Multi-Location Field Evaluation of Bambara Groundnut (*Vigna subterranean* (L) Verdc) for Agronomic Performance and Seed Protein.

By

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Abstract

Bambara groundnut (*Vigna subterranea*) is one of the most important legumes cultivated primarily for food by smallholder farmers in Africa. It is an affordable source of protein and contributes to income generation as well as soil fertility. Despite its importance, it is cultivated largely for subsistence purposes in South Africa. Growers use landraces. The agronomic performance of the traditional varieties depends on environmental factors prevailing in a particular area. In Limpopo and Mpumalanga Provinces, there is no adequate information regarding the performance of bambara groundnut germplasm. The objectives of the study were to (i) determine the agronomic performance of Bambara groundnut across three contrasting locations in Limpopo and Mpumalanga provinces over two cropping seasons (ii) determine the genotypic variation in the seed protein level among 42 bambara groundnut genotypes. Forty-two bambara groundnut genotypes were evaluated under three different environmental conditions (Syferkuil, Thohoyandou and Nelspruit) over two (2013/2014, 2014/2015) seasons in a 7 × 6 rectangular lattice design replicated three times. Eight agronomic traits including dry shoot weight (DSW), number of pods per plant (NPP), pod length (PL), number of seed per pod (NSP), pod weight per plant (PWT), seed weight per plant (SWT), 100 seed weight (100-SWT) and seed yield (SYLD) were measured. The results showed that there were significant genotype x location interactions which demonstrated that the prevailing agro-ecological conditions at the test locations were distinct from each other. Five genotypes (‘BGN-19‘, ‘BGN-11‘, ‘BGN-12‘, ‘BGN-4‘and ‘BGN-34‘) attained >25.0% seed yield advantage over the local check ‘BGN-39‘. The results also showed that light brown coloured genotypes attained relatively higher seed yield compared to the other seed colours types. The cultivar superiority index (CSI) showed that three genotypes (‘BGN-12‘, ‘BGN-19’ and ‘BGN-34’) were the most stable across the test locations and attained >900.0 kg/ha on average. There were significantly high positive correlations between PWT and each of the three other attributes (SWT, 100 SWT and SYLD). In terms of seed protein, the results showed a poor relationship between seed yield and protein levels. ‘BGN-12’ which produced the highest seed yield, attained the lowest percent seed protein while genotype. On average, the genotypes contained 21.72% protein. The highest and lowest seed protein quantities were attained by the genotypes ‘BGN-42’ (25.17%) and ‘BGN-12’ (19.89%) respectively.

Keywords: bambara groundnut; agronomic performance; stability; seed protein; principal component analysis

Dedication

This dissertation is dedicated in memory of my late father Nkhwela Samuel Mogale. His words of inspiration and encouragement in pursuing my dreams still live within me. I also dedicate it to my mother Kgabo Emission Mogale who always supports me and constantly reminds me that with hard work, it is possible to achieve beyond my dreams.

Declaration

I, Mogale Tlou Elizabeth, hereby declare that this dissertation, for the Master Science in Agriculture (Plant Production) in the Department of Plant Production at the University of Venda, hereby submitted by me, has not been submitted previously for a degree at this or any other University. It is my own work, design and execution. All reference material contained therein has been acknowledged accordingly.

Signature: …… Date:……...

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As supervisor, I agree to the submission of this dissertation:

Signature: …… Date:……...

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Co-Supervisor: Prof. M. Zhou

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List of Abbreviations

ANOVA	=	analysis of variance
AOAC	=	Association of Official Analytical Chemists
ARC-GCI	=	Agricultural Research Council and Grain Crop Institute
BGN	=	bambara groundnut
CSI	=	cultivar superiority index
DSW	=	dry shoot weight
FAOSTAT	=	Food and Agriculture Organisation of the United Nation Statistics
NIRS	=	near infrared reflectance spectroscopy
NPP	=	number of pods per plant
PCA	=	principal component analysis
PL	=	pod length
PWT	=	pod weight per plant
SWTP	=	seed weight per plant
SYLD	=	seed yield
SAS	=	statistical analysis software system
100-SWT	=	hundred seed weight

1.0 CHAPTER ONE: GENERAL INTRODUCTION

1.1 Introduction

Bambara groundnut (*Vigna subterranea*) is an important food legume grown in many parts of Africa. It originated from West African (Baudoin and Mergeai, 2001). At present, bambara groundnut is cultivated in several countries in sub-Saharan Africa. It is mainly grown for human consumption (Mazahib *et al.,* 2013) as well as livestock feeds (Bamashaiye *et al.,* 2011). The crop also contributes to the improvement of soil fertility through nitrogen fixation (Yakubu *et al.,* 2010). Surplus bambara groundnut is often traded in informal markets in Africa thus generating income for smallholder growers (Azam-Ali *et al.,* 2001).

Bambara groundnut contains relatively high (>18%.0) seed protein (Mazahib *et al.,* 2013). Hence, it can be used to improve the nutritional status in both human and livestock (Massawe *et al.,* 2002). In addition, it contains essential amino acids such as lysine, tryptophan as well as methionine (Minka and Bruneteau, 2000).

The production of bambara groundnut in Africa is generally high in the west Africa region. For instance, Mali produced the highest amount of bambara groundnut during 2014(Table 1.1). In South Africa, the major bambara groundnut producing areas are Limpopo, Mpumalanga and KwaZulu-Natal Provinces (Mabhaudhi and Modi, 2013). However, the reliable production quantities are difficult to obtain partly because the crop is grown for subsistence purposes. In addition, growers often intercrop bambara groundnut with cereals.

Table 1.1 The main bambara groundnut producing countries in west Africa in 2014

Country	Area(ha)	Average Yield(t/ha)	Production(Mt)
Mali	175 850.0	0.82	145 240.0
Niger	70 505.0	0.45	32 383.0
Burkina Faso	52 420.0	1.10	57 890.0
Cameroon	43 430.0	0.88	38 410.0

Source: FAOSTAT, 2014

The production of bambara groundnut in Africa is constrained by several factors. For example, there are no improved commercial cultivars of bambara groundnut hence farmers rely on traditional landraces (Mabhaudhi and Modi, 2013). The traditional varieties that are cultivated by smallholder farmers in many African countries evolved from their wild relatives (Massawe *et al.,* 2005), which are generally low yielding. Secondly, poor germination which leads to poor crop establishment results in diminished yields (Legwaila *et al.,* 2013). Thirdly, bambara groundnut production is affected by poor seed storage. The seed is susceptible to bruchids which results in reduced seed quality (Adu-Dapaah and Sangwan 2004). In some regions, nematodes reduced the yield of bambara groundnut significantly (Hillocks *et al.,* 2012).

1.2 Problem Statement

The agronomic performance of traditional varieties depends on the environmental factors prevailing in a particular area. The agronomic performance of our current bambara groundnut germplasm (i.e. in the bambara groundnut breeding programme at the School of Agriculture, University of Venda) in both Limpopo and Mpumalanga Provinces has not been determined adequately. In addition, there is a dearth of information on the seed protein levels of the traditional varieties grown by local smallholder farmers in Limpopo. In the germplasm ear-marked for developing new improved cultivars of bambara groundnut, both the seed protein levels and agronomic performance have not been evaluated across diverse agro-ecologies in the target production region. Therefore, selection of elite stable cultivars for these local agro-ecological conditions is difficult.

1.3 Justification of the Study

The genetic improvement of crops requires adequate information on the performance of current germplasm of the specific crop of interest. At present, there is no sufficient information about the agronomic performance of our current bambara groundnut germplasm in the target production area encompassing the semi-arid region of Limpopo and Mpumalanga Province (South Africa). In addition, high protein cultivars that produce optimum yields in traditionally low-input smallholder production systems are desirable in the target production area. In this study, a multi-location evaluation of local germplasm of bambara groundnut will generate useful information for selecting superior cultivars in terms of both seed protein and agronomic performance for the farmers. The improved cultivars will benefit end-users in the region.

1.4 Objectives of the Study

The broad objective of this study was to evaluate bambara groundnut germplasm in contrasting environments. The specific objectives of the study were to determine:

(i) the agronomic performance of 42 genotypes of bambara groundnut using three contrasting test locations

(ii) the stability of the bambara groundnut genotypes

(iii) the genotypic variation in the seed protein level among 42 bambara groundnut genotypes.

1.5 Hypotheses

The study tested the following null hypotheses:

(i) the agronomic performance was similar among the bambara groundnut genotypes that were evaluated at three test locations in Limpopo and Mpumalanga Provinces

(ii) the stability was similar among the bambara groundnut genotypes that were evaluated at three test locations in Limpopo and Mpumalanga Provinces

(iii) the seed protein levels were similar among the 42 bambara groundnut genotypes.

1.6 Dissertation outline

This dissertation is divided into six chapters. The first chapter introduces the crop and defines the problem statement as well as objectives of the study. Chapter two focuses on reviewing some relevant aspect of bambara groundnut production and seed protein levels. In chapter three, the genetic materials as well as the methods that were used in the study are described. The results of the study are presented in chapter four followed by a discussion of the results in chapter five. The last chapter (six) outlines a summary of the findings from the study. A combined list of references for all the chapters is followed by a list of appendices at the end of the main body of the dissertation.

2.0 CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

In this chapter, several aspects of literature including morphological characteristics and the major uses of bambara groundnut are reviewed. The chapter further outlines the production, agronomic performance as well as the nutritional attributes of bambara groundnut with emphasis on seed protein.

2.2 Origin and distribution of bambara groundnut

Bambara groundnut is an indigenous African legume crop that originated from West Africa around Mali. Currently, it is cultivated in many other parts of the world including South America, Asia and Oceania (Baudoin and Mergeai, 2001). It is produced in several African countries in western, eastern and southern Africa (Hillocks *et al.,* 2012). In southern Africa, the crop is cultivated mainly in Botswana (Karikari, 2000), South Africa (Mabhaudi and Modi, 2013) and Zimbabwe (Makanda *et al.,* 2009b) among other countries.

2.3 Morphological characteristics of bambara groundnut

Bambara groundnut is a herbaceous, annual crop with a stem that forms a crown at the surface (Bamashaiye *et al.,* 2011). The plant has as a well-developed taproot with alternate and trifoliate leaves that develop from the stem (Karikari, 2000). It consists of about ten running stems with short internodes, from where the roots develop. The crop has auxiliary peduncles that extend from the nodes on the stem and each peduncle forms one or three flowers (Gibbon and Pain, 1985). The bambara groundnut plant can grow up to about 0.3.0m tall. It consists of underground pods that can grow up to 3.7 cm depending on the number of seeds (usually ≤ 3) per pod (Basu *et al.,* 2007). The seed of bambara groundnut occurs in a variety of colours particularly white, cream, red, black and dark brown.

2.4 Major uses

Bambara groundnut is consumed in a variety of forms. The fresh immature seed is often boiled and consumed as a snack while the dry mature seed is cooked also for human consumption. In African countries, the dry grain is often milled into flour and used in preparing food (Bamashaiye *et al.,* 2011). Bambara groundnut can also be processed into highly nutritious milk, (Murevanhema and Jideane, 2013). Surplus grain of bambara groundnut is often traded at local informal markets thus generating income for smallholder farmers in many African countries (Makanda *et al.,* 2009a). The foliage of bambara groundnut can also be used for livestock feeds (Adeparusi and Agbede, 2009). The leaves and stem rich in nitrogen and phosphorus (Tibe *et al.,* 2007). According to Yakubu *et al.,* (2010), bambara groundnut can fix

about 28.4 kg/ha of atmospheric nitrogen. Hence, bambara groundnut is frequently intercropped with cereals in many smallholder cropping systems in Africa as it improves the soil fertility. The crop residue is often incorporated into the soil in order to add organic matter (Giller and Cadissch, 1995).

2.5 Production of bambara groundnut

The correct planting date of bambara groundnut is dependent on the location. In some parts of Limpopo Province (South Africa), it is planted only in January for cultural reasons. In Botswana and Zambia, planting usually commences in November at the beginning of each cropping season (Bamashaiye *et al.,* 2011). Bambara groundnut performs well under well-drained sandy loam soils with the pH range of 5.0 to 6.5 (Basu *et al.,* 2007). The crop requires a deep ploughed, fine seedbed to allow the underground pods to grow (Baudoin and Mergeai, 2001). It requires optimum temperatures of about 30.0 to 35.0 ^{o}C in order to allow good seed germination (Bamashaiye *et al.,* 2011). The plant density of bambara groundnut depends on the cultivar. However, generally it requires about 60.0 kg/ha of seed (Basu *et al.,* 2007). Weeding and hilling (earthing-up) are usually done simultaneously using hand hoe. In most smallholder cropping systems in Africa, bambara groundnut is cultivated in harsh environments without agronomic inputs such as fertilisers, irrigation or pesticides (Massawe *et al.,* 2005).

One of the important factors in the production of bambara groundnut is the availability of good quality seed that can germinate quickly and uniformly. In the majority of the smallholder cropping systems particularly in Africa, the seed is generally of poor quality since there is no commercial production of the seed. Therefore, it is essential to determine the seed quality prior to planting. In general, accelerated aging of seed is caused by exposure to high temperatures (>40^{o} C) and humidity. It is also important to determine the seedling viability.

2.6 Agronomic performance

The field performance is often used to assess the genetic potential and adaptation of bambara groundnut landraces. In these field evaluations, yield components such as pod load, duration to maturity and seed size are often positively correlated to the grain yield of bambara groundnut (Ouedraogo *et al.,* 2008; Mabhaudhi and Modi, 2013; Kouassi and Zorobi, 2011). These characters are also useful in the selection of potential new cultivars. In a study involving the field evaluation of bambara groundnut landraces, early maturity was positively correlated with high yield (Toure *et al.,* 2012).

In another study comparing the productivity of cultivars differing in growth habit, the bunched types attained higher yield than the spreading types (Dje *et al.,* 2005). A study conducted in South Africa reported that red coloured genotypes matured earlier and produced >30% higher yield than the brown coloured types (Mabhaudhi and Modi, 2013). Nonetheless, these studies did not attempt to evaluate the stability of the cultivars across different environments and cropping seasons. In the case of the bambara groundnut germplasm utilized in this study, there is no adequate information regarding their yield potential in the region particularly in Limpopo and Mpumalanga Provinces. Furthermore, it is unclear if the relatively high yielding genotypes are stable across the region.

2.7 Environmental influence

Plant breeders often aim at developing cultivars that produce optimum stable yields across multiple environments. The yield of a genotype can differ with environments and seasons (Gurmu *et al.,* 2009). Therefore, genotype x environment interaction is used to determine the stability among the genotypes (Sabaghnia *et al.,* 2012). Understanding the genotype x environment interaction among the bambara groundnut genotypes will help to identify the most stable genotypes across different environments in the target production areas of Limpopo and Mpumalanga Provinces.

The yield stability can be measured using different methods. Eberthart and Russell (1966) proposed the estimated variance of genotype deviations from regressions as a measure of stability. In 1988, Linn and Binns derived a stability concept which uses the cultivar superiority index (CSI) which measures the squared difference between a cultivar's yield and the maximum yield within each environment averaged over all environments. In this method, the cultivars with broader adaptation have lower CSI values because they yield closer to the maximum within each environment (Shiringani and Shimelis, 2011).

2.8 Breeding in bambara groundnut

The breeding of bambara groundnut crop is limited by difficulties to perform crosses. This is because of the small size of flowers and low seed set (Massawe *et al.,* 2005). Identification of landraces within segregating populations that produce optimum stable yields is currently one of the common approaches breeders are utilizing to improve bambara groundnut (Kouassi and Zorobi, 2011). However, in this approach, the germplasm requires field evaluation in the target production area in order to identify and validate the performance of the superior genotypes.

Agronomic attributes particularly yield, yield components and seed protein are important selection criteria in bambara groundnut breeding (Massawe *et al.,* 2005). However, environmental factors influence these quantitative attributes particularly the grain yield. Therefore, these attributes require multi-location evaluation in order to determine their stability from one season to the other in a prospective production region. Apart from selecting for yield components, high seed protein would be desirable since bambara groundnut is used primarily for human consumption. The evaluation of nutritional components such as the essential amino acids (for instance lysine, methionine or tryptophan) could be valuable in the genetic improvement of bambara groundnut. However, the analysis of amino acids is generally expensive compared to that for crude protein.

In some communities, the popularity of bambara groundnut depends on the seed coat (testa) colour. For instance, in Burkina Faso, creamy white bambara groundnut is the most popular on the market due to its homogenous colour (Ouedraogo *et al.,* 2008). In addition, some smallholder farmers rely on the seed colour to identify their traditional varieties (Massawe *et al.,* 2005). Also, seedling vigour as an indicator of the potential for crop establishment could be a useful criterion in the development of improved cultivars of bambara groundnut.

2.8.1 Principal component analysis

The principal component analysis (PCA) is a multivariate method often used to change correlated variables into uncorrelated variables called the principal components (Rymuza *et al*., 2012). The method is used to differentiate the relationships among the traits and also decide which agronomic variables contribute to the grain yield (Mohammadi *et al*., 2014). The PCA method can be explained using the eigenvalues. When the eigenvalue is greater than one, it is considered significant (Esposito *et al*., 2007). The phenotypic variation among bambara groundnut genotypes can be explained using the PCA biplots. When the genotypes are scattered in all the quadrants in a PCA biplot there is a large genetic variation for the traits. When the genotypes are closer to each other in a principal component axes, it means that they have similar relationships for the variables (Esposito *et al*., 2007).

The PCA method was successfully used to classify quantitative and qualitative variations in bambara groundnut (Shegro *et al*., 2013). In the study, the variation among the agronomic traits of bambara was attributed partly to grain characteristics such as seed weight. In another study, the PCA method grouped closely related genotypes of peanut based on their field performance (Punto and Lantican 1983).

2.9 Nutritional attributes of bambara groundnut

The seed of bambara groundnut contains considerable amounts of protein (14.0 to 24.0%), carbohydrates (60.0%) and oil (6.0 and 12.0%) (Olugbemi and Adebosin, 2014). In addition, bambara groundnut contains about 30.0% essential amino acids such as lysine, tryptophan as well as methionine (Minka and Bruneteau, 2000).

Several methods are often used for quantifying grain protein in legumes such as bambara groundnut. For instance, the Kjeldahl method is used to determine the nitrogen concentration from organic and inorganic compounds (Pomeranic and Meloan, 1978). The protein content present in a sample is calculated from the nitrogen concentration (Fijihara *et al.,* 2001). The method has been used previously to determine the protein level in many leguminous crops such as chickpea (Qayyum *et al.,* 2012) broad and kidney beans (Afiukwa *et al.,* 2013) as well as cowpea (Animasaum *et al.,* 2015). The grain protein of bambara groundnut genotypes was also determined by near infrared reflectance spectroscopy (NIRS) (Schmilovitch *et al.,* 2000). The NIRS is a fast, non-destructive method and does not involve chemicals or reagents. In another study, the grain protein was determined using nuclear magnetic resonance (Pople *et al.,* 1957). The technique was used to analyse protein and oil quantity in soybean (Gwata and Nziramasanga, 2001; Weir *et al.,* 2005). Apart from these three methods, grain protein can be determined using Duma's method which is a combustion method that measures nitrogen gas involved in the total destruction of the sample (AOAC, 1995). However, in each of these methods, grain protein varied significantly within each leguminous species.

2.10 Summary of the literature review

(i) Bambara groundnut is a multi-purpose crop largely cultivated by smallholder growers in Africa.

(ii) In general, the crop is difficult to breed particularly using conventional approaches due to the small size of the flowers and breeders resort to selection from landrace populations.

(iii) In South Africa, there are no improved commercial cultivars of Bambara groundnut at present.

(iv) Poor seed germination which leads to poor crop establishment is one of the constraints in bambara groundnut production

(v) Multi-location evaluation of experimental lines was used successfully for identifying genotypes with optimum and stable yield in specific agro-ecologies.

(vi) The grain protein in bambara groundnut showed considerable varietal differences.

3.0 MATERIALS AND METHODS

3.1 Test locations

Three test locations were used for the field evaluation of the bambara groundnut germplasm in this study (Table 3.1). The test locations were distinct from each other in terms of the agro-ecological conditions.

Table 3.1 Prevailing agro-ecological conditions at three test locations that were used for evaluating bambara groundnut genotypes.

Location	Altitude (m)	Soil type	Annual rainfall (mm)	Mean summer temperature (°C)	References
Syferkuil	1 325	Sandy loam	350-500	28 °C to 30 °C	Moshia *et al*., 2008
Thohoyandou	764	Red-well drained	450-900	22 °C to 38 °C	Mzezewa *et al*., 2012
Nelspruit	1 781	Sandy loam	600-800	18 °C to 29 °C	Paterson, 2012

3.2 Genetic materials

Forty-one genotypes and one local check were used in the study. All the genotypes were selected previously from the landrace germplasm collection in the bambara groundnut breeding program at the School of Agriculture (University of Venda) (Rikhotso *et al*., 2013). The germplasm consisted of a diverse range of seed colour types and size including black, light brown, cream (white) and red (Fig. 3.1). Most of the genotypes (50%) consisted of the cream (white) testa colour (Table 3.2). The hylum color among the genotypes was predominantly white (Table 3.2). Thirteen genotypes including the local check ('BGN-39') possessed a light brown testa while only three genotypes ('BGN-4'; 'BGN-7' and 'BGN-34') and five genotypes were classified as black and red seeded types (Table 3.2).

Fig.3.1 The four major seed types based on testa color among 42 bambara groundnut genotypes evaluated across three locations in Limpopo and Mpumalanga Provinces (South Africa). [clockwise: 1a = Cream / white (plain hylum); 2 = Light brown; 1b = Cream / white (dark brown hylum); 3 = Red; 1c = Cream / white (brown hylum); 4 = black].

Table 3.2 Bambara groundnut experimental lines that were evaluated for agronomic performance at three test locations.

Code of experimental line	Testa color	Hylum color and /or morphological features
BGN-1	Cream (White)	Brown; speckled
BGN-2	Light brown	White
BGN-3	Cream (White)	Brown
BGN-4	Black	White
BGN-5	Cream (White)	White
BGN-6	Light brown	White
BGN-7	Black	White
BGN-8	Cream (White)	Brown
BGN-9	Cream (White)	White; speckled
BGN-10	Red	White
BGN-11	Light brown	White
BGN-12	Red	White
BGN-13	Cream (White)	White; speckled
BGN-14	Red	White
BGN-15	Cream (White)	White surround by dark color
BGN-16	Cream (White)	White
BGN-17	Red	White
BGN-18	Cream (White)	White
BGN-19	Light brown	White
BGN-20	Red	White
BGN-21	Cream (White)	White surround by dark color
BGN-22	Cream (White)	White surround by dark color
BGN-23	Red	White
BGN-24	Cream (White)	White surround by dark color
BGN-25	Red	White
BGN-26	Cream (White)	White surround by dark color
BGN-27	Cream (White)	White surround by dark color
BGN-28	Cream (White)	White surround by dark color
BGN-29	Cream (White)	White surround by dark color
BGN-30	Red	White
BGN-31	Red	White
BGN-32	Cream (White)	White surround by dark color
BGN-33	Light brown	White
BGN-34	Black	White
BGN-35	Cream (White)	White surround by dark color
BGN-36	Cream (White)	White surround by dark color
BGN-37	Light brown	White
BGN-38	Light brown	White
BGN-39 (Check)	Light brown	White
BGN-40	Cream (White)	White surround by dark color
BGN-41	Light brown	White
BGN-42	Cream (White)	White surround by dark color

3.3 Planting, trial management and measurements

Healthy seed of each genotype was planted manually at each test location in a two-row field plot. In each row, the seed was planted at approximately 4.0 cm deep and 25.0 cm intra-row. The rows were 2.0 m long each and spaced at 0.6 m apart. There was no treatment of seed with commercial inoculants prior to planting. During the season, standard agronomic management practices (such as weeding) were followed.

At maturity, the plants were harvested manually by excavating the pods followed by sun-drying to a constant weight. For each plot, five plants were selected at random for determining the following agronomic attributes:

(i) dry shoot weight per plant (DSW)
(ii) number of pods per plants (NPP)
(iii) pod weight per plant (PWT)
(iv) pod length (PL)
(v) number of seeds per pod (NSP)
(vi) 100 seed weight (100-SWT)
(vii) seed weight per plant (SWTP) and
(viii) seed yield (SYLD)

3.4 Determination of seed protein

The seed protein was determined using the Kjeldahl method (AOAC, 1990). In the method, 1.0g of the seed sample of each genotype was mixed with 25.0ml H_2SO_4 in a heating tube prior to the addition of sodium sulphate and allowed to undergo digestion for 9 to 10 hours after which distillation was carried out. The digested mixture (Fig. 3.2) was distilled thereafter and collected into 4.0% boric acid containing two drops of methyl red indicator and titrated using direct titration method in order to determine the N concentration which was subsequently converted (x 6.025 conversion factor) into the protein concentration. The process was repeated (duplicated) for each sample.

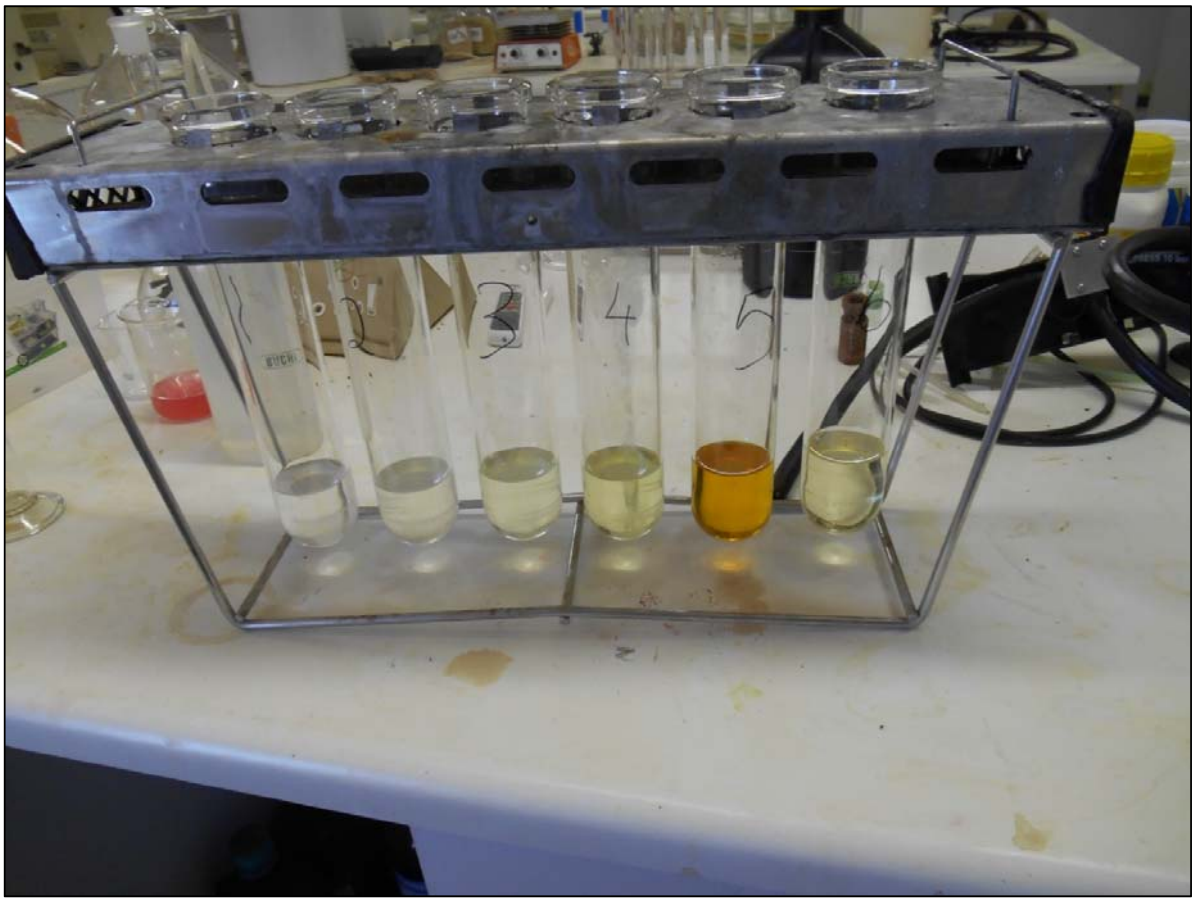

Fig. 3.2 An example of digested mixtures (test tubes 1 to 6) during seed protein analysis in the laboratory using Kjedahl method.

3.5 PCA and cluster analysis

The PCA was conducted to reduce a number of variables into a few uncorrelated components using PROC PRINCOMP of SAS Institute (2014). The cluster analysis was done on a genetic distance matrix of the genotypes to predict their grouping pattern. PROC CLUSTER of SAS institute (2014) using unweighted pair of group method was employed (Habtamu *et al*., 2011).

3.6 Experimental design and data analysis

At each location, the field trial was arranged as a 7 × 6 rectangular lattice design replicated three times. Data sets were subjected to analysis of variance (ANOVA) procedure with SAS (SAS Institute, 2014) using the following statistical linear model:

$$Y_{ijkl} = \mu + L_i + S_j + RL_{ik} + G_l + SG_{jl} + LG_{il} + SLG_{ijl} + \varepsilon_{ijkl}$$

where:

Y_{ijkl} = the value of the observed trait for genotype *i* in crop season *j* in replicate *k* within the environment *l*

μ = the overall mean

L_i = the effect of the i^{th} environment

S_j = the effect of the j^{th} crop season

RL_{ik} = the random effect of the i^{th} environment within the k^{th} replicate

G_l = the effect of the l^{th} genotype

SG_{jl} = the interaction between the j^{th} season and the l^{th} genotype

LG_{il} = the interaction between the i^{th} environment and the l^{th} genotype

SLG_{ijl} = the interaction among the i^{th} environment and the j^{th} season and the l^{th} genotype

ε_{ijkl} = the residual error

The stability of each genotype over the two seasons was determined using the Cultivar Superiority Index (CSI) (Shiringani and Shimelis, 2011; Lin and Binn, 1998). The CSI measured the squared difference between each genotype's yield and the maximum yield within each environment averaged over all environments. Therefore the genotypes with broader adaptation had relatively lower CSI values because they yielded closer to the maximum within each environment. The data set for the seed protein content were analysed (using the ANOVA procedure) as a randomized complete block with two replications. The means were separated using the Duncan's procedure (Duncan, 1956).

4.0 CHAPTER FOUR: RESULTS

This chapter presents the findings of the study covering all the three specific objectives. In the first part, the chapter presents the results of the evaluation of agronomic performance of bambara groundnut genotypes under different environmental conditions. The results of the stability of the genotypes as well as their seed protein are also presented.

4.1 Field evaluation

There were significant (P<0.05) differences for both dry shoot weight (DSW) and seed yield (SYLD) among the bambara groundnut genotypes at Syferkuil over the two seasons (Table 4.1). In addition, there were highly significant (P<0.01) differences for all the traits, with the exception of pod length (PL) between the two seasons at Syferkuil. However, at Thohoyandou, there were highly significant (P<0.01) differences among the genotypes for all the six agronomic traits (Table 4.1). Overall, the number of seeds per pod (NSP) and number of pods per plant were similar among the genotypes (hence the actual values were not shown in the summary tables). Nonetheless, some individual genotypes, such as 'BGN-34' showed high pod load and shoot biomass particularly at Nelspruit (Fig 4.1). The genotype x season interaction was significant (P < 0.05) for only three agronomic attributes namely pod weight per plant (PWT), seed weight per plant (SWTP) and 100-seed weight (100-SWT). In comparison, the genotype x season interaction was highly significant (P < 0.01) at Thohoyandou only for DSW, PWTP and SWTP (Table 4.1). At Nelspruit, the genotypes showed highly significant (P<0.01) differences for only three traits namely PL, 100-SWT and SYLD (Table 4.1)

The combined analysis of variance across the three locations showed that there were highly significant (P<0.01) differences between the genotypes for all the six agronomic traits that were evaluated (Table 4.2). Similarly, the genotypes showed highly significant (P<0.01) differences for all the traits. The location x genotype and location x season interactions were highly significant (P<0.01) for all the six traits (Table 4.2). However, there were no significant seasonal effects on PL. In contrast, the genotype x season interaction was highly significant (P<0.01) for PWT, SWTP, 100-SWT and SYLD (Table 4.2). Similarly, the location x season interaction was highly significant (P<0.01) for all the traits except for PL.

Table 4.1 Mean squares for six agronomic traits of bambara groundnut genotypes evaluated at each of three locations in Limpopo and Mpumalanga Provinces (South Africa). (DSW=dry shoot weight; PL=pod length; PWT=pod weight per plant; SWTP=seed weight per plant; 100-SWT=100 seed weight; SYLD=seed yield).

Source	df	Traits					
		DSW (g)	PL (cm)	PWT (g)	SWTP (g)	100-SWT (g)	SYLD (kg/ha)
Syferkuil							
Rep	2	4545.7948	0.1667	2652.1297	1390.5710	1144.4278	396124.2030
Block (Rep)	18	150.1219	0.1563	238.6511**	127.1804**	727.4501**	214781.1300**
Genotype	41	285.5370*	0.2243	151.0608	78.5357	678.0480**	166107.1040*
Rep x Genotype	70	86.0904	0.2349	65.7085	35.0575	210.1808	56579.3600
Season	1	23973.3762**	0.0648	29396.5315**	15789.6648**	4558.4376**	5418708.0130**
Genotype x Season	41	202.8322	0.1296	192.9209*	99.6828*	358.2087*	136803.2070
Thohoyandou							
Rep	2	74.5149	0.8703	102.75	74.7707	90.6707	629830.2200
Block (Rep)	18	70.7505**	0.1790**	41.5788**	22.4382**	73.8885	119580.1200**
Genotype	41	54.7358**	0.2905**	64.0598**	36.5444**	317.4535**	329891.4200**
Rep x Genotype	70	65.4924**	0.1216	25.8315*	16.0741*	88.1723	78030.7900
Season	1	2663.5053**	6.8673**	213.2376**	0.6380	4961.6131**	8507.4700
Genotype x Season	41	54.1231**	0.1144	35.0418**	19.6380**	94.6069	59246.8300
Nelspruit							
Rep	2	2876.7867	0.3717	2460.1720	1391.6916	99.7687	1249961.5300
Block (Rep)	25	187.5782	0.3294**	112.6008	57.5193	335.1312	189391.7000*
Genotype	41	176.3749	0.4069**	123.3651	74.2006	747.3446**	432436.5100**

**; * = significant at the 1.0% and 5.0% probability levels, respectively.

Fig. 4.1 High pod load and shoot biomass in an individual plants of bambara groundnut genotype ‘BGN-34’ (top) and ‘BGN-7’ (bottom) during the 2013/2014 cropping season.

Table 4.2 Mean squares for six agronomic traits of bambara groundnut genotypes evaluated across three locations in Limpopo and Mpumalanga Provinces (South Africa). (DSW=dry shoot weight; PL=pod length; PWT=pod weight per plant; SWTP=seed weight per plant; 100-SWT=100 seed weight; SYLD=seed yield).

Source	df	Trait					
		SDW (g)	PL (cm)	PWTP (g)	SWTP (g)	100-SWT (g)	SYLD (kg/ha)
Location (L)	2	5146.4568**	7.2723**	8337.8410**	5579.4775**	26314.8063**	7513352.4700**
Rep(L)	5	1848.1472**	0.4318**	1103.2623**	586.7296**	567.0311**	720010.1500**
Genotype (G)	41	211.1427**	0.4269**	138.4241**	83.2894**	783.7333**	483010.6800**
L x G	82	317.8963**	0.2192**	189.2403**	105.1468**	253.6023**	133479.7400**
Rep x G(L)	205	58.6012	0.1338*	39.8851	22.2758	129.8104*	75648.6000
Season (S)	1	2845.4328**	6.0589	8816.0709**	5977.7789**	188.8253	2120370.1400**
L x S	1	23879.9718**	2.5110**	20777.5920**	9812.1017**	9224.8363**	3299842.0600**
G x S	41	92.7932	0.1273	93.6530**	52.9119**	209.6093*	106931.8400*
L x G x S	41	180.5421**	0.1395	154.5920**	75.0082**	251.5969**	113345.6500**

**; * = significant at the 1.0% and 5.0% probability levels, respectively.

At Syferkuil, at least six genotypes achieved >50.0% higher DSW than the check genotype (Fig. 4.2). The highest DSW was attained by the genotype 'BGN-40' (44.95 g) while the check genotype ('BGN-39') achieved only 21.70 g (Table 4.3). The longest pods (2.90 cm) were observed for genotype 'BGN-40' but the heaviest pods (31.31 g) were developed by the genotype 'BGN-18' (Table 4.3). In comparison with the check genotype, 'BGN-18' attained a two-fold higher SWTP. On average, the seed size of the genotypes was >50.0 g at Syferkuil (Table 4.3). In terms of seed yield, only genotype 'BGN-11' achieved >1.0 t/ha while the trial mean at the location was 540.36 kg/ha. Nonetheless, >40.0% of the genotypes attained <500.0 kg/ha but genotype 'BGN-21' achieving the lowest (191.0 kg/ha) SYLD (Table 4.3).

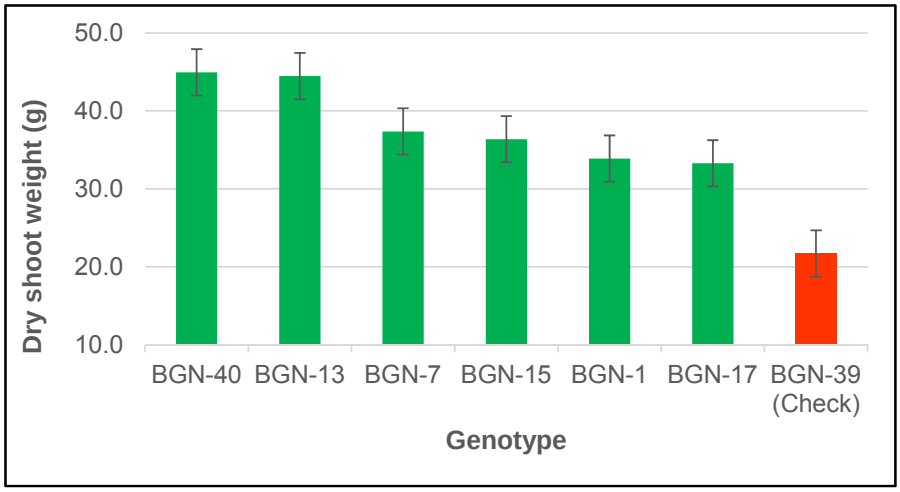

Figure 4.2 Dry shoot weight of the top six bambara groundnut genotypes in comparison with the check genotype (red) that were evaluated at Syferkuil during 2013/2014 cropping season.

Table 4.3 Means of six agronomic attributes of bambara groundnut genotypes evaluated at Syferkuil during 2013/2014 and 2014/2015 cropping seasons. (DSW=dry shoot weight; PL=pod length; PWT=pod weight per plant; SWTP=seed weight per plant; 100-SWT=100 seed weight; SYLD=seed yield).

Genotype	Trait					
	DSW (g)	PL (cm)	PWT (g)	SWTP (g)	100-SWT (g)	SYLD (kg/ha)
BGN-40	44.95 a	2.90 a	10.59 h-j	5.59 ij	38.40 no	469.40 d-m
BGN-13	44.47 ab	1.98 e-f	26.27 a-e	17.01 a-f	55.60 d-n	512.00 c-m
BGN-7	37.37 a-c	2.28 b-f	24.68 a-f	17.09 a-f	64.97 a-i	790.90 a-e
BGN-15	36.37 a-d	2.00 d-f	27.39 a-c	19.73 a-d	62.98 c-j	511.10 c-m
BGN-1	33.89 a-e	2.08 c-f	13.35 f-j	9.00 f-j	50.84 g-o	371.70 f-m
BGN-17	33.28 a-f	2.30 b-f	23.77 a-g	16.55 a-g	67.15 a-g	514.70 c-m
BGN-18	32.29 a-g	2.68 ab	31.31 a	22.60 a	58.18 c-l	392.80 f-m
BGN-33	31.55 a-g	1.92 ef	16.76 c-j	12.08 b-j	76.08 ab	744.50 a-f
BGN-20	30.42 a-h	2.56 a-c	24.33 a-g	18.28 a-e	71.31 a-e	612.00 b-l
BGN-8	29.90 a-h	2.00 d-f	18.99 b-j	11.65 c-j	69.03 a-f	697.10 a-h
BGN-9	29.51 a-h	2.42 a-e	12.25 g-j	8.69 f-j	59.47 b-k	585.80 b-l
BGN-32	28.86 b-h	1.98 ef	7.96 j	4.73 j	39.27 m-o	300.50 k-m
BGN-27	28.40 c-h	1.92 ef	10.18 ij	6.30 h-j	14.05 p	251.50 lm
BGN-31	28.34 c-h	2.08 c-f	19.68 a-j	13.93 a-i	66.46 a-h	683.10 a-j
BGN-16	28.10 c-h	2.10 c-f	24.45 a-g	14.28 a-i	51.67 f-o	381.10 f-m
BGN-34	27.52 c-h	1.94 ef	22.80 a-h	16.69 a-g	81.67 a	926.00 ab
BGN-35	27.27 c-h	2.06 c-f	16.18 c-j	10.14 e-j	37.11 p	258.40 l-m
BGN-4	27.15 c-h	2.36 b-f	19.60 a-j	14.39 a-i	52.28 f-o	813.60 a-d
BGN-23	26.33 c-h	2.20 b-f	19.92 a-j	12.34 b-j	63.24 b-j	687.30 a-i
BGN-21	25.80 c-i	1.92 ef	13.67 f-j	9.26 e-j	54.69 e-n	191.10 m
BGN-41	25.65 c-i	2.20 b-f	12.40 g-j	8.42 f-j	68.25 a-g	674.90 a-j
BGN-24	25.40 c-i	1.84 f	9.84 ij	5.95 i-j	56.64 c-m	192.90 m
BGN-30	25.39 c-i	2.02 d-f	14.79 d-j	10.48 e-j	58.77 b-l	511.40 c-m
BGN-19	25.30 c-i	2.10 c-f	20.58 a-i	16.30 a-g	72.11 a-d	648.10 a-k
BGN-42	25.22 c-i	2.28 b-f	23.88 a-g	13.21 b-j	39.05 no	310.50 j-m
BGN-6	24.66 c-i	2.18 b-f	29.70 ab	21.12 ab	63.07 b-j	719.10 a-g
BGN-2	23.95 c-i	2.28 b-f	15.18 d-j	10.90 d-j	49.33 h-o	318.70 i-m
BGN-11	23.66 c-i	1.96 ef	26.79 a-d	20.35 a-c	66.30 a-i	1016.70 a
BGN-25	23.05 c-i	2.52 a-d	21.96 a-i	14.06 a-i	69.95 a-e	604.70 b-l
BGN-39 (Check)	21.70 c-i	2.14 c-f	14.81 d-j	10.36 e-j	41.81 l-o	421.40 e-m
BGN-5	21.32 d-i	2.22 b-f	18.29 b-j	12.72 b-j	48.99 i-o	485.40 c-m
BGN-29	20.02 e-i	2.28 b-f	12.60 f-j	8.93 f-j	57.29 c-l	384.00 f-m
BGN-26	19.94 e-i	1.96 ef	12.50 g-j	8.82 f-j	41.96 l-o	326.60 h-m
BGN-3	18.74 e-i	1.90 ef	15.85 c-j	11.40 c-j	47.14 j-o	480.70 c-m
BGN-10	18.54 e-i	2.12 c-f	16.88 c-j	11.54 c-j	63.48 b-j	693.60 a-h
BGN-28	17.69 f-i	2.00 d-f	10.59 h-j	7.64 g-j	39.46 m-o	498.80 c-m
BGN-12	16.65 g-i	2.16 b-f	14.43 e-j	10.87 d-j	73.18 a-c	848.40 a-c
BGN-37	16.56 g-i	2.18 b-f	16.24 c-j	12.02 c-j	51.20 g-o	548.40 c-m
BGN-14	16.38 g-i	2.30 b-f	15.88 c-j	11.67 c-j	66.44 a-h	694.30 a-h
BGN-36	14.68 hi	2.04 c-f	15.65 c-j	10.85 d-j	43.58 k-o	676.30 a-j
BGN-38	14.49 hi	2.26 b-f	11.12 h-j	8.25 f-j	60.11 b-k	595.80 b-l
BGN-22	10.10 i	2.22 b-f	21.54 a-i	15.19 a-h	55.05 d-n	349.80 g-m
Grand mean	25.73	2.16	17.99	12.41	56.38	540.36
C.V.(%)	48.26	19.04	52.91	56.88	24.07	53.95
R^2(%)	91.76	84.84	94.65	94.46	92.08	89.63

In each column, means followed by a different letter are significant at the 5.0% probability level.

At Thohoyandou, the highest (29.56 g) and lowest (11.66 g) DSW was achieved by genotype 'BGN-13' and 'BGN-22' respectively (Table 4.4). Overall, the mean DSW at Thohoyandou (21.03 g) was only 7.0% more than that of the check genotype ('BGN-39'). 'BGN-40' produced the longest pods (2.74 cm) while the shortest pods (1.72 cm) were observed for genotype 'BGN-21' (Table 4.4). At the same location, genotype 'BGN-13' produced both the heaviest pods per plant (23.12 g). The average seed size (46.72 g per 100 seed) at the location was at least 10.0% higher than for the check genotype. The highest SYLD (982.0 kg/ha) was achieved by 'BGN-19' and the trial mean was 639.01 kg/ha (Table 4.4).

In the single season that the genotypes were evaluated at Nelspruit, the highest DSW (24.87 g) was 47.0% heavier than the trial mean (Table 4.5). The genotype 'BGN-40' also attained the longest pods (2.81 cm) at Nelspruit. The mean SWTP (9.14 g) at the location was 40.0% lower than the highest seed weight per plant that was achieved by the genotype 'BGN-13' (Table 4.5). However, the largest seed (55.38 g per 100 seed) was observed for genotype 'BGN-19' while the highest SYLD (1 206.80 kg/ha) at the location was attained by genotype 'BGN-19' (Table 4.5). The average SYLD across the three locations was <1.00 t/ha (Table 4.6)

Table 4.4 Means of six agronomic attributes of bambara groundnut genotypes evaluated at Thohoyandou during 2013/2014 and 2014/2015 cropping seasons. (SDW=dry shoot weight; PL=pod length; PWT=pod weight per plant; SWTP=seed weight per plant; 100-SW=100 seed weight; SYLD=seed yield).

Genotype	Trait					
	DSW (g)	PL (cm)	PWT (g)	SWTP (g)	100-SWT (g)	SYLD (kg/ha)
BGN-13	29.56 a	1.91 f-k	23.12 a	16.69 a-c	40.53 h-m	596.90 e-m
BGN-32	29.31 a	1.89 g-k	12.84 de	8.80 c-e	39.01 j-m	411.30 k-n
BGN-34	27.60 ab	2.07 b-j	22.65 a-c	16.34 ab	59.02 ab	908.40 a-c
BGN-40	25.64 a-c	2.74 a	13.36 b-e	8.78 c-e	44.33 e-m	460.90 i-n
BGN-16	25.50 a-c	2.17 b-i	19.41 a-e	12.68 a-e	41.47 g-m	391.20 l-n
BGN-18	24.92 a-c	2.14 b-i	20.82 a-d	15.34 a-d	42.18 f-m	431.50 j-n
BGN-17	24.79 a-c	2.11 b-i	16.94 a-e	12.02 a-e	54.08 a-f	739.80 a-g
BGN-7	24.66 a-c	2.11 b-i	18.51 a-e	13.18 a-e	47.78 b-l	697.70 b-i
BGN-35	24.40 a-d	2.14 b-i	20.40 a-d	13.93 a-e	40.46 i-m	465.50 i-n
BGN-38	23.93 a-e	2.03 c-k	22.78 ab	17.02 a	49.87 a-k	827.30 a-e
BGN-3	23.11 a-e	1.77 jk	19.34 a-e	14.19 a-d	41.57 g-m	478.90 g-n
BGN-42	23.06 a-e	2.01 c-k	17.94 a-e	11.26 a-e	34.74 m-n	431.90 j-n
BGN-30	22.68 a-e	2.03 c-j	16.65 a-e	12.21 a-e	52.91 a-g	657.20 c-k
BGN-33	22.55 a-e	1.93 e-k	17.52 a-e	13.00 a-e	54.41 a-f	872.00 a-d
BGN-27	22.41 a-e	1.97 e-k	10.29 e	6.78 e	24.40 n	323.60 n
BGN-20	22.15 a-e	2.31 b-d	20.75 a-d	15.83 a-c	56.49 a-e	793.40 a-f
BGN-24	21.79 a-f	1.92 f-k	13.82 a-e	9.71 b-e	42.85 f-m	402.10 k-n
BGN-15	21.79 a-f	1.98 e-k	19.07 a-e	14.20 a-d	45.47 c-m	472.70 h-n
BGN-25	21.76 a-f	2.31 bc	19.64 a-e	13.44 a-e	57.56 a-c	852.40 a-e
BGN-41	21.68 a-f	2.05 c-j	17.01 a-e	11.92 a-e	52.06 a-i	603.80 e-m
BGN-4	21.13 a-f	1.98 e-k	19.33 a-e	14.44 a-d	45.32 d-m	921.90 a-b
BGN-31	20.93 a-f	1.98 e-k	17.33 a-e	12.24 a-e	51.58 a-j	735.70 a-h
BGN-1	20.72 a-f	2.08 b-j	13.15 c-e	9.67 b-e	40.65 h-m	488.20 g-n
BGN-8	20.58 a-f	1.99 e-k	15.36 a-e	10.27 a-e	52.76 a-h	612.00 d-l
BGN-19	20.32 a-f	2.01 c-k	19.09 a-e	15.60 a-c	62.06 a	982.00 a
BGN-23	20.02 a-f	2.08 b-j	18.66 a-e	12.90 a-e	51.58 a-j	815.00 a-f
BGN-29	19.94 a-f	2.20 b-g	14.68 a-e	10.80 a-e	46.30 c-m	612.20 d-l
BGN-21	19.66 a-f	1.72 k	13.96 a-e	9.81 b-e	46.45 c-m	346.20 m-n
BGN-2	19.65 a-f	2.36 b	14.32 a-e	10.45 a-e	45.51 c-m	594.90 e-m
BGN-39 (Check)	19.57 a-f	1.88 h-k	13.85 a-e	9.68 b-e	42.37 f-m	702.10 b-i
BGN-28	18.58 b-f	2.06 b-j	14.31 a-e	10.35 a-e	38.01 k-m	558.40 f-n
BGN-9	18.49 b-f	2.21 b-f	11.72 de	8.41 d-e	46.16 c-m	663.90 b-k
BGN-14	18.39 b-f	2.19 b-h	17.70 a-e	13.06 a-e	52.58 a-i	763.40 a-f
BGN-6	18.27 b-f	1.92 f-k	19.68 a-e	14.11 a-d	53.03 a-g	790.30 a-f
BGN-11	17.72 b-f	1.86 i-k	20.20 a-d	15.23 a-d	54.23 a-f	974.70 a
BGN-37	17.02 c-f	2.03 c-j	17.06 a-e	12.24 a-e	43.88 f-m	656.90 c-k
BGN-26	16.78 c-f	1.91 g-k	13.93 a-e	10.16 a-e	43.65 f-m	680.60 b-j
BGN-5	16.51 c-f	2.01 c-k	12.75 de	9.02 c-e	36.46 l-n	469.80 i-n
BGN-36	15.99 c-f	1.98 e-k	15.55 a-e	10.94 a-e	40.37 i-m	657.50 c-k
BGN-12	14.27 d-f	2.23 b-e	16.21 a-e	12.51 a-e	57.36 a-d	926.50 ab
BGN-10	13.86 ef	2.00 d-k	16.67 a-e	12.44 a-e	46.94 b-m	689.40 b-j
BGN-22	11.66 f	1.95 e-k	14.70 a-e	10.62 a-e	43.19 f-m	378.50 l-n
Grand mean	21.03	2.05	16.98	12.17	46.72	639.01
C.V.(%)	65.18	20.35	75.43	79.11	35.28	55.54
R^2(%)	15.34	22.05	13.07	13.11	21.75	27.63

In each column, means followed by a different letter are significant at the 5.0% probability level

Table 4.5 Means of six agronomic attributes of bambara groundnut genotypes evaluated at Nelspruit during 2013/2014 cropping season. (SDW=dry shoot weight; PL=pod length; PWT=pod weight per plant; SWTP=seed weight per plant; 100-SWT=100 seed weight; SYLD=seed yield)

Genotype	Trait					
	DSW (g)	PL (cm)	PWT (g)	SWTP (g)	100-SWT (g)	SYLD (kg/ha)
BGN-35	24.87 a	2.07 b-f	16.33 b-d	11.73 b-g	41.13 b-g	560.60 g-o
BGN-13	24.49 ab	1.86 b-j	23.22 a	15.58 a	28.37 k-n	564.90 g-n
BGN-34	23.86 a-c	2.04 b-g	18.22 b	12.36 a-d	45.21 b-e	853.40 b-f
BGN-16	22.04 a-d	2.09 b-e	9.46 j-m	6.11 n-p	30.01 j-n	293.40 o-q
BGN-42	21.26 a-e	1.83 c-k	10.40 h-m	6.68 m-p	28.40 k-n	355.50 m-q
BGN-30	20.67 a-f	1.99 b-h	14.27 b-i	10.50 b-k	47.14 a-c	647.20 e-l
BGN-18	20.37 a-f	1.68 h-l	10.99 g-m	8.01 h-p	27.44 l-n	342 40 m-q
BGN-14	19.83 a-g	2.01 b-g	16.24 b-e	11.86 b-e	40.57 b-h	666.90 c-k
BGN-17	19.83 a-g	1.94 b-i	11.87 d-m	8.18 g-p	41.92 b-g	825.50 b-g
BGN-28	19.09 a-g	1.91 b-j	9.38 j-m	6.15 n-p	30.96 i-n	520.50 i-q
BGN-41	18.97 b-g	1.96 b-h	15.52 b-f	10.91 b-i	38.36 b-j	524.10 i-q
BGN-20	18.66 c-h	2.11 b-d	17.90 b	13.59 ab	47.10 a-c	909.50 b-e
BGN-2	18.32 c-i	2.17 b	8.90 k-m	5.93 n-p	37.52 d-k	575.00 g-m
BGN-33	18.15 c-i	1.76 f-l	17.94 b	13.19 a-c	37.87 c-j	755.40 b-i
BGN-27	18.14 c-i	1.77 e-k	9.37 j-m	6.38 m-p	26.00 mn	257.80 q
BGN-39 (Check)	18.00 d-i	1.80 d-l	12.96 c-k	9.20 d-n	37.56 d-k	747.10 b-j
BGN-38	16.89 d-j	1.73 g-k	16.13 b-e	11.30 b-h	40.15 b-i	779.90 b-i
BGN-37	16.82 d-j	1.94 b-i	16.38 b-d	11.74 b-f	36.07 e-l	565.90 g-n
BGN-21	16.66 d-k	1.50 l	8.11 l-m	4.84 p	36.30 e-l	346.90 m-q
BGN-6	16.18 e-k	1.78 e-l	13.84 b-j	9.83 c-m	43.93 b-f	648.80 d-l
BGN-3	16.11 e-k	1.61 j-l	16.80 bc	11.67 b-g	36.09 e-l	482.80 j-q
BGN-11	15.93 e-k	1.77 e-k	15.30 b-g	10.92 b-i	42.67 b-g	812.90 b-h
BGN-19	15.88 e-k	1.86 b-j	16.33 b-d	12.84 a-c	55.38 a	1206.80 a
BGN-25	15.86 e-k	2.03 b-g	10.89 g-m	8.25 f-p	47.58 ab	916.70 b-d
BGN-7	15.77 e-k	1.90 b-j	11.43 f-m	7.75 h-p	33.88 g-m	458.90 k-q
BGN-26	15.42 f-k	1.63 i-l	11.72 e-m	8.48 e-o	44.36 b-f	926.60 bc
BGN-36	15.36 f-k	1.91 b-j	11.42 f-m	7.98 h-p	34.51 g-m	537.60 i-p
BGN-24	15.25 f-k	1.84 c-j	14.70 b-h	10.56 b-j	30.79 j-n	466.60 k-q
BGN-29	15.09 f-k	2.17 b	10.99 g-m	7.69 i-p	35.72 f-l	593.90 f-m
BGN-23	14.91 f-k	1.84 c-j	14.10 b-i	10.44 b-k	45.35 b-e	1015.10 ab
BGN-31	14.90 f-k	1.91 b-j	12.17 d-l	8.66 e-o	36.99 d-k	549.80 h-o
BGN-32	14.86 f-k	1.80 d-l	10.10 i-m	7.03 j-p	33.69 g-n	305.20 n-q
BGN-9	14.84 f-k	2.04 b-g	10.12 i-m	6.96 k-p	33.97 g-m	584.20 g-m
BGN-8	14.43 g-k	2.01 b-g	10.93 g-m	7.29 j-p	36.36 e-l	393.20 l-q
BGN-4	14.14 g-k	1.84 c-j	14.20 b-i	10.28 b-l	37.15 d-k	829.00 b-g
BGN-12	14.10 g-k	2.13 bc	13.72 b-j	10.30 b-l	45.94 b-d	929.50 bc
BGN-15	13.04 h-k	1.80 d-l	7.56 m	5.69 n-p	31.69 h-n	363.50 m-q
BGN-40	13.03 h-k	2.81 a	11.07 f-m	7.17 j-p	42.96 b-g	392.90 l-q
BGN-1	12.70 i-k	1.87 b-j	10.72 h-m	7.63 i-p	30.81 j-n	439.90 k-q
BGN-22	12.05 j-k	1.51 k-l	7.54 m	5.59 o-p	30.97 i-n	278.00 p-q
BGN-5	12.02j-k	1.86 b-j	9.33 j-m	6.78 l-p	24.44 n	399.40 k-q
BGN-10	10.86 k	1.83 c-k	13.36 c-k	9.80 c-m	31.74 h-n	542.80 i-p
Grand mean	16.90	1.90	12.90	9.14	37.26	599.19
C.V. (%)	32.52	15.77	33.09	36.78	23.64	42.32
R^2 (%)	82.54	81.32	80.58	79.15	79.88	80.38

In each column, means followed by a different letter are significant at the 5.0% probability level

Table 4.6 Means of six agronomic attributes of bambara groundnut genotypes evaluated across three locations during 2013/2014 cropping season. (SDW=dry shoot weight; PL=pod length; PWT=pod weight per plant; SWTP=seed weight per plant; 100-SWT=100 seed weight; SYLD=seed yield).

Genotype	Trait					
	DSW (g)	PL (cm)	PTWT (g)	SWTP (g)	100-SWT (g)	SYLD (kg/ha)
BGN-13	32.84 a	1.92 g-m	2.20 a	16.43 a	41.50 g-m	557.93 c-n
BGN-40	27.87 ab	2.82 a	11.67 ef	7.18 g-i	41.90 f-m	441.07 f-n
BGN-34	26.33 a-c	2.02 e-k	21.22 ab	15.13 a-d	61.97 a	895.93 a-c
BGN-17	25.97 a-c	2.12 b-h	17.53 b-e	12.25 a-f	54.38 a-e	693.33a-k
BGN-7	25.93 a-c	2.17 c-i	18.21 a-e	12.67 a-f	48.88 b-k	649.17 a-l
BGN-18	25.86 a-c	2.17 b-f	21.04 a-c	15.32 a-c	42.60 e-m	388.90 h-n
BGN-35	25.51 a-d	2.09 c-i	17.64 a-e	11.93 a-g	39.57 j-m	428.17 g-n
BGN-16	25.21 a-d	2.12 b-g	17.77 a-e	11.02 d-i	41.05 h-m	355.23j-n
BGN-32	24.34 b-e	1.89 i-l	10.30 f	6.85 hi	37.32 k-m	339.00 l-n
BGN-33	24.08 b-e	1.87 j-m	17.41 b-e	12.76 a-f	56.12 a-d	790.63 a-f
BGN-20	23.74 b-e	2.33 b	20.99 a-c	15.90 ab	58.30 a-c	771.63 a-g
BGN-15	23.73 b-e	1.93 g-m	18.01 a-e	13.21 a-e	46.71 c-l	449.10 f-n
BGN-42	23.18 b-f	2.04 e-k	17.41 b-e	10.38 d-i	34.06 m	365.97 i-n
BGN-27	22.98 b-f	1.89 i-m	9.95 f	6.49 i	21.48 n	277.63 n
BGN-30	22.91 b-f	2.01 e-k	15.24 b-f	11.06 b-i	52.94 a-g	605.27 a-n
BGN-1	22.44 b-g	2.01 e-k	12.41 d-f	8.77 e-i	40.77 i-m	433.27 g-n
BGN-41	22.10 b-g	2.07 c-j	14.98 b-f	10.42 d-i	52.89 a-g	600.93 a-n
BGN-8	21.64 b-g	2.00 e-k	15.09 b-f	9.74 e-i	52.72 a-h	567.43 c-n
BGN-31	21.39 b-g	1.99 f-k	16.39 b-f	11.61 a-h	51.68 a-i	656.20a-l
BGN-9	20.95 b-g	2.22 b-e	11.36 ef	8.02 f-i	46.53 d-l	611.30 a-n
BGN-24	20.81 b-g	1.87 j-m	12.79 d-f	8.74 e-i	43.43e-m	353.87 k-n
BGN-4	20.81 b-g	2.06 d-j	17.71 a-e	13.04 a-e	44.92 d-m	854.83 a-d
BGN-21	20.71 b-g	1.71 m	11.91 d-f	7.97 f-i	45.81 d-m	294.73 mn
BGN-2	20.64 b-g	2.27 b-d	12.80 d-f	9.09 e-i	44.12 e-m	496.20 e-n
BGN-19	20.50 b-g	1.99 f-k	18.67 a-d	14.92 a-d	63.18 a	945.63 a
BGN-23	20.42 b-g	2.04 e-k	17.56 b-e	11.89 a-g	53.39 a-f	839.13 a-e
BGN-25	20.22 b-g	2.29 bc	17.45 b-e	11.92 a-g	58.36 ab	791.27 a-f
BGN-39 (Check)	19.76 b-g	1.94 g-l	13.87 d-f	9.74 e-i	40.58 i-m	623.53 a-n
BGN-6	19.70 b-g	1.96 f-l	21.07 a-c	15.02 a-d	53.34 a-g	719.40 a-i
BGN-3	19.32 c-g	1.76 l-m	17.33 b-e	12.42 a-f	41.60 f-m	480.80 f-n
BGN-11	19.10 c-h	1.86 j-m	20.76 a-c	15.50 a-c	54.40 a-e	943.77 ab
BGN-28	18.45 c-h	1.99 f-k	11.43 ef	8.05 f-i	36.14 ml	525.90 d-n
BGN-38	18.44 c-h	2.01 e-k	16.68 b-f	12.19 a-f	50.04 b-j	734.33 a-h
BGN-29	18.35 c-h	2.22 b-e	12.76 d-f	9.14 e-i	46.44 d-l	530.03 d-n
BGN-14	18.20 c-h	2.17 b-f	16.61 b-f	12.20 a-f	53.20 a-g	708.20 a-j
BGN-26	17.38 d-h	1.83 k-m	12.72 d-f	9.15 e-i	43.32 e-m	644.60 a-m
BGN-37	16.80 e-h	2.05 e-k	16.56 b-f	12.00 a-f	43.72 e-m	590.40 b-n
BGN-5	16.62 e-h	2.03 e-k	13.46 d-f	9.51 e-i	36.63 ml	451.53 f-n
BGN-36	15.34 f-h	1.98 f-l	14.21 c-f	9.92 e-i	39.49 j-m	623.80 a-n
BGN-12	15.01 f-h	2.17 b-f	14.79 b-f	11.23 b-i	58.83 ab	901.47 a-c
BGN-10	14.42 gh	1.98 f-k	15.64 b-f	11.26 e-i	47.39 b-l	641.93a-m
BGN-22	11.27 h	1.89 h-m	14.59 b-f	10.47 d-i	43.07 e-m	335.43 l-n
Grand mean	21.22	2.04	15.96	11.25	46.78	592 85
C.V. (%)	19.18	5.48	21.29	21.44	12.58	17.97
R^2 (%)	72.92	84.48	67.56	69.63	85.13	82.72

In each column, means followed by a different letter are significant at the 5.0% probability level

4.1.1 Relative agronomic performance in three selected agronomic attributes

Across the three locations, 10 genotypes attained >20.0% SDW over the check genotype (Table 4.7). However, genotype 'BGN-13' which attained the highest (59.05%) increase in SDW relative to the check did not perform proportionately high in terms of both 100-SWT and SYLD. In contrast, 'BGN-34' which produced >30.0% higher SDW than the check, achieved 52.71% and 43.69% advantage over the check genotype in 100-SWT and SYLD, respectively. In addition, genotype 'BGN-19' produced the largest seed (100-SW = 63.18 g) and relatively high SYLD (945.63 kg/ha) (Table 4.7). Furthermore, based on the testa color, genotype 'BGN-19' was also classified as light brown seed type which was similar to that of the check genotype 'BGN-39' while 'BGN-34' was in the black seeded type category (Table 4.8). The red seeded type ('BGN-25') also achieved a relatively good yield (852.39 kg/ha).

4.1.2 Relationship between agronomic attributes

There was a highly significant ($P<0.01$) correlation between PWT and each of SWTP, 100-SWT and SYLD (Table 4.9). The results also showed a highly significant ($P<0.01$) positive correlation between SWTP and SYLD. In contrast, PL showed a positive but not significant ($P > 0.05$) relationship with PWTP, SWTP, 100-SWT and SYLD (Table 4.9). At least >90.0% of the change in SWTP was explained by PWT (Fig. 4.3). However, the results indicated that only 60.0% of the change in SYLD was accounted for by the seed size as measured by the 100-SWT.

Table 4.7 The relative agronomic performance in three agronomic attributes of the top 10 bambara groundnut genotypes evaluated across three locations in Limpopo and Mpumalanga Provinces (South Africa).

Genotype		Mean	% Advantage Over Check
Shoot dry weight (g)			
1	BGN-13	32.84	66.19
2	BGN-40	27.87	41.04
3	BGN-34	26.33	33.25
4	BGN-17	25.97	31.43
5	BGN-7	25.93	31.22
6	BGN-18	25.86	30.87
7	BGN-35	25.51	29.10
8	BGN-16	25.21	27.58
9	BGN-32	24.34	23.18
10	BGN-33	24.08	21.86
Check	BGN-39	19.76	-
100-seed weight (g)			
1	BGN-19	63.18	55.69
2	BGN-34	61.97	52.71
3	BGN-12	58.83	44.97
4	BGN-25	58.36	43.81
5	BGN-20	58.30	43.67
6	BGN-33	56.12	38.29
7	BGN-11	54.40	34.06
8	BGN-17	54.38	34.01
9	BGN-23	53.39	31.57
10	BGN-6	53.34	31.44
Check	BGN-39	40.58	-
Seed yield (kg/ha)			
1	BGN-19	945.63	51.66
2	BGN-11	943.77	51.36
3	BGN-12	901.47	44.58
4	BGN-34	895.93	43.69
5	BGN-4	854.83	37.10
6	BGN-23	839.13	34.58
7	BGN-25	791.27	26.90
8	BGN-33	790.63	26.80
9	BGN-20	771.63	23.75
10	BGN-38	734.33	17.77
Check	BGN-39	623.53	-

Table 4.8 Mean 100-seed weight (100-SWT) and seed yield (SYLD) in the best performing genotypes in each of the four distinct testa color seed categories.

Testa Color	Genotype	100-SWT (g)	SYLD (kg/ha)
Light brown	BGN-19	63.18	945.63
Black	BGN-34	61.97	895.93
Red	BGN-25	58.36	791.27
Cream / white	BGN-26	43.32	644.60
Light brown	BGN-39 (Check)	40.58	623.53

Table 4.9 The Pearson correlations of six agronomic attributes of bambara groundnut genotypes evaluated across three locations in Limpopo and Mpumalanga Provinces (South Africa). [SDW=dry shoot weight; PL=pod length; PWT=pod weight per plant; SWTP=seed weight per plant; 100-SW=100 seed weight SYLD=seed yield].

	DSW (g)	PL (cm)	PWTP (g)	SWTP (g)	100-SWT (g)	SYLD (kg/ha)
DSW (g)	1.0000					
PL (cm)	0.1187	1.0000				
PWTP (g)	0.3839 *	0.0245	1.0000			
SWTP (g)	0.2313	0.0400	0.9498 **	1.0000		
100-SWT (g)	0.0583	0.1709	0.4789 **	0.5682 **	1.0000	
SYLD (kg/ha)	0.1546	0.0735	0.4684 **	0.5799 **	0.7804 **	1.0000

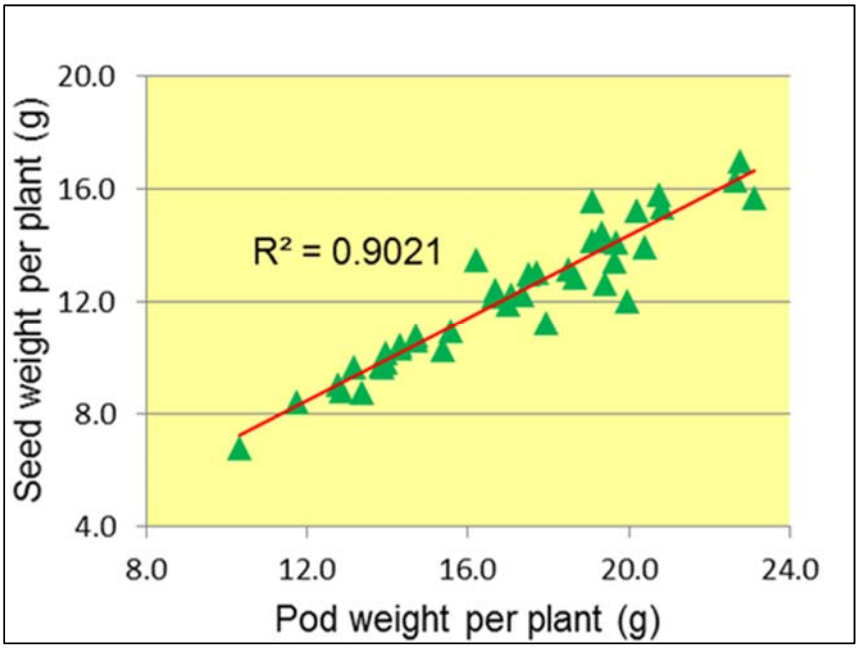

Fig. 4.3 The relationship between seed weight per plant and pod weight per plant of bambara groundnut genotypes evaluated across three locations in Limpopo and Mpumalanga Provinces (South Africa).

4.1.3 Principal component analysis

The principal component analysis (PCA) for the eight agronomic traits (including NPP and NSP)from the combined cropping seasons 2013/2014 and 2015/2016 indicated that two principal components (PCs) with eigen values greater than one accounted for 66.77% of the total variation among the genotypes (Table 4.10). PC1, which was dominated by SWTP, explained most of the variation (42.28%) while PC2 accounted for 24.49% of the total variation. Two of the traits, namely SDW and NPP showed negative correlations for PC2. In addition, PC2 was correlated mainly with PL and NSP but both SDW and NPP showed negative correlations. Based on these two principal components, the PCA also produced generally two clusters (groups) of the genotypes one of which included at least 50.0% of the top 10 performing genotypes that achieved >20.0% seed size advantage over the check (Fig. 4.4). However, genotype 'BGN-34' which consistently attained more than 25.0% higher SDW, 100-SWT and SYLD than the check, fell outside either group.

Table 4.10 Eigenvectors and eigenvalues of the first two principal components of eight agronomic attributes of bambara groundnut genotypes that were evaluated at Limpopo and Mpumalanga provinces. (SDW=shoot dry weight; NPP=number of pods per plant; PL=pod length; PWT=pod weight per plant; NSP=number of seeds per plant; SWTP=seed weight per plant; 100-SW=100 seed weight; SYLD=seed yield).

Trait	PC1	PC2
SDW	0.17	-0.16
NPP	0.43	-0.28
PL	-0.07	0.53
PWTP	0.57	0.04
NSP	-0.13	0.54
SWTP	0.52	0.02
100-SWT	0.33	0.37
SYLD	0.33	0.42
Eigen value	3.38	1.96
% of variation	42.28	24.49
% of variation cummulative	4.28	66.77

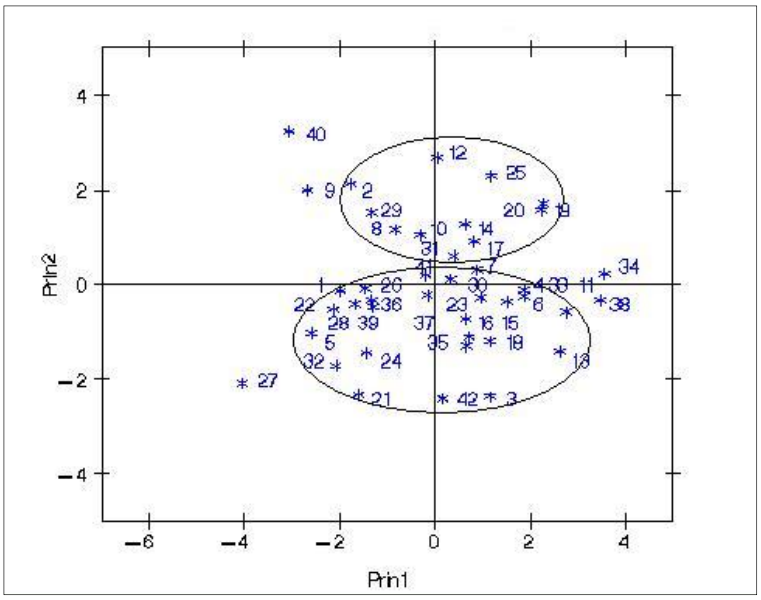

Fig. 4.4 Two clusters of bambara groundnut genotypes based on two principal components.

4.1.4 Cluster analysis

Further analysis produced a dendrogram clustering the genotypes into six well defined groups (Fig. 4.5). The number of genotypes per cluster ranged from two to twelve (Table 4.11). The seed color types were distributed throughout the six groups indicating that the clustering was independent of this qualitative trait, hence it was not considered in the process. It was interesting to note that two relatively high yielding light brown genotypes ('BGN-11' and 'BGN-12') as well as a black seeded genotype ('BGN-4') were clustered together in group VI (Fig. 4.5). The number of genotypes per cluster ranged from two to twelve (Table 4.11). Group V contained the highest number of genotypes while both groups II and V had no genotype that was classified as high yielding (Table 4.11). The latter group was dominated by the cream (white) seeded types.

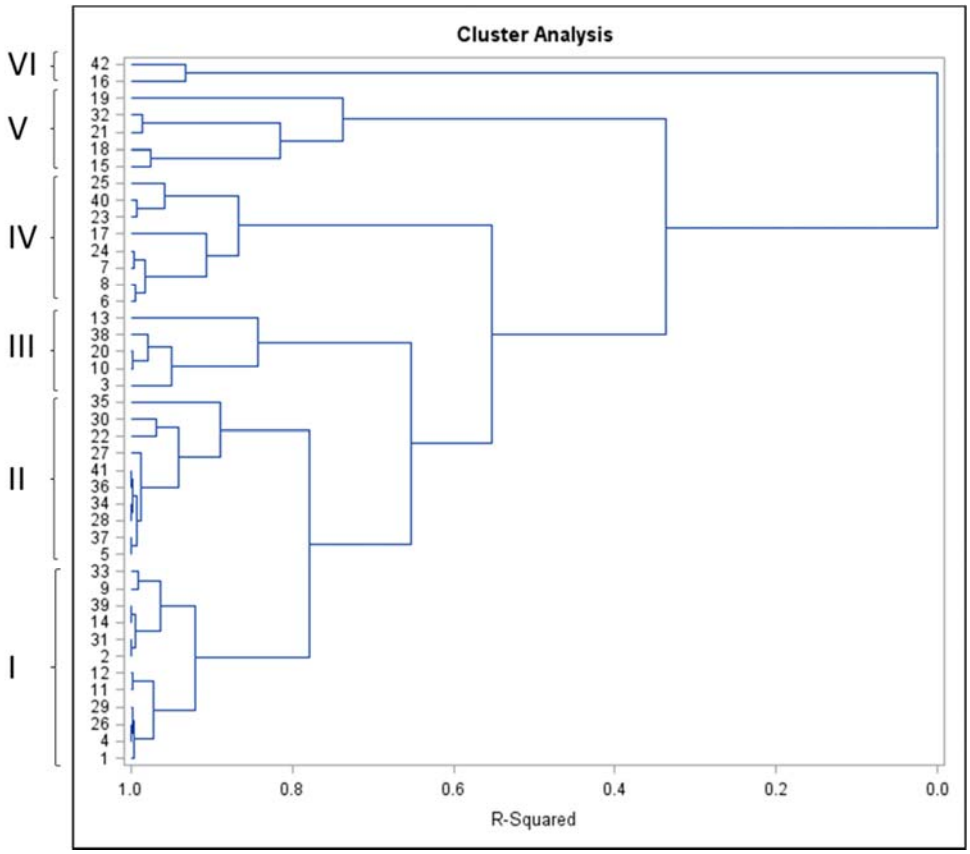

Fig. 4.5 Dendrogram of 42 bambara groundnut genotypes evaluated for agronomic performance in the field across three locations in Limpopo and Mpumalanga Provinces (South Africa).

Table 4.11 Groups of bambara groundnut genotypes determined by cluster analysis based on agronomic attributes. (Seed size as determined by 100-seed weight (g): Large > 55.0; medium = 45.0 – 55.0; small < 45.0; Seed yield (kg/ha): high > 780.0; medium = 680 - 780; low < 680.0).

Group	Genotype	Total	Notes		
			Seed size	Seed yield	Seed color
I	BGN-42	2	Small	Low yield	Cream/White
	BGN-16		Small	Low yield	Cream/White
II	BGN-19	5	Large	High yield	Light brown
	BGN-32		Small	Low yield	Cream/White
	BGN-21		Small	High yield	Cream/White
	BGN-18		Small	Low yield	Cream/White
	BGN-15		Small	Low yield	Cream/White
III	BGN-25	8	Large	High yield	Red
	BGN-40		Small	Low yield	Cream/White
	BGN-23		Medium	High yield	Red
	BGN-17		Medium	Moderate yield	Red
	BGN-24		Small	Low yield	Cream/White
	BGN-7		Medium	Low yield	Black
	BGN-8		Medium	Low yield	Cream/White
	BGN-6		Medium	Moderate yield	Light brown
IV	BGN-13	5	Small	Low yield	Cream/White
	BGN-38		Medium	Moderate yield	Light brown
	BGN-20		Large	Moderate yield	Light brown
	BGN-10		Medium	Low yield	Light brown
	BGN-3		Small	Low yield	Cream/White
V	BGN-35	10	Small	Low yield	Cream/White
	BGN-30		Medium	Low yield	Red
	BGN-22		Small	Low yield	Cream/White
	BGN-27		Small	Low yield	Cream/White
	BGN-41		Medium	Low yield	Light brown
	BGN-36		Small	Low yield	Cream/White
	BGN-34		Large	High yield	Black
	BGN-28		Medium	Low yield	Light brown
	BGN-37		Small	Low yield	Cream/White
	BGN-5		Small	Low yield	Cream/White
VI	BGN-33	12	Medium	High yield	Light brown
	BGN-9		Medium	Low yield	Cream/White
	BGN-39 (Check)		Small	Moderate yield	Light brown
	BGN-14		Medium	Moderate yield	Light brown
	BGN-31		Medium	Low yield	Red
	BGN-2		Medium	Low yield	Light brown
	BGN-12		Large	High yield	Light brown
	BGN-11		Medium	High yield	Light brown
	BGN-29		Medium	Low yield	Cream/White
	BGN-26		Small	Low yield	Cream/White
	BGN-4		Medium	High yield	Black
	BGN-1		Small	Low yield	Cream/White

4.2 Stability of genotypes

The results showed that genotype 'BGN-12' was the most stable followed by 'BGN-19' (Table 4.12). In addition, 15 genotypes (approximately 35.0% of the germplasm used in the study) were relatively more stable than the check ('BGN-39') across the locations (Appendix I). Despite a large proportion (50.0%) of cream (white) seeded genotypes used in the study, these types were all less stable than the check. However among the relatively stable genotypes, the light brown and black seeded types constituted 66.0% and 13.0% respectively. The top five most stable genotypes included at least one black seeded ('BGN-34') and one red ('BGN-23') seeded types while the rest were light brown ('BGN-12', 'BGN-19' and 'BGN-11') seeded types (Table 4.12). In contrast, 'BGN-27' was the most unstable genotype.

Table 4.12 Cultivar superiority index of the top 10 most stable bambara groundnut genotypes that were evaluated for agronomic performance in the field across three locations in Limpopo and Mpumalanga Provinces (South Africa).

Genotype	CSI	Rank
BGN-12	36100.14	1
BGN-19	45288.65	2
BGN-34	46178.34	3
BGN-11	51736.83	4
BGN-23	57714.08	5
BGN-4	62531.49	6
BGN-25	90232.72	7
BGN-20	95913.11	8
BGN-33	96651.60	9
BGN-38	127777.50	10
BGN-39	214683.40	16

4.3 Seed protein

There were significant ($P<0.05$) differences in seed protein content among the bambara groundnut genotypes and 28.0% of the genotypes were inferior to the check in terms of seed protein (Table 4.13). However, in comparison with the check, the genotype 'BGN-42' achieved 19.74% higher percent seed protein (Table 4.13). On average, the genotypes contained 21.72% protein. In comparison with the check, only three genotypes ('BGN-42', 'BGN-7' and 'BGN-32') achieved >10.0% higher seed protein. The percent seed protein ranged from 19.89% ('BGN-12') to 25.17% (in 'BGN-42') (Table 4.13). In general the cream (white) seeded genotypes showed a relatively high % seed protein compared to the other seed color types (Table 4.14). A poor relationship existed between % seed protein and seed size (Fig. 4.6).

Table 4.13 Mean percent seed protein of bambara groundnut genotypes.

	Genotype	% Seed Protein	Seed Color Type	% Increase Over Check
1	BGN-42	25.17 a	Cream / White	19.74
2	BGN-7	23.19 ab	Black	10.32
3	BGN-32	23.13 abc	Cream / White	10.04
4	BGN-41	23.09 abc	Light brown	9.85
5	BGN-19	22.88 bcd	Light brown	8.85
6	BGN-15	22.85 bcd	Cream / White	8.71
7	BGN-24	22.65 bcde	Cream / White	7.75
8	BGN-35	22.54 bcdef	Cream / White	7.23
9	BGN-3	22.51 bcdef	Cream / White	7.09
10	BGN-13	22.38 bcdfg	Cream / White	6.47
11	BGN-11	22.34 bcdefg	Light brown	6.28
12	BGN-40	22.32 bcdefg	Cream / White	6.18
13	BGN-27	22.24 bcdefg	Cream / White	5.80
14	BGN-30	22.19 bcdefg	Red	5.57
15	BGN-2	22.13 bcdefg	Light brown	5.28
16	BGN-37	22.07 bcdefgh	Cream / White	5.00
17	BGN-17	22.06 bcdefgh	Red	4.95
18	BGN-36	22.05 bcdefgh	Cream / White	4.90
19	BGN-28	22.04 bcdefgh	Light brown	4.85
20	BGN-26	21.94 bcdefgh	Cream / White	4.38
21	BGN-25	21.86 bcdefgh	Red	4.00
22	BGN-14	21.47 bcdefgh	Light brown	2.14
23	BGN-38	21.69 bcdefgh	Light brown	3.19
24	BGN-9	21.60 bcdefgh	Cream / White	2.76
25	BGN-31	21.44 bcdefgh	Red	2.00
26	BGN-5	21.25 bcdefgh	Cream / White	1.09
27	BGN-6	21.23 bcdefgh	Light brown	1.00
28	BGN-8	21.21 bcdefgh	Cream / White	0.90
29	BGN-22	21.15 bcdefgh	Cream / White	0.62
30	BGN-39 (Check)	21.02 bcdefgh	Light brown	0.00
31	BGN-1	20.93 cdefgh	Cream / White	-0.43
32	BGN-18	20.92 cdefgh	Cream / White	-0.48
33	BGN-23	20.83 defgh	Red	-0.90
34	BGN-16	20.75 defgh	Cream / White	-1.28
35	BGN-21	20.54 efgh	Cream / White	-2.28
36	BGN-4	20.49 efgh	Black	-2.52
37	BGN-34	20.48 efgh	Black	-2.57
38	BGN-20	20.45 efgh	Light brown	-2.71
39	BGN-33	20.45 efgh	Light brown	-2.71
40	BGN-29	20.43 fgh	Cream / White	-2.81
41	BGN-10	20.29 gh	Light brown	-3.47
42	BGN-12	19.89 h	Cream / White	-5.38
Grand mean		21.72	-	-
C.V. (%)		5.04	-	-

Means followed by a different letter are significant at the 5.0% probability level

Table 4.14 Percent seed protein among the main seed color types of 42 bambara groundnut genotypes.

Attribute	Seed Color Type				
	Black	Cream / White	Light brown	Red	Total
Mean % seed protein	21.87	21.86	19.9	21.68	-
Mean % seed protein of top three genotypes	21.87	23.72	22.77	22.19	-
Range % seed protein	20.48 - 23.19	**20.43 - 25.17**	19.89 - 23.09	20.83 - 22.19	-
% Increase over check	10.32	**19.74**	9.85	5.57	-
Number of entries	3	20	14	5	42

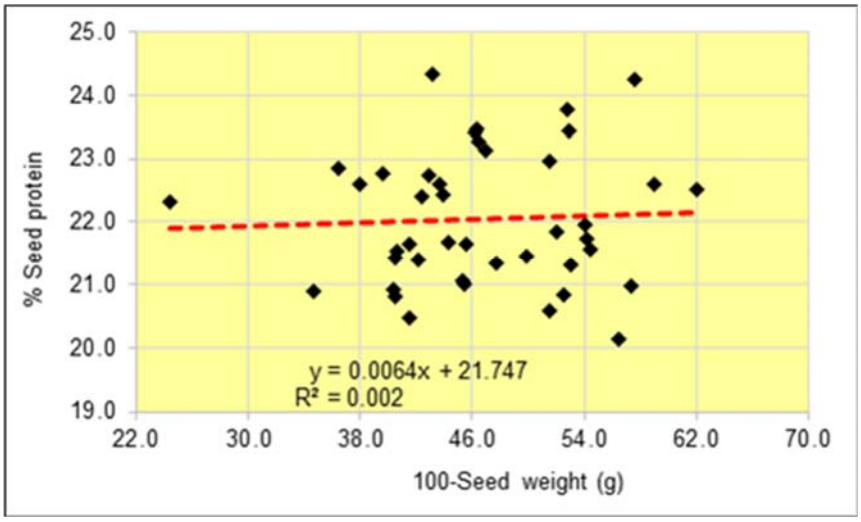

Fig. 4.6 A week correlation between percent seed protein and seed size in diverse bambara groundnut germplasm raised at Thohoyandou (Limpopo Province, South Africa).

CHAPTER FIVE: DISCUSSION

The results of this study revealed new insight in the agronomic performance of Bambara groundnut in the representative agro-ecologies that were used. Firstly, the results showed significant seasonal variations among the genotypes for the majority of the agronomic variables at both Syferkuil and Thohoyandou. This confirmed that genotypic performance in Bambara groundnut is generally sensitive to environmental conditions. This observation was in agreement with the findings reported previously in bambara groundnut (Alake *et al*., 2016; Jonah *et al*., 2012; Pungulani *et al*., 2012) However, it was interesting that pod length (PL) was generally not influenced by the seasons. This suggested that while the seasons effects were able to discriminate the genotypes in terms of the majority of quantitative traits that were measured, the seasonal variations were not sufficiently distinct to separate the genotypes in terms of the PL.

The results also showed that the location effects were significant between Nelspruit and the other two test locations particularly for yield (SYLD) with the former showing markedly higher yield potential (>1.0 t/ha versus < 0.6 t/ha). This observation suggested that both Syferkuil and Thohoyandou were similar and therefore, in future, there will be little merit, if any, in utilizing both of them simultaneously for identifying superior genotypes of bambara groundnut. However, in this regard, inclusion of the test locations at Nelspruit together with either Syferkuuil or Thohoyandou will be useful. Many studies involving multi-location evaluations of the agronomic performance of various crops including legumes (Dehghaui *et al*., 2011; Yan and Tinker, 2006) and cereals (Anley *et al*., 2013; Kaya *et al*., 2006) have been able to identify the best or most ideal test locations (or test environment). In most of these studies, the genotypes plus the genotype x environmental interaction bi-plot method was used widely partly because of its ability to map graphical presentations that can be interpreted easily (Yan and Wu, 2008).

It appears that the bambara germplasm that was used in this study possessed inherently low yield potential. Results from a recent study showed grain yield range of 703–2256 kg/ha as well as a moderate N fixation capacity (28.0 kg/ha) (Yakubu *et al*., 2010). The low yield reported in this study could be partly because the germplasm was originally selected from traditional varieties that were not improved genetically. In the same breath, a quantitative determination of the crop establishment in the study could have provided more information possibly accounting for, at least in part, the yield levels that were observed. Significant variation in seedling vigour in the present germplasm was previously reported (Mogale, 2015) (Appendix II). However, the seedling vigour was determined under laboratory conditions. Nonetheless, in comparison with control, the results indicated a significant yield improvement.

In addition, the study was able to identify relative good performing genotypes in the other seed colour categories apart from the light brown type. At any rate, the relatively low yield associated with the germplasm also suggested strongly that more work aimed at the improvement of seed yield will be merited.

In this study, differential cultivar performance could be attributed probably to differences between the location at Nelspruit and the other test locations. Nonetheless, cultivars 'BGN-19' produced the highest SYLD at both Nelspruit and Thohoyandou suggesting that it can be considered for further evaluation at more locations over more seasons. Similarly, four other cultivars that attained >25% yield advantage over the check ('BGN-11'; 'BGN-12'; 'BGN-4'; and 'BGN-34') could also be considered for further evaluation. Ideally, final field evaluation stage could involve the participation of growers in the target production area in order to enhance the adoption of the new cultivars. Farmers participation in cultivar evaluation was used routinely recently for a broad range of crops (Horn *et al*., 2016; Thapa *et al*., 2009; Tiwari *et al*., 2009; Vom Brocke *et al*., 2010; Witcombe *et al*., 2001). This approach could also resolve the end-use preferred traits such as seed color types or seed size. For instance, 'BGN-34' consists of black large seed while genotype 'BGN-19' consists also of large but light brown seed. Therefore, it will be merited to involve growers to determine their preferred set of trait combinations as represented by each of the individual potential cultivars.

The results also showed that based on the cultivar superiority index, genotype 'BGN-19' was the most stable after 'BGN-12' thus suggesting that this potential cultivar has a wide ecological amplitude in which it can be cultivated. Nonetheless, as indicated above more test-locations and seasons will be required for these genotypes especially in Mpumalanga where the crop was evaluated for only one season. In this regard, it is also important to exercise caution about the observed relatively high yield potential in individual genotypes partly because the on-farm productivity of the crop is generally lower than that from field experimental trails. On the other hand, it will be interesting to evaluate the yield potential of these genotypes under irrigated conditions of which higher seed yield (>3.0 t/ha) for light brown genotypes of the crop were reported previously (Mabhaudi and Modi, 2013).

The results from this study also indicated genotypic difference in dry matter accumulation as measured by the dry shoot weight (DSW). The three genotypes ('BNG-13'; 'BGN-32'; 'BGN-34') that attained at least 40% higher DSW than the check genotypes could be useful in the production of fodder for instance. Alternatively, the crop residues from these genotypes that remains in the field after harvesting could provide a good source of organic matter if it is incorporated in the soil. Previous studies with groundnut for instance, showed that the crop

residue that was not removed from the field supplied a considerable amount of organic matter to the soil (Giller and Cadish, 1995). In a similar study involving soybean, there was asignificant improvement in the grain yield of corn that was grown in the same field during the following season (Kasasa *et al*., 1998). However, in this study, there was no significant correlation between DSW and SYLD implying that in general, genotypes that produced high DSW did not necessarily produce high seed yield. Nonetheless, there were some high yielding individual genotypes (for example 'BGN-19') that also achieved high (>25.0g) DSW.

Both the weight of the pods per plant as well as the weight of seed per plant showed significant positive relationships with the SYLD which was in agreement with other studies reported previously (Abu and Buah, 2011; Amarainghe *et al*., 2016; Karikari and Tabona, 2004). From a practical plant breeding stand point, the positive correlation between the pod weight per plant (PWT) and SYLD presented a relatively more rapid and less laborious method for screening for high yielding genotypes since both manual excavation and the subsequent shelling of Bambara groundnut pods is quite cumbersome. In general, the medium to large cultivars were present in the cream (white), light brown and red types which provides seed colour type options for end-users.

In terms of crude seed protein, the cream seeded types showed a considerably higher level than the rest of the seed colour types suggesting that breeding programs aimed at improving this attribute in bambara groundnut need to consider cream seeded germplasm. Ideally, the quality of the seed protein in particular, the amino acid profiles would be of interest in future studies. While this additional information is limited partly by the cost of laboratory (chemical) analysis, it is valuable to determine the amino acid profiles of the genotypes. However, the findings regarding seed protein levels were consistent with the results from similar studies elsewhere. For instance, previous studies indicated that bamabra groundnut could possess as high as 25.0% crude protein (Mahala and Mohammed, 2010). In another study that was conducted to determine amino acid levels in the seed of bambara groundnut, two essential amino acids lysine and methionine achieved 6.6% and 1.3% respectively (Temple and Aliyu, 1994). In this study, the amino acid profiles were not determined but a preliminary survey of minerals in the germplasm that was conducted separately (Mogale and Gwata, 2016; *unpublished*) indicated distinct variability in both iron (Appendix III) and zinc (Appendix IV).

There will also be merit in future in investigating the genetic control of the nutritional attributes such as the essential amino acid, minerals, vitamins and crude seed protein in order to design effective breeding approaches to manipulate these traits. Recently, mutation breeding was used successfully to improve both seed protein and the amino acid, methionine in bambara

groundnut (Bharatkumar *et al*., 2015). Other approaches that have been used successfully in the genetic enhancement of bambara groundnut include tissue culture (Lacroix *et al*., 2003; Kone *et al*., 2007) and marker assisted selection (Ho *et al*., 2016; Ho *et al*., 2017). New improved bambara groundnut cultivars that are high yielding and highly nutritional will be desirable particularly for resources poor rural communities in South Africa. Another drawback in the study was the absence of exotic germplasm originating from other bambara groundnut production regions such as West Africa or countries in southern Africa for the purposes of comparison. A comparison of the performance of a broad range of genotypes would be useful for designing genetic improvement programs (Aliyu *et al*., 2014) for the local bambara groundnut end-users since this legume popular and is often marketed informally (Appendix V). Nonetheless, the results of this study were useful in revealing the agronomic potential and seed protein levels in the currently available bambara groundnut germplasm.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

The results of this study showed that the agronomic potential of bambara groundnut genotypes varied depending on the prevailing agro-ecological conditions under which they were cultivated. The highest seed yield (1.2 t/ha) was observed for genotype 'BGN-19' at Nelspruit. At least five of the experimental genotypes attained >30.0% higher dry shoot weight and 100-seed weight than the local check genotype while five of the newly identified potential cultivars also achieved at least >25.0% higher seed yield than the check genotype. Three of the superior genotypes consisted of light brown seed while the remainder were black seeded types. This variation in seed colour types is important in terms of technology adoption by growers. The highest amount of crude seed protein (25.17%) was observed for the cream (white) seeded genotype 'BGN-42'. The cluster determination using the principal component analysis showed that the 42 genotypes could be grouped into six distinct categories among which the highest number of genotypes (12) was classified together with the check genotype. Three of the five most stable genotypes were of the light brown seeded types and the remaining two were either black or red seeded types.

There were significant positive relationships among some of the agronomic attributes notably between seed yield and each of pod weight per plant, seed weight per plant as well as 100-seed weight. Overall, there was considerable variation in the amount of seed crude protein among the genotypes. The cream seeded types showed the highest amount of percent seed protein (25.17%) with an advantage (19.74%) over the check genotype.

In future, there will be merit in the further evaluation of the best 10 performing genotypes over more test locations in the target production areas. In addition, it will be useful to consider the participation of bambara groundnut growers in the selection of desirable genotypes that possess the preferred combinations of end-use traits. This will facilitate cultivar adoption by the growers as well as provide reasonably sufficient quantities of the initial seed to the growers. Furthermore, quantitation of the amino acids in the germplasm will be useful in future. This will enhance the chances for the genetic improvement of both the nutritive and agronomic attributes of bambara groundnut for the local end-users. The application of molecular techniques which was reported previously (Lacroix *et al*., 2003; Kone *et al*., 2007; Ho *et al*., 2016; Ho *et al*., 2017) offers more options to bambara groundnut breeders in future since the small size of the flowers and sensitivity to emasculation will not limit the use of molecular methods in improving cultivar development. In practice, these approaches work efficiently

when they are combined with conventional breeding methods in many field crops including legumes (Atemkeng et al., 2011).

REFERENCES

Abu HB and Buah SSJ (2011). Characterization of bambara groundnut landraces and their evaluation by farmers in the upper west region of Ghana. *Journal of Developments in Sustainable Agriculture*. **6**: 64-74.

Adeparusi EO and Agbede JO (2009) Evaluation of leucaena and gliricidia leaf protein concentrate as supplements to bambara grundnut (*Vigna subterranean* (L.) Verdc) in the diest of oreochromis niloticus. *Aquaculture Nutrition* **12**: 335-342.

Adu-Dapaah HK and Sangwan RS (2004). Improving bambara groundnut productivity using gamma irradiation and *in vitro* techniques. *African Journal of Biotechnology* **3**: 260-265.

Afiukwa CA, Ubi BE, Kunert KJ, Emmanuel TF and Akusu JO (2013). Seed protein content variation in cowpea genotypes. *World Journal of Agricultural Sciences* **1**: 094-099.

Alake CO and Alake OO (2016). Genetic diversity for agro-nutritional traits in African landraces of vigna subterranean germplasm. *Journal of Crop Improvement*. **30**: 378-397.

Aliyu S, Massawe F and Mayes S (2014). Beyond landraces: developing improved germplasm resources for underutilized species - a case for Bambara groundnut. *Biotechnology and Genetic Engineering Reviews* **30**: 127-41.

Amarasinghe YPJ, Wijesinghe G and Pushpakumara RW (2016). Multivariate analysis, genetic diversity and phenotypic correlation of nineteen exotic groundnut accessions. *Journal of Agrisearch*. **3**:7-12.

Animasaun DA, Oyedeji S, Azeez YK, Mustapha OT and Azeez MA (2015). Genetic variability study among ten cultivars of cowpea (*Vigna unguiculata* L. Walp) using morpho-agronomic traits and nutritional composition. *The Journal of Agricultural Sciences* **10**: 119-130.

Anley W, Zeleke H and Dessalegn Y (2013). Genotype x environment interaction of maize (*Zea mays*) across North Western Ethopian. *Journal of Plant Breeding and Crop Science* **5**: 171-181.

AOAC international (1990). The official methods of analysis. Arlington, VA: AOAC international AOAC international (1995). The official methods of analysis. Arlington, VA: AOAC international.

Atemkeng MF, Muki TJ, Park J and Jifon J (2011). Integrating molecular tools with conventional breeding strategies for improving phosphorus acquisition by legume crops in acid soils of Sub-Saharan Africa. *Biotechnology and Molecular Biology Review* **6**: 142-154.

Azam-Ali SN, Sesay A, Karikari SK, Massawe FJ, Aguilar-Manjarrez J, Bannayan M and Hampson KJ (2001). Assessing the potential of an underutilized crop: a case study using bambara groundnut. *Journal of Experimental Agriculture* **37**: 433-472.

Bamashaiye OM, Adegbola JA and Bamishaiye EI (2011). Bambara groundnut: an under-utilized nut in Africa. *Advances in Agricultural Biotechnology* **1**: 60-72.

Baudoin JP and Mergeai G (2001). Bamabara groundnut (*Vigna subterranea* (L) Verdc*). Crop Production in Tropical Africa* **3**: 313-317.

Basu S, Mayes S, Davey M, Roberts JA, Azam-Ali SN, Mithen R and Pasquet RS (2007). Inheritance of 'domestication' traits in bambara groundnut (*Vigna subterranea (*L) Verdc). *Euphytica* **157**: 59-68.

Berchie JN, Sarkodie-Addo J, Adu-Dapaah H, Agyemang A, Addy S, Asare E and Donkor J (2010). Yield evaluation of three early maturing bambara groundnut (*Vigna subterranea* L. Verdc) landraces at the CSIR-Crops Research Institute, Fumesua-Kumasi, Ghana. *Journal of Agronomy* **9**: 175-179.

Bharatkumar C, Nandini R, Bhanuprakash K, Dhanapal GN, Shashidhar HE and Savithramma DL (2015). Genetic enhancement of protein and methionine content in bambara groundnut (*Vigna subterranea* (L.) Verdc.) through mutation breeding. *International Journal of Research in Agriculture and Forestry* **2**: 8-13.

Dehghani H, Ebadi A and Yousef A (2006). Biplot analysis of genotype by environment interaction for barley yield in Iran. *Journal of Agronomy* **98**: 388-393.

Djè Y, Béket SB and Zoro IA (2005). Preliminary variability observation between some bambara groundnut landraces (*Vigna subterranea* (L) Verdc Fabaceae) of Ivory Coast. *Biotechnology, Agronomy, Society and Environment* **9**: 249-258.

Duncan DB (1956). Multuple range test F test. *Biometrics* **11**: 1-42.

Eberhart SA and Russell WA (1966). Stability parameters for comparing crop varieties. *Crop Science* **6**: 36–40.

Esposito MA, Milanesi LA, Martin EA, Cravero VP, Anido FSL and Cointry EL (2007). Principal component analysis based on morphological characters in pea (*Pisum sativum* L). *Internationa Journal of Plant Breeding* **1**: 135-137.

FAOSTAT: Food of Agriculture Organization of the United Nations Statistics Division (2014). The production quantities of bambara groundnut in Africa.

Fijihara S, Katsuga A and Aoyayi Y (2001). Nitrogen-to-protein conversion factors for common vegetables in Japan. *Journal of Food Science* **66**: 412-415.

Gibbon D and Pain A (1985). Crops of the drier regions of the tropics, longman scientific and technical longman group UK Ltd.

Giller KE and Cadissch (1995). Future benefits from biological nitrogen fixation: an ecological approach to agriculture. *Developments in Plant Research* **174**: 225-277.

Gurmu F, Mohammed H and Alemaw G (2009). Genotype x environment interactions and stability of soybean for grain yield and nutrition quality. *African Crop Science Journal* **17**: 87-99.

Gwata ET and Nziramasanga N (2001). Seed protein and oil content in Zimbabwean soybean (*Glycine max* L) varieties. *Plant Varieties Seeds* **14**: 125-128.

Habtamu A, Tsige G, Tadesse D and Landuber W (2011). Multivariate diversity, heritability and genetic advance in *Tef* landraces in Ethiopia. *African Crop Science Journal* **19**: 201-212.

Hillocks RJ, Bennert C and Mponda OM (2012). Bambara nut: a review of utilization, market potential and crop improvement. *African Crop Science Journal* **20**: 1-16.

Ho WK, Chai HH, Kendabie P, Ahmad NS, Jani J, Massawe F, Kilian A and Mayes S (2017). Integrating genetic maps in bambara groundnut [*Vigna subterranea* (L) Verdc.] and their syntenic relationships among closely related legumes. *Genome* **18**: 1-9.

Ho WK, Muchugi A, Muthemba S, Kariba R, Mavenkeni BO, Hendre P, Song B, Van Deynze A, Massawe F and Mayes S. (2016). Use of microsatellite markers for the assessment of bambara groundnut breeding system and varietal purity before genome sequence. *Genome* **59**: 427-31.

Horn LM, Ghebrehiwot HM and Shimelis AH (2016). Selection of neovel cpwpea genotypes derived through gamma irradiation. *Fontiers in Plant Science* **7**: 262-273.

Jonah PM, Aliyu B, Kadams AM and Jibungh GG (2012). Variation in pod yield characters and heritability estimates in some accessions of bambara groundnut (*Vigna subterranean*). *Global Research Journal of Agriculture and Biological Science* **3**: 360-369.

Karikari SK (2000). Variation between local and exotic bambara groundnut landraces in Botswana. *African Journal of Crop Science* **8**: 153-157.

Karikari SK and Tabona TT (2004). Constitutive traits and selective indices of Bambara groundnut (*Vigna subterranea*) landraces for drought tolerance under Botswana conditions. *Physics and Chemistry of the Earth* **29**: 1029-1034.

Kasasa P, Mpepereki S and Giller KE (1998). Nodulation and yield of promiscuous soybean (*Glycine max* (L) Merr.) varieties under field conditions. In: *Proceedings of the Soil Fertility Network Results and Planning Workshop*. Waddington, S.R., Murwira.,H.K., Kumwenda, J.D.T., Hikwa, D. and Tagwira, F. (Eds.), pp. 99-103. CIMMYT, Harare, Zimbabwe.

Kaya Y, Akcura M and Taaner S (2006). GGE biplot analysis of multi-environment yield trials in bread wheat. *Turkish Journal of Agriculture and Forestry* **30**:325-337.

Kone M, Patat-Ochatt EM, Conreux C and Samgwan RSSJ (2007). In- vitro morph genesis from cotyledon and epicotyls explants and flow cytometary distinction between landrace of bambara groundnut (*Vigna subterranea* (L.) Verdc) an under-utilizied grain legume. *Plant Cell Tissue Organ Culture* **88**: 61-75.

Kouassi NJ and Zorobi IA (2011). Evaluation of agronomic performance of two landraces of bambara groundnut (*Vigna subterranea*) in woodland savannahs of Cote d'Ivoire. *African Journal of Agricultural Research* **6**: 4852-4856.

Lacroix B, Assoumou Ndong Y and Sangwan, RS (2003). Efficient in vitro direct shoot regeneration systems in bambara groundnut (*Vigna subterranea* L. Verdc.). *Plant Cell Report* **21**: 1153-1158.

Legwaila GM, Karikari KS, Mogamisi K and Mathowa T (2013). Response of three bambara groundnut landraces to pre-sowing hydration in Botswana. *Agriculture and Biology Journal of North America* **4**: 430-434.

Lin CS and Binns MR (1988). A method for analyzing cultivar × location × year experiments: a new stability parameter. *Theoretical Applied Genetics* **76**: 425–430.

Mabhaudhi T and Modi AT (2013). Growth, phenological and yield responses of a bambara groundnut (*Vigna subterranea* (L) Verdc) landrace to imposed water stress under field conditions. *South African Journal of Plant and Soil Sciences* **27**: 299-304.

Mahala AG and Mohammed AAA (2010). Nutritive evaluation of bambara groundnut (Vigna subterranean) pods, seeds and hull as animal feed. *Journal of applied sciences research*. **6**: 383-386.

Makanda I, Tongoona P, Madamba R, Icishahayo D and Derera J (2009a). Evaluation of bambara groundnut varieties for off-season production in Zimbabwe. *African Journal of Crop Science* **16**: 175-183.

Makanda I, Tongoona P, Madamba R, Icishahayo D and Derera J (2009b). Path coefficient analysis of bambara groundnut pod yield components at four planting dates. *Research Journal of Agriculture and Biological Sciences,* **5**: 287-292.

Massawe FJ, Dickinson M, Roberts JA and Adam-Ali SN (2002). Genetic diversity in Barbara groundnut (*Vigna subterranean* (L) Verdc) landraces revealed by AFLP markers. *Genome* **45**: 1175-1180.

Massawe FJ, Mwale SS, Azam-Ali SN and Roberts JA (2005). Breeding in bambara groundnut (*Vigna subterranea* (L) Verdc): strategic considerations. *African Journal of Biotechnology* **4**: 463-471.

Mazahib AM, Nuha MO, Salawa IS and Babiker EE (2013). Some nutritional attributes of bambara groundnut as influenced by domestic processing. *International Food Research Journal* **20**: 1165-1171.

Minka SR and Bruneteau M (2000). Partial chemical composition of bambara pea (*Vigna subterranean* (L). Verdc). *Food Chemistry* **68**: 273-276.

Moshia ME, Mashatola MB, Shaker P, Fouché PS and Boshomane MAW (2008). Land suitability assessment and precision farming prospects for irrigated maize-soybean intercropping in Syferkuil experimental farm using geospatial information technology. *Journal of Agriculture and Social Research* **8**: 138-147.

Mohammadi MJ, Movahedi Z and Sabaghnia N (2014). Multivariate statistical analysis of some traits of bread wheat for breeding under rainfed conditions. *Journal of Agricultural Science* **59**: 1-14.

Murevanhema YY and Jideani VA (2013). Potential of Bambara groundnut (*Vigna subterranea* (L.) Verdc) milk as a probiotic beverage-a review. *Journal of Food Science and Nutrition* **53**: 87-90.

Mzezewa J, Misi T and Van Rensburg LD (2012). Characterisation of rainfall at semi-arid ecotope in the Limpopo Province (South Africa) and its applications for suitable crop production. *Water South Africa* **36**: 1816-7950.

Olugbemi PW and Adebosin WG (2014). Adaptability of bambara groundnut (*Vigna subterranea* (L.) Verdc) to sub-humid environment of Epe local government of Lagos State. *Academia Journal of Agricultural Research* **2:** 43-48.

Ouédraogo M, Ouédraogo JT, Tigneré JB, Balma D, Dabiré CB and Konaté G (2008). Characterization and evaluation of accessions of bambara groundnut from Burkina Faso. *Science Nature* **5**: 191-197.

Paterson DG (2012). Soils and agricultural potential for the proposed p166 road, near Mbombela, Mpumalanga province. *Journal of Natural Sciences* **48**: 1-14.

Pomeranic Y and Meloan EC (1978). A recommended method for kjeldahl techniques in total nitrogen. *Journal of Food Technology* **32**: 69-73.

Pople JA, Bernstein HJ and Schneider WG (1957). The analysis of nuclear magnetic resonance spectra. *Journal of chemistry* **35**: 65-81.

Pungulani L, Kadyampakeni D, Nsapoto L and Kachapila M (2012). Selection of high yielding and farmer's preferred genotypes of bambara nut (*Vigna subterranean L. Verdc*) in Malawi. *American Journal of Plant Sciences*. **3**: 1802-1808.

Punto NG and Lantican RM (1983). Genotype x environment interaction for the yield in field legumes: Peanut Arachis hypogaea . *Crop Science Society of the Phillipines* **1**: 94-100.

Qayyum MMN, Butt MS, Anjum FM and Nawaz H (2012). Composition analysis of some selected legumes for protein isolates recovery. *The Journal of Animal & Plant Sciences* **22**: 1156-1162.

Rikhotso T, Gwata E and Mzezewa J (2013). Preliminary selection of early bambara groundnut for urban markets in Limpopo Province (South Africa). *Tropentag.* Book of abstracts, 17-19 September,Hohenheim, Germany pp 405.

Rymuza K, Turska E, Wielogorska G and Bombik A (2012). Use of principal component analysis for the assessment of spring wheat characteristics. *Journal of Agriculture* **11**: 79-90.

Sabaghnia N, Karimizadeh R and Mohammadi M (2012). Genotype × environment interaction and stability analysis for grain yield of lentil genotypes. *Journal of Agriculture* **99**: 305-312.

SAS Institute Inc. (2014). SAS 9.1.3: ETL *Studio: User's guide*. Cary, NC: SAS institute.

Schmilovitch Z, Mizrah A, Hoffman A, Egozi H, and Fuchs Y (2000). Determination of mango physiological indices by near-infrared spectrometry. *Post harvesting and Biological Technology* **19**: 245-252.

Shegro A, Jansen van Rensburg WS and Adebola PO (2013). Assessment of genetic variability in bambara groundnut (*Vigna subterrenea* L) using morphological quantitative traits. *Academic Journal of Agricultural Research* **1**: 45-51.

Shiringani RP and Shimelis HA (2011). Yield response and stability among cowpea genotypes at three planting dates and test environments. *African Journal of Agricultural Research* **6**: 3259-3263.

Temple VJ and Aliyu R (1994). Proximate composition of the cream coloured decorticated seeds of bambara groundnut (Voandzeia subterranea L. Thouars.). *Bioscience Research Communication* **6**:51 - 54.

Tibe O, Amarteifio JO and Njogu RM (2007). Trypsin inhibitor activity and condensed tannin content in bambara groundnut (*Vigna subterranea* (L) Verdc) grown in Southern Africa. *Journal of Applied Science and Environmental Management* **11:** 159-164.

Tiwari TP, Virk DS, Sinclair FL (2009) Rapid gains in yield and adoption of new maize varieties for complex hillside environments through farmer participation. I. Improving options through participatory varietal selection (PVS). *Field Crops Research* **111**:137–143.

Toure Y, Kone M, Tanoh HK and Kone D (2012). Agromorphological and phenological variability of 10 bambara groundnut (*Vigna subterranea* (L) Verdc Fabaceae) landraces cultivated in the Ivory Coast. *Tropicultura* **30**: 216-221.

Thapa DB, Sharma RC, Muswari A. Ferrara G, Sharma S, Basnet RK, Witcombe JR, Virk DS and Joshi KD (2009). Identifying duperior cultivars in participatory research on respurce poor farms. *Field Crop Research* **112**: 124-130.

Vom Brocke K, Trouche G, Weltzien E, Barro-kondombo CP, Goze E and Chantereau J (2010). Participatory varietal development for sorghum in Burkin Faso: farmer's selection and farmer's criteria. *Field Crop Research* **119**: 183-194.

Weir AD, Omielan J, Lee EA and Rajcan I (2005). Use of NMR for Predicting Protein Concentration in Soybean Seeds Based on Oil Measurements. *Journal of American Oil Chemists Society* **82**: 87-92.

Witcombe JR, Joshi KD, Rana RB and Virk DS (2001). Increasing genetic diversity by participatory varietal selection in high potential production systems in Nepal and India. *Euphytica*, **122**, 575–588.

Yakubu H, Kwari JD and Sandabe MK (2010). Effect of phosphorus fertilizer on nitrogen fixation by some grain legume varieties in Sudano – Sahelian Zone of North Eastern Nigeria. *Nigerian Journal of Basic Applied Science* **18**: 19-26.

Yan and Tinker (2006). Biplot analysis of multi-environment trial data: principles and applications. *Canadian Journal of Plant Science* **86**: 623-645.

Yan W and Wu HX (2008). Application of GGE biplot to evaluate genotype (G), environment € and GxE interaction on pinus radiate: case study. *Newzealand Journal of Forestry Science* **38**: 132-142.

APPENDICES

Appendix I

Cultivar stability indices of 42 bambara groundnut genotypes evaluated across three locations in Limpopo and Mpumalanga Provinces (South Africa).

Genotype	CSI	Rank
BGN-1	415999.68	33
BGN-2	345407.22	28
BGN-3	354860.54	29
BGN-4	**62531.49**	**6**
BGN-5	398841.10	30
BGN-6	145559.55	11
BGN-7	230388.85	21
BGN-8	300329.71	25
BGN-9	224831.06	20
BGN-10	210301.46	15
BGN-11	**51736.83**	**4**
BGN-12	**36100.14**	**1**
BGN-13	271686.57	24
BGN-14	147739.91	12
BGN-15	408724.25	31
BGN-16	529110.52	38
BGN-17	152018.18	13
BGN-18	479829.61	35
BGN-19	**45288.65**	**2**
BGN-20	**95913.11**	**8**
BGN-21	608428.34	41
BGN-22	557212.43	40
BGN-23	**57714.08**	**5**
BGN-24	520942.16	37
BGN-25	**90232.72**	**7**
BGN-26	215197.34	17
BGN-27	639874.20	42
BGN-28	306221.69	27
BGN-29	304235.91	26
BGN-30	224658.43	19
BGN-31	201200.55	14
BGN-32	550507.83	39
BGN-33	**96651.60**	**9**
BGN-34	**46178.34**	**3**
BGN-35	419788.53	34
BGN-36	223000.35	18
BGN-37	245249.24	23
BGN-38	**127777.50**	**10**
BGN-39	**214683.40**	**16**
BGN-40	411171.90	32
BGN-41	241980.59	22
BGN-42	508680.05	36

Appendix II

A Short Communication prepared for local presentation (2017)

Variation in Seedling Vigour Among Diverse Bambara Groundnut Genotypes

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Abstract

Bambara groundnut (*Vigna subterranea*) is an annual herbaceous plant that is native to Africa. It is cultivated mainly for its seeds that are used for human consumption. It is ranked the third most important leguminous crop in many parts of Africa after cowpea (*Vigna unguiculata*) and peanut (*Arachis hypogaea*). It is well adapted to semi-arid regions and tolerant to marginal soils. In southern Africa, it is cultivated mainly by smallholder growers who retain seed from one harvest to the next planting. The seed is often poor in quality, leading to poor germination and crop establishment and consequently reduction in yield. This study was designed to determine the variation in seedling vigour and identify superior bambara groundnut genotypes. The study was conducted under laboratory conditions at room temperature at Thohoyandou () (Limpopo Province, South Africa). Five seeds of each of 42 genotypes of bambara groundnut were planted separately in plastic containers using a 7X6 rectangular lattice design with three replications. Four seedling vigour traits namely the primary root length (PRL), secondary root length (SRL), shoot height (SHT) and dry root weight (DRW) were measured 18 d after planting. There were highly significant ($P<0.01$) differences among the genotypes for all traits. 'BGN-40' produced a two-fold increase in SHT, SRL and DRW over the check ('BGN-39'). Similarly, 'BGN-31' and 'BGN-9' attained a two-fold increase in SRL and DRW over the check. The results indicated the potential of phenotypic based selection for the traits in bambara groundnut germplasm which was used in the study.

Key words: bambara groundnut; genotype; seedling; selection; vigour.

1.0 Introduction

Barbara groundnut (*Vigna subterranea*) is a leguminous crop which is cultivated in many African countries. The seed is rich in protein (25.0%) and minerals. Apart from utilisation as human food, the stover is useful for animal feeds. The crop is also useful particularly in smallholder cropping systems in Africa partly because of its ability to fix atmospheric nitrogen. However, in South Africa, there are no commercial improved bambara groundnut cultivars, hence the growers utilize seed of traditional landraces selected from previous harvests (Hillocks *et al*., 2011). The seed is often poor in quality, leading to poor germination and crop establishment thus a reduction in yield (Mabhaudhi, 2009). Ideally, good quality seed of high genetic and physical purity, uniform and with high percent germination as well as free from seed borne diseases is desirable in order to achieve optimum yield.

Limited research work focussing on seed viability and seedling vigor in bambara groundnut has been reported. In a study conducted with seed of different testa colors, Chibarabada *et al*., (2015) reported that black speckled types outperformed cream types in percent germination. Using in vitro conditions, Kone *et al*. (2015) attributed low germination partly to the seed coat. In other legumes such as french bean (Vishvanath *et al*., 2006) and lentil (Hojjat, 2011), seed size influenced percent germination. Apart from measuring percent germination, several studies measured seedling traits in order to determine the seedling vigour (Chibarabada *et al*., 2015; Razpour *et al*., 2013; Hojjat, 2011; Peksen *et al*., 2004.). In the present study, the objectives were to determine the variation in seedling vigour and identify superior bambara groundnut genotypes.

2.0 Materials and methods

Genetic materials

Forty-one experimental lines and one local check were used in the study. All the experimental lines were selected from the landrace germplasm previously in the bambara groundnut breeding program at the School of Agriculture (University of Venda) (Rikhotso *et al*., 2013). The germplasm consisted of a diverse range of seed colour types and size.

Planting and measurements

The seed samples of each genotype was germinated in the laboratory at room temperature. The seed was germinated in plastic jars measuring 7.5 cm x 8.0 cm in order to raise the seedlings (Gwata *et al*., 2016). Prior to germination, the inside of the base of each jar was lined with moist filter paper before placing 10 seeds and ensuring that each individual seed was free from contact with other seeds in the jar. Three plants from each jar per replicate were

measured for primary root length (PRL), secondary root length (SRL), shoot height and dry root weight (DRW).

Experimental design and data analysis

The experiment was laid out as randomized complete block design replicated three times. Data sets were subjected to standard analysis of variance procedures (SAS Institute, 2014) followed by mean separation (Duncan, 1956).

3.0 Results and Discussion

The results showed significant ($P<0.05$) differences among bambara groundnut genotypes for all the seedling parameters that were measured (Table A-1). Four genotypes ('BGN-27', 'BGN-22', 'BGN-29', 'BGN-32' and 'BGN-16') failed to germinate suggesting that prior to large-scale field planting, seed germination tests will be necessary. The longest primary roots (156.67 cm) were produced by 'BGN-9'. There were phenotypic differences in root attributes (Fig. A-1). Some genotypes germinated but did not develop viable shoots (Fig. A-2). On average, the PRL among the genotypes (82.16 cm) exceeded the mean SRL (45.30 cm) by >80.0%. The genotypes 'BGN-21' and 'BGN-40' attained the highest SRL (96.17 cm) and SHT (55.33 cm), respectively (Table A-1). The shortest shoots (5.0 cm) were observed for genotype 'BGN-27' which suggested that early seedling growth varied considerably in bambara groundnut. The genotype also showed proportionately short primary and secondary roots. These observations were consistent with the highly significant correlation that was found between SHT and both PRL and SRL (Table A-2). Interestingly, the DRW exhibited a negative relationship with all the other seedling traits. The genotypic variation in PRL and SHT among the top 10 performing genotypes differed markedly (Fig. A-3). Similar findings in studies involving these traits in legumes were also reported (Chen *et al*., 2017; Gwata *et al*., 2016; Sarker *et al*., 2005).

In comparison with the check genotype ('BGN-39'), the greatest relative performance for both PRL (38.22%) and SHT (118.44%) were observed for genotype 'BGN-9' but genotype 'BGN-7' showed no relative advantage of the check in both the SHT and DSW (Table A-3). Overall, genotypes 'BGN-9', 'BGN-14' and 'BGN-31' were superior in terms of the seedling parameters that were measured. The three genotypes were from three distinct seed colour types. This was potentially useful in cultivar adoption since the preference for seed colour types varies among smallholder bambara groundnut growers. In contrast, dark colored seeds produced more vigorous seedling than light colored seeds (Berchie *et al*., 2010; Sinefu, 2011; Zulu and Modi, 2010). In other studies involving legumes, the variation in seedling vigour was attributed to seed size. For instance, Ambika *et al*., (2014) observed that genetic variation influences

Table A-1 Means of four seedling vigour parameters of bambara groundnut genotypes evaluated under laboratory conditions. (PRL = primary root length; SRL = secondary root length; SHT = shoot height; DRW = dry root weight).

Genotype	PRL(cm)	SRL(cm)	SHT(cm)	DRW(g)
BGN-9	156.67a	82.00a-c	32.00a-g	0.07a-c
BGN-14	145.50ab	88.00ab	54.33ab	0.08a
BGN-7	145.50ab	50.50a-f	20.83c-h	0.03a-e
BGN-23	124.00a-c	54.17a-f	35.67a-f	0.04a-e
BGN-40	122.17a-c	57.33a-f	55.33a	0.03a-e
BGN-31	118.83a-c	87.33ab	45.33a-d	0.08a
BGN-17	117.83a-c	47.67a-f	22.17c-h	0.05a-e
BGN-21	117.17a-c	96.17a	27.50a-h	0.06a-c
BGN-20	115.83a-c	53.50a-f	45.83a-c	0.04a-e
BGN-33	114.17a-c	77.67a-d	17.67c-h	0.05a-e
BGN-39(Check)	113.83a-c	40.33a-f	25.33b-h	0.03a-e
BGN-25	111.33a-c	53.33a-f	35.83a-f	0.04a-e
BGN-34	106.50a-c	46.17a-f	20.33c-h	0.04a-e
BGN-8	104.50a-c	72.17a-e	29.00a-h	0.05a-d
BGN-6	103.50a-c	26.17c-f	29.50a-h	0.04a-e
BGN-37	102.00a-c	66.17a-e	30.83a-g	0.05a-e
BGN-38	100.00a-c	39.83a-f	20.17c-h	0.04a-e
BGN-12	94.00a-c	55.50a-f	42.00a-e	0.05a-e
BGN-10	92.50a-c	47.50a-f	21.67c-h	0.03a-e
BGN-30	92.50a-c	55.17a-f	23.17c-h	0.05a-e
BGN-15	91.83a-c	56.50a-f	28.33a-h	0.06a-c
BGN-11	91.67a-c	64.17a-e	25.33b-h	0.05a-e
BGN-3	91.33a-c	54.50a-f	31.50a-g	0.05a-e
BGN-24	84.00a-c	41.83a-f	19.67c-h	0.05a-e
BGN-36	83.33a-c	39.83a-f	14.50e-h	0.05a-e
BGN-2	77.83a-c	32.50b-f	18.17c-h	0.03a-e
BGN-1	77.00a-c	29.17c-f	11.67f-h	0.04a-e
BGN-28	74.67a-c	31.67b-f	15.67d-h	0.04a-e
BGN-13	69.67a-c	39.00a-f	19.50c-h	0.03b-e
BGN-5	64.00a-c	47.17a-f	25.33b-h	0.04a-e
BGN-4	60.67a-c	96.33a	20.17c-h	0.04a-e
BGN-41	58.83a-c	41.17a-f	33.83a-g	0.05a-e
BGN-19	57.00a-c	30.17c-f	13.33e-h	0.05a-e
BGN-18	42.67a-c	40.33a-f	26.50a-h	0.04a-e
BGN-35	37.17a-c	22.50d-f	21.33c-h	0.03a-e
BGN-26	29.83a-c	14.67ef	12.67e-h	0.02b-e
BGN-42	24.00bc	20.67d-f	12.83e-h	0.02c-e
BGN-27	6.67c	3.33f	5.00gh	0.00e
BGN-22	0.00c	0.00f	0.00h	0.00e
BGN-29	0.00c	0.00f	0.00h	0.00e
BGN-32	0.00c	0.00f	0.00h	0.00e
BGN-16	0.00c	0.00f	0.00h	0.00e
Mean	82.16	45.30	23.57	0.03

The means followed by different letters in the same column are significantly different at the 5.0% probability level.

Fig. A-1 Phenotypic variation in seedling vigour in bambara groundnut.

Fig. A-2 Poor shoot growth in some seedlings of bambara groundnut genotypes.

variation in seed size between varieties and is due to flow of nutrients into the seed at the mother plant which eventually impacts on mobilization of food reserves to growing seedlings.

Future studies could determine the seedling vigour under field conditions (Kolasinska, 2000). and various abiotic stresses including salinity (Tsegay and Gebreslassie, 2014), pH (Mandic *et al*., 2012), temperature (Antonio *et al*., 2016; Porch, 2006; Awal and Ikeda, 2002; Covell *et al*., 1986) and low moisture (Serraj *et al*., 2004). This will enhance the selection of elite germplasm that can be exploited in the genetic improvement of seedling vigour in bambara groundnut. There will be merit in testing the seedling vigour also under a variety of soil types.

Table A-2 The Pearson correlation coefficients among bambara groundnut genotypes which were evaluated for seedling vigour under laboratory conditions.

	PRL(cm)	**SRL(cm)**	**SHT(cm)**	**DRW(g)**
PRL(cm)	1.0000			
SRL(cm)	0.6592**	1.0000		
SHT(cm)	0.5508**	0.5548**	1.0000	
DRW(g)	-0.4464*	-0.5894**	-0.6007**	1.0000

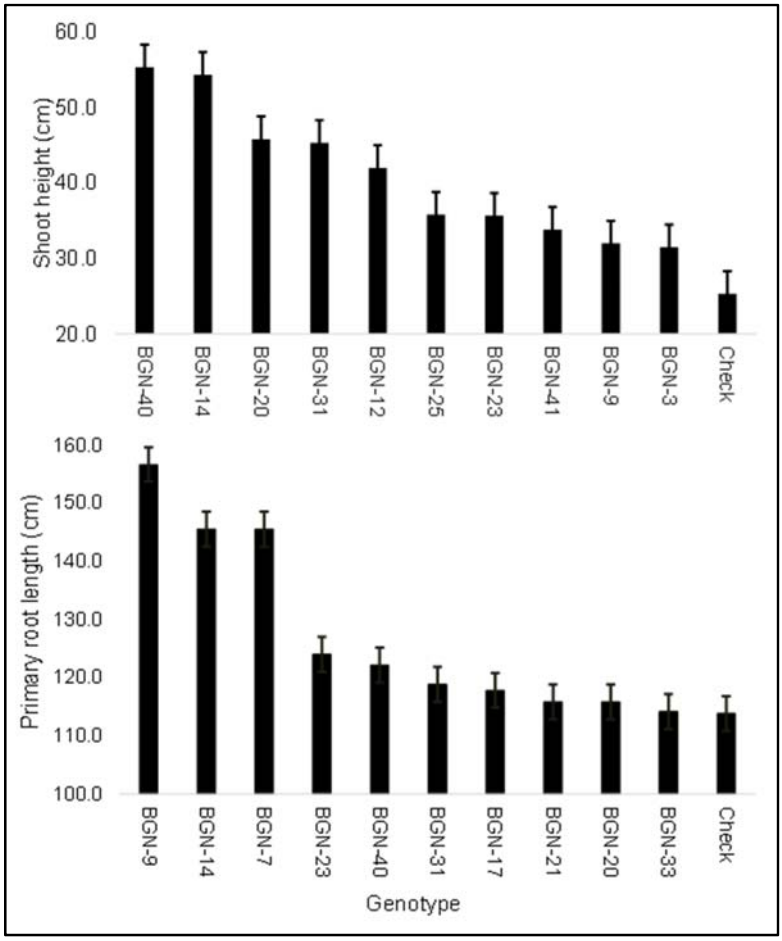


Fig. A-3 Genotypic variation in shoot height and primary root length in the top 10 bambara groundnut genotypes that were evaluated for seedling vigour under laboratory conditions.

Table A-3 Means of the top genotypes in each of the seed colour types of bambara groundnut germplasm which was evaluated for seedling vigour under laboratory conditions. (Values in bold represent the relative percent advantage over the check genotype).

Genotype	PRL(cm)	SRL(cm)	SHT(cm)	DSW(g)	Seed color
BGN-9	156.67 (**38.22%**)	82.00 (**103.32%**)	55.33 (**118.44%**)	0.07 (**133.33%**)	Cream/white
BGN-14	145.50 (**28.56%**)	88.00 (**118.20%**)	54.33 (**114.49**%)	0.08 (**166.67%**)	Light brown
BGN-7	145.50 (**28.56%**)	50.50 (**25.22%**)	20.83 (**-17.76%**)	0.03 (**0.00%**)	Black
BGN-31	118.83 (**5.00%**)	87.33 (**116.54%**)	45.33 (**78.96%**)	0.08 (**166.67%**)	Red
BGN-39 (Check)	113.18	40.33	25.33	0.03	Light brown

References

Ambika S, Manonmani V and Somasundaram G (2014). Review on effect of seed size on seedling vigour and seed yield. *Research Journal of Seed Science* **7**: 31-38.

Awal MA and Ikeda T (2002). Effects of changes in soil temperature on seedling emergence and phenological development in field-grown stands of peanut (*Arachis hypogaea*). *Environmental and Experimental Botany* **47**: 101-113.

Basra AS (2005). Handbook of seed science and technology, New York; Harworth Press.

Berchie JN, Sarkodie –Addo J, Adu-Dapaah H, Agyemang A, Addy S, Assare E and Donkor J (2010). Yield evaluation of three early maturing bambara groundnut (*Vigna subterranea (L) Verdc*). *Journal of Agronomy* **9**: 175-179.

Chen Y, Ghanem ME and Siddique KHM (2017). Characterising root trait variability in chickpea (*Cicer arietinum* L.) germplasm. *Journal of Experimental Botany* **68**: 1987-1999.

Chibarabada TP, Modi AT and Mabhaudhi T (2014). Seed quality characteristics of a bambara groundnut (*Vigna subterranea* L.) landrace differing in seed coat color. *South African Journal of Plant and Soil* **31**: 219-226.

Chibarabada TP, Modi AT and Mabhaudhi T (2015). Water use characteristics of a bambara groundnut (*Vigna subterranean* L Verdc) landrace during seedling establishment. *Water South Africa* **41**: 472-482.

Covell S, Ellis RH, Roberts EH and Summerfield RJ (1986). The influence of temperature on seed germination rate in grain legumes. I. Acomparison of chickpea, lentil, soybean and cowpea at constant temperatures. *Journal of Experimental Botany* **37**: 705–715. 10.1093/jxb/37.5.705 [Cross Ref].

Duncan DB (1956). Multiple range and multiple F tests. *Biometrics* **11**: 1-42.

Gwata ET, Shimelis H and Matova PM (2016). Potential of Improving Agronomic Attributes in Tropical Legumes Using Two Mutation Breeding Techniques in Southern Africa. *In*: (ed.) Konvalina P. Alternative Crops and Cropping Systems, InTech, DOI: 0.5772/62262. ISBN 978-953-51-2279-1, pp. 71-85.

Hojjat SS (2011). Effect of seed size on germination and seedling growth of some lentil genotypes (*Lens culnaris* Medik.). *International Journal of Agriculture and Crop Science* **3**: 1-5.

Hillocks RJ, Bennett C and Mponda OM (2011). Bambara nut: a review of utilization, market potential and crop improvement. *African Journal of Crop Science* **20**: 1-16.

Kolasinska K, Szyrmer J, Dul S (2000). Relationship between laboratory seed quality tests and field emergence of common bean seed. *Journal of Crop Science* **40**: 470-475.

Koné M, Koné T, Silué N, Soumahoro AB, and Kouakou TH (2015). In vitro seed germination and seedling growth of bambara groundnut (*Vigna subterranea* (L.) Verdc. (Fabaceae)) *The Scientific World Journal* 1-8.

Kouassi NJ and Zorobi IA (2011). Evaluation of agronomic performance of two landraces of bambara groundnut (*Vigna subterranea*) in woodland savannahs of Cote d'Ivoire. *African Journal of Agricultural Research* **6**: 4852-4856.

Mabhaudhi T (2009). Response of landrace maize (*Zea mays* L.) to water stress compared with commercial hybrids. MSc thesis, University of KwaZulu-Natal, South Africa.

Mandic V, Krnjaja V, Tomic Z, Bijelic Z, Zujovic M, Simic A, Prodanovic S (2012). Genotype, seed age and pH impacts on germination of alfalfa. *Romanian Biotechnological Letters* **17**: 7205-7211.

Peksen E, Peksen A, Bozoglu H, and Gulumser A (2004). Some seed traits and their relationships to seed germination and field emergence in pea (*Pisum sativum* L.). *Journal of Agronomy* **3**: 243-246.

Porch TG (2006). Application of stress indices for heat tolerance screening of common bean (*Phaseolus vulgaris*). *Journal of Agronomy and Crop Science* **192**: 390–394.

Rezapour R, Kazemi-arbat H, Yarnia M and P Zafarani-Moatta P (2013). Effect of seed size on germination and seed vigour of two soybean (*Glycine max* L.) cultivars. International Research *Journal of Applied and Basic Sciences* **4**: 3396-3401.

Rikhotso T, Gwata E and Mzezewa J (2013). Preliminary selection of early bambara groundnut for urban markets in Limpopo Province (South Africa). *Tropentag.* Book of abstracts, 17-19 September, Hohenheim, Germany pp 405.

Sarker A, Erskine W and Singh M (2005). Variation in shoot and root characteristics and their association with drought tolerance in lentil landraces. *Genetic Resources and Crop Evolution* **52**: 89–97

SAS Institute Inc. (2014). SAS 9.1.3: ETL *Studio: User's guide*. Cary, NC: SAS institute.

Serraj R, Krishnamurthy L, Kashiwagi J, Kumar J, Chandra S, Crouch JH (2004). Variation in root traits of chickpea (*Cicer arietinum* L.) grown under terminal drought. *Field Crops Research* **88**: 115-127.

Sinefu F (2011). Bambara groundnut response to controlled environment and planting date associated water stress. *Journal of Crop Science* **31**: 816-822.

Tsegay BA and Gebreslassie B (2014). The effect of salinity (NaCl) on germination and early seedling growth of *Lathyrus sativus* and *Pisum sativum* var. *abyssinicum*. *African Journal of Plant Science* **8**: 225-231.

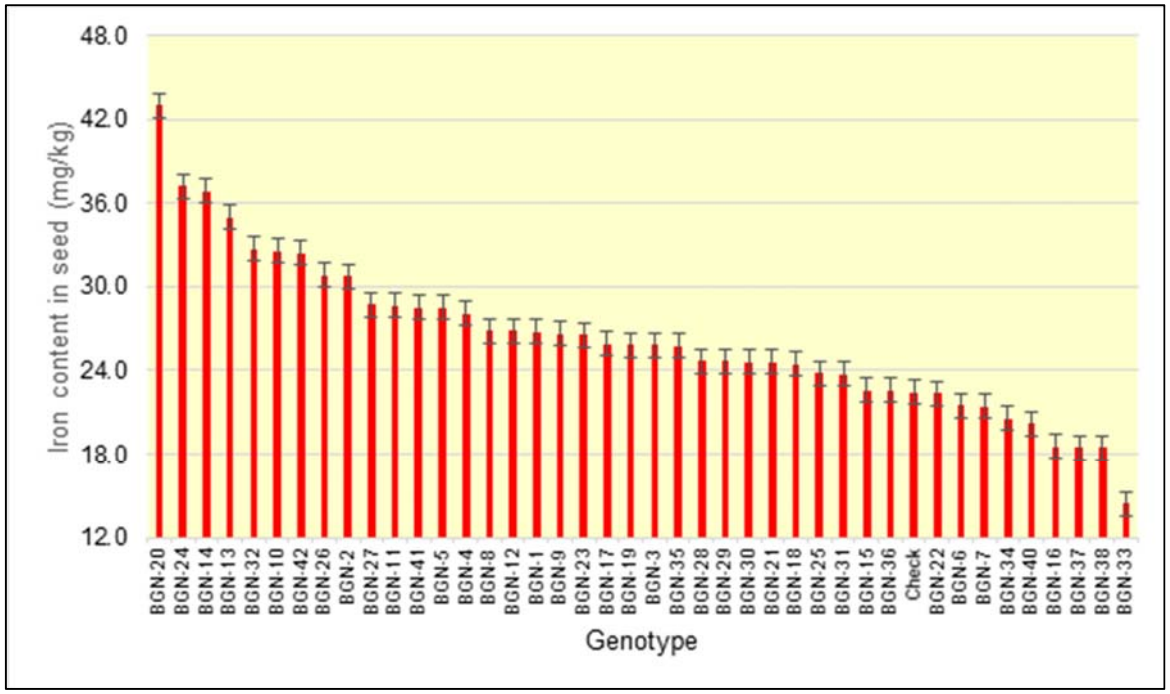
Vishvanath K, Kalappa VP and Prasad SR (2006). Standardisation of screen sizes for French bean seed processing. *Seed Research* **34**: 77-81.

Zulu NS and Modi AT (2010). A preliminary study to determine water stress tolerance in wild melon (*Citrillus lanatus* L). *South African Journal of Plant and Soil* **27**: 334-336.

Appendix III

Variability in iron content in the seed of bambara germplasm raised at Thohoyandou (Limpopo Province, South Africa).

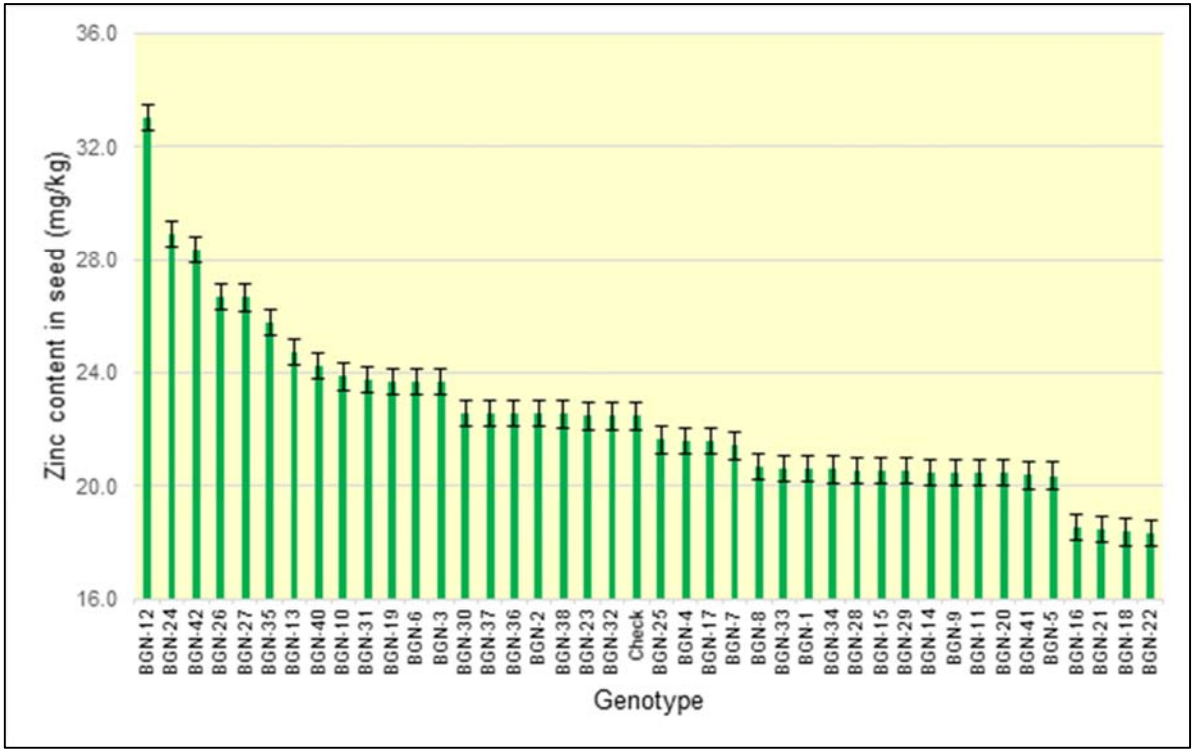
Source: Mogale and Gwata, 2016; unpublished).



Appendix IV

Variability in zinc content in the seed of bambara germplasm raised at Thohoyandou (Limpopo Province, South Africa).

Source: Mogale and Gwata, 2016; *unpublished*).



Appendix V

Informal marketing of bambara groundnut in Makhado (Limpopo Province, South Africa)