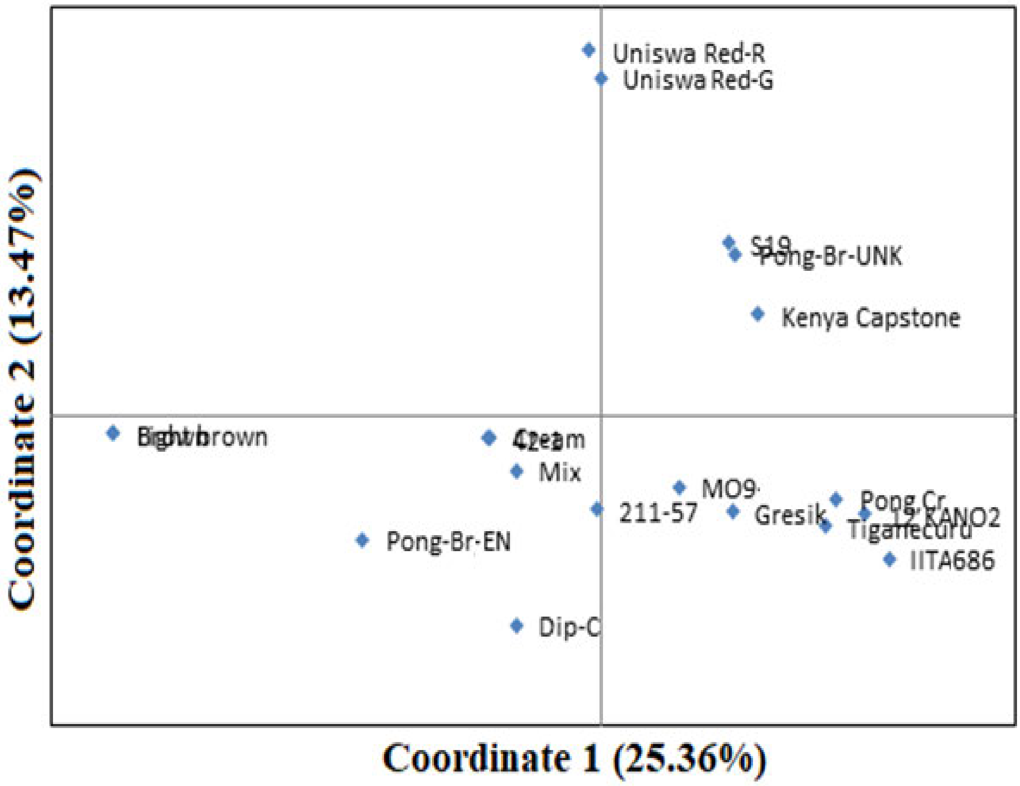

Figure 4.2: Principal coordinate analysis showing clustering of the different genotypes based on the genetic distance

4.3.3 Morphological variability analysis

The mean performance of the genotypes was assessed over two environments in two seasons (Table 4.5). The emphasis was on the overall performance and not the inherent variation in genotype performance over different locations or seasons. The lines showed significant variation in their agronomic performance especially for yield, pod mass, leaf number and leaf diameter. The chlorophyll content and stomatal conductance were significantly different among the lines, but both showed narrow genetic variation. The flowering period and maturity duration were not significantly variable among the genotypes. The highest seed mass of 104 g $plant^{-1}$ was obtained in line IITA686 followed by Mix (78.1 g $plant^{-1}$) and Brown (76.7 g $plant^{-1}$) while Pong-Br-UNK and Pong-Cr attained less than 25 g $plant^{-1}$.

Table 4.5: Mean agro-morphological characteristics of the 19 Bambara lines used in this study

Line	PH	EMG	LN	MDL	MDW	CD	RDM	PDM	SDM	SNP	HSP	SM
211-57	15.10	86.50	28.80	6.70	1.40	32.20	37.30	117.10	79.70	60.50	38.60	44.90
42-1	16.40	84.90	29.20	6.30	1.70	31.10	45.30	107.70	62.20	53.90	42.00	44.10
Brown	15.20	88.90	25.20	6.40	1.80	28.90	20.10	68.40	48.30	73.00	59.60	76.70
Cream	16.10	82.80	25.30	6.60	2.00	30.20	17.10	60.40	43.30	102.10	99.10	63.80
DIP-C	14.60	72.80	20.40	6.20	1.80	27.80	18.30	52.50	35.80	55.90	48.60	35.20
Gresik	14.80	76.50	25.30	6.80	1.80	29.80	23.10	81.60	58.50	57.60	55.70	68.70
IITA686	15.30	74.40	22.70	6.20	1.90	29.90	30.30	80.50	50.20	90.00	69.80	104.00
KANO2	14.60	82.00	26.20	6.90	1.90	28.90	35.50	117.00	81.40	86.70	65.60	75.60
Kenya Capstone	17.00	72.80	26.50	6.60	1.80	30.30	29.90	129.90	100.10	79.40	70.60	63.30
Light brown	14.90	79.00	22.30	7.10	1.80	30.90	20.10	60.80	40.70	50.60	36.30	51.30
Mix	15.60	86.90	24.10	6.20	1.90	30.40	22.40	106.60	84.10	119.20	102.20	78.10
MO9	15.20	98.00	24.80	7.00	1.50	30.40	45.80	107.00	61.10	79.00	63.70	63.40
Pong-Br-EN	15.10	79.20	24.90	6.70	1.80	29.80	18.90	67.60	48.70	79.80	69.40	42.20
Pong-Br-UNK	14.40	85.40	26.40	7.20	1.80	30.40	18.70	55.60	36.90	49.40	38.70	20.50
Pong-Cr	14.20	82.70	23.20	6.50	1.80	29.40	20.70	70.70	50.00	64.50	52.30	21.30
SI9	15.00	85.20	23.80	6.60	2.00	29.90	19.30	75.70	56.40	96.00	71.70	62.80
Tiganecuru	14.20	45.10	25.10	6.40	1.60	29.40	17.20	44.10	26.80	62.40	45.70	47.00
Uniswa Red-G	14.90	78.40	26.50	6.60	1.90	29.70	28.90	113.90	85.10	64.80	49.20	75.00
Uniswa Red-R	15.90	64.70	28.10	6.80	2.00	30.50	18.90	80.20	61.40	110.10	89.10	69.50
Mean	15.18	79.27	25.20	6.62	1.80	29.99	25.67	84.07	58.46	75.52	61.47	58.28
Lsd (5%)	0.94	16.52	2.90	0.30	0.15	2.00	18.03	12.00	16.10	3.74	23.18	14.70
CV (%)	17.40	17.95	17.40	12.50	22.70	18.30	52.13	57.00	52.61	5.50	38.00	26.90

PH=plant height, EMG=final emergence rate, LN=leaf number per plant, MDL=mid-leaf diameter, MDW=mid-leaf diameter, CD=canopy diameter, RDM=root dry mass, PDM=pod dry mass, SDM=shoot dry mass, SNP=seed number per plant, HSP=healthy seed number per plot, SM=seed mass per plot

4.3.4 Selection of potential genotypes for improvement

The genotypes-trait biplot was constructed to show the relative performance of each genotype based on principle component analysis (Figure 4.3). Genotypes plotted closer to a trait vector were highly correlated with that trait while the vector length signified the relative performance in that trait. The best performing genotypes based on seed mass (SM) and shoot dry mass (SDM) traits were IITA_686, DIP-C, Brown and KANO2. These genotypes exhibited preferable agronomic traits such as early flowering, although they had low pod mass, canopy diameter and leaf numbers.

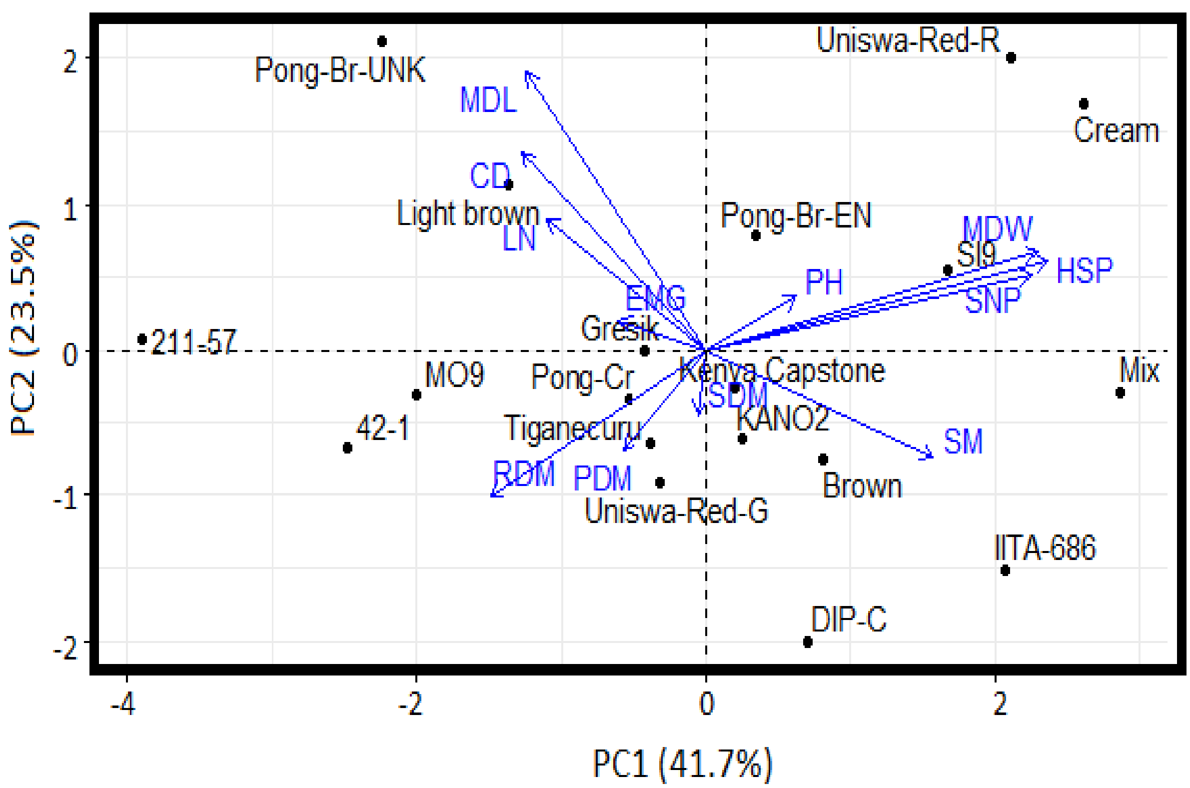

Figure 4.3: Genotypes-trait biplot showing the relative performance of each genotype based on principle component analysis.

4.4 Discussion

The number of effective alleles exhibited a wide range (1.0 to 8.6) showing that there could be essential allelic polymorphisms that contribute to genetic diversity. Similarly, Somta *et al*. (2011) found that the mean number of effective alleles in Bambara groundnuts to be 6.8. Other self-pollinating legumes were also found to have effective number of alleles between 6.0 and 9.2 (Blair *et al*., 2008). The number of effective alleles found in a panel of germplasm is also influenced by the number of SSR markers used. For instance, Wang *et al*. (2006) found a higher number of effective alleles using 60 SSR markers while Odongo *et al*. (2015) reported a lower number of effective alleles concomitant with lower number of SSR markers (12 SSR markers were used in their study). In essence, the effective alleles found in this study were adequate to highlight the genetic differences in the Bambara groundnut lines in the germplasm used. The mean number of effective alleles of 3.38 in this study was comparable to 3.64 reported by Rungnoi *et al*. (2012). The low number of effective alleles detected suggests that the number of SSR markers used in this study may have to be increased in order to discriminate a larger pool of germplasm. The lack of allelic diversity in the markers could constrain identification of variation in diverse germplasm comprised of accessions from different source populations. The differences in allelic diversity reported in different studies reflect differences in populations under investigation and the markers and choices of primers used.

The average expected heterozygosity (He) of 0.57 and polymorphic information content (PIC) of 0.57 obtained in this study showed that the available variation was relatively moderate similar to He (0.50) and PIC (0.42) values reported by Molosiwa *et al*. (2015) in Botswana. Over 60% of the markers in this study had PIC values above 0.60 showing that they had high ability to discriminate among the individual genotypes. Alternatively, the high PIC values are indicators of the available genetic diversity among the genotypes in the panel under investigation. The PIC is considered to estimate chances of detecting a specific allele in the offspring and has application in detecting marker linkage in heritability studies (Elston, 2005).

Genetic variation in a population may be attributed to the breeding system of the species and the ecological factors that influence transmission of genes among individual genotypes in the population. Ecological factors can lead to geographical isolation of populations promoting or restricting entry of new alleles into the gene pool. The breeding system of Bambara groundnut is highly autogamous (Nandino *et al*., 2015), which encourages inbreeding leading to lower observed heterozygosity relative to the expected heterozygosity. The lack of a significant difference between the observed and expected heterozygosity values obtained in this study signify that there could have been some random mating that occurred within this population. The mean Shannon index of 1.15 found in this study is concomitant with autogamous species, which indicates low variation due to inbreeding suppression. Comparison of individual markers revealed that markers P21 and D8 had the lowest Shannon indices of zero showing that they were either not useful or there was no allelic diversity at their loci or they were only detected on a single locus. The Shannon index is known to tend towards zero when there is no allelic variation (Glasenapp *et al*., 2015). Thus, markers such as P32 and P15 with high PIC, Shannon information index and allelic diversity have enhanced ability to distinguish different genotypes.

While the genetic variation revealed by the genetic parameters is important, the interrelationships among the genotypes is also essential in selecting parental lines for use in generating crosses. Selection of genetically distant and high performing genotypes for use in generating crosses can reduce inbreeding depression and increase achievable genetic gain. The clustering of the genotypes using the UPGMA method grouped the 19 genotypes into three distinct groups based on their genetic similarity showing that the genetic differences were significant. The genetic clustering did not conform to sources of collection showing that each genotype was unique and did not share similarities with localization. Although inbreeding may have occurred in genotypes collected from similar locations, the autogamous nature of the Bambara groundnut maintained the unique genetic constitution of individual genotypes. In addition, the genotypes collected from a single source may not have been separated long enough to evolve into a unique and distinct population. Genetic and morphological divergence occur due to reproductive isolation of a population in time and space (Worsham *et al*., 2017). Genotypes Light brown and Brown, and Uniswa-Red R and Uniswa-Red G showed close relatedness and could be due to the different nomenclature used during germplasm conservation. It is possible that they are the same genotypes with morphological divergence due to a mutation at a locus or a few genomic regions. The use of genetic markers is thus more reliable in ensuring that closely related genotypes are not used in hybrid development. The UPGMA and PCoA analyses were congruent in identifying Pong-BR UNK, Pong-Br-EN and Pong-CrPong-Cr as divergent genotypes by placing them in different clusters and quantifying large genetic distances among them. The similarity in nomenclature may have been influenced by morphological attributes but the underlying genetic composition showed that they are uniquely different genotypes. Other researchers have found divergence in genotypes that were morphologically or named similar (Mayes *et al*., 2019; Mohammed, 2014).

Differences in the genetic make-up of the lines contribute to variation observed in the morphological performance since agro-morphological performance is an aggregate of genetic and environmental interaction. The Bambara groundnut genotypes showed wide and significant variation in pod mass, leaf number and diameter, chlorophyll content, stomatal conductance and ultimately yield providing opportunities for selecting high performing genotypes for recommendation or and breeding. The poor performing genotypes can also be investigated for other attributes such as pests and disease reaction or be used in further genetic analysis for recessive alleles that may not be expressed in heterozygotes. Genotypes IITA686, Brown, Cream and KANO2 were selected as the best performing genotypes for further evaluation and development as pure lines for breeding purposes.

4.5 Conclusions

The current study found the existence of moderate genetic variability among the Bambara groundnut genotypes, which explained the observed morphological and physiological differences that could be exploited for agricultural use and Bambara groundnut improvement. The results revealed that 60% of the selected SSR markers were highly polymorphic and sufficiently distinguished the tested Bambara groundnut genotypes. The cluster analysis classified the 19 Bambara genotypes into three distinct genetic groups. Genotypes Light brown, Brown, Uniswa Red G, Uniswa Red R, Dip-C and Pong, Br, EN showed unique genetic patterns and relationships suggesting that they may have different genetic makeup from the rest of the genotypes. These can be used as sources of novel genes in Bambara groundnut improvement programs. Hence, the information generated will contribute significantly to Bambara improvement in South Africa.

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Chapter 5 Evaluating nutritional content among Bambara groundnut lines

Abstract

Bambara groundnut (*Vigna subterranea* (L.) Verdc) is a legume crop with potential to address food insecurity in sub-Sahara Africa. However, a lack of nutritional information hinders its full potential utilization. The objective of this study was to evaluate nutritional content in 19 Bambara groundnut lines. Ash, fat, proteins, starch, calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), phosphorous (P), sodium (Na), copper (Cu), and zinc (Zn), acid detergent fiber (ADF) and neutral detergent fiber (NDF) in the grain were analysed. The nutritional content varied significantly ($p<0.05$) among the lines with lines S19, Gresik, Pong-Br-UNK, Pong-Cr exhibiting the highest means for starch and protein ranging from 11.05 to 11.94%. Nutrients that showed high variation were starch, NDF, and protein, which had coefficient of variation of 32.08, 24.01, and 20.36%, respectively. Cu was the least abundant element, with an average mean of 6.05% across all the lines. The first principal component (PC) accounted for 21.9% of the variation and was strongly correlated with K (which contributed 20.1%), ash (19.5%), NDF (16.4%) and ADF (14.8%). The second PC explained 16.1% of the variation and was significantly correlated to moisture content (21.5%), Mg (19.7%), starch (17.3%) and protein (10.7%). This information is essential to select superior lines for developing breeding populations and lines for improving nutrition among poor households in SSA. The negative correlations among some of the nutritional content would be a challenge for simultaneous selection to breed nutritious Bambara groundnut lines. Further, an analysis of anti-nutrient chemicals is recommended.

Keywords: Bambara groundnut, correlation, food security, nutrition, utilization, variation

5.1 Introduction

A majority of South African households lack healthy food that contains essential nutrients, vitamins, minerals, and, most importantly, proteins. This has led to poor health and increased the proportion of the population facing food insecurity challenges in the country. Legumes are a source of dietary minerals and nutrients, such as carbohydrates, dietary fiber and proteins (Duke, 2012). The nutritional composition of legumes is generally higher than commodity cereal crops, which dominate the diets of many people in developing countries. Bambara groundnut (*Vigna subterranea* (L.) Verdc) is a legume that can provide essential nutrients and improve the dietary needs of food insecure households. The seed contains 65% carbohydrate, 18% protein 6.5% oil and essential minerals such as calcium, iron, potassium and sodium (Mazahib *et al*., 2013). These nutrients are necessary for the food insecure communities who cannot afford animal-based protein due to the high cost of meat. The per capita consumption of meat in sub-Sahara Africa is significantly lower at about 10 kg per year compared to 50 kg per year for part of Asia and over 90 kg per year for most first world countries (Alexandratos, 2015). In addition, Bambara groundnut is relatively more adaptable to environments with poor soils and low moisture availability and can still produce reasonable yield compared to commodity cereal crops such as maize, wheat or rice (Berchie *et al*., 2012). The ability of Bambara groundnut to tolerate drought and marginal soils makes it an ideal crop for production under smallholder agro-systems that are consistently challenged by lack of inputs and management. Despite the high nutritional content of Bambara groundnut grains, much of the work on Bambara groundnut improvement has concentrated on agro-morphological characteristics, and less focus has been paid to characterize the nutritional content of Bambara groundnut. Nutritional characterization will enable the recommendation of highly nutritious lines for production or use in breeding programs.

Crop improvement plays a considerable role in increasing production and ensuring food availability to meet the growing demand for food. The food supply deficit is widening due to rapid population growth and decreasing crop productivity due to climate change. The impact of climate has also adversely affected the nutritional quality of crops, which necessitates continuous assessment and identifying lines with superior nutritional composition (Lake *et al*., 2012). Small scale farmers use landrace seeds that have unknown and variable potential for nutrient accumulation. The farmers cultivate landraces due to a non-availability of improved cultivars because there are currently a limited number of breeding programs to develop improved cultivars. Most of the landraces available have been evaluated for agro-morphological traits and quality traits such as cooking or processing time. Thus, there is a need to assess Bambara for essential nutrients such as protein, starch, amino acids, zinc (Zn), and iron (Fe).

Proteins, starch and mineral elements are some of the essential nutrients required for balanced human diets. The variation in the nutritional composition of Bambara groundnut has been investigated using standard and established protocols at research laboratories. Studies conducted have shown that Bambara groundnut has the potential to be a supplementary food to provide balanced diets in food-insecure communities.

For instance, Akpalu. (2010) assessed five landraces for nutritional quality in Ghana and found that the landraces contained adequate protein, carbohydrate, fat and fiber to meet human dietary requirements. In addition to protein, Alake and Alake. (2016) found that Bambara also contained generous amounts of essential minerals such as Zn, magnesium (Mg), potassium (K), Fe and calcium (Ca). They further asserted that the Bambara lines could be improved to develop cultivars with high protein or mineral elements content. Amarteifio *et al*. (2010) evaluated nine landraces from Botswana, Namibia and Swaziland for ash, crude protein, crude fat, neutral detergent fibre (NDF) and acid detergent fibre (ADF), and found that the landraces from Botswana had more protein content compared to those from Namibia and Swaziland. Similarly, Atoyebi and Adebawo. (2017) found that 20 Bambara accessions could be grouped into high, medium and low content categories based on their protein, moisture, carbohydrate, crude fiber, total sugar, fat, starch ash and mineral contents. They further reiterated that the accessions in the low content group could be used as animal feed as they contained below the required levels for human diet. Nutritional content in Bambara groundnut would be expected to vary across different agro-ecologies, possibly because of genotype by environment interaction but the reports from other studies provide useful guidelines. Differences in nutritional contents among different Bambara groundnut genotypes have been reported depending on the germplasm, origin or environmental conditions. However, most studies focus on investigating a few nutrients in a limited number of genotypes sourced from a single source or from similar genetic backgrounds. Very few studies focused on a number of nutrients and mineral elements among divergent lines sourced from different countries and combining variable agro-morphological characteristics. This study focuses on different lines sourced from different countries to determine the nutritional content variation for selection of cultivars with an optimum nutritional content combination. The objective of this study was to evaluate nutritional content diversity among 19 Bambara groundnut lines.

5.2 Materials and methods

5.2.1 Seed Materials

The germplasm used used in this study was made up of 19 Bambara groundnut lines obtained from different sources (Table 5.1). The seeds were sourced from subsistence farmers in the Tugela Ferry (28°45’ S; 30°27’E) and Deepdale (30°33' S; 29°54' E) areas inKwaZulu-Natal in South Africa, and from the University of Nottingham in the United Kingdom (UK). The nutrient content was analysed in mature seed. The mature seeds were harvested from a previous field screening trial that was conducted in the 2019 planting season.

Table 5.1: List of Bambara groundnut lines used in the study showing their origin and seed coat colour.

Line	Source	Country of Origin	Seed coat colour
IITA686	University of Nottingham	Tanzania	Light brown
DIP-C	University of Nottingham	Botswana	Cream
S19	University of Nottingham	Namibia	Black
Uniswa Red-G	University of Nottingham	Swaziland	Dark red
Uniswa Red-R	University of Nottingham	Swaziland	Red
Kenya Capstone	Capstone Seed company	South Africa	Cream
Gresik	University of Nottingham	Indonesia	Black
Brown	Local Farmers	South Africa	Brown
Light brown	Local Farmers	South Africa	Light, brown
Cream	Local Farmers	South Africa	Cream
M09-4	University of KwaZulu-Natal	Zimbabwe	Brown
211-57	Capstone Seed company	South Africa	Dark cream
Tiganecuru	University of Nottingham	Botswana/Mali	White
Jan-42	University of KwaZulu-Natal	Zambia	Black
Mix	Local Farmers	South Africa	Mixture of colours
KANO2	University of Nottingham	Nigeria	Light cream
Pong-Br-EN	Local farmers	Unknown	Light brown
Pong-Br-UNK	Local farmers	Unknown	Light brown with sparkle
Pong-Cr	Local farmers	Unknown	Cream

5.2.2 Seed biochemical analysis

The chemical assay was conducted in three replicates for each line after oven drying. The dried seeds were ground into a fine powder using an electric blender. The fine powder was then sent for analysis at the analytical laboratory of the KwaZulu-Natal Department of

Agriculture and Rural Development at Cedara. The different nutrients were determined using various methods appropriate for each nutrient. The carbohydrates content was determined using acid hydrolysis method while starch was determined using enzymatic catalysis. The ash content was determined using a dry ashing technique. Crude protein was determined using the Dumas method. The Hunter method was used to quantify mineral content and the amount of fat was evaluated using the ether extraction method. Lastly, acid detergent fiber (ADF) and neutral detergent fiber (NDF) were deduced using the Van Soest method.

5.2.3 Data Analyses

Means, standard deviations, minimums, maximums, and coefficients of variation were computed for each nutrient trait. The data was subjected to analysis of variance (ANOVA) using GenStat® Version 18 (VSN International, United Kingdom) and genotype means separated at 5% probability level using the Fischers' Unprotected least significant difference. Subsequently, Pearson correlations, principal component, and hierarchical analyses were conducted to deduce trait and line associations using R software (R Core Team, 2019).

5.3 Results

5.3.1 Summary statistics of nutrients measured in 19 Bambara groundnut lines

The summary statistics revealed that ADF had a minimum value of 5.28, with a mean of 7.96 and a maximum of 13.35 (Table 5.2). Fat and ADF exhibited almost the same range of values. Maximum values for sodium and magnesium were less than 1% on average. Protein and NDF had mean values of 20 and 23%, respectively. The most significant constituents of Bambara groundnut seeds were starch (32.33%), and NDF (24.13%). The largest coefficients of variation were exhibited by Ca (126.34%) and Na (49.47%), showing that there was a high variation for these nutrient traits.

5.3.2 Mean concentration of mineral elements in 19 Bambara groundnut lines

All the lines exhibited significant differences in all the nutrients analyzed (Table 5.3). Lines S19, Gresik, Pong-Br-UNK, Pong-Cr had the highest means for starch and protein that ranged from 11.05 to 11.94 (Table 5.4). Conversely, lines 211-57, Light brown, Pong-Cr, and S19 had the lowest means for Ca. Lines Uniswa Red-G and Pong-Br-UNK exhibited the lowest means for Na and Mg whereas, lines S19, Pong-Cr, Light brown, Gresik, Pong-Br-UNK, and Tiganecuru had high means for starch, P, Zn, Mn, Ash, K and protein nutritional content.

5.3.3 Bivariate correlations of nutrient variables

The bivariate correlations revealed that the nutrients exhibited variable correlations amongst each other (Table 5.5). Ash was negatively correlated to ADF ($r=-0.52$, $p<0.05$) and NDF ($r=-0.51$, $r<0.05$), which showed that ADF and NDF had a negative impact on ash content. However, it was observed that K was positively correlated to ash ($r=0.82$, $r<0.01$), while NDF was positively correlated to ADF ($r=0.79$, $p< 0.001$). The strongest positive correlations among the mineral nutrients were observed between Fe and P ($r=0.53$, $p<0.05$), moisture and Mn ($r=0.53$, $p<0.05$) and protein and P ($r=0.53$, $p<0.01$) while NDF and K ($r=-0.52$, $p<0.05$) exhibited the strongest negative correlation. Moisture and Fe ($r=0.45$, $p<0.01$) showed the weakest positive correlations. In contrast, the weakest and negative associations were between moisture and K ($r=-0.45$, $p<0.05$). Fats, proteins, starch, Ca, Cu and Na were not significantly associated with any of the other nutrients

Table 5.2: Summary statistics of nutrients measured in 19 Bambara groundnut lines

Statistic	ADF	Ash	Ca	Cu	Fat	Fe	K	Mg	Mn	Moisture	NDF	Na	P	Protein	Starch	Zn (ppm)
Minimum	5.28	4.30	0.00	2.03	5.50	12.04	1.10	0.16	14.00	2.43	15.96	0.00	0.34	13.48	22.51	23.00
Mean	7.96	5.44	0.08	6.05	7.52	20.00	1.30	0.20	15.60	3.47	24.13	0.01	0.44	20.34	32.33	25.34
Median	7.94	5.53	0.06	6.06	7.54	17.09	1.26	0.20	16.00	3.40	22.88	0.01	0.43	20.21	33.00	25.01
Maximum	13.35	7.02	0.49	8.08	8.89	46.07	1.71	0.28	17.08	4.70	39.81	0.03	0.55	24.55	40.56	29.05
Quartile 1	6.77	5.09	0.04	5.07	6.96	15.08	1.19	0.19	14.02	3.01	20.09	0.01	0.41	19.26	29.33	25.00
Quartile 3	8.41	5.76	0.08	7.07	8.02	21.07	1.41	0.21	17.01	3.97	27.55	0.02	0.47	21.53	34.99	27.00
SEM	0.22	0.07	0.01	0.17	0.10	1.01	0.02	0.00	0.18	0.07	0.79	0.00	0.01	0.32	0.54	0.23
CV (%)	21.09	9.87	126.34	21.63	9.77	37.94	11.88	10.77	8.75	16.19	24.76	49.47	11.15	11.78	12.51	6.96
Skew	1.44	0.23	3.61	-1.28	-0.46	2.15	0.84	1.06	-0.10	0.41	1.07	0.05	0.22	-0.64	-0.47	0.64
Kurtosis	3.12	0.51	12.13	2.21	0.04	4.88	0.17	2.12	-1.79	-0.51	0.81	0.21	0.17	1.67	0.37	-0.15

CV=coefficient of variation, SEM=standard error of mean, Ca=calcium (%),Fe=iron(%), K=potassium(%), Mg=magnesium(%), Mn=manganese(ppm), P=phosphorous(%), Na=sodium(%), Cu=copper(ppm), Zn=zinc(ppm), ADF=acid detergent fiber (%)and NDF=neutral detergent fiber (%)

Table5.3: Mean squares and significance tests for 19 Bambara groundnut lines

SOV	df	ADF	Ash	Ca	Cu	Fat	Fe	K	Mg	Mn	Moisture	NDF	Na	P	Protein	Starch	Zn
Rep	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.10	0.00	0.00	0.10	0.00	0.00
Lines	18	8.6***	0.7***	0.03***	5.3***	1.5***	179.2***	0.07***	0.001***	5.3***	0.9***	101.6***	0.0***	0.01***	17.3***	50.8***	7.6***
Residual	36	0.10	0.10	0.00	0.00	0.10	0.00	0.00	0.00	0.30	0.10	4.80	0.00	0.00	0.30	0.00	1.00
Total	56	2.80	0.30	0.00	1.70	0.50	57.60	0.00	0.00	1.90	0.30	35.70	0.00	0.00	5.70	16.30	3.10
Mean		8.00	5.40	0.10	6.10	7.50	20.00	1.30	0.20	15.60	3.50	24.10	0.00	0.40	20.30	32.30	25.30
lsd (5%)		0.50	0.50	0.00	0.10	0.50	0.10	0.00	0.00	0.80	0.40	3.60	0.00	0.00	0.80	0.20	1.70
CV%		3.50	5.20	18.20	0.50	3.70	0.20	1.10	7.20	3.30	6.80	9.00	18.20	6.60	2.50	0.30	4.00
se		0.30	0.30	0.00	0.00	0.30	0.00	0.00	0.00	0.50	0.20	2.20	0.00	0.00	0.50	0.10	1.00

CV=coefficient of variation(%), LSD=least significant difference, SE=standard error, Ca=calcium (%),Fe=iron(%), K=potassium(%), Mg=magnesium(%), Mn=manganese(ppm), P=phosphorous(%), Na=sodium(%), Cu=copper(ppm), Zn=zinc(ppm), ADF=acid detergent fiber (%)and NDF=neutral detergent fiber (%)

Table5.4: Mean concentration of nutrients in 19 Bambara groundnut lines

Line	Ash	Fat	ADF	Ca	Mg	K	Cu	Fe	Moisture	NDF	Protein	Starch	Na	P	Zn	Mn
KANO2	5.70	5.75	8.20	0.09	0.21	1.38	7.06	16.06	3.06	24.75	19.92	36.75	0.02	0.43	27.03	16.03
211-57	5.91	7.92	7.77	0.02	0.20	1.41	7.06	16.06	2.87	23.90	20.01	28.66	0.00	0.46	25.02	14.02
42-1	5.65	8.39	6.06	0.05	0.18	1.23	7.06	15.06	3.57	17.69	18.71	33.00	0.01	0.40	25.02	17.02
Brown	5.85	8.03	8.09	0.08	0.21	1.32	5.06	12.06	3.56	26.13	21.08	34.86	0.01	0.43	25.03	17.03
Cream	5.25	7.76	7.91	0.48	0.19	1.22	6.03	14.03	3.02	21.80	21.60	31.35	0.01	0.43	25.01	14.01
DIP-C	4.41	7.93	13.28	0.07	0.21	1.14	5.05	17.05	4.10	35.86	13.57	31.84	0.02	0.39	25.03	15.03
Gresik	5.63	7.60	9.66	0.06	0.19	1.24	6.07	21.07	3.63	22.86	23.29	35.02	0.01	0.46	23.02	17.02
IITA686	5.46	7.04	5.51	0.07	0.21	1.42	8.06	19.06	4.34	17.32	24.44	29.29	0.02	0.51	25.02	17.02
Kenya Capstone	5.85	6.96	8.21	0.07	0.21	1.57	7.04	21.04	2.89	22.66	21.23	31.46	0.01	0.47	27.04	16.04
Light Brown	5.69	6.79	7.67	0.02	0.19	1.42	5.06	46.06	4.45	20.28	19.75	33.07	0.01	0.51	23.69	16.36
Mix	5.06	8.31	8.11	0.04	0.19	1.14	5.05	14.05	3.42	23.43	20.23	40.54	0.01	0.34	23.03	14.03
MO9	5.41	7.31	6.56	0.04	0.20	1.42	6.01	21.01	3.19	18.51	19.85	28.21	0.01	0.43	25.00	14.00
Pong-Br-EN	6.60	7.18	6.36	0.07	0.17	1.69	2.07	23.07	3.72	18.65	19.93	33.09	0.01	0.44	27.01	15.01
Pong-Br-UNK	5.06	8.73	8.22	0.06	0.18	1.25	7.07	17.07	4.09	28.47	20.35	35.47	0.00	0.45	23.04	17.04
Pong-Cr	4.79	6.76	8.68	0.02	0.19	1.12	7.05	29.05	3.78	25.93	21.40	32.85	0.01	0.42	26.35	14.69
S19	5.73	8.26	8.16	0.06	0.19	1.28	6.05	27.05	3.42	27.60	19.44	34.41	0.01	0.44	29.02	17.02
Tiganecuru	5.06	7.50	9.39	0.07	0.26	1.11	6.05	20.05	2.52	39.75	24.49	26.04	0.00	0.48	25.00	14.00
Uniswa Red -G	5.41	7.19	6.58	0.03	0.19	1.23	5.04	14.04	2.83	20.70	19.21	22.56	0.01	0.36	27.04	14.04
Uniswa Red-R	4.93	7.47	6.78	0.07	0.20	1.18	7.05	17.05	3.44	22.26	17.98	35.78	0.00	0.41	25.02	17.02
Lsd	0.46	0.46	0.46	0.02	0.02	0.02	0.05	0.05	0.39	3.61	0.84	0.15	0.00	0.05	1.69	0.85

LSD=least significant difference at 5% level, Ca=calcium (%),Fe=iron(%), K=potassium(%), Mg=magnesium(%), Mn=manganese(ppm), P=phosphorous(%), Na=sodium(%), Cu=copper(ppm), Zn=zinc(ppm), ADF=acid detergent fiber (%) and NDF=neutral detergent fiber (%).

Table5.5: Pearson correlation coefficients among nutrients of 19 Bambara groundnut lines

	Ash	Fat	ADF	Ca	Mg	K	Cu	Fe	Moisture	NDF	Protein	Starch	Na	P	Zn	Mn
Ash	-															
Fat	-0.18	-														
ADF	-0.52*	0.13	-													
Ca	-0.07	0.06	0.02	-												
Mg	-0.26	-0.3	0.31	-0	-											
K	0.82***	-0.4	-0.44	-0.1	-0.22	-										
Cu	-0.34	-0.1	-0.1	-0	0.23	-0.28	-									
Fe	0.13	-0.3	0.01	-0.3	-0.16	0.23	-0.1	-								
Moisture	-0.12	0.09	0.05	-0.2	-0.45*	0.07	-0.1	0.45*	-							
NDF	-0.51*	0.2	0.79***	-0.1	0.62**	-0.52*	0	-0.08	-0.19	-						
Protein	0.26	-0.2	-0.35	0.14	0.36	0.11	0.26	0.05	-0.19	-0.1	-					
Starch	-0.01	0.17	0.14	0	-0.37	-0.08	-0	0.02	0.38	-0.1	-0.15	-				
Na	-0.09	-0.2	0.36	0.14	-0.06	0.02	-0.2	-0.01	0.23	-0.1	-0.33	-0.02	-			
P	0.32	-0.3	-0.09	-0	0.26	0.42	0.26	0.53**	0.22	0.01	0.53**	-0.2	-0.2	-		
Zn	0.33	-0.4	-0.14	0	0.06	0.31	-0.1	0.04	-0.34	-0	-0.14	-0.26	0.26	-0.1	-	
Mn	0.18	0.11	-0.13	-0.2	-0.22	0.11	0.3	0.14	0.53*	-0.2	0.03	0.45	-0	0.33	-0	-

Ca=calcium (%), Fe=iron (%), K=potassium (%), Mg=magnesium (%), Mn=manganese (ppm), P=phosphorous (%), Na=sodium (%), Cu=copper (ppm), Zn=zinc (ppm), ADF=acid detergent fiber (%) and NDF=neutral detergent fiber (%).

5.3.4 Multivariate analysis

The first five principal components (PC) with Eigen values ≥1 explained about 71% of the variation in the nutrient composition among the 19 Bambara groundnut lines (Table 5.6). The first, second, third, and fourth PCs accounted for 21.9, 16.5, 13.9, and 11.6% of the variation, respectively. The traits that contributed the most to PC1 were K (20.1%), ash (19.5%), NDF (16.4%) and ADF (14.8%), each contributing more than the overall average of 13% to the variation explained by PC1. On the second PC, moisture, Mg, protein, and starch accounted for 10 to 21% each of the variation. Mostly P (which contributed 21.5%), Cu (13.4%), Zn (10.1%), and Mn (10.5%) contributed to the variation explained by PC3. For PC4, ADF, Fat, Fe, and Na contributed more than 13% each to the variation. Cu and Na contributed 29.9 and 21.8% of the variation attributable to PC5, respectively. Only Ca had no significant contribution on either PC, failing to contribute above the threshold of 10%, which was the average contribution if all the traits were to contribute equally.

Table 5.6: Principal components, the proportion of variance and contributions of different nutrients among 19 Bambara groundnut lines

	PC1	PC2	PC3	PC4	PC5
Eigen value	3.51	2.64	2.22	1.86	1.21
Proportion of variance	21.92	16.51	13.9	11.63	7.59
Cumulative variance	21.92	38.43	52.33	63.95	71.55
Ash	19.52	0.75	2.39	0.1	3.44
Fat	3.85	6.09	0.04	13.36	9.69
ADF	14.79	0.63	0.35	14.77	0.59
Ca	0.47	1.05	1.81	3.78	9.37
Mg	4.64	19.75	4.51	2.34	0.04
K	20.1	0.52	1.71	1.63	1.09
Cu	0.36	1.07	13.94	4.2	29.93
Fe	4.67	0.78	5.67	18.81	4.14
Moisture	1.28	21.46	5.72	4.36	0.28
NDF	16.4	1.68	2.83	5.84	5.46
Protein	2.58	10.71	10.73	4.23	0.02
Starch	0	17.28	0.92	0.83	0.36
Na	0.36	1.59	7.24	15.96	21.89
P	6.32	3.38	21.52	3.47	0.28
Zn	1.6	4.56	10.06	6.31	5.64
Mn	3.06	8.7	10.56	0.01	7.76

PC=principal component, Ca=calcium (%), Fe=iron (%), K=potassium (%), Mg=magnesium (%), Mn=manganese (ppm), P=phosphorous (%), Na=sodium (%), Cu=copper (ppm), Zn=zinc (ppm), ADF=acid detergent fiber (%) and NDF=neutral detergent fiber (%).

Figure 5.1 shows the multiple relationships among lines and traits. Lines whose vectors are plotted close to a vector for a particular nutrient are highly correlated to that nutrient, while the length of the vector for a line will estimate its mean for that specific nutrient. Thus, a line plotted with a long vector indicates that it has a high mean for the associated nutrient. Lines Gresik and 42-1 were closely associated with the vector for moisture content. Lines that were associated with protein were Kano-2, 211-57, Kenya-Capstone, while Pong-Br-UNK and Gresik were in close proximity to the vector for starch and Uniswa- Red-R and Mix associated with fat. The lines S19 and Pong-Br-EN were in close proximity to the vector for Fe. The other lines, such as MO9, were associated with P, while Mg and Ca correlated to lines Uniswa Red-G.

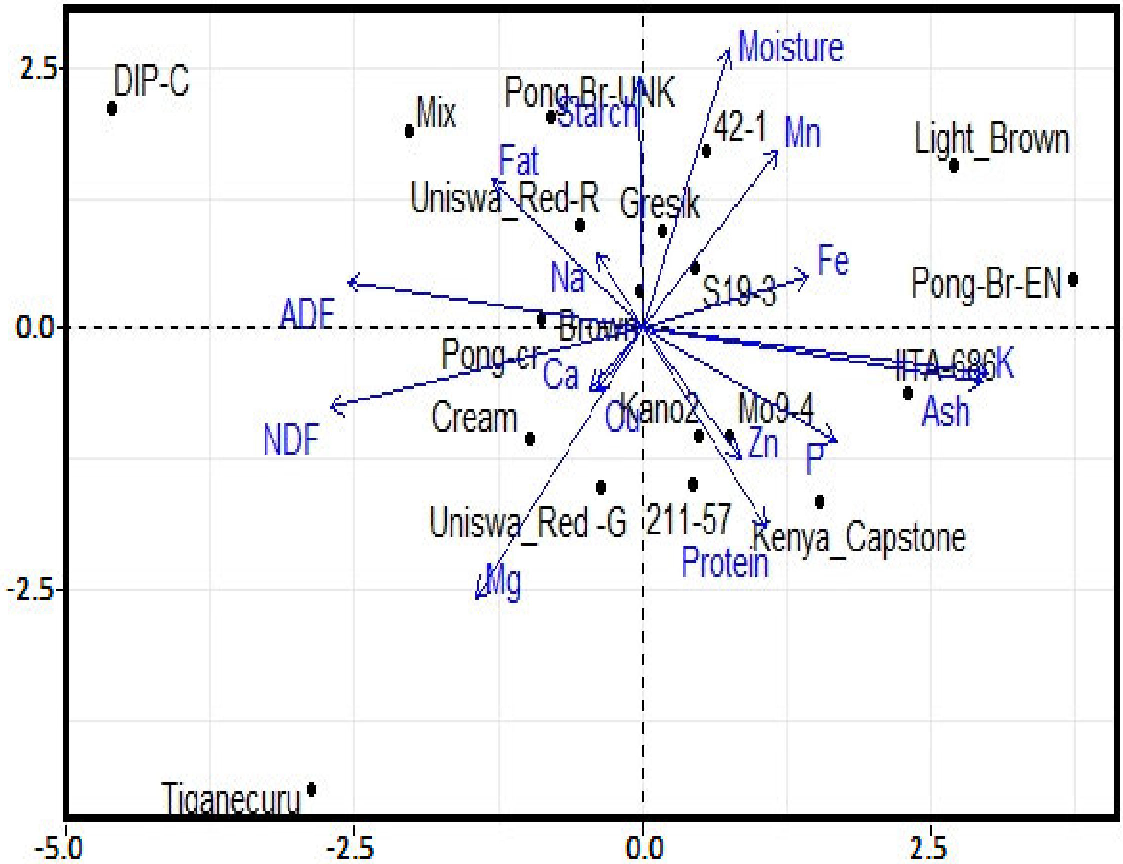

Figure 5.1: Multivariate associations depicting the line-trait relationship among the nutrient variables and 19 Bambara groundnut lines

The UPGMA dendrogram revealed that the 19 lines could be grouped into five distinct clusters based on similarity in their nutritional properties (Figure 5.2). There were two clusters that were comprised of a high number of lines than the rest of the clusters. The first cluster contained six lines, while the third cluster was the largest with nine lines. While the smaller clusters only contained two lines and one line each. The cluster that contained Mix, Pong-Br-UNK, 42-1, Gresik, Uniswa Red-R, and Brown was characterized by high mean values for starch, Na, fat, moisture Mn and ADF. The other cluster that contained Kenya Capstone, 211-57, MO9, KANO2, S19, IITA686, Cream, Uniswa Red-G, and Pong-Cr exhibited high mean values for, P, ash, NDF, Mg, Ca, Fe and protein.

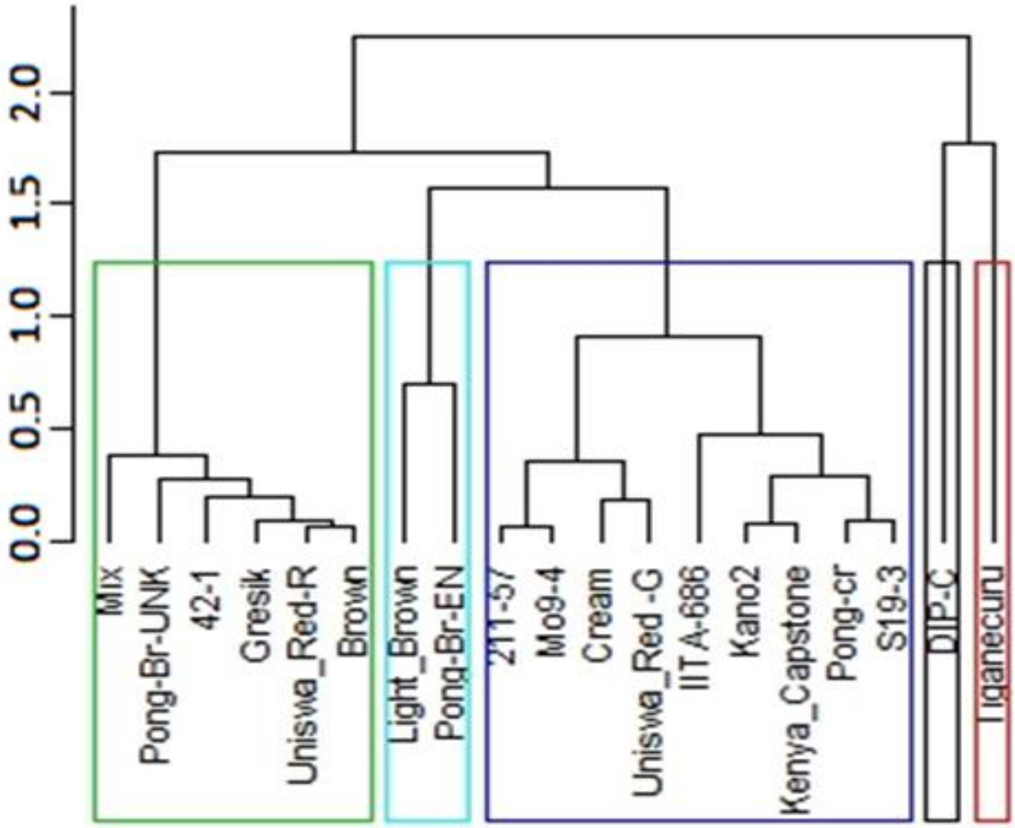

Figure 5.2: Dendrogram depicting the interrelatedness of 19 Bambara groundnut lines based on nutrient content

5.4 Discussion

5.4.1 Nutrient content variability in Bambara groundnut

The summary statistics results revealed that the nutrient concentrations varied among the 19 Bambara groundnut lines. Furthermore, the summary statistics showed that Bambara groundnut seed contained higher amounts of ADF, NDF and fat compared to the other nutrients. However, the higher coefficients of variation for Ca and Na showed that the lines exhibited a much wider variety in the accumulation of these nutrients compared to the others. Previous studies have reported variation in nutritional composition among

Bambara groundnut lines. For instance, Aremu *et al*. (2006) reported that Bambara groundnut accumulated protein 11.05%, carbohydrate of 11.56%, and 4.28% ash, which were comparable to those reported in this study. In contrast, Yao *et al*. (2015) found significantly higher values for Cu, Zn, and Mn than those found in this study. The differences in nutritional content reported in different studies emanate from variations in the evaluated Bambara groundnut germplasm and may be influenced by environmental conditions under which the germplasm was evaluated.

5.4.2 Genotypic variation in nutritional content

The significant differences in nutrient content exhibited by the lines open an opportunity for selection for cultivar recommendation or parental line selection during breeding for nutritional improvement. Line S19, Gresik, Pong-Br-UNK, Pong-Cr had the highest means for starch and proteins that ranged from 11.05 to 11.94%, respectively, indicating that Bambara groundnut can be utilized as a more affordable source of proteins and starch for energy for the food insecure households. Similarly, Kaptso *et al*. (2015) observed that Bambara groundnut flour contained appreciable amounts of proteins (24.0–25.5 g/100 g), carbohydrates (57.9–61.7 g/100 g) with marginal differences between two varieties. The results in this study further revealed that the highest means were for starch compared to the other nutrient traits, which substantiates the observation that Bambara groundnut is widely used for porridge and pap making, which are affordable staple meals that provide carbohydrates in some African countries (Swanevelder, 1998). Danbaba *et al*. (2016) evaluated different instant porridges formulated from broken fractions of rice blended with Bambara groundnut flour through extrusion cooking and reported that the optimum product contained high protein and minerals, and was appropriate for weaning to reduce protein malnutrition in Africa. The high content of NDF displayed by some of the lines shows that they can be adopted and improved for use as animal feed. Atoyebi *et al*. (2017) reiterated that Bambara groundnut lines with low nutritional content for human diets could be useful as animal feed. However, the low content for nutrients such as Na, P, Mg exhibited by some lines would be a cause for concern to prevent hidden hunger or low provision of essential elements. The low content in other vital nutrients and excessively high Cu content in Bambara groundnut was also reported by Amarteifio *et al*. (2006).

5.4.3 Associations among nutrient variables

The bivariate correlations revealed that ADF and NDF were negatively correlated to ash, which could be attributed to anti-nutrient properties of lignin, hemicellulose, cellulose, and silica that make up a large proportion of ADF and NDF (Ijarotim and Esho, 2009). Ash is required for human consumption, and lines that contain high ADF and NDF may not be suitable for human diets. The ADF and NDF are anti-nutrients that reduce the bioavailability of nutrients. The positive correlation between K and ash was expected since ash is made up of residual macro-nutrients (Ayeni *et al*., 2008). Bambara groundnut seeds that contained high levels of moisture had high levels of Fe and Mn but low Mg content. This negative correlation represents a challenge for simultaneous identification of lines with favorable content for all the nutrients based on moisture accumulation. However, the positive correlation between P and protein was beneficial for simultaneous selection. The positive correlation between P and protein is characteristic of protein-rich foods, which is essential for growth and development for young children (Barrere *et al*., 2006).

5.4.4 Multivariate associations among lines and nutrient variables

Five principal components (PC) with Eigen values ≥1 explained about 71% of the variation in the nutrient traits among the 19 Bambara groundnut lines. The first two PCs were the most important for explaining the variation. The nutrients that accounted for a high variation on PC1 were K, Ash, NDF, and ADF, indicating that differences in accumulation of these nutrients were the most discriminatory among the lines. The high contribution of these nutrients on the first PC shows that they must be used as target traits for evaluation and discriminating Bambara groundnut lines for characterization and conservation purposes. However, this importance is limited to characterization or conservation purposes and not significant for dietary requirements since all nutrients are equally crucial for diet. Moisture, Mg, protein, and starch were the second most important group of nutrients in terms of discriminating among the lines as they were highly correlated to the second PC. The lack of significant contribution by Ca shows that it was the least discriminatory among the nutrients probably because the lines contained almost similar amounts of Ca. Zhao *et al*. (2013) also found that mineral elements had different contributions to PCs, and the differences in the importance of traits on different PCs reported by various researchers can be attributed to differences in germplasm and experimental conditions.

Multi-variate approaches allow for simultaneous selection of lines with desirable performance in several traits, unlike the univariate methods that examine a single trait at a time (Flores *et al*., 1998; Montesinos-López *et al*., 2018). For instance, the lines KANO2, 211-57, Kenya-Capstone Pong-Br-UNK, Gresik Uniswa- Red-R and Mix were in close proximity to vectors of nutrients such as protein, starch and fat, showing that they can be selected for these nutrients either for a recommendation for production or as parental lines for breeding. Conversely, lines that were not in close proximity to a vector of any particular nutrient did not accumulate significant proportions of the nutrients. Lines such as DIP-C and Tiganecuru were not associated with any particular trait vector showing that these lines need to be improved in several nutrients. However, this does not imply that these lines cannot be used for other purposes. Lines that have below-average content of most nutrients could still be selected for recommendation or breeding if they have other desirable characteristics such as good cooking and processing qualities or low anti-nutrient elements such as phytic acids. The low P in these lines could potentially mean that they have low phytic acid since high P is responsible for forming the group of phosphate that chelates with protein and other nutrients, making them unavailable for human nutrition (Urbano *et al*., 2000).

The hierarchical clustering was consistent with the line-trait association biplot where Mix, Pong-Br-UNK, 42-1, Gresik, Uniswa Red-R, and Brown were clustered together based on their high starch, Na, fat, moisture Mn and ADF content. The other cluster that contained Kenya Capstone, 211-57, MO9, KANO2, S19, IITA686, Cream, Uniswa Red-G, and Pong-Cr exhibited high mean values for, P, ash, NDF, Mg, Ca, Fe and protein. Lines DIP-C and Tiganecuru, which had a high concentration of ADF and Mg, had long vectors and in close proximity relative to the vectors for these nutrients while Light brown and Pong-Br-EN were farther in terms of their association with the Fe vector. Clustering based on biochemical traits has been used successfully to identify divergence among lines (e.g. Song *et al*., 2011; Singh *et al*., 1989). The selection of lines for crossing should focus on divergent lines to avoid inbreeding depression while recommending a number of lines for production can spread the risk posed by biotic and abiotic stresses, especially in stress-prone environments of sub-Sahara Africa.

5.5 Conclusion

The nutrient content of the Bambara groundnut varies with genotypic differences among the lines. Genotypes Mix, Pong-Br-UNK, 42-1, Gresik, Uniswa Red-R, and Brown were clustered together based on their high starch, Na, Ca, fat, moisture Mn and ADF contents. These lines can be recommended for production or breeding to increase nutrient availability among poor communities who require cheaper sources of nutrients such as protein and carbohydrates. The lines that exhibited poor performances for nutritional content must be evaluated for other traits such as agronomic performance before they can be disregarded in breeding or production programs. The nutritional information obtained in this study provides baseline information for recommending Bambara groundnut for nutritional intervention. Also, it opens opportunities to investigate the bio-availability of the nutrient in human or animal diets.

5.6 References

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Chapter 6 Assessment of mineral element composition of diverse Bambara groundnut lines using electron miscroscopy

Abstract

Bambara groundnut (*Vigna subterranea* (L.) Verdc) has the potential to contribute to the food security status in many developing countries of sub-Sahara Africa. It is thus imperative to assess the nutritional composition of Bambara groundnut lines for recommendation and for identifying germplasm for crop improvement programs. Electron microscopy provides an alternative option for qualitative and quantitative assessment of nutrient content in plant matter. Thus, 19 Bambara groundnut lines were evaluated for micronutrient content and structural composition using electron microscopy. The microscopy showed that the lines were different in structural composition and quantified variations among the lines in micronutrient content. Carbon (C) and oxygen (O) were the most abundant nutrients, while sulphur (S) and phosphorous (P) were the least available nutrient in Bambara groundnut. Lines 211-57, Pong-Br-EN, Uniswa Red-G and Uniswa Red-R contained the highest concentrations of elements such as aluminium (Al), chloride (Cl), potassium (K), magnesium (Mg), nitrogen (N) and O. On the other hand, lines such as KANO2, DIP-C, IITA686 and Kenya Capstone were the poorest in terms of nutrient accumulation. The first and second PCs accounted for 31.18% and 19.82% of the total variation among the lines, respectively. The elements N (which accounted for 24.1%), C (19.9%), Cl (13.4%) and Mg (11.3%) accounted for the most variation explained by the first principal component (PC). On the second PC, K and S accounted for 39.9% and 41.8% each of the variation. The lines with high content for multiple nutritional elements such as 211-57, Pong-Br-EN and Uniswa Red-G were

recommended for production, while the poor nutrient accumulators can be assessed for other important attributes such as forage.

Keywords: Bambara groundnut, food security, micronutrient, microscopy, mineral elements

6.1 Introduction

Production of crops in adequate quantity and suitable quality is essential to ensure food security. The major thrust of crop production in many systems has centred on increasing quantity. However, it is necessary to evaluate the nutritional and mineral content of the cultivated crops to ensure that human dietary needs are addressed. Minerals form an essential part of the human diet and are required in a range of quantities (Gharibzahedi and Jafari, 2017). Minerals and nutrients for human diet are obtained mainly from crops. Some of the essential minerals required by humans are calcium, chromium, copper, iron, magnesium, manganese, molybdenum, potassium, sodium and zinc (Kitadi *et al*., 2020). These are essential and have diverse functions for human growth, development and health (Rutherfurd-Markwick, 2012). Therefore, there is a need to identify affordable and accessible sources of these essential minerals. Bambara groundnut (*Vigna subterranea* (L.) Verdc) is one of the crop species that has been identified for improving food and nutrition security in the SSA region due to its adaptation to low input agricultural systems (Mubaiwa *et al*., 2018). Sub-Sahara Africa is characterized by a wide distribution of moisture-constrained environments that are problematic for the production of staple crops such as maize, wheat and rice, leading to widespread food insecurity, especially among the rural population. Bambara groundnut contains appreciable amounts of minerals such as potassium (K), magnesium (Mg), phosphorous (P), iron (Fe), chlorine

(Cl), manganese (Mn), aluminium (Al) and calcium (Ca), making it suitable for mitigating hunger in rural communities (Abdulsalami and Sheriff, 2010).

Bambara groundnut has been defined as a famine staple crop with high potential to contribute to food and nutrition security in semi-arid areas (Cleasby *et al*., 2016; William *et al*., 2016). Despite strong assertions of its contribution to food security, the nutritional composition of Bambara groundnut is less documented. A substantial proportion of poor rural households suffer from hidden hunger due to a lack of essential minerals (Burchi *et al*., 2011). Hidden hunger is a state where people suffer from a disease caused by a lack of one or several trace elements in their diets. Protein and mineral deficiencies are common problems among children and pregnant women, resulting in wasting, stunted growth, anaemia, weight loss and compromised immunity. In developing countries, the use of fortified foods to mitigate malnutrition and mineral deficiencies has been largely ineffective due to their high cost and limited access by rural communities. There is a need to assess nutrient and mineral composition of Bambara groundnut to formulate appropriate and affordable intervention strategies for food security, crop production and Bambara groundnut improvement.

The variation in mineral composition of Bambara groundnut has been investigated using standard and established protocols. For instance, Amarteifio *et al*. (2006) evaluated Bambara groundnut landraces from three countries in SSA and found that they contained appreciable amounts of Ca, K, Mg, P and Fe. In a separate study, Olaleye *et al*. (2013) also found that the same elements in addition to sodium (Na) and zinc (Zn) were available in Bambara groundnut in sufficient amounts for human diet. Protein, fat, fiber and ash were also found in Bambara groundnut (Amarteifio and Moholo, 1998). However, most studies have used destructive sampling and combustion methods to quantify nutritional content in Bambara. While these methods are useful, they have the disadvantage of using large sample sizes. The use of microscopy in assessing nutritional content in Bambara groundnut has several advantages, including the need for single seed for analysis, being rapid, and allowing the seeds to be viewed in their natural state without grinding or combustion.

The scanning microscopy can detect differences in surface structure, reveal spatial differences in nutritional composition and provide qualitative analyses (Adebiyi *et al*., 2019; Kaptso *et al*., 2015; Silva *et al*., 2016). These properties allow scanning microscopy to produce topographical, morphological and compositional information that is not possible using the combustion procedures that only generate quantitative variables. Information generated from scanning microscopy is applicable in different fields of science including health based on structure, which affects bioavailability of nutrients, and in plant breeding by selecting lines with a favourable structure of nutrients. The rapid assessment provided by scanning miscroscopy and the need to evaluate a number of lines that were previously evaluated using the combustion procedure necessitated this study. Therefore, the objective of this study was to assess mineral element diversity among 19 Bambara groundnut lines using scanning electron microscopy.

6.2 Materials and methods

6.2.1 Plant Materials

Bambara groundnut germplasm used in this study was made up of 19 lines obtained from different sources (Table 6.1). The seeds were sourced from subsistence farmers from Tugela Ferry (28°45’ S; 30°27’E) and Deepdale (30°33' S; 29°54' E) in the KwaZulu-Natal province of South Africa, a private seed company Capstone Seeds and the University of Nottingham in the United Kingdom (UK). The nutrient content was analysed in mature seed material. The mature seeds were harvested from a previous field screening trial that was conducted in the 2018 planting season.

Table 6.1: List of Bambara groundnut lines used in the study showing their origin and seed coat colour.

Line	Source	Country of Origin	Seed coat colour
IITA686	University of Nottingham	Tanzania	Light brown
DIP-C	University of Nottingham	Botswana	Cream
S19	University of Nottingham	Namibia	Black
Uniswa Red-G	University of Nottingham	Swaziland	Dark red
Uniswa Red-R	University of Nottingham	Swaziland	Red
Kenya Capstone	Capstone Seed company	South Africa	Cream
Gresik	University of Nottingham	Indonesia	Black
Brown	Local Farmers	South Africa	Brown
Light brown	Local Farmers	South Africa	Light, brown
Cream	Local Farmers	South Africa	Cream
M09-4	University of KwaZulu-Natal	Zimbabwe	Brown
211-57	Capstone Seed company	South Africa	Dark cream
Tiganecuru	University of Nottingham	Botswana/Mali	White
42-1	University of KwaZulu-Natal	Zambia	Black
Mix	Local Farmers	South Africa	Mixture of colours
KANO2	University of Nottingham	Nigeria	Light cream
Pong-Br-EN	Local farmers	Unknown	Light brown
Pong-Br-UNK	Local farmers	Unknown	Light brown with sparkle
Pong-Cr	Local farmers	Unknown	Cream

6.2.2 Microscopy and Microanalysis Unit (MMU)

The mineral content and composition of different elements were evaluated under Zeiss EVO Scanning Electron Microscope (SEM) using Energy Dispersive X-Ray

Spectrotomy (EDX) technique. Three seeds of each line were cryo-fractured in liquid nitrogen and split into two halves. The seed halves were mounted on stubs and secured using a carbon double-sided insulating tape. The microscopy quantified the proportion of each element in percentage of the total mineral concentration in each sample. In addition, the microscopy produced surface and spatial images of the cross-section of each sample. The surface roughness and morphology of the cross-section of the seed were measured on area of 1 μm× 1 μm. Subsequently, the cross-section of the seeds was imaged SEM under the Zeiss EVO SEM in high vacuum mode at a scanning speed of 5 kV.

6.2.3 Data analyses

Means, standard deviations, minimums, maximums, and coefficients of variation were computed for the concentration of each mineral element. The data was subjected to analysis of variance (ANOVA) using GenStat® Version 19 (VSN International, United Kingdom) and means were separated at 5% probability level using the Fischers' unprotected least significant difference (LSD) at 0.05 significance level. Subsequently, the principal component analysis (PCA) was conducted to deduce multivariate and line-trait associations. The PCA was conducted using the "FactoMineR" package (Le *et al.*, 2008) in R software (R CoreTeam, 2019). The SEM images were compared for structural differences among the lines.

6.3 Results

6.3.1 Summary statistics of essential elements measured in 19 Bambara groundnut lines

The summary statistics showed that the minimum aluminum (Al) content across all the lines was 0.04%, with an average value of 0.76% and a maximum value of 1.74% (Table 6.2). Phosphorus (P) and sulphur (S) content was almost similar across the lines. On average, P, S and magnesium (Mg) content values were 0.87%. The mean values of chlorine and nitrogen were 11.24% and 10.42%, respectively. Carbon (C) with 58.06% and oxygen (O) with 38.42% were the most abundant constituents of Bambara groundnut seeds.

6.3.2 Mean concentration of essential elements measured in 19 Bambara groundnut lines

The Bambara groundnut lines exhibited significant ($p<0.001$) differences in Al, C, Cl and K, although the variation in C content was very narrow (Figure 6.1). The range in C content among the lines was between 54% recorded in line IITA686 and 60% recorded for line Brown content. Line KANO2 had the lowest levels of Cl content (0.23%) while 211-57 attained more than 12.39% Cl content. High concentration levels of element K were recorded for lines 211-57, DIP-C, Pong-Br-UNK and Uniswa Red-G.

Significant differences ($p<0.001$) among the Bambara groundnut lines were observed in Mg, Mo, P and S content (Figure 6.2). There was wide variation observed on the levels of Mg among the lines. Tiganecuru had the lowest Mg content (mean Mg content was <1%) than the other lines. The Mg contents of lines Brown, 211-57, Pong -Br-EN, S19, Pong-Br-UNK, Uniswa Red-G and Uniswa Red-R was almost similar at 1.03%. The Mo content for the majority of the lines ranged between 7.4 and 7.9%. The line Dip-C had the lowest molybdenum (Mo) content (0.65%), whereas 211-57 had the highest (7.95%). There were low variation P and S content among the lines. The P and S contents of the line's ranges were below 1%.

There were significant differences ($p<0.01$) observed among the Bambara groundnut lines for N and O contents (Figure 6.3). Most lines had roughly similar N concentrations ranging between 10 and 11%. In contrast, the average N content among lines IITA686, KANO2 and Kenya-Capstone was 7.4%. The oxygen content among the lines exhibited a narrow range of variation, with line 211-57 having the highest O concentration of 40.38% and Pong cr had the least (34.6%).

Table 6.2: Summary statistics of essential elements in percentage measured in 19 Bambara groundnut lines

Statistic	Al	C	Cl	K	Mg	Mo	N	O	P	S
Minimum	0.04	61.22	0.21	1.21	0.01	0.61	6.94	34.68	0.14	0.14
Mean	0.76	58.06	11.24	1.84	0.55	7.01	10.42	38.42	0.43	0.35
Median	0.45	58.3	11.71	1.69	0.28	7.57	11	38.73	0.45	0.32
Maximum	1.74	61.22	0.21	3.33	1.23	7.96	11.54	40.39	0.66	0.77
Quartile 1	0.34	57.17	11.32	1.52	0.22	7.44	10.86	38.01	0.35	0.23
Quartile 3	1.44	59.12	12.34	1.97	0.97	7.8	11.2	39.16	0.49	0.47
SEM	0.08	0.22	0.36	0.07	0.05	0.22	0.18	0.18	0.02	0.02
CV (%)	75.47	2.89	23.94	28.02	74.8	23.18	12.82	3.61	29.03	45.1
Skewness	0.6	-0.41	-3.67	1.55	0.39	-3.24	-1.75	-1.01	-0.18	1.03
Kurtosis	-1.27	-0.25	12.32	1.89	-1.52	9.88	1.41	0.83	-0.02	0.57

CV=Coefficient of variation SEM=standard error of mean, Al: aluminum (%); C: carbon(%), K: potassium(%); Mg: magnesium(%); Mo: molybdenum(%); N:nitrogen(%); O:oxygen(%); P:phosphorous(%); S:sulphur(%)

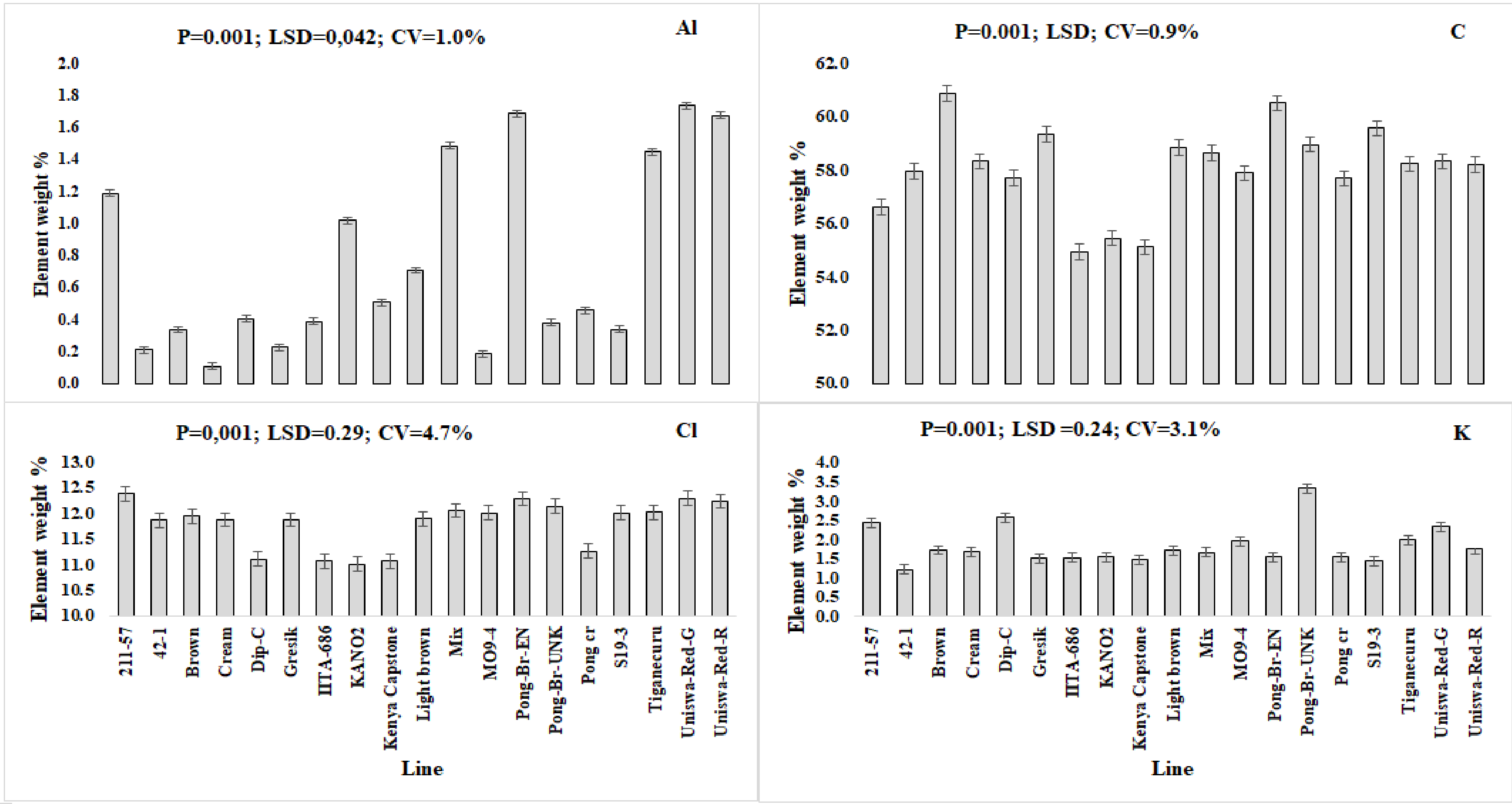

Figure 6.1: A comparison of aluminium, carbon, chlorine and potassium element weight % among 19 Bambara groundnut lines.

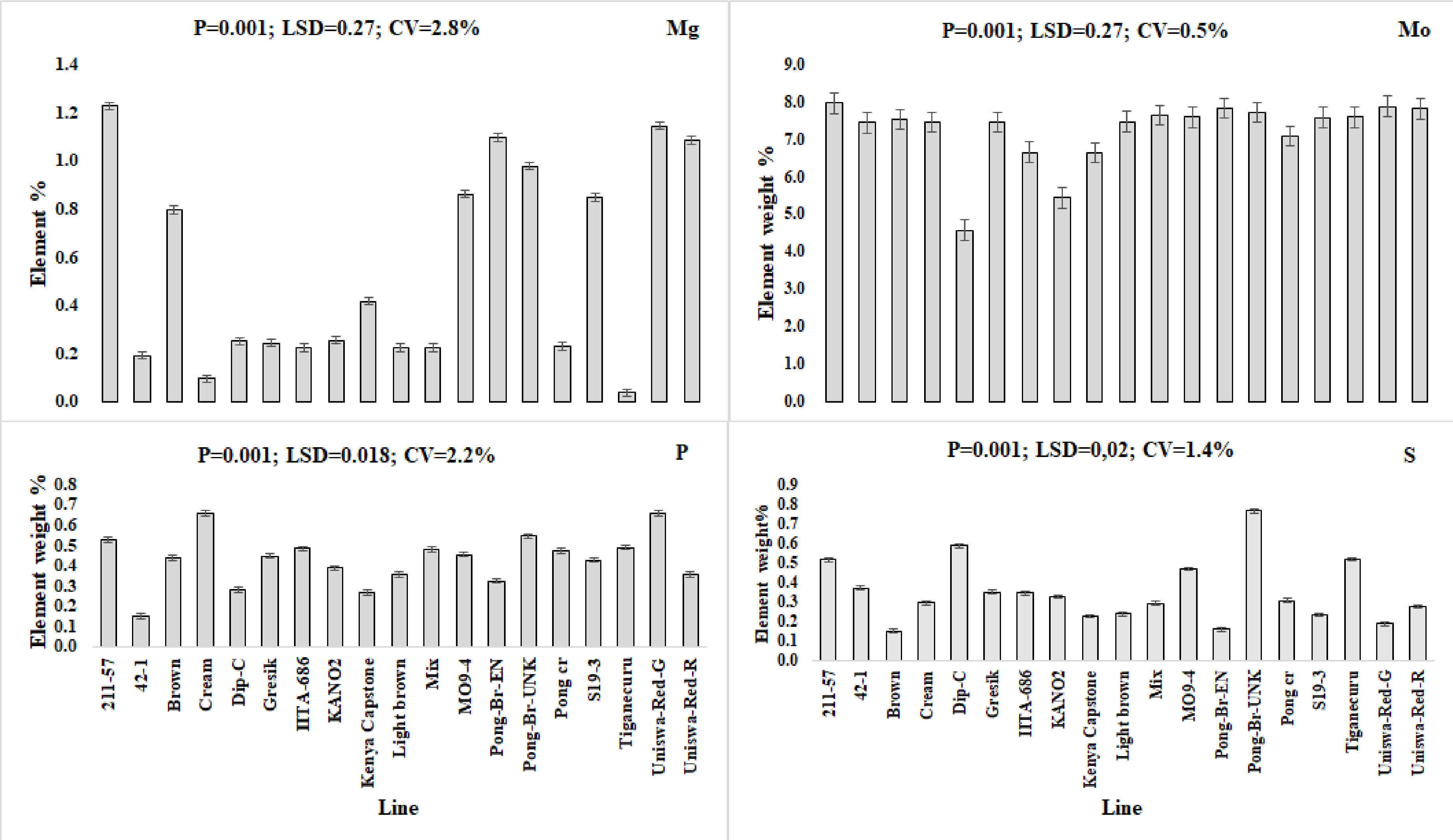

Figure 6.2: A comparison of magnesium, molybdenum, phosphorous and sulphur element weight % among 19 Bambara groundnut lines.

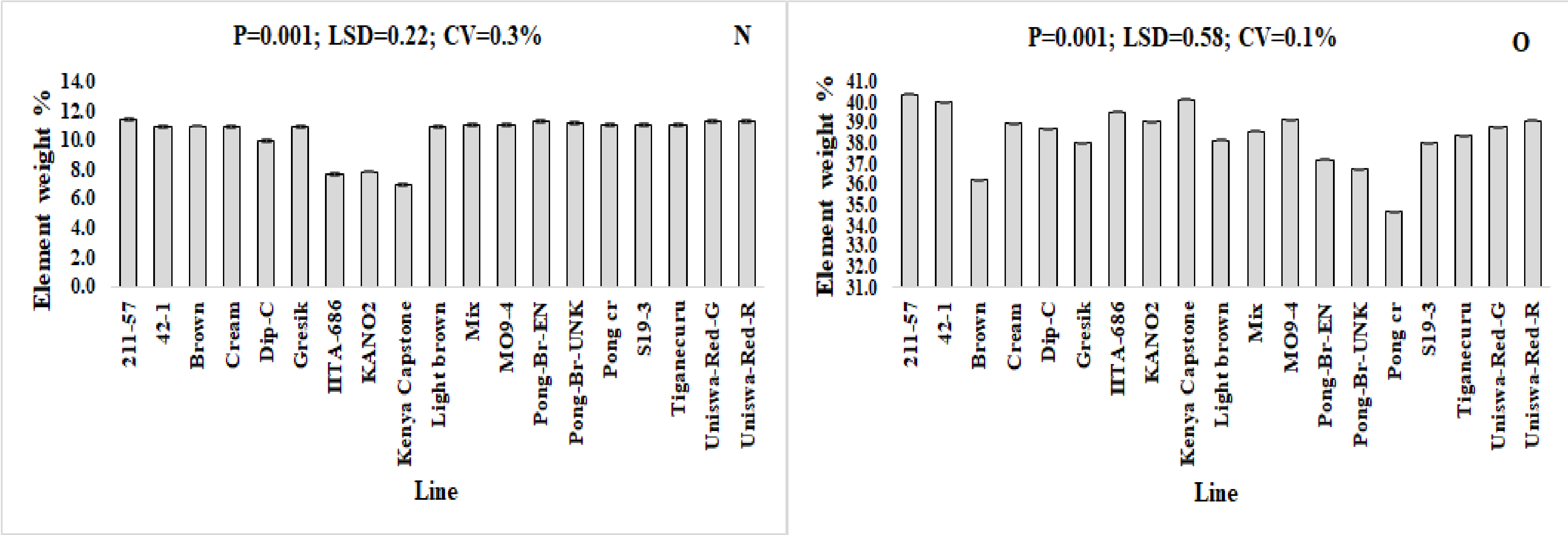

Figure 6.3: A comparison of nitrogen and oxygen element weight% among 19 Bambara groundnut lines.

The different lines were viewed under the scanning microscope and the scanned images showed variation in surface roughness and compositional structure of the lines. Figure 6.4 compares the lines KANO2 and IITA686, which had the lowest concentrations for most elements. The scanning microscope images of these two lines did not show distinct differences in surface roughness, which could be concomitant with similarities in their element composition. The elements were also visualized on a refractance spectrum, which showed that the elements in the two lines peaked at similar spectra (Figure 6.5).

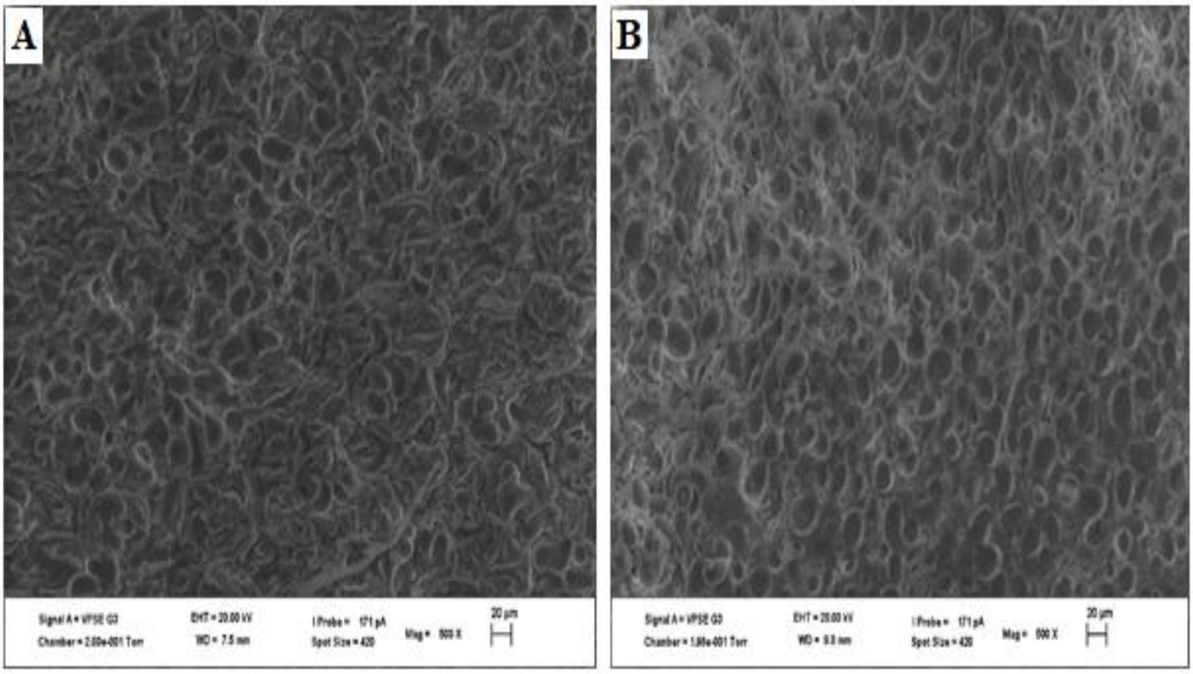

Figure 6.4: Images of (A) KANO2 and (B) IITA686 Bambara groundnut lines viewed under the scanning electron microscopy (SEM)

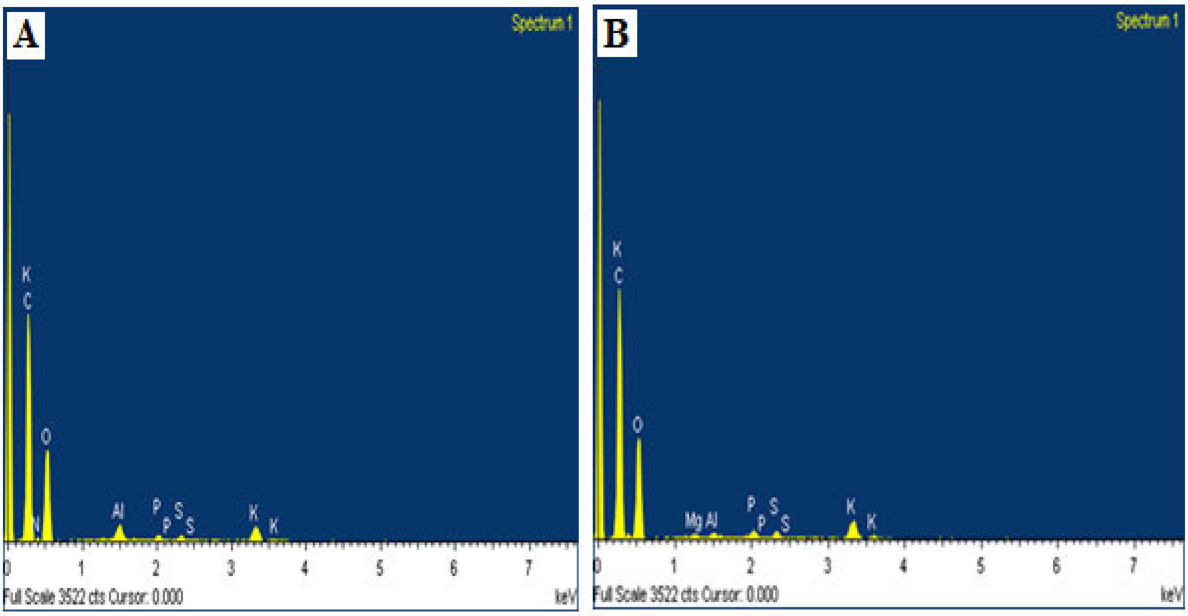

Figure 6.5: Spectrums of line (A) KANO2 and (B) IITA686 Bambara groundnut lines viewed under scanning electron microscopy (SEM)

There was a sharp contrast between KANO2 and IITA686 lines with the similar concentration of elements and lines such as Uniswa Red-R and UniswaRed-G, that had higher element concentrations (Figure 6.6). The surface roughness of Uniswa Red-R and Uniswa Red-G were desnse and rougher, showing a dense composition of mineral elements. The spectrum of elements for Uniswa Red-R and Uniswa Red-G lines shows similarities in their element composition (Figure 6.7) and a clear distinction from lines that had significantly lower element composition.

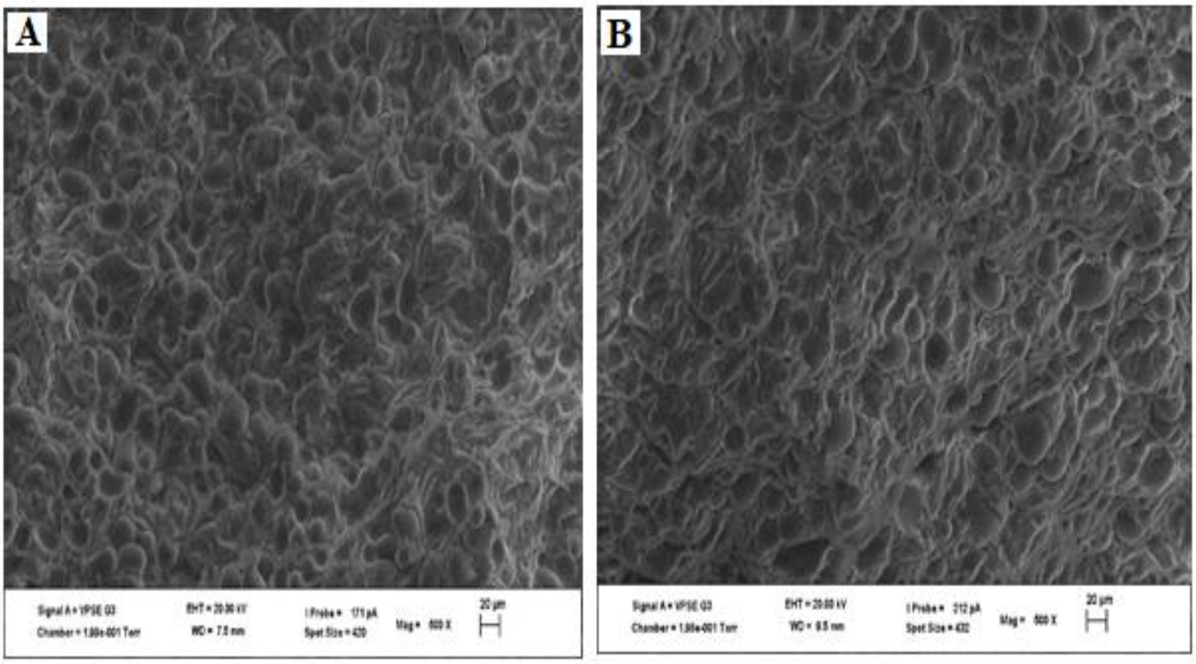

Figure 6.6: Images of line Uniswa Red-R (A) and Uniswa Red-G (B) Bambara groundnut lines taken under Scanning Electron Microscopy (SEM)

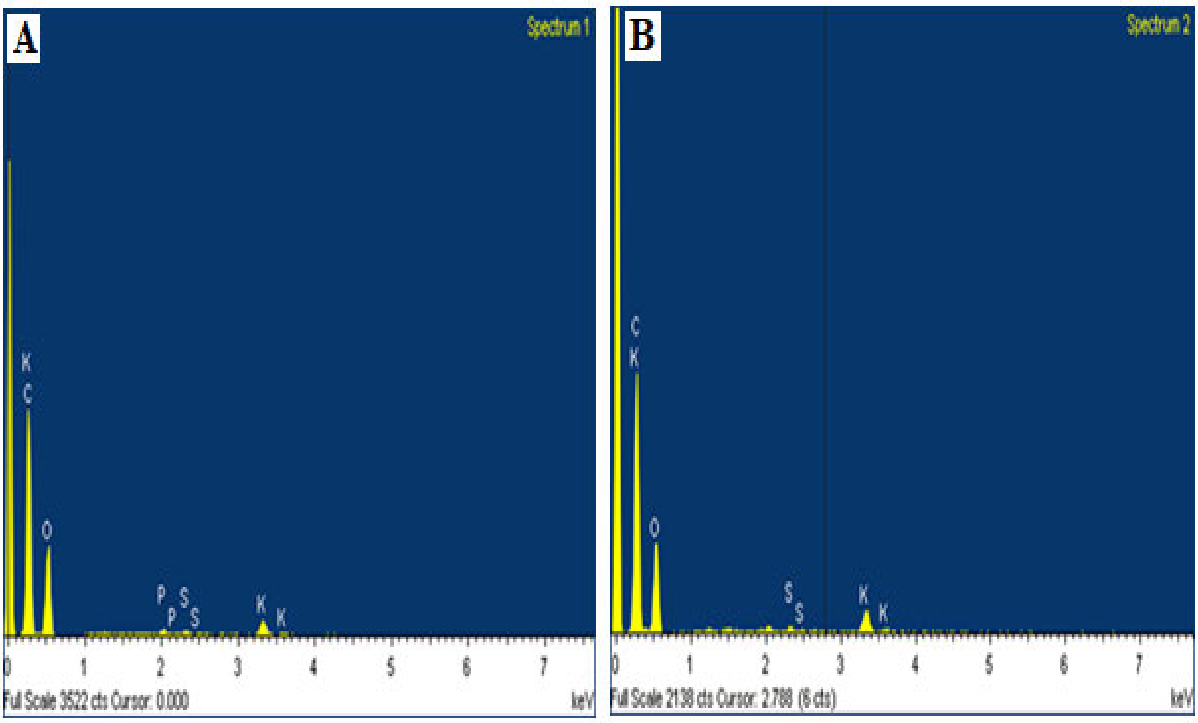

Figure 6.7: Spectra for UniswaRed-R (A) and UniswaRed-G (B) Bambara groundnut lines taken under Scanning Electron Microscopy (SEM)

6.3.3 Multivariate analysis

The mean element composition of the different lines was subjected to PCA to identify lines that had considerably high amounts of multiple elements. The PCA revealed that the first three principal components (PC) with Eigen values ≥1 explained about 66% of the variation in the element composition among the 19 Bambara groundnut lines (Table 6.3). The first PC accounted for 31.18% of the total variation followed by the second PC with 19.82% of the variation. Nitrogen accounted for 24.1% of the variation on the first PC followed by C (19.9%), Cl (13.4%) and Mg (11.3%). The variation on the second PC was dominated by S and K, which accounted for 39.9 and 41.8% of the variation on this PC, respectively. The third PC accounted for 14.72% of the total variation among the Bambara groundnut lines with Al (33.3 %), O (21.8%), Mg (17.4%), and C (10.7%) being the major determinants of variation on this PC. Elements such as Mo and P accounted for insignificant proportion of variation on the three PCs.

Table 6.3: Principal components, the proportion of variance and contributions of different elements among 19 Bambara groundnut lines

	PC1	PC2	PC3
Eigen value	3.12	1.98	1.47
Variance (%)	31.18	19.82	14.72
Cumulative variance (%)	31.18	51.01	65.73
Al	2.66	0.82	33.26
C	19.99	3.02	10.66
Cl	13.42	0.00	1.70
K	4.31	39.96	0.59
Mg	11.28	0.72	17.37
Mo	9.69	9.46	7.28
N	24.91	0.02	1.87
O	7.05	0.68	21.79
P	6.68	3.55	3.93
S	0.00	41.78	1.54

Ca=calcium (%), Fe=iron (%), K=potassium (%), Mg=magnesium (%), Mn=manganese (ppm), P=phosphorous (%), Na=sodium (%), Cu=copper (ppm), Zn=zinc (ppm), ADF=acid detergent fiber (%) and NDF=neutral detergent fiber (%).

Figure 6.8 shows the multiple relationships among lines and traits. Line and element vectors in close proximity signify a strong correlation between the particular line and the element. On the other hand, the length of the line vector estimates its mean for that specific element. Thus, a line plotted with a long vector indicates that it has a high mean for the associated element. Lines Pong-Br-UNK and 211-57 were closely associated with the vector for K. Lines that were associated with P, Mg, N, Cl were Tiganecuru and Uniswa Red-G while cream, KANO2 and DIP-C were in close proximity to the vector for O. The lines Pong-Cr, Brown, S19 and Pong-Br-EN, Uniswa- Red-R and Mix were in close proximity to the vector for Al, C and Mo. The elements S and K were associated with the line MO9. The other lines, such as, IITA686, Kenya Capstone, 42-1, Gresik and Light brown were not associated with any particular element vector.

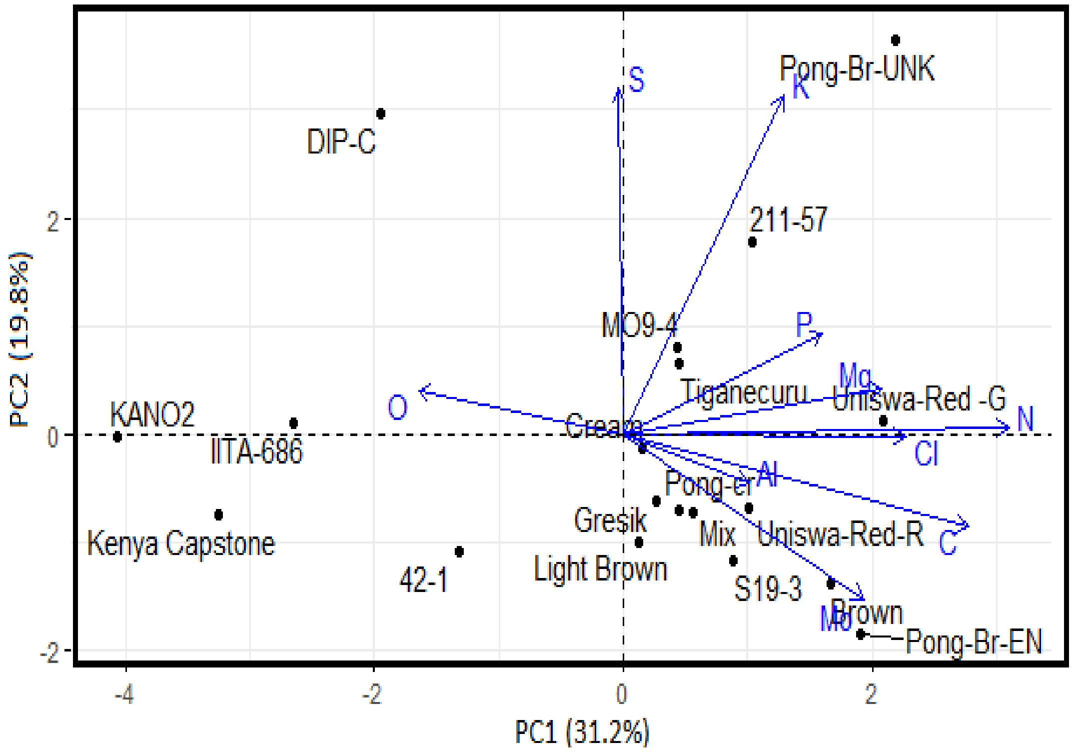

Figure 6.8: Multivariate associations depicting the line-trait relationship among the element variables and 19 Bambara groundnut lines

6.4 Discussion

6.4.1 Element content variability in Bambara groundnut

The summary statistics results revealed that the element concentrations varied among the 19 Bambara groundnut lines, signifying that there were significant genetic differences among the lines. Variation in element accumulation is concomitant with differences in genetic make-up of the Bambara groundnut lines that include accessions and landraces collected from different sources. The genotypic differences among the lines would allow for the selection of superior lines for recommendation in nutritional programs, or for crop production and Bambara groundnut improvement programs. Genetic variation among crop genetic resources is a basis for crop improvement (Upadhyaya *et al*., 2011) while differences in nutritional content will allow appropriate structuring of food security intervention strategies (Gross *et al*., 2000). Furthermore, the summary statistics showed that Bambara groundnut seed contained higher amounts of C, O and N compared to the other elements, which could be related to the high energy and protein content in Bambara groundnut. The high energy and protein content of Bambara groundnut promoted its use in the provision of energy and protein to vulnerable communities (Adzawla *et al*., 2016). The analysis also showed that there was wider variation in Cl accumulation among the lines, which would allow for selection of lines with suitable Cl content for human and livestock diets. Oyeleke *et al*. (2012) also reported that there was significant variation in mineral composition among Bambara groundnut seeds, and found that on average Bambara groundnut contained potassium with 1,70%, phosphorus 0,74%, magnesium 0,35%, and calcium 0,26%, which were comparable to those reported in this study.

6.4.2 Line variation in element content

The significant differences in element contents exhibited by the 19 Bambara groundnut lines open opportunities for selection for cultivar recommendation or parental line selection during breeding for nutritional improvement. Line 211-57 had high concentrations for most elements, namely Al, P, Cl, K, Mg, Mo, O, S and N, making it the most suitable line to supplement human dietary intake. Alternatively, 211-57 could be recommended for breeding purposes provided it is evaluated for other desirable characteristics such as high yield and agronomic performance. The differences in element concentrations in among the lines can be explained by the differences in genetic makeup and the origin of the line. Some studies have shown that landraces may surpass other biological types of Bambara groundnut in nutritional composition. For instance, Amarteifio *et al*. (2006) found that landraces grown in Swaziland were good sources of Ca, K, Mg, P and Fe compared to those grown in Botswana. Line 211-57 has shown that it has potential in mineral element accumulation, making it potentially highly nutritious. Major elements are considered crucial to the human diet and are required in relatively larger quantities compared to the minor elements. The high carbon content observed among all the 19 Bambara groundnut lines shows that the lines are good energy sources. Carbon is an integral component of seed biomass and carbohydrates required for cellular respiration to release energy (Hartmann *et al.,* 2018). Carbon, hydrogen, and oxygen make up carbohydrates, which are sources of energy for cellular processes. Oxygen and nitrogen were also abundant among the lines, which combine as building block for protein content. Like many other legumes, Bambara groundnut is grown for its appreciably high levels of protein for supplementing cereal-dominated diets of SSA countries (Duodu and Apea, 2017).

The majority of the lines had high levels of C, Cl, Mo, N, O and K making the lines good sources of these elements, which are vital for regulating blood pressure, digestion, energy production, and nerve signals (Soetan *et al*., 2010). Bambara groundnut has also been grown for medical purposes. The large concentrations of Cl, Mo, Mg, and K make Bambara groundnut a potentially important source of nutrients for populations in developing countries where balanced diets containing carbohydrates, protein and mineral nutrients are scarce. Soetan *et al*. (2010) also noted the importance of mineral elements in maintaining health, which would justify the inclusion of foods such as Bambara groundnut to avert hidden hunger among the poor populace.

Lines 42-1, brown, cream, DIP-C, Gresik, IITA686, KANO2, Kenya Capstone, Light brown, Mix, MO9, Pong-Br-EN, Pong-Br-UNK, S19, Tiganecuru, Uniswa Red-R, Uniswa Red-G were deficient in P and S making these genotypes poor in terms of supplementing dietary P and S requirements. Such lines will need to be improved through selection and breeding.

6.4.3 Multivariate associations among lines and element variables

The variation among the Bambara groundnut lines could be explained largely by three principal components, showing that most of the variation could be compressed into three integral variables. The elements that accounted for a high variation on the first PC were N, C, Cl and Mg, indicating that variation in the concentration of these elements provided a means for differentiating the lines. The high contribution of these elements on the first PC shows that they could be used primarily for selection among the Bambara groundnut lines for characterization or conservation purposes. The elements S and K were the second most important group of nutrients in terms of discriminating the lines as they accounted for much of the variation on the second PC. This means that genotypes that may not be discriminated using the first set of elements on the first PC could be differentiated by their difference in S and K accumulation. The third PC was correlated to Al, O, Mg and C and likewise would be useful for distinguishing the lines after using the elements associated with the first and second PCs. The elements Mo and P had non-significant contribution on any of the three PC, showing that the lines accumulated similar amounts of these elements. Similarly, Sarker *et al*. (2018) also found that some nutritional elements did not vary among 43 vegetable amaranth (*Amaranthus tricolor*).

Studies have shown that the use of multivariate analyses allow for better and simultaneous selection of genotypes with desirable performance in multiple characteristics (Linnen *et al*., 2013; Montesinos-López *et al*., 2018) making such tools more suitable in crop evaluation. The Bambara groundnut lines with high concentration of several elements would be ideal for addressing food and nutrition security. The lines Pong-Cr, Brown, S19 and Pong-Br-EN, Uniswa- Red-R and Mix were identified to be associated with several elements including Al, C and Mo, and would thus be ideal for production or use in breeding programs to enhance nutritional content in Bambara groundnut. These lines would need to be evaluated for anti-nutrient elements to ascertain the bioavailability of their high nutritional content. It has been reported previously that despite the high nutritional quality of Bambara, some of the benefits are limited by the presence of anti-nutrient elements such as cyanogenic glycosides and trypsin inhibitors (Atoyebi *et al*., 2018). On the contrary, lines such as, IITA686, Kenya Capstone, 42-1, Gresik and Light brown were not particularly superior in accumulating any particular element indicating that they need to be improved in several nutrients or they can be used for other purposes such as fodder or soil restitution. They may be selected for recommendation or breeding if they have other desirable characteristics such as processing qualities, easy to shell and cooking time. For instance, some lines with high nutritional content were found to also contain anti-nutrient elements that limited the bioavailability of the nutrients during human or ruminant digestion (Atoyebi *et al*., 2018). If the lines with low nutritional content exhibit low anti-nutrient elements, they would be useful for breeding to reduce the anti-nutrient elements in the nutritionally superior lines.

6.5 Conclusion

The element content of the Bambara groundnut varies with genotypic differences among the lines. Lines 211-57, Pong-Br-EN, Uniswa Red-G and Uniswa Red-R, contained the highest number of elements such as aluminium, chloride, potassium, magnesium, nitrogen and oxygen. These lines can be recommended for production or breeding to increase nutrient availability among poor communities who require cheaper sources of essential elements. On the other hand, lines such as KANO2, DIP-C, IITA686 and Kenya Capstone that displayed poor performances for element content must be evaluated for other traits such as agronomic performance so they can be grouped into different desirable groups that can be used for further studies and breeding programs. This study can be used as a reference to provide baseline information for recommendations for the inclusion of Bambara groundnut as a source of essential elements and improve the effectiveness of using Bambara groundnut in food and nutrition security intervention programs.

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Chapter 7 Correlation, regression and path coefficient analyses of agronomic traits and nutritional composition in Bambara groundnut

Abstract

Bambara groundnut (*Vigna subterranea* (L.) Verdc) is a legume with potential to improve food and nutrition security in sub-Sahara Africa. Identifying genotypes with desirable agronomic performance and high nutritional content is important for improving productivity and food and nutrition security. The objectives of this study were to determine interrelations among agronomic traits and nutritional content with seed mass for indirect selection. Field experiments were conducted at University of KwaZulu–Natal's Controlled Environment Facility (CEF) and Ukulinga Farm to assess agronomic performance. Nutritional content was determined by standard combustion procedure and electron microscopy. The data was subjected to correlation, regression and path coefficient analyses to deduce interrelationships and direct and indirect effects of the traits on seed mass production. The agronomic traits and nutrients exhibited variable correlations with seed mass and amongst themselves. Among the agronomic traits, number of seeds (NS), number of healthy seeds (NHS) and plant height (PH) exhibited the strongest associations with and direct effects on seed mass. Regression analysis showed that nutrients such as NDF, MG and starch, and mineral elements such as nitrogen (N), oxygen (O) and phosphorous (P) were the most important predictors of seed mass productivity in Bambara groundnut. Path analysis identified NS, NHS, PH, Mg, oxygen and carbon contents had positive effects on seed mass. The findings provide a means to facilitate indirect selection of genotypes with high seed mass productivity via

proxy traits such as agronomic performance and nutrient content for production and improvement.

Keyword: agronomic performance; food security; interdependence; selection; yield productivity,

7.1 Introduction

Bambara groundnut (*Vigna subterranea* (L.) Verdc) is an orphan legume crop that has huge potential as an additional source of food for people in marginalized communities. The crop is a source of energy and essential nutrients such as carbohydrate (65%), protein (18%), oil (6.5%) and essential minerals such as calcium, iron, potassium and sodium (Mazahib *et al.*, 2013). The Bambara groundnut seeds can be utilized for extracting milk or processed as flour, boiled or roasted while its leaves are consumed as a vegetable delicacy (Hillocks *et al.*, 2012; Murevanhema and Jideani, 2013). Farmers in sub-Sahara Africa (SSA) have been encouraged to consider cultivating Bambara groundnut for diversification due to its high nutritional content, drought tolerance and ecosystem services such as nitrogen fixation and green manure provision and the high nutritional content will improve food security among poor communities whose diets are mostly cereal based. Its high drought tolerance makes it suitable for production under marginal environments due to its adaptability to semi-arid conditions and low input and management requirement.

In general, crop production in SSA is limited mainly by drought stress and poor soil fertility leading to starvation. Bambara groundnut is a low input crop, with relatively high tolerance to drought and salinity stresses prevalent in many parts of the sub-Sahara African region (Benson *et al.*, 2015). Chai *et al.* (2016) reported that Bambara groundnut used a combination of mechanisms that include maintaining high stomatal conductance, and high leaf water status that ensure the perpetuation of high levels of photosynthesis under drought stress. These capabilities render Bambara groundnut to be adaptable to smallholder agriculture systems that mainly depend on seasonal rainfall and sunlight. It would be prudent to assess agronomic performance in traits such as earliness to maturity, stomatal conductance, chlorophyll content, leaf are indices and canopy diameter, which are among the agronomic traits affecting yield productivity.

While yield is the ultimate goal of crop production, grain quality is also an important determinant of the value of a crop. Nutrient content in Bambara groundnut is affected by several factors including the cultivars under production, the production practices and environments conditions (Alake and Alake, 2016). The importance of legumes is to supply protein while Bambara groundnut has a substantial amount of carbohydrates for energy supply. Identifying genotypes with superior nutritional qualities is vital for alleviating hunger and starvation in poor communities. It will be prudent to understand interrelationships among agronomic traits and nutritional content and their impact on grin yield productivity. Such information will be vital for recommending superior cultivars for production while for Bambara groundnut improvement programs it will assist in devising appropriate selection strategies for improvement of yield, agronomic performance and nutritional content.

Interrelationships among agronomic traits and nutritional content and their impact ion grain yield can be assessed using methods such as correlation, regression and path co-efficient analysis have been used to identify target traits (Bankole *et al*., 2019). Correlation measures simple pairwise relationship between traits (Nyholt, 2004). Traits that exhibit favourable correlations can be improved simultaneously by selecting either one of them. Like in other crops, information on correlations is important in Bambara groundnut for yield improvement via indirect selection of agronomic traits (Alake and Ayo-Vaughan, 2017).

However, correlations cannot provide adequate description of relative importance of individual traits and how each contributes to the final yield especially when there are multiple traits with direct and indirect effects on yield (Laidig *et al.*, 2017). Thus, a more detailed analysis of the interactions would be required to understand the importance of each trait and rank their importance for target selection. Regression analysis has been employed to derive predictions for based on the relative importance of each trait. Traits that exhibit strong predictors for grain yield would be selected and identified as target traits for indirect selection for grain yield. In addition, path coefficient analysis can be employed to trace the direct and indirect pathways in which each individual trait affects grain yield. The path coefficient analysis partitions the dependent variable into its contributory independent variable (Mengistu *et al*., 2020). Thus, the objectives of this study were to conduct correlation, regression and path coefficient analyses to deduce the relationship among agronomic traits and nutrients and their impact on grain yield in Bambara groundnut to facilitate the ranking of agronomic traits and nutrients for targeted and indirect selection of grain yield.

7.2 Materials and Methods

7.2.1 Planting material

This study evaluated agronomic traits and nutrient content in 19 Bambara groundnut lines. The seeds were sourced from subsistence farmers of Tugela Ferry (28°45’ S; 30°27’E), Deepdale (30°33' S; 29°54' E) and Capstone Seed company in KwaZulu-Natal, and the University of Nottingham in the United Kingdom (Table 7.1).

Table 7.1: List of Bambara groundnut lines used in the study showing their origin and seed coat colour.

Line	Source	Country of Origin	Seed coat colour
IITA686	University of Nottingham	Tanzania	Light brown
DIP-C	University of Nottingham	Botswana	Cream
S19	University of Nottingham	Namibia	Black
Uniswa Red-G	University of Nottingham	Swaziland	Dark red
Uniswa Red-R	University of Nottingham	Swaziland	Red
Kenya Capstone	Capstone Seed company	South Africa	Cream
Gresik	University of Nottingham	Indonesia	Black
Brown	Local Farmers	South Africa	Brown
Light brown	Local Farmers	South Africa	Light, brown
Cream	Local Farmers	South Africa	Cream
M09-4	University of KwaZulu-Natal	Zimbabwe	Brown
211-57	Capstone Seed company	South Africa	Dark cream
Tiganecuru	University of Nottingham	Botswana/Mali	White
42-1	University of KwaZulu-Natal	Zambia	Black
Mix	Local Farmers	South Africa	Mixture of colours
KANO2	University of Nottingham	Nigeria	Light cream
Pong-Br-EN	Local farmers	Unknown	Light brown
Pong-Br-UNK	Local farmers	Unknown	Light brown with sparkle
Pong-Cr	Local farmers	Unknown	Cream

7.2.2 Field Trial

7.2.3 Site description and trial establishment

The field trials were conducted under at sites over a period of two years. The first field trial was conducted at the University of KwaZulu–Natal's Controlled Environment Facility (CEF) in Pietermaritzburg (29°37'34.0"S 30°24'13.4"E). The second field trial was conducted at the University of KwaZulu-Natal's Ukulinga Research Farm in

Pietermaritzburg (29° 37′ S; 30° 16′ E; 775 m a. s. l.). The first field trial was planted in December in 2017 while the second trial was planted in December in 2018. The land was disc ploughed and rotovated to achieve a fine tilth. Two seeds were planted per hole at 3cm depth. The experiments were laid out as a complete randomized block design replicated three times. The plots were single rows of 0.5 × 0.5m size. Plants in a row were planted 15cm apart. The moisture content of the soil was deduced from digital moisture sensors using the ML3 Theta Kit (Delta T Devices UK) and tensiometers sunk at 30 and 60 cm depths at several points in the field. Supplementary irrigation was supplied to maintain adequate soil moisture (average 80% of field capacity) three times per week. Weeds were removed manually when necessary while the other agronomic practices were as per standard Bambara groundnut production practice in South Africa (DAFF 2010).

7.2.4 Data Collection

7.2.4.1 Agronomic traits

The plant growth and physiology parameters were measured during the vegetative growth period until harvest maturity. Three plants per plot were measured for agronomic performance. Plant height (PH) was measured from the soil surface to the tip of the fully matured leaf using a tape measure (Stanley 3m Power lock steel). Mid-leaf length (MLL) and mid-leaf width, (MLW) were measured on the middle leaf of the trifoliate. Canopy diameter (CD) was measured on three random plants in a plot using a measuring tape. Leaf number was counted as the number of fully developed trifoliate leaves in a plot. Stomatal conductance (SC) was determined using the Model SC–1 steady state leaf porometer (Decagon Devices, Inc., USA). A portable chlorophyll meter, the SPAD-502

Plus (Konica Minolta, Japan) was used to measure chlorophyll content index (CCI) on the fully expanded trifoliate and solar radiation exposed leaves.

7.2.4.2 Yield parameters

The plants were harvested for yield parameters. The entire plot was harvested by digging the entire plant from the ground. After harvesting shoot, roots, pod and whole plant mass from each experimental plot were weighed with a digital sensitive balance (Masskot, FX320, and Switzerland) and average mass (g) per plot was recorded. After shelling the crop, seed mass (SM) per plant was weighed with a digital sensitive balance (Masskot, FX320, Switzerland). Thereafter, number of seeds were counted and categorised into damaged (D) and number of healthy seeds (NHS) from the pods.

7.2.4.3 Nutrient content determination

The chemical assay was conducted in three replicates for each line after oven drying. The dried seeds were ground into a fine powder using an electric blender. The fine powder was then sent to Cedara analytical laboratory for the nutrient assay. The different nutrient traits were determined using various methods appropriate for each nutrient. The carbohydrate content was determined using acid hydrolysis method while starch was determined using enzymatic catalysis. The ash content was determined using a dry ashing technique. Crude protein was determined using the Dumas method. The Hunter method was used to quantify mineral content and the amount of fat was evaluated using the ether extraction method. Lastly, acid detergent fiber (ADF) and neutral detergent fiber (NDF) were deduced using the Van Soest method.

7.2.4.5 Microscopy and Microanalysis Unit (MMU)

The mineral content and composition of different elements were evaluated under Zeiss EVO Scanning Electron Microscope (SEM) using Energy Dispersive X-Ray Spectrotomy (EDX) technique. Three seeds of each line were cryo-fractured in liquid nitrogen and split into two halves. The seed halves were mounted on stubs and secured using a carbon double-sided insulating tape. The microscopy quantified the proportion of each element in percentage of the total mineral concentration in each sample. In addition, the microscopy produced surface and spatial images of the cross-section of each sample. The surface roughness and morphology of the cross-section of the seed were measured on area of 1 μm× 1 μm. Subsequently, the cross-section of the seeds was imaged SEM under the Zeiss EVO SEM in high vacuum mode at a scanning speed of 5 kV.

7.2.5 Data Analyses

All data analyses were conducted using packages hosted in R software (R Core Team 2020). The Pearson correlation coefficients were deduced by the "FactorMiner" package in R software (Le et al., 2008). The agronomic traits, nutrients and mineral elements were regressed as independent variables on seed mass, which was the dependent variable. The following regression model was used:

$$Y = \alpha + \beta X + \varepsilon$$

Where Y=yield response of the genotype (dependent variable)

α=yield response when the independent variable X=0

β=rate of change for Y for each unit of X

X=value of the independent variable

ε= the error associated with prediction of Y from X

Path analysis was performed using the “lavaan” package in R software (Yves, 2012) to deduce direct and indirect effects of secondary traits on seed mass of the lines. Path coefficient analysis was based on the phenotypic correlation coefficients based on the following model (Dewey and Lu, 1959):

$$r_{ij} = P_{ij} + \sum r_{ik} P_{kj}$$

where, rij = the correlation between the independent variable (i) and respondent variable, seed mass (j). Pij = the direct effects of the independent variable (i) and Σ r_{ik} P_{kj} = the sum of indirect effects of an independent variable (i) on the respondent variable (j) through the other independent variables (k). The residual effects of unknown factors were calculated as:

$$R = \sqrt{1 - \sum r_{ij} P_{ij}}$$

Where R=residual effects and $\sum r_{ij} P_{ij}$ = the sum of all direct and indirect effects of the independent variables on the respondent variable. Ideally, residual values must be smaller when the independent variables in the model can explain the variability in the respondent variable. Pictograms were constructed to illustrate the direct and indirect pathways influencing seed mass productivity in Bambara.

7.3 Results

7.3.1 Bivariate correlations among agronomic traits and nutrient content

The agronomic traits exhibited variable correlations with seed mass (Table 7.2). Seed mass was positively correlated to NS ($r=0.58$, $p<0.01$), NHS ($r=0.51$, $p<0.05$) and PH ($r=0.45$, $r<0.05$), which showed that NS, NHS and PH had a positive impact on seed mass. The agronomic traits also exhibited variable correlations amongst themselves. For instance, NS and NHS exhibited the strongest correlations ($r=0.96$, $p<0.001$). Similarly, LN and CD exhibited positive correlation ($r=0.56$, $p<0.01$) while it was observed that CCI was negatively correlated to SC ($r = -0.46$, $r<0.05$).

The nutrients did not exhibit any significant correlations with seed mass (Table 7.3). The most important correlation among nutrients were observed between Mg and NDF ($r=0.64$, $p<0.01$), starch and Mn ($r=0.45$, $p<005$) and K and NDF ($r=-0.52$, $p<0.05$).

Among the mineral elements, only S exhibited significant correlation with seed mass ($r=-0.51$, $p<0.05$) (Table 7.4). There were significant correlations among the mineral elements. For instance, Cl ($r =0.47$, $p<0.05$) and N ($r =0.78$, $r<0.001$), were positively correlated to C. The strongest correlation among the mineral nutrients was observed between S and K ($r =0.74$, $p<0.001$). However, some of the mineral elements like Al, Mg, Mo and P had no associations with the other elements.

Table 7.2: Pearson correlation coefficients among agronomic traits of 19 Bambara groundnut lines

	SM	NS	NHS	PH	LN	MLL	MLW	CD	SC	CCI
SM	1									
NS	0.58**	1								
NHS	0.51*	0.96***	1							
PH	0.45*	0.31	0.37	1						
LN	0.03	-0.02	-0.06	0.44	1					
MLL	-0.18	-0.24	-0.25	-0.17	0.22	1				
MLW	0.3	0.39	0.44	0.15	-0.26	-0.09	1			
CD	0.09	-0.1	-0.17	0.43	0.56**	0.36	-0.36	1		
SC	-0.11	-0.09	-0.02	-0.2	-0.38	0.13	-0.32	-0.16	1	
CCI	0.01	-0.2	-0.24	-0.16	-0.26	-0.07	0.31	-0.09	-0.46*	1

SM=seed mass, NS=number if seeds, NHS=number of heathy seeds, PH=plant height, LN=number of leaves, MLL=mid-leaf length, MLW=mid-leaf width, CD=canopy diameter, SC=stomatal conductance, CCI=chlorophyll content, *, * and ***=significance at 0.05, 0.01 and 0.001, respectively.

Table 7.3: Pearson correlation coefficients among nutrients of 19 Bambara groundnut lines

	SM	Fat	NDF	Protein	Starch	Mg	K	Fe	Zn	Mn
SM	1									
Fat	-0.25	1								
NDF	-0.40	0.20	1							
Protein	0.32	-0.18	-0.10	1						
Starch	0.00	0.17	-0.05	-0.15	1					
Mg	0.19	-0.21	0.64**	0.31	-0.35	1				
K	0.15	-0.36	-0.52*	0.11	-0.08	-0.24	1			
Fe	-0.25	-0.34	-0.08	0.06	0.02	-0.13	0.23	1		
Zn	0.06	-0.35	-0.02	-0.14	-0.26	0.02	0.31	0.04	1	
Mn	0.17	0.11	-0.17	0.03	0.45*	-0.19	0.12	0.14	-0.04	1

SM=seed mass, NDF= Neutral detergent fiber, Mg=magnesium, K=potassium, Fe=iron, Zn=zinc, Mn=manganese, *, * and ***=significance at 0.05, 0.01 and 0.001, respectively.

Table 7.4: Pearson correlation coefficients among mineral elements of 19 Bambara groundnut lines

	SM	Al	C	Cl	K	Mg	Mo	N	O	P	S
SM	1										
Al	0.08	1									
C	-0.24	0.07	1								
Cl	-0.21	-0.03	0.47*	1							
K	0.44	0.1	0.07	0.17	1						
Mg	-0.09	0.38	0.29	0.26	0.4	1					
Mo	0.14	0.22	0.29	0.32	-0.2	0.34	1				
N	-0.42	0.21	0.78***	0.57**	0.27	0.37	0.38	1			
O	0.39	0.09	-0.57*	-0.11	-0.08	-0.02	-0.09	-0.37	1		
P	0.17	0.12	0.08	0.13	0.39	0.18	0.35	0.27	-0.18	1	
S	-0.51*	-0.21	-0.19	0.02	0.74***	-0.04	-0.31	0.09	0.09	0.09	1

SM=seed mass, Al = aluminium, C= carbon, Cl=chloride, K = potassium, Mg = magnesium, Mo =molybdenum, N =nitrogen, O=oxygen, P= phosphorous, S=sulphur, *, * and ***=significance at 0.05, 0.01 and 0.001, respectively.

7.3.2 Regression of agronomic traits, nutrients and mineral elements on seed mass

All the traits exhibited significant ($p<0.05$) impact on SM (Table 7.5). Traits NS, NHS and PH were the most important predictors of SM. For every unit increase in NS, the SM was predicted to increase by 2.05 units. It was observed that the PH had a higher impact on SM as for every unit increase in PH the SM was predicted to increase by an estimate of 15.08. Other traits such as LN, SC and MLW were also substantial predictors of seed mass. LN and CD had negligible impact on seed mass. The model predicted that the independent variables explained 0.58 of the variation in seed mass as indicated by the coefficient of determination (R^2).

Table 7.5: Regression of secondary agronomic traits on grain yield

Independent trait	Estimate	Standard error	z-value	P(>|z|)
Intercept	-249.84	377.07	-0.66	0.52
NS	2.05	0.05	39.46	0.00
NHS	1.81	0.06	30.52	0.00
PH	15.08	0.50	30.20	0.00
LN	-1.31	0.16	-8.35	0.00
MLL	2.92	1.03	2.84	0.00
MLW	12.65	2.15	5.90	0.00
CD	-1.92	0.38	-5.10	0.00
SC	0.23	0.04	6.31	0.00
CCI	0.81	0.23	3.54	0.00
R^2	0.58			

R^2=coefficient of determination, NS=number if seeds, NHS=number of healthy seeds, PH=plant height, LN=number of leaves, MLL=mid-leaf length, MLW=mid-leaf width, CD=canopy diameter, SC=stomatal conductance, CCI=chlorophyll content

All the nutrient traits were significant ($p<0.05$) predictors of SM. Nutrients such as NDF, starch, Mg and Fe were the most important predictors of SM. For every unit increase in NDF, the SM was predicted to increase by 3.78 units. Also, for every unit increase in starch, SM was predicted to increase by 1.32 units. The element Fe had the least impact on SM. About 64% of the variation in seed mass was explained by the independent variables as exhibited by the R^2 (Table 7.6).

Table 7.6: Regression of secondary nutrient traits on grain yield

Independent trait	Estimate	Standard error	z-value	P(>|z|)
Intercept	-150.63	178.24	-0.85	0.42
Fat	1.30	0.46	2.82	0.01
NDF	3.78	0.07	51.42	0.00
Protein	0.40	0.11	3.60	0.00
Starch	1.31	0.07	18.11	0.00
Mg	986.53	23.16	42.60	0.00
K	-23.68	1.86	-12.73	0.00
Fe	0.55	0.04	15.85	0.00
Zn	2.47	0.17	14.24	0.00
Mn	1.49	0.20	7.31	0.00
R^2	0.64			

R^2=coefficient of determination, NDF= Neutral detergent fiber, Mg=magnesium, K=potassium, Fe=iron, Zn=zinc, Mn=manganese

All of the mineral elements except Cl and S exhibited significant ($p<0.05$) impact on SM (Table 7.7). The mineral elements such as C, K, N and O were the most important predictors of SM. It was observed that for every unit increase in K, SM was predicted to increase by 23.17 units which was the highest unit increase compared for the other traits.

The least predictor of SM was by O, as for every unit increase in O, SM was predicted to increase by 7.70 units. Mineral elements such as Mo and S were negative predictors of SM as exhibited by their negative estimates and z-values. The coefficient of determination for the model predicting seed mass using these independent variables was 0.72.

Table 7.7: Regression of secondary mineral element traits on grain yield

Independent trait	Estimate	Std.Err	z-value	P(>|z|)
Intercept	-583.94	371.69	-1.57	0.15
Al	3.41	0.44	7.69	0.00
C	8.13	0.28	28.66	0.00
Cl	0.15	0.10	1.56	0.12
K	23.17	1.17	19.89	0.00
Mg	6.69	0.78	8.60	0.00
Mo	-1.44	0.18	-7.87	0.00
N	12.12	0.34	35.45	0.00
O	7.69	0.19	41.40	0.00
P	106.65	2.42	44.12	0.00
S	-1.86	3.24	-0.58	0.57
R^2	0.72			

Al = aluminium, C= carbon, Cl=chloride, K = potassium, Mg = magnesium, Mo =molybdenum, N =nitrogen, O=oxygen, P= phosphorous, S=sulphur

7.3.3 Path coefficients of agronomic traits, nutrients and mineral elements on seed mass

The correlations were further partitioned into direct and indirect effects. The agronomic traits had variable direct effects on SM (Table 7.8 and Figure 7.1). The trait NS had the highest direct and positive effects of 2.03 on seed mass. The second highest positive direct effects on seed mass were exhibited by NHS (1.72). In contrast, LN and NHS had negative directs effects on seed mass of -0.17 and -10, respectively. The traits also exhibited indirect effects on seed mass through other traits. The trait NS exhibited the highest indirect effects on seed mass (1.96) through NHS through NS. However, the indirect effects of NHS through NS were negative and very high (-1.66). The rest of the indirect effects are depicted in Figure 6.1. The trait NS had the highest total effects on seed mass of 0.58 followed by NHS (0.51) and PH (0.45). The least total effects on seed mass were exhibited by CCS while SC and MLL had negative total effects on seed mass. The residual variance of the direct and indirect effects on seed mass was 0.42 (Figure 7.1).

Table 7.8: Direct and indirect effects of agronomic traits on grain yield

Traits	NS	NHS	PH	LN	MLL	MLW	CD	SC	CCI	Total effects	P value
NS	**2.04**	-1.66	0.18	0.00	-0.01	0.04	0.01	-0.01	-0.01	0.58	0.00
NHS	1.96	**1.72**	0.22	0.01	-0.01	0.05	0.02	0.00	-0.01	0.51	0.00
PH	0.63	-0.63	**0.60**	-0.07	-0.01	0.02	-0.04	-0.02	-0.01	0.45	0.00
LN	-0.04	0.11	0.26	**-0.17**	0.01	-0.03	-0.06	-0.05	-0.02	0.03	0.00
MLL	-0.49	0.44	-0.10	-0.04	**0.04**	-0.01	-0.03	0.02	0.00	-0.18	0.00
MLW	0.80	-0.75	0.09	0.04	0.00	**0.11**	0.03	-0.04	0.02	0.30	0.00
CD	-0.21	0.29	0.26	-0.09	0.02	-0.04	**-0.10**	-0.02	-0.01	0.09	0.00
SC	-0.18	0.04	-0.12	0.06	0.01	-0.03	0.02	**0.12**	-0.03	-0.11	0.00
CCI	-0.40	0.42	-0.09	0.04	0.00	0.03	0.01	-0.06	**0.06**	0.01	0.00

NS=number if seeds, NHS=number of heathy seeds, PH=plant height, LN=number of leaves, MLL=mid-leaf length, MLW=mid-leaf width, CD=canopy diameter, SC=stomatal conductance, CCI=chlorophyll content

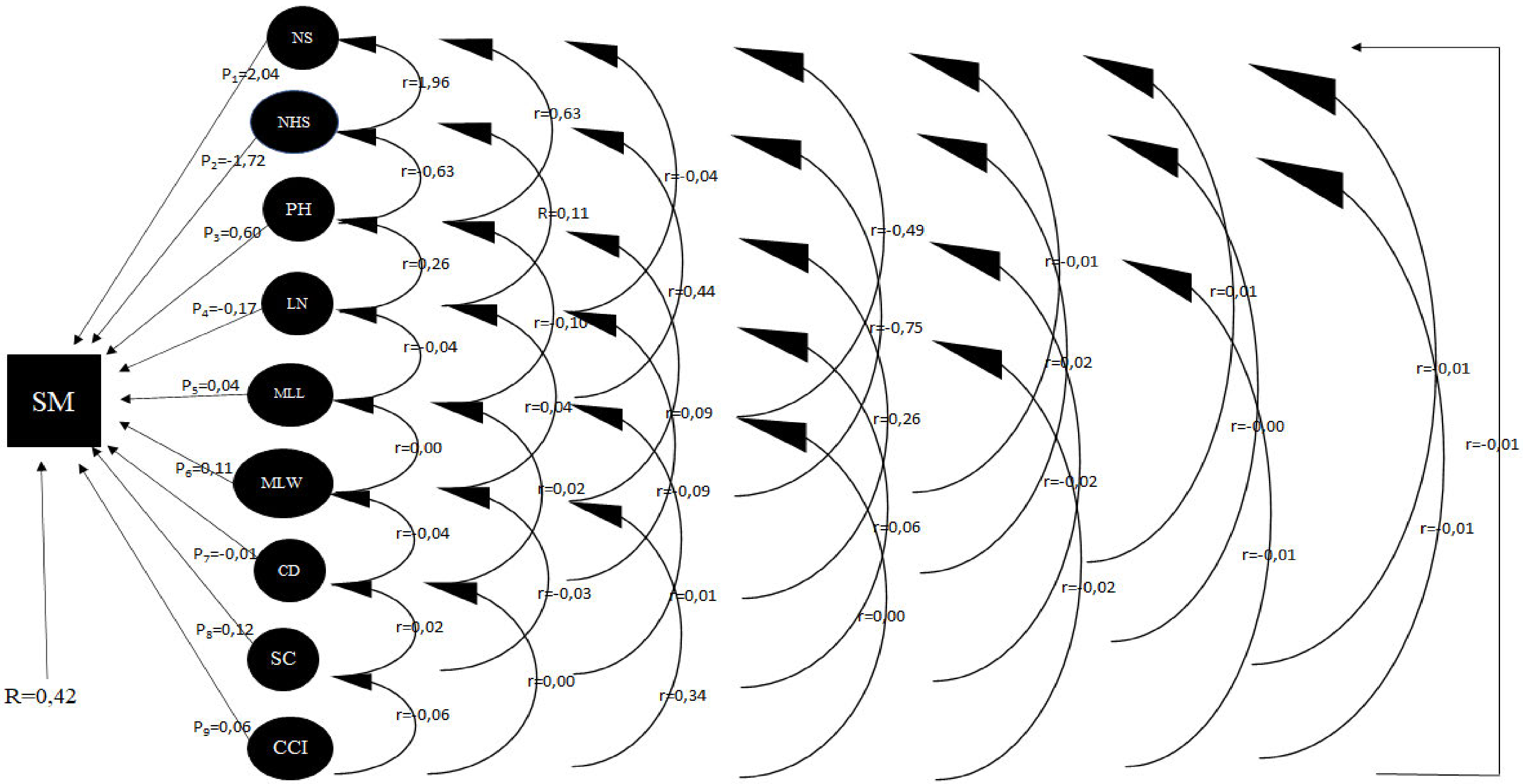

Figure 7.1: Nature of association of agronomic traits in Bambara groundnut based on path coefficient analysis.

Note: NS = number of seeds, NHS=number of healthy seeds, PH = plant height, LN =leaf number, MLL =mid-leaf length, MLW =mid-leaf width, CD =canopy diameter, SC =stomatal conductance, CCI = chlorophyll content, R = residual, r = correlation coefficient, p = unilateral pathway, SM = Seed mass. Double arrowed lines denote mutual association as measured by correlation coefficients, while single arrowed lines denote direct influence and as measured by the path coefficient analysis

The nutrient had variable direct effects on SM. Mg had the highest direct effects of 0.89 on seed mass followed by starch whose direct effects on seed mass were 0.27. Other nutrients such as protein, Mg, Zn and Mn had notable positive direct effects on seed mass. The strongest negative direct effects on seed mass were exhibited by NDF (-1.05), followed by Fe (-0.20) and K (-0.18). High indirect effects on SM were exhibited by Mg through NDF (0.67). On the other hand, Mn had negative indirect effects (-0.16) on seed mass through Mg. The highest positive total effects on SM were recorded for protein (0.032) followed by Mg (0.19) and Mn (0.17). Nutrients such as NDF (-0.40), Fe (-0.25) and fat (-0.25) had negative total effects on seed mass. Overall, the model had residual variance of 0.36 and the complex direct and indirect pathways are depicted in (Figure 7.2).

Table 7.9: Direct and indirect effects of nutrients on grain yield

Traits	Fat	NDF	Protein	Starch	Mg	K	Fe	Zn	Mn	Total effects	P values
Fat	**0.04**	-0.21	-0.01	0.04	-0.19	0.06	0.07	-0.07	0.01	-0.25	0.01
NDF	0.01	**-1.05**	0.00	-0.01	0.57	0.09	0.02	0.00	-0.02	-0.40	0.00
Protein	-0.01	0.10	**0.05**	-0.04	0.28	-0.02	-0.01	-0.03	0.00	0.32	0.00
Starch	0.01	0.05	-0.01	**0.26**	-0.31	0.01	0.00	-0.05	0.04	0.00	0.00
Mg	-0.01	-0.67	0.01	-0.09	**0.89**	0.04	0.03	0.00	-0.02	0.19	0.00
K	-0.02	0.55	0.00	-0.02	-0.21	**-0.18**	-0.05	0.06	0.01	0.15	0.00
Fe	-0.02	0.09	0.00	0.01	-0.11	-0.04	**-0.20**	0.01	0.01	-0.25	0.00
Zn	-0.02	0.02	-0.01	-0.07	0.01	-0.05	-0.01	**0.19**	0.00	0.06	0.00
Mn	0.00	0.17	0.00	0.12	-0.16	-0.02	-0.03	-0.01	**0.09**	0.17	0.00

NDF= Neutral detergent fiber, Mg=magnesium, K=potassium, Fe=iron, Zn=zinc, Mn=manganese

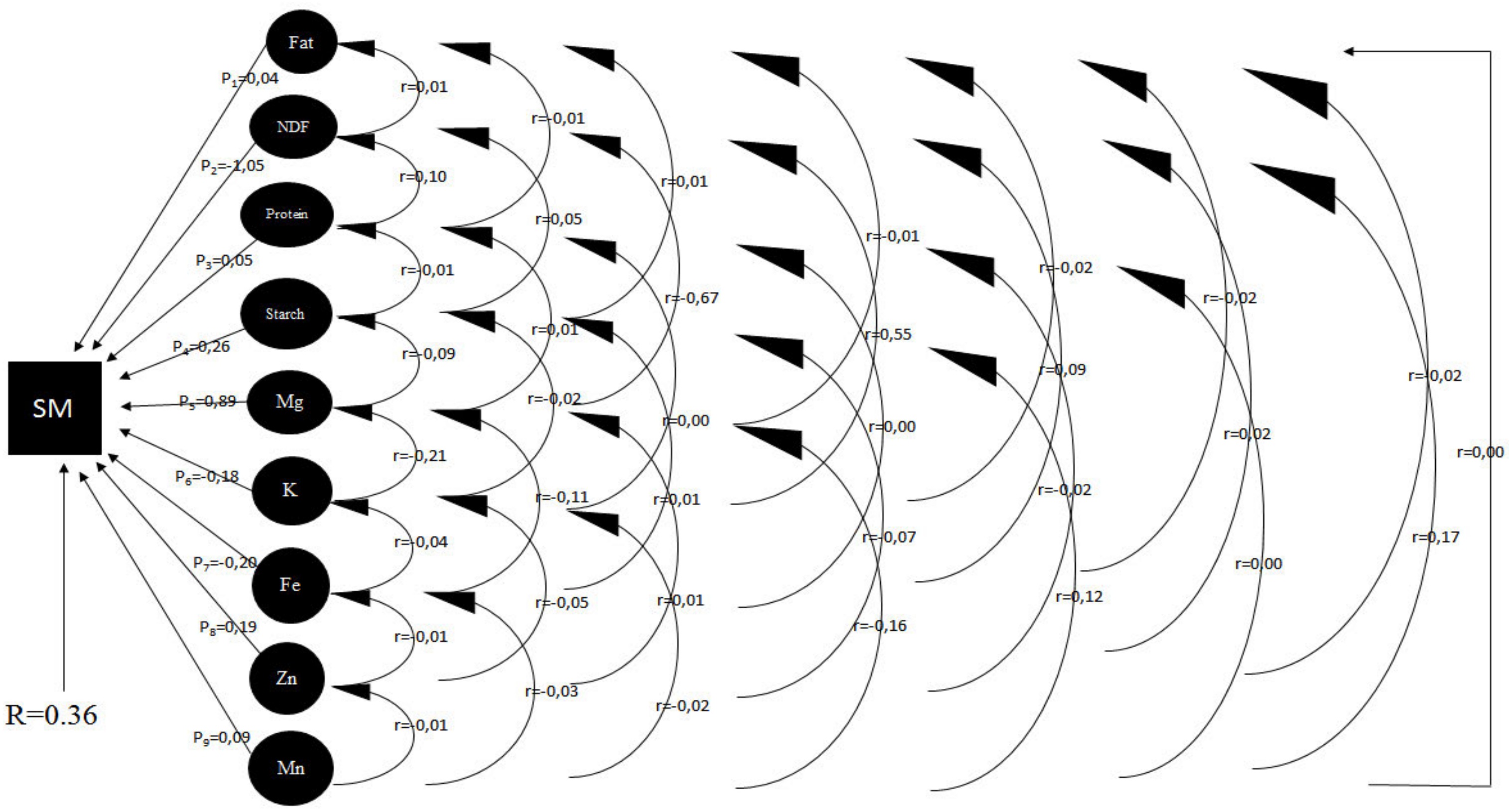

Figure 7.2: Nature of association of agronomic traits in Bambara groundnut based on path coefficient analysis.

Note: NDF = Neutral detergent fiber, Mg= magnesium, K = potassium, Fe =iron, Zn =zinc, Mn = manganese content, R = residual, r = correlation coefficient, p = unilateral pathway, SM = Seed mass. Double arrowed lines denote mutual association as measured by correlation coefficients, while single arrowed lines denote direct influence and as measured by the path coefficient analysis

The path analysis revealed that, P (0.65) C (0.63) and O (0.52) had the highest positive direct effects on seed mass. Mineral elements that exhibited the highest negative direct effects on seed mass were N (-0.78) and K (-0.56). The highest positive indirect effects on seed mass were exhibited by N (0.49) through C and Cl (0.29) through C. On the contrary, C (-0.61) exerted the highest negative indirect effects on seed mass through N. Most minerals exerted negative indirect effects through N while P provided a positive pathway to increase seed mass. The total effects showed that O (0.39) had the highest positive effects followed by P (0.17). On the other hand, S and K had the highest negative effects on seed mass of -0.51 and -0.44, respectively. The different pathway showing a residual variance of 0.28 were depicted in (Figure 7.3).

Table 7.10: Direct and indirect effects of secondary mineral elements on grain yield

Traits	Al	C	Cl	K	Mg	Mo	N	O	P	S	Total effects	P values
Al	**0.09**	0.04	0.00	-0.05	0.05	-0.02	-0.16	0.05	0.08	0.00	0.08	0.00
C	0.01	**0.63**	0.01	-0.04	0.04	-0.03	-0.61	-0.30	0.05	0.00	0.24	0.00
Cl	0.00	0.29	**0.02**	-0.10	0.04	-0.04	-0.44	-0.05	0.08	0.00	-0.21	0.12
K	0.01	0.04	0.00	**-0.56**	0.05	0.02	-0.21	-0.04	0.25	-0.01	-0.44	0.00
Mg	0.04	0.18	0.01	-0.22	**0.13**	-0.04	-0.29	-0.01	0.12	0.00	0.09	0.00
Mo	0.02	0.18	0.01	0.11	0.05	**-0.11**	-0.29	-0.05	0.22	0.00	0.14	0.00
N	0.02	0.49	0.01	-0.15	0.05	-0.04	**-0.78**	-0.19	0.18	0.00	-0.42	0.00
O	0.01	-0.36	0.00	0.05	0.00	0.01	0.29	**0.52**	-0.11	0.00	0.39	0.00
P	0.01	0.05	0.00	-0.22	0.02	-0.04	-0.21	-0.09	**0.65**	0.00	0.17	0.00
S	-0.02	-0.12	0.00	-0.41	-0.01	0.03	-0.07	0.05	0.06	**-0.01**	-0.51	0.57

Al = aluminium, C= carbon, Cl=chloride, K = potassium, Mg = magnesium, Mo =molybdenum, N =nitrogen, O=oxygen, P= phosphorous, S=sulphur

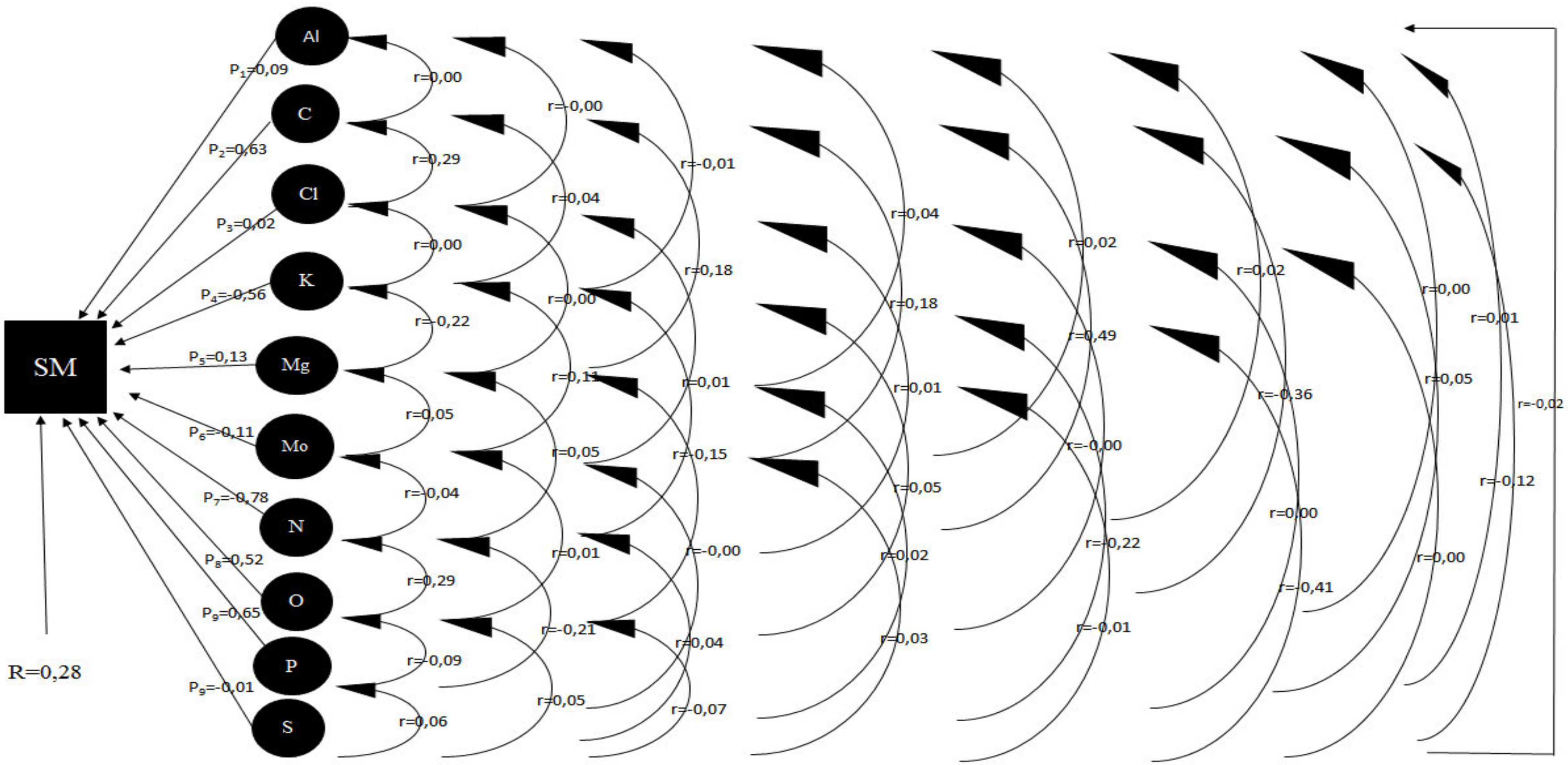

Figure 7.3: Nature of association of agronomic traits in Bambara groundnut based on path coefficient analysis.

Note: Al = aluminium, C= carbon, Cl=chloride, K = potassium, Mg = magnesium, Mo =molybdenum, N =nitrogen, O=oxygen, P= phosphorous, S=sulphur, R = residual, r = correlation coefficient, p = unilateral pathway, SM = Seed mass. Double arrowed lines denote mutual association as measured by correlation coefficients, while single arrowed lines denote direct influence and as measured by the path coefficient analysis

7.4 Discussion

7.4.1 Associations between variables and seed mass productivity

Agronomic traits, nutrients and mineral elements exhibited variable correlations with seed mass showing that their different impact of yield productivity in Bambara groundnut. The agronomic traits such as NS, NHS and PH that exhibited positive correlations with seed mass could be selected for indirect improvement on seed yield in Bambara groundnut. The ability to accumulate a high number of seeds and preventing seed rots prior to harvest are vital determinants of final harvestable yield in crops including Bambara groundnut. It is imperative to ensure that proper crop protection practices are implemented to maintain seed healthy to prevent accumulation of toxins and physical damage, which compromise yield quantity and quality. Taller Bambara groundnut genotypes have increased advantage in solar radiation interception for increased photosynthesis, which could explain the positive relations between height and seed mass found in this study. Some studies have shown that there is a positive relationship between yield and plant height (Misangu *et al.,* 2007). The other trait exhibited variable correlations with seed mass although they were not statistically significant. For instance, leaf mid-length was negatively correlated to seed mass while mid-leaf width exhibited positive correlations with seed mass. This could imply that genotypes with narrow and long leaves may not be efficient in photosynthesis for seed mass accumulation. Narrow leaves may be efficient at radiation interception and gaseous exchange in high density planted crops (Willey and Heath, 1969) but a reduction in leaf size would result in decline in biomass partitioning and ultimately low yield potential (Sesay *et al.,* 2010). The nutrients and mineral elements did not exhibit any significant correlations with seed mass showing that their relationship with yield may not be explained by simple correlations and would need to be further expounded by stronger analytical tools. Traits and nutrients that exhibit desirable correlations with seed mass could be used for indirect selection for seed mass during Bambara groundnut improvement. On the other hand, desirable correlations between seed mass and nutrients would imply that increasing seed yield in Bambara groundnut would not compromise its nutritional quality especially protein, magnesium and potassium. However, the negative direct and total effects of nitrogen on seed mass have been identified as a major contributor to low yield potential in legumes compared to cereals. Low yield productivity in legumes has been attributed to high energy requirements to produce protein rich grains compared to cereals and other crops (Munier-Jolain and Salon, 2005).

7.4.2 Predictors of seed mass

Regression analysis reveals which correlations between the independent traits and seed mass (respondent variable) are of significant importance to facilitate an effective selection process for yield improvement. The regression analysis revealed that all the investigated agronomic traits had impact on seed mass. However, the most important predictors of seed mass were total number of seeds, number of healthy seeds and the plant height. Similarly, Aghili *et al.* (2012) found plant height and the number of seeds number per pod were important determinants for grain yield in lentils. Salimi *et al.* (2012) used regression analysis and also found that the number of seeds per plant explained 84% of the variation in grain yield and concluded that the number of seeds was the most important trait. Thus, tall genotypes that produce a high number of healthy seeds could be selected for yield improvement or recommended for production to improve Bambara groundnut productivity. Among the nutrients assessed in Bambara, NDF, Mg and Fe explained much of the variation in seed mass. Although protein and starch are known to be the major constituents of Bambara groundnut, their range of variation was narrow among the genotypes to explain the variation in seed mass. This implies that the genotypes can be selected based on their NDF, Mg and Fe compositions. For instance, NDF is known to constitute lignin, hemicellulose, cellulose and silica that have anti-nutrient properties (Ijarotim and Esho, 2009). Genotypes with low NDF would be ideal for human consumption while those containing high levels of NDF could be useful as feedstock in biofuel generation. The selection for genotypes should target genotypes with high Mg and Fe content to improve nutrient availability. Nitrogen, oxygen and phosphorous content were the most important mineral elements for predicting seed mass productivity among the genotypes. These elements are important constituents of protein and selection of genotypes with high levels of these would improve protein content. Bambara groundnut genotypes containing high levels of mineral elements would be useful in supplementing human diets, given that minerals are vital for proper functioning of the human health system (Gharibzahedi and Jafari, 2017). The coefficients of determination for the models for agronomic traits, nutrients and mineral elements were high showing that the traits captured much variation and could be used effectively to predict yield variability in Bambara groundnut.

7.4.3 Direct and indirect effects of variables on seed mass productivity

Path-coefficient has been used to investigate the direct and indirect effects of independent complex traits towards the dependent trait. The study found that the strongest direct effects on seed mass were exhibited by agronomic traits such as NS, NHS and PH, nutrients such as Mg and starch, and mineral elements such as C and P showing that these variables should be prioritised. Seed mass is a quantitative trait that is an aggregate of secondary traits that have contributory effects on yield (Aliyu and Makinde, 2016). Hence, it is important to deduce the contributory effects in order to understand their relationships with yield and devise suitable selection protocols. Traits that exhibit high and positive effects on another trait can be selected resulting in increase in the other trait (Aremu *et al.,* 2019). The selection of tall genotypes with high number of seeds, high Mg and starch content would improve yield productivity of the evaluated germplasm. Hashemi and Shahani. (2019) also found that the number of seeds was a vital determinant of seed yield in *Hibiscus sabdariffa*. Selection of genotypes with high mean values for traits such as MLL and SC, nutrients such as fat, NDF and Fe and mineral elements such as Cl, K and S that exhibited negative direct and total effects on seed mass would compromise yield productivity in Bambara groundnut. Traits that exhibit unfavourable effects on seed yield should not be investigated for negative drag to devise suitable strategies to break such unfavourable drag. For instance, mutation breeding could be used to create new genetic variation and break unfavourable genetic linkage that may exist between these traits and seed mass productivity. Dwivedi *et al*. (2003) bemoaned a lack of improvement in disease resistance in the legume groundnut (*Arachis hypogea* L) by introgression of genes from wild *Arachis* sp due to unfavourable linkage with low shelling ability, reticulation and constriction in the pods. However, mutation breeding has been employed successfully in Ghana (Adu-Dapaah *et al.,* 2003), showing that it could be used in this population to generate genetic variation for simultaneous improvement of traits and nutrients that exhibited negative effects on seed mass.

7.5 Conclusion

Overall, the study was able to identify the relationships between the investigated agronomic traits, nutrients and mineral elements, and seed mass. The relationships revealed the potential of selecting each individual variable and their combinations to improve seed yield selection of tall genotypes that produce a high number of healthy seeds with high levels of magnesium, phosphorous, oxygen and nitrogen will be emphasized to improve grain yield during development of improved cultivars.

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Chapter 8 General Discussion, Conclusions and Recommendations

8.1 Introduction

Most countries in sub-Saharan Africa (SSA) experience water scarcity and malnutrition. Crop production in SSA is dominated by cereal crops such as maize, wheat and sorghum that are carbohydrate rich but lack other nutrients like protein and micronutrients, which contribute to poor diets among many people in rural households. There is a need to increase dietary diversity to alleviate malnutrition. Grain legumes such as Bambara groundnut are rich sources of protein, starch and micronutrients but yet they still remain under-explored. Therefore, there is a need to promote the production of neglected and underutilised grain legumes to diversify nutrition and improve food security. Bambara groundnut is known to be ideal for the semi- and arid tropics, where water scarcity and poor soil fertility limit agricultural productivity The promotion of Bambara groundnut, especially considering that it is an underutilised legume with no improved cultivars readily available on the market, will require knowledge on its adaptation to environments via assessing its diversity for agro-morphology, genetic make-up and nutritional value. It is imperative to investigate Bambara groundnut lines in-order to generate baseline information for breeding and varietal selection for the development of improved cultivars that will be suitable for production under SSA conditions and will be high in nutritional content.

In this study, it was hypothesised that the Bambara groundnut lines were diverse in agronomic performance and physiological traits, they consisted of genetically divergent individuals, their nutritional content varied and the multivariate relationships among the traits and yield would allow indirect selection. To test these hypotheses, field experiments were conducted under different environmental conditions at two sites. Further, analyses were conducted using the seeds harvested for nutritional composition and lastly multivariate trait relationships were deduced among the traits.

8.2 Findings from the Review

A critical literature review (Chapter 2) was conducted to identify opportunities and challenges for successful development and promotion of Bambara groundnut lines into mainstream agriculture. It was revealed that research and development on legumes mainly focused on a few prominent legumes such as common bean and soya bean based on their economic value and demand leading to Bambara groundnut lagging behind and being neglected. There is a need to diversify crop production in the changing environment due to climate change and diverse dietary requirements. However, there is limited documented information on Bambara groundnut especially on specialised developed cultivars that are high yielding, nutritious, adaptable and readily available on the market for production and for consumption. Therefore, there is need for more research and development on Bambara groundnut to attract farmers and consumers as well as to increase competitive advantage over other and major grain legumes. The main findings of the review were:

➢ Bambara groundnut is one of the neglected legume crops in the SSA region

➢ It is characterized as a drought tolerant species with capability to produce reasonable yield under moisture stress and nitrogen deficient soils

- The high protein source, starch and mineral elements content in Bambara groundnut makes it suitable for food and nutrition security intervention in areas where cereals are dominant constituents of diets
- It has potential to contribute to food and nutritional security in SSA, especially amongst smallholder farmers.
- Smallholder farmers face production challenges and a lack of proper adaptable improved cultivars leading to low production and productivity of the crop
- To improve productivity of Bambara groundnut under smallholder farming, and for the crop to penetrate the mainstream agriculture, there is a need to identify superior lines and develop improved cultivars that will be available to farmers.

8.3 Research findings in brief

8.3.1 Agro-Morphological diversity of Bambara groundnut lines evaluated under field conditions

Nineteen Bambara groundnut lines obtained from subsistence farmers of Tugela Ferry (KwaZulu-Natal), Deepdale (KwaZulu-Natal), and University of Nottingham (UK) were used for this study. The field trials were conducted under two sites over a period of two years the first field trial under site one was conducted at the University of KwaZulu–Natal's Controlled Environment Facility (CEF) Pietermaritzburg. The second field trial under site two was conducted at the University of KwaZulu-Natal's Ukulinga Research Farm in Pietermaritzburg. The main findings of the study were as follows:

- The Bambara groundnut lines exhibited significant ($p<0.05$) variation for agronomic performance and grain yield production, indicating differential response of genotypes necessary for selection of superior lines and for breeding purposes.

- The variability among the Bambara groundnut lines confirmed the initial hypothesis that the lines were genetically divergent and would exhibit phenotypic differences in response to environmental conditions.
- The result provides information necessary for future breeding programmes and varietal selection studies on Bambara groundnut to develop adaptable cultivars suitable for different environments.

8.3.2 Assessment of the genetic diversity of Bambara groundnut genotypes using SSR markers

The study evaluated genetic diversity in a set of germplasm forms as basis for crop improvement. The genetic variation of 19 Bambara groundnut lines was characterised using 20 polymorphic SSR markers. The main findings of the study were as follows:

- The results revealed that 60% of the selected SSR markers were highly polymorphic and sufficiently distinguished the tested Bambara groundnut genotypes.
- Genotypes Light brown, Brown, Uniswa Red-G, Uniswa Red-R, Dip-C and Pong-Br-EN showed unique genetic pattern and relationship suggesting that they may have different genetic makeup from the rest of the genotypes.

8.3.3 Evaluating Nutritional Content among Bambara Groundnut Lines

The study evaluated nutritional content in 19 Bambara groundnut lines. A chemical assay was conducted, with three replicates for each line after oven dried seeds, which were ground into a fine powder using an electric blender. The main findings of the study were as follows:

- The nutritional content varied significantly ($p<0.05$) among the lines with lines S19, Gresik, Pong-Br-UNK, Pong-Cr exhibiting the highest means for starch and protein ranging from 11.05 to 11.94%.
- Nutrients that showed high variation were starch, NDF, and protein, which had high coefficient of variations among each other.
- Lines Mix, Pong-Br-UNK, 42-1, Gresik, Uniswa Red-R, and Brown were clustered together based on their high starch, Na, Ca, fat, moisture Mn and ADF contents.

8.3.4 Assessment of mineral element composition of diverse Bambara groundnut lines using electron microscopy

This study used electron microscopy to provide an alternative option for qualitative and quantitative assessment of mineral elements content in plant matter. The mineral elements that were determined were Al, C, Cl, K, Mg, Mo, N, O, P and S. Thus, 19 Bambara groundnut lines were evaluated for micronutrient content and structural composition using electron microscopy. The main findings of the study were as follows:

- Carbon (C) and oxygen (O) were the most abundant nutrients, while sulphur (S) and phosphorous (P) were the least available nutrient in Bambara groundnut.
- The lines with high content for multiple nutritional elements such as 211-57, Pong-Br-EN and Uniswa Red-G were recommended for production, while the poor nutrient accumulators can be assessed for other important attributes such as forage.

8.3.5 Correlation, regression and path coefficient analyses of agronomic traits and nutritional composition in Bambara groundnut

This study assessed interrelations among agronomic traits and nutritional content with seed mass for indirect selection. Field experiments were conducted at University of KwaZulu–Natal's Controlled Environment Facility (CEF) and Ukulinga Farm to assess agronomic performance. Nutritional content was determined by standard combustion procedure and electron microscopy. The data was subjected to correlation, regression and path coefficient analyses to deduce interrelationships and direct and indirect effects of the traits on seed mass production. The main findings of the study were as follows:

- The seed mass number of seeds (NS), number of healthy seeds (NHS) and plant height (PH) exhibited the strongest associations and direct effects on seed mass.
- Regression analysis showed that nutrients such as NDF, Mg and starch, and mineral elements such as nitrogen (N), oxygen (O) and phosphorous (P) were the most important predictors of seed mass productivity in Bambara groundnut.
- The relationships revealed the potential of selecting each individual variable and their combinations to improve seed yield.

8.4 Implications of findings

- The negative correlations among some of the traits would present challenges for simultaneous identification of lines with favorable nutritional content, agronomic performance and genetic divergence for selection and breeding.
- The environment also played a critical role in condition the agro-morphological differences, growth and development among the Bambara groundnut lines showing that superior lines could be identified for specific and broad environments.

- The observed low content for nutrients such as Na, P, Mg exhibited by some lines would be a cause for concern to prevent hidden hunger or low provision of essential elements.
- The low number of effective alleles detected suggests that the number of SSR markers used in this study may need to be increased in order to discriminate a larger pool of germplasm.
- The lack of a significant difference between the observed and expected heterozygosity values obtained in this study signify that there could have been some random mating that occurred within this population.
- Lines IITA686, Kenya Capstone, 211-57, KANO2, Uniswa Red-G, Uniswa Red-R 42-1, MO9, Brown, Cream and Mix were selected for high agronomic performance in traits such as number of seeds, plant biomass, plant height and seed mass accumulation.
- For high protein, carbohydrate, starch and fat content, lines S19, Gresik, Pong-Br-UNK, Pong-Cr, IITA686, KANO2, Uniswa Red-R, 42-1, MO9, Dip-C, Brown, Cream, Tiganecuru and Mix were identified.
- It will be important to elucidate genetic control of nutrient accumulation in order to devise suitable breeding strategies. Bambara groundnut is a highly autogamous species, which is difficult to cross in mating designs. However, other researchers have managed to generate crosses from divergent Bambara groundnut lines. Mutation breeding could also play an important role in improving these traits and creating new variation for exploitation.

8.4 Recommendations and Future Directions

Based on the observations made in this study, the following technical and research recommendations are given;

- The Bambara groundnut improvement program at UKZN should be expanded and include germplasm from diverse geographical sources with improved seed storage facilities and organization of the seed sources.
- The superior genotypes identified must be evaluated in multiple locations to assess stability and performance of the genotypes and genotype x environment interactions.
- Genetic studies must also be extended to include genome wide coverage by markers such as a single nucleotide polymorphisms (SNP) to increase chances of identifying underlying genetic control of traits.
- Multiple location trial will also be recommended to enable calculation of trait heritability estimates that are important for devising suitable strategies for trait improvement.
- Few lines that showed late maturing should be planted early (November) to avoid poor yield caused by late planting and soil type has an influence on the spreading of the pods the use of sandy soils improves the pod formation and spreading.
- There should be studies to identify the best genotypes for different cropping systems as intercropping, double cropping and crop rotation so as to improve soil health to increase nitrogen fixation benefits from the legume.
- Future studies should explore effects of factors such as management practices and environmental stresses fertilizer levels, plant density water stress and heat stress.