Morphological diversity, heritability, and selection of the first F_7 improved Bambara groundnut (*Vigna subterranea* L. Verdc) evaluated in South Africa.

by

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Discipline of Crop Science

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PREFACE

The candidate completed the research in this thesis while based in the Discipline of Crop Science, School of Agricultural, Earth and Environmental Sciences of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

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

[redacted]

Signed: Prof. Tafadzwa Mabhaudhi (**Supervisor**)

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DECLARATION: PLAGIARISM

I, **Mametja Stuat**, declare that,

1. This thesis, titled "Morphological diversity, heritability, and selection of the first F_7 improved Bambara groundnut (*Vigna subterranea* L. Verdc) evaluated in South Africa", is the result of my own and has not been submitted for any degree or examination at any other university.
2. All sources have been appropriately cited in accordance with the guidelines of the University of KwaZulu-Natal.
3. This thesis does not contain data, pictures, graphs, or other information from other researchers unless expressly acknowledged as being sourced from others.

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SC Mametja (Candidate)

GENERAL ABSTRACT

Bambara groundnut (*Vigna subterranea* L. Verdc) is an underutilised legume crop that can improve food and nutrition security, especially in low-input farming systems. The specific objectives of the study were: (i) to evaluate the agro-morphological diversity of Bambara groundnut (BGN) recombinant inbred lines (RILs) using quantitative and qualitative traits, (ii) to determine genetic variance components among BGN RILs to guide future selection. The first study assessed the genetic diversity present among BGN genotypes using agro-morphological traits. Fifty-four BGN genotypes comprising 44 RILs developed from a cross between single genotype Tiga Nicuru (maternal) and Dip-C (paternal) lines and ten local checks were evaluated in three environments in the KwaZulu Natal province of South Africa. The genotypes were laid out using 6 x 18 alpha lattice block design replicated twice. The combined analysis of variance (ANOVA) showed significant ($P < 0.01$) difference among the genotypes for most of the agro-morphological traits, except for days to 50% emergence (DTE), days to 50% flowering (DTF), and plant spread (PS). The genotype by environment interaction effects were highly significant ($P < 0.001$) for seed length (SL), seed width (SW), grain yield (GY), unshelled grain yield (UGY), grain yield per plant (GYPP) and shelling percentage (SHLP). Principal component analysis (PCA) showed that the first four principal components accounted for 79% of the total phenotypic variance observed. Hierarchical cluster analysis assigned the 54 BGN genotypes into six clusters. The second study assessed genetic variance parameters among the 44 RILs and ten local checks. Germination percentage recorded the highest broad-sense heritability (H^2) estimate of 65%, while days to 50% flowering recorded the highest H^2 (65%).GY recorded the highest phenotypic coefficient of variation (69.4%), genotypic coefficient of variance (44.8%), and genetic advance (59.5%). The best linear unbiased prediction **(**BLUP) estimates revealed that the genotypes P3, G23, G1, G8 and LL4 were superior and high yielding. These genotypes should be recommended for further multilocational evaluations.

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ABBREVIATIONS

σ^2e	Error variance
σ^2E	Environmental variance
σ^2G	Genotypic variance
σ^2P	Phenotypic variance
%CV	Percentage coefficient of variance
ARC	Agriculture Research Council
ANOVA	Analysis of variance
BGN	Bambara groundnut
CRDA	Cedara College of Agriculture
DTE	Days to 50% emergence
DTF	Days to 50% flowering
E	Environment
ENV	Environment
F7	Seventh generation
GA	Genetic advance
GCV	Genetic coefficient of variance
GH	Growth habit
GGE	Genotype and genotype by environment interaction
GEI	Genotype by environment interactions
GP	Germination percentage
GY	Grain yield
GYPP	Grain yield per plant
H^2	Broad sense heritability
MET	Multi environmental trial
PC	Principal component
PCA	Principal component analysis
PCV	Phenotypic coefficient of variance
PH	Plant height
PS	Plant spread
RD	Relative difference
RILs	Recombinant inbred lines
REML	Restricted maximum likelihood model
SHLP	Shelling percentage
SL	Seed length
SW	Seed width
SSA	sub-Saharan Africa
NUS	Neglected and underutilised species
WUE	Water use efficiency

Chapter 1: Introduction

1.1 Background

An Indigenous legume known as Bambara groundnut (*Vigna subterranea* (L) Verdc.), native to sub-Saharan Africa, is grown at modest levels by many farmers as a part of family food and nutritional security crop (Mayes et al., 2019). The Bambara groundnut (BGN) is a valuable source of essential amino acids, fatty acids, and minerals. It is a mineral-rich crop because it possesses a significant amount of potassium (K) (11.44–19.35 mg/100 g), iron (Fe) (4.9–48 mg/100 g), sodium (Na) (2.9–12.0 mg/100 g), and calcium (Ca) (95.8–99 mg/100 g) (Khan et al., 2021a). In terms of consumption and socioeconomic status, it is ranked the third most crucial legume in sub-Saharan Africa (SSA) after *Arachis hypogea* (groundnuts) and *Vigna unguiculata* (cowpea) (Maphosa et al., 2022).

The legume BGN originated in West Africa (Paliwal et al., 2021). It is reported that the crop also has centres of diversity in Asian countries such as Malaysia, Bangladesh, India, Thailand, and Indonesia (Khan et al., 2021b). Globally, West African countries are the primary producers of BGN, and the top three global BGN-producing countries are Burkina Faso, Niger, and Cameroon (Majola et al., 2021). In South Africa, BGN is primarily cultivated in Limpopo, Mpumalanga, and KwaZulu-Natal provinces (Adedayo et al., 2021). In 2018, an estimated 0.2 million tonnes of BGN were produced globally, mainly by small-scale farmers across the SSA. Hence, it is challenging to precisely quantify the total production and size of production areas in SSA (Majola et al., 2021).

Global food security in SSA has recently been compromised by weather variability (Soumare et al., 2021). Rainfall and temperature are unpredictable in the semi-arid tropics, resulting in unreliable environmental conditions for crop growth (Aruna et al., 2016). Rain has become unevenly distributed, falling mainly in coastal areas (Chivenge et al., 2015), and showing decreasing trend in the Southern hemisphere (Mabhaudhi et al., 2018). In addition, raising temperatures, frequent droughts, flooding, severe pest and disease outbreaks , and soil degradation significantly affect growth and performance of main crop species such as maise (*Zea mays)* and wheat (*Triticum aestivum*) (Muhammad et al., 2020).

Recent studies have confirmed the exceptional drought tolerance (Jørgensen et al., 2010; Jørgensen et al., 2011; Mabhaudhi et al., 2013) and water use efficiency (WUE) (Chibarabada et al., 2015a, b) of the BGN. The BGN uses several mechanisms to escape, avoid, and tolerate drought (Paliwal et al., 2021). In severe droughts, it can reduce canopy size and reach maturity early while maintaining excellent water use efficiency without significantly affecting grain yield (Mabhaudhi et al., 2013). Despite the resilience of the crop, it is among the world's neglected and underutilised crops.

Many factors forestall the transformation of the undeveloped BGN value chain throughout SSA, and chief among them is the lack of improved cultivars. Most BGN farmers rely on landraces, which are stable but low-yielding because they are a heterogeneous mix of several homozygous individuals. BGN hardly benefited from breeding programs targeting the development of improved cultivars.

The complex anatomy of the BGN's reproductive system has been a major impediment to the improvement of the legume (Ajilogba et al., 2022; Olanrewaju, 2022). The crop has minute florets, which are recalcitrant to emasculation and hand pollination. However, scientists at the University of Nottingham recently proved the amenability of Bambara to artificial hybridisation. This novel discovery, coupled with the elucidation of the annotated Bambara genome, means that careful trait selection can now commence, leading to the development of high-yielding Bambara cultivars (Mayes et al., 2019). The sustainable promotion and improvement of BGN require developing and deploying improved cultivars adapted to the new climate conditions.

Moreover, developing new cultivars requires a clear understanding of the existing diversity to inform breeding programs and management strategies. With that regard, Bambara pure lines representing the available worldwide germplasm were recombined by a team of scientists at the University of Nottingham (Ahmad et al., 2016; Chai et al., 2016), to develop recombinant inbred lines (RILs), which were further advanced from F_3 and F_6 in South Africa for selection. Hence, this study is the first to evaluate the performance of true breeding Bambara lines in South Africa and the region.

1.2 Problem statement

Purified genetic lines for BGN are scarce, as are quantitative and comparative data on morphological traits deemed necessary by farmers (Feldman et al., 2015). Farmers still rely on retained BGN landrace seeds bulked from the previous year for production. The literature shows no evidence of commercialised registered BGN lines across sub-Saharan Africa, including South Africa. As a result, farmers use landrace seeds that are heterogeneous, poor and have unstable grain yield. Hence, the study aims to evaluate BGN RILs' performance to select the best-performing BGN pure lines.

1.3 Justification

BGN is a promising future legume that can withstand and combat the challenges of climate change. Bambara is well known for its drought tolerance and the ability to grow in marginal soils where other staple crops fail. The legume has the potential to improve the economic status of small-scale farmers. BGN is a complete food legume dense in nutrients like protein, carbohydrates, and minerals; it can solve malnutrition and food insecurity across rural areas. The primary step in detailing well-performing BGN RILs is to phenotype and select the best-yielding lines. Hence, the study aims to evaluate the first newly developed BGN RILs for their agro morphological performance and diversity in South Africa.

1.4 Study aim

This study aimed to evaluate the performance of BGN RILs to select the best-performing BGN pure lines.

1.5 Hypothesis

i. BGN recombinant inbreed lines will outperform the ten parental landrace cheks as they are a recombinant of two world best-performing pure lines.

ii. BGN genotypes will show highly significant genetic variability.

1.6 Specific objectives

The specific objectives of the study were:

i. To evaluate the agro-morphological diversity of BGN recombinant inbred lines using quantitative and qualitative traits.
ii. To determine genetic variance components among BGN RILs to guide future selection.

1.7 Thesis structure

The thesis comprises five chapters, as demonstrated in Table 1.1.

Table 1.1: Outline of thesis structure

Chapter	Title
1	The introduction chapter provides the background, problem statement, justification, study aim and objectives, and thesis structure.
2	Status of Bambara groundnut (*Vigna subterranea*n L.) production: A scoping review
3	An experimental chapter on the assessment of agro-morphological diversity of BGN RILs based on quantitative and qualitative traits.
4	An experimental chapter to determine the genetic variance components among BGN RILs to guide future breeding.
5	This chapter provides a general overview and summary of findings for each chapter and conclusions.

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Chapter 2: Status of Bambara groundnut (*Vigna subterranean* L.) production: A scoping review

Abstract

In sub-Saharan Africa (SSA), subsistence farmers are the leading producers of the Bambara groundnut (*Vigna subterranea* L. Verdc., 2n = 2x = 22); the legume is regarded as neglected and contributes to food and nutritional security in the SSA. A scoping review methodology was used to review the literature on the current recommended Bambara groundnut's (BGN) production practice to inform future crop improvement breeding objectives. Two electronic search engines (Scopus and Web of Science) were used to search published articles related to the current trends, constraints, and potential strategies that can be implemented to improve BGN in the SSA. After screening, 36 relevant studies were identified and included for data extraction and synthesis. The results show that several environmental factors influence the agronomic performance of BGN. Some abiotic, biotic, and agronomic factors significantly influencing BGN production include temperature, photoperiod, drought, sowing time, mounding period, soil type, and seed quality. The lack of commercially improved seeds for BGN cultivars suggests the urgent need to address the problem through breeding.

Keywords: Bambara groundnut; production, constraints, biotic and abiotic stress.

2.1 Introduction

Recently, climate change has been widely acknowledged as a critical concern that severely impacts food security and livelihoods across the region (Connolly-Boutin and Smit, 2016). Some implications associated with climate change are now visible, including rising global average temperatures and the change in precipitation patterns, leading to the destruction of the ecosystem, biodiversity, and human systems worldwide. Sub-Saharan Africa is more vulnerable to climate change as it mostly depends on rain-fed agricultural systems (Kotir, 2011). Heat and drought are the major stresses impacting yields of major crops, such as maise (*Zea mays* L.), rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), and soybean (*Glycine max*) (Fahad et al., 2017).

However, neglected and underutilised crops' (NUS) have the potential to overcome these challenges because of their climate resilience (Karunaratne et al., 2015). Most NUS crops have the potential to build sustainable food systems that can adapt to the effects of climate change, especially in rural economies (Mubaiwa et al., 2018). There is also the need to increase the food supply to meet the needs of the rising world population by focusing on improving minor crops (Maphosa et al., 2022). This can be done by focusing on crops that thrive in low-input agriculture systems, such as the Bambara groundnut (*Vigna subterranea*).

The annual BGN is similar to the groundnut (*Arachis hypogaea*) regarding growth habit and cultivation; for many Africans, it is among the top five sources of protein (Vietmeyer, N. (1978); Basu et al., 2007). It has a protein content of about 15 - 27%, comparable to that of cowpea (*Vigna unguiculata* (L.) and peanuts (*Arachis hypogaea*) (Arise et al., 2017), but more than that of other legumes, including chickpea (*Cicer arietinum*), soybean (*Glycine max*), and faba bean (*Vicia faba*) (Azman Halimi et al., 2019; Hillocks et al., 2012). In addition, the crop has a well-balanced proportion of essential nutrients, including starch (up to 53% of seed), amylose (15.7 - 35.3%), and dietary fiber 10.3% (Azman Halimi et al., 2019).

The legume fixes up to 28.42 kg/ha N due to its symbiotic relationship with the rhizobia bacteria (Wafula et al., 2021), making it ideal for low-input agricultural systems. The BGN can also grow well on poor soils where other allied legumes would fail (Barimalaa et al., 2005). Most BGN accessions are tolerant to water-deficit stress compared to other legumes (Nautiyal et al., 2017) and can avoid and escape drought depending on the severity and length of stress in a particular location (Mayes et al., 2019b). This makes the BGN an exemplary model crop for resilient food systems in changing climates.

Despite BGN being drought tolerant and nutrient-dense legume, the crop has an undeveloped value chain, which limits its productive potential. Currently, BGN is often produced on an average area of 0.4 ha by relatively elderly farmers. The absence of improved agronomic practices and high-yielding cultivars also forestalls the transformation of BGN. This is because BGN has never benefitted from any breeding programmes targeting the development of improved cultivars. Chibarabada et al. (2015a) reported that a lack of seed availability for improved BGN seeds leads farmers to rely on landraces and seeds preserved from previous harvests. In addition, traits such as susceptibility to fungi and bacterial wilts, “hard to cook” traits, photoperiod sensitivity, and many other socio-economic factors also affect the potential of BGN (Berchie *et al.*, 2010).

Information on present opportunities and challenges needs to be synthesised to upscale the BGN value chain in SSA. Furthermore, knowledge of production systems and farmers' practices suffice to set product target profiles for BGN and other NUS crops. Hence, the scoping literature review methodology adopted by Arksey and O'Malley (2005) can synthesise the existing information and knowledge gaps on factors constraining or influencing BGN production, which helps devise strategies to promote the legume. Therefore, this review aims to (i) highlight the production of BGN in sub-Saharan Africa, (ii) identify constraints affecting BGN production, and (iii) propose a roadmap for the efficient improvement of BGN.

2.2 Materials and methods

The current study adopted a scoping review methodology framework proposed by Arksey and O'Malley (2005). The scoping review framework follows five stages, which are: (1) identifying the research question, (2) identifying relevant studies, (3) study selection, (4) charting data, and (5) collating, summarising, and reporting the results. It was stated that a scoping study tends to address a broader topic where many different study designs might be applicable, and it is less likely to seek to address specific research questions nor, consequently, to assess the quality of included studies. The study aimed to consolidate and disseminate research findings while identifying gaps in the current literature on BGN.

2.2.1 Research question

In the current study, the following research question was adopted: “What are the current BGN production trends, production constraints and potential strategies used for improving BGN production in sub-Saharan Africa, and how can an efficient roadmap be developed to improve its cultivation and utilisation?” The question was developed to match the review goal which is to "assess the production requirements of BGN to guide the breeding for broad and specific adaptation of new and improved BGN cultivars.

2.2.2 Identification of relevant studies, data sources, and search strategy

Two search engines, Scopus, and Web of Science (WOS), were used to source relevant publications that addressed the review’s aim. PubMed and Google Scholar were also used to identify the existing literature that might have been missed by the other two search engines. The syntax: "Bambara groundnut" AND "Environment" AND "Production" was used to search for the relevant studies conducted in African, subdivided into five regions: Northern Africa, Western Africa, Eastern Africa, central Africa, and Southern Africa.

2.2.3 Study selection

The search results were exported as "RIS" file type and imported into EndNote version 20.2.1; the EndNote software was used to isolate and exclude duplicates before the screening. EndNote was further used to search and download full-text articles. The software was further used to screen all relevant and irrelevant studies. An Excel sheet was created to capture extracted information from the Endnote software.

2.2.4 Exclusion of irrelevant publications

The screening process was carried out systematically, namely title screening, abstract screening, and full-text screening. Data was only extracted from full-text screened materials; all studies identified were screened and kept on each screening selection. Book chapters, article papers that address "Bambara groundnut, production, and breeding,", and other articles relevant to the study objective were kept for further screening. The following material types were included in the study, book chapters, journals, articles, and review papers (Table 2.1).

Table 2.1: Inclusion and exclusion summary table.

Variant	Inclusion	Exclusion
Language	English	Non-English
Region	African regions	Non-African regions
Focus	Address one of the following: BGN, production, breeding, and field evaluation of NUS.	Address none of these: BGN, production, breeding, and field evaluation of NUS.
Species	Address BGN	Do not address BGN
Availability of full-text paper	If full-text paper is available	If full-text paper is not available

2.2.5 Data extraction, synthesis, and reporting

The data collection groups comprised authors, study title, study theme, publication year, region where the study was carried out in Africa and the key results/conclusion. The data were compiled in a spreadsheet using Microsoft Excel 2019. The review was recapitulated into specific themes based on the identified studies. When summarising the results, the alternative grey literature sourced from Google Scholar was incorporated to support the main findings of the results. The findings in this review were reported using a systematic narrative approach. The overview, key themes, trends, and gaps in knowledge were reported to cover the depth of the available evidence and strengthen the findings of this review.

2.3 Results

2.3.1 Summary of identified studies

One thousand two hundred thirty-eight publications were identified on Scopus and Web of Science (WOS) electronic databases, and eight duplicates were removed before the screening, keeping 1230 publications. After title, abstract, and full-text screening, 36 studies were kept for data extraction (Figure 2.1). These publications were used to write the study review, and data was extracted from the 36 studies. Grey literature in this study was sourced from Google Scholar and the internet to support the findings.

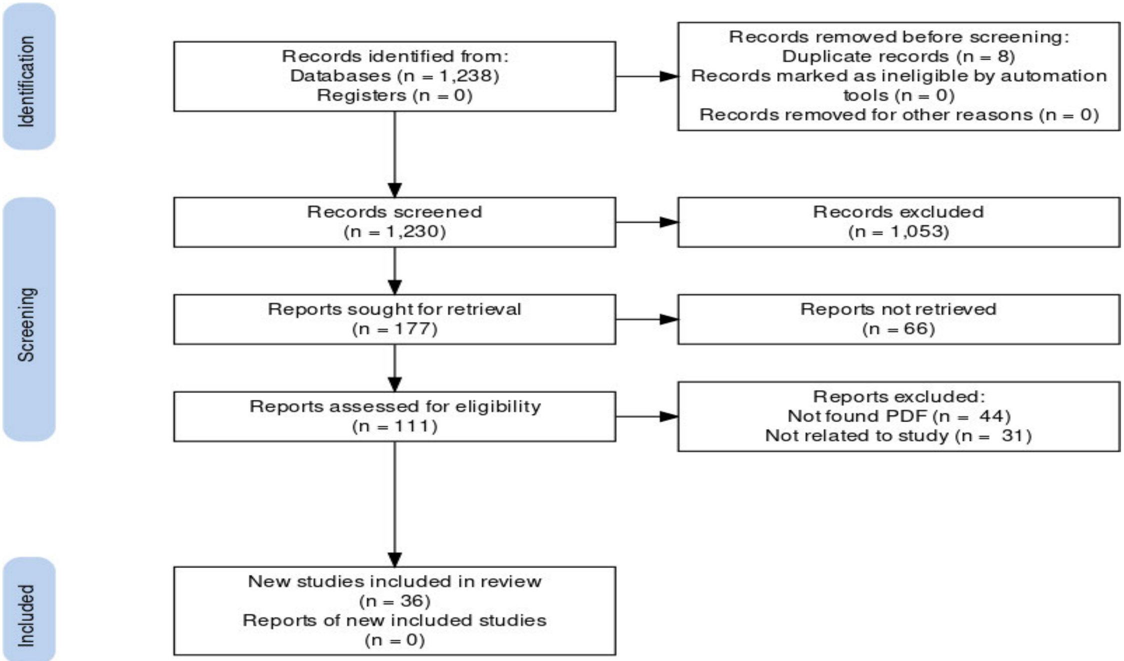

Figure 2.1: Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram of the literature screening process

2.3.2 Regional and timeline of published articles on BGN production

The findings indicate that the Southern African region has the most publications on BGN's production, followed by Western Africa (WA), Eastern Africa (EA), Northern Africa (NA), and Central Africa (CA) (Figure 2.2). These publications were from 2004 - 2022, with 2017 recording the highest publications (Figure 2.3).

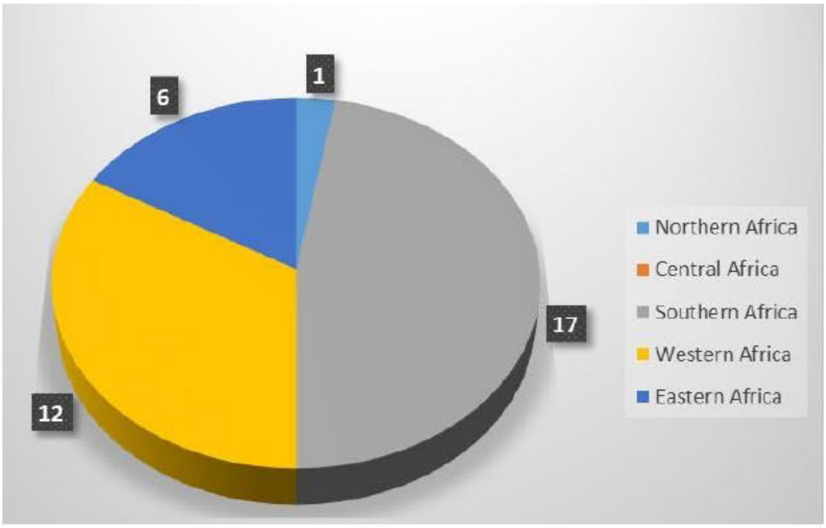

Figure 2.2: Number of publications identified per region in Africa for environmental production of Bambara groundnut

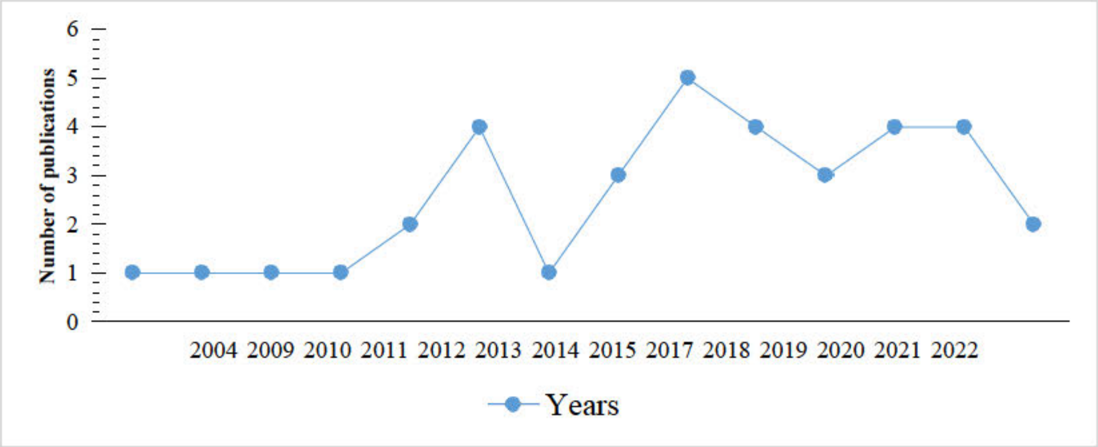

Figure 2.3: The line graph illustrating 36 article publications from 2004 to 2022 related to BGN

2.3.3 Breakdown of studies identified in different regions in Africa on BGN crop

In West Africa, a total of 12 studies were identified. Seven studies from the 12 focused on breeding, and the remaining papers addressed the production and value chain of BGN. In the SA, studies focused more on productivity in relation to drought and water use efficiency (WUE). South Africa had the most publications in Southern Africa, with a total of 12 articles out of 17 identified publications (Table 2.2). Some of the studies in Southern Africa addressed issues related to WUE, drought, adaptability and productivity associated with the crop BGN. The studies in East Africa focused on utilisation, food security, nutrition, salinity, and genetic variation, and most of the studies recorded from that region were done in Kenya. Only a single study was reported from Northern Africa.

Table 2.2: Summary of identified articles on the environmental production of BGN explored

	Factor investigated	Conclusion	Region	Citation
Agronomy	Water use efficiency	BGN landraces, characterised by reduced canopy size, early flowering, maturity, and high WUE under stress, exhibit varying responses to drought, indicating the influence of seed coat colour.	SA	Mabhaudhi et al. (2013)
		Under water stress, dark-coloured BGN seeds perform better than light-coloured BGN seeds.	SA	Chibarabada et al. (2015a)
		BGN output was lower in rainfed than irrigated conditions. BGN demonstrated a degree of phenological flexibility in response to drought.	SA	Chibarabada et al. (2015b)
		Climatic and edaphic factors impact yield and nutrient content in BGN, suggesting breeding for nutrient uniformity and providing insights into limited legume production environments.	SA	Chibarabada et al. (2017)
		Crops responded to reduced soil moisture by stomatal adjustment and canopy size and time reduction.	SA	Chibarabada et al. (2019)
	Drought	The traits, canopy spread, root shoot ratio, 100-seed weight, and number of seeds per pod can be used to identify the drought tolerance BGN genotypes.	SA	Karikari and Tabona. (2004)
		BGN demonstrated drought avoidance and escape strategies by restricting water loss through stomatal closure and decreasing plant height, leaf number, and leaf area index.	SA	Mabhaudhi and Modi. (2013)
		The model indicates that the red BGN landraces may be a potential drought-tolerant crop.	SA	Mabhaudhi et al. (2014)
		Understanding morphological processes involved in NUCS responses is vital to discovering drought-resistant crops. Well-calibrated and authorised models could aid as selection tools for drought tolerance NUCS.	SA	Chivenge et al. (2015)
		The study identified 13 neglected and underutilised crops (NUS) and suggested limiting resources to improve the NUS.	SA	Mabhaudhi et al. (2017)
	Cultural practice	A population density of 250,000 plants per hectare appeared to give the highest BGN grain yield and plant biomass per unit area, regales of seedbed type.	WA	Kouassi and Zoro BI. (2010)
		The yield of BGN is influenced by the planting time, with February and March sowings yielding more than April and June sowings (in Ghana).	WA	Berchie et al. (2012)
		BGN field mounding should be done early (about two weeks after sowing) or late (about seven weeks after sowing) but not during flowering, as it results in yield reduction	WA	Ouedraogo et al. (2013)
		Farm yield manure application reduces fusarium wilt distribution in a BGN field, reducing disease incidence and severity.	EA	Wakhungu et al. (2017)
		The study found that the accumulation of phosphorus in biomass, grain, and biological yields of BGN was influenced by genotype, phosphorus levels, and genotype-phosphorus interaction.	EA	Wafula et al. (2021)
Breeding	Pre-breeding	The study concluded that Zimbabwe lacks improved commercial BGN seeds, posing a significant challenge for farmers	EA	Mubaiwa et al. (2018)
		Draft genomes of 28 crops highlight production issues, germplasm conservation, seed systems, and marketing channels.	EA	Kamenya et al. (2021)
		A high diversity of African BGN landraces was revealed in this study, providing a broad gene pool for future crop improvement.	NA	Soumare et al. (2021)
	Germplasm selection	Different BGN landraces have varying levels of salinity tolerance. Leaf area and seed germination can be used to screen salinity tolerance.	EA	Ambede et al. (2012)
		BGN is a highly variable crop with high genetic diversity; by using molecular markers and phenotypic descriptors, its centre was identified as Nigeria and Cameroon	WA	Olukolu et al. (2012)

		The study found a significant positive correlation between harvest index and grain yield, indicating that yield system traits significantly influence BGN variation.	EA	Alake and Ayo-Vaughan, (2017)
		It was concluded that variability between BGN landraces in their agro-nutritional traits responds to production years.	WA	Alake, (2018)
		The BGN population showed a significant phenotypic variability in agro-morphological markers, indicating a clear correlation between agro-morphological diversity and geographical origins.	WA	Bonny et al. (2019)
		Significant genetic variability in BGN accessions was found, with 83.37% of the variability observed in agro morphological and yield traits.	WA	Yekeen et al. (2020)
		The study found significant differences among BGN genotypes, suggesting that BGN with high grain yield can be selected for mass selection and germplasm conservation.	SA	Mohammed et al. (2020)
		Increased testing diversity and agroecological zones can enhance precise conclusions since some BGN genotypes perform well in different environments, indicating varying performance from different genotypes.	WA	Olanrewaju et al. (2021)

EA; East Africa, **SA**; Southern Africa, **NA**; Northen Africa, **WA**; West Africa

2.3.4 Bambara groundnut production constraints

Several studies have reported several production constraints of the BGN (Table 2.3). The main constraints identified include long cooking time, lack of improved commercial seeds, cucumber mosaic virus, root-knot nematodes, and insects (*Collosobruchus maculutus* Fab and *Callosobruchus subinnotatus* Pic). Some of the abiotic constraints highlighted include salinity and planting date; these parameters influence the yield of BGN.

Table 2.3: Identified BGN production constraints

Constraints	**Region**	**Citation**
Long cooking time	WA	Berchie et al. (2010)
Lack of improved BGN seeds	WA	Berchie et al. (2010)
Salinity	EA	Ambede et al. (2012)
Time of sowing	WA	Berchie et al. (2012)
Insects (*Callosobruchus maculatus* Fab. and *Callosobruchus subinnotatus* Pic.)	WA	Sankara et al. (2016)
Cucumber mosaic virus	WA	Buhari et al. (2022)
Root-knot nematodes (*Meloidogyne* spp.)	WA	Ayeni et al. (2022)

WA; West Africa, **EA**; East Africa

2.4 Discussion

The study was conducted to highlight the status of BGN production, its constraints, and a proposed roadmap to commercialise this neglected BGN legume in sub-Saharan Africa (SSA). Thirty-six studies were identified, where data was extracted and synthesised following the standard reporting procedures of systematic reviews and meta-analyses (PRISMA).
Among the five African sub-regions, Southern region had the highest number of articles (47%), followed by western Africa (33%), eastern Africa (16.6%) and northern Africa (2.7%), respectively (Figure 2.2). The results display an exponential increase in publications on BGN from 2004 to 2022. This gradual exponential increase proved growing interest in this underutilised crop. Aliyu et al. (2014) mentioned that BGN has been gaining prominence in the research community in recent years due to rising evidence suggesting the effects of climate change on traditional crops. Scientists are keen on exploiting the nutrition, and drought tolerance of the crop to alleviate food and nutrition insecurity (Majola et al., 2021).

BGN is cultivated throughout Sub-Saharan Africa (SSA), and the West Africa leads in production, with annual production of 0.14 million tons from an estimated area of 0.18 million hectares (Majola et al., 2021), from countries such as Nigeria, Burkina Faso, Côte d'Ivoire, Ghana, Mali, Senegal, and Niger, respectively (Soumare et al., 2021). Zimbabwe is the largest producer of BGN in Southern Africa, contributing about 2000–3000 tons per year (Majola et al., 2021).

In general, all the included articles assessed in this study were classified in either agronomy or breeding. Publications grouped under agronomy, were further classified under three main research themes comprising water use efficiency, cultural practice, and drought. Studies under breeding mainly focused on BGN pre-breeding and germplasm selection. Five studies investigated the status of BGN in relation to water use efficiency (WUE). Mabhaudhi et al. (2013) discovered that BGN exhibits high WUE under water stress, with some evidence of the potential influence of genetic variation due to seed coat colour. In support of this claim, Chibarabada et al. (2015a) suggested that dark-coloured BGN landraces performed better under moisture stress environments than light-coloured BGN landraces, implying that dark-coloured BGN have high WUE.

Chibarabada et al. (2017) studied the nutritional water productivity of selected grain legumes including BGN and showed that BGN had the lowest macro and micronutrient content compared to other legumes. It was also found that nutritional content varied across the same BGN landrace. Climatic and edaphic factors in different environments influence the development, growth, and yield of BGN. The variability in climate, temperature, and rainfall significantly influences the production of BGN in various environments and regions across Africa. Environments with lower temperatures (~ 16 to 25

°C) and higher rainfall (> 450 mm) cause BGN to flower and mature earlier while producing poor grain yield (Alake, 2018). Bambara performs well under rain-fed conditions, however more potential is realised under rain-fed environments supplemented by irrigation (Mabhaudhi and Modi, 2013). Two experiments were conducted under rain-fed conditions in the Sudan–Sahel agro-ecological zone of Burkina Faso to investigate the effect of mounding at different BGN growing stages, and it was concluded that mounding should not coincide with the flowering stage as it impacts grain yield of BGN (Ouedraogo et al., 2013). In addition, the effect of planting dates on BGN yield revealed that more pod yield is produced by establishing the crop on irrigation just before the onset of rain fall periods.

According to Mubaiwa et al. (2018), BGN is challenged by "hard to cook" quality because some most landraces are difficult to mill and take a long time to cook. Genetic variation in cooking time exists suggesting that its potential for improvement through breeding. Cooking time has been addressed in breeding in legumes such as the common bean (*Phaseolus vulgaris*), and cowpea (*Vigna unguiculata*). The main abiotic constraints that affected BGN grain yield were unpredictable rainfall, temperature extremes (extraordinarily high and low), altitude and poor soils, as mentioned. BGN production varies depending on soil types as revealed by Chibarabada et al. (2018), that the legume produces good yield when planted in sandy soil. However, sandy soil had a negative influence on crop emergence. The production of BGN is affected by high salinity concentration which reduces emergence (Ambede et al (2012).

On the other hand, Chibarabada et al. (2015b) reported that dark-coloured seed coat had better seedling emergence in contrast to a light-coloured seed coat. Mabhaudhi et al. (2014) revealed that red BGN landraces are too tolerant to drought. Even though several environmental factors influence BGN, it remains a resilient crop compared to most traditional crops. Franke (2021) demonstrated that in a climate model, BGN may improve yield in the presence of climate change challenges.

Although Bambara is a tolerant crop, one major factor highlighted by Maphosa et al. (2022b) are the biotic stresses; our study noted that viruses, nematodes, and insects contribute to yield loss for BGN legumes. Cucumber mosaic virus, rot-not nematodes and insects significantly reduces BGN yields in West Africa. Other BGN limitations identified by the literature is hard-to-cook (HTC) traits and photoperiod, coupled with a lack of improved commercial BGN seeds. Akintayo et al. (2021) suggested the need to tackle hard-to-cook traits in BGN for energy conservation and encourage increased consumption. Biotechnology can alleviate such challenges as suggested by Soumane et al. (2022).

2.5 Limitations

The literature review was limited to studies completed exclusively in five African regions to narrow the scope to only the African continent. Studies outside Africa relating to our research problem were not examined since the setting was restricted to published work within the African continent. The research used two databases (Scopus and Web of Science) to investigate and search the literature; specific grey literature and non-published works related to the status of BGN may have been omitted when searching and screening the literature.

2.6 Conclusion

A scoping review was conducted to summarise and disseminate research findings and identify gaps in existing literature for BGN's production in specific environments. The literature has documented and explored several factors that negatively and positively influence the development and performance of BGN production. Environmental factors such as soil type, extreme high and low temperature, photoperiod, rainfall, drought, topography, salinity, landrace type, pests, diseases, and regional classification influence the production of BGN. Management and cultural practices also influence performance and BGN production; some noted factors in the literature address sowing dates, hard-to-cook, sowing density, and mounding time. The results show no evidence of released commercialised improved BGN genotype in Africa. The lack of commercialised improved seeds for BGN remains a significant constraint that limits farmers from producing this legume, affecting food production worldwide.

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Chapter 3: Agro-morphological diversity of Bambara groundnut recombinant inbred lines evaluated across three environments in the KwaZulu-Natal province of South Africa

Abstract

Bambara groundnut (*Vigna subterranea* L. Verdc.) is an underutilized native African legume renowned for its exceptional nutrient content and multiple-stress tolerance. However, like most orphaned crops, the Bambara groundnut (BGN) hardly ever benefited from breeding, and most farmers cultivate landraces. Hence, the rationale of this study is to evaluate the agro-morphological diversity of F_7 BGN recombinant inbred lines (RILs) developed from a cross between single genotype Tiga Nicuru (maternal) and DipC (paternal) lines. The experiments were conducted across three different environments in KwaZulu- Natal using 6 x 18 alpha lattice block design, replicated twice. Eleven quantitative and five qualitative traits were recorded following BAMNET criteria. Data was subjected to variance analysis, principal component analysis, Pearson's correlation coefficients, and cluster analysis to assess the diversity among the genotypes. The analysis of variance revealed highly significant differences ($p < 0.01$) amongst the RILs for most traits except for days to 50% emergence, days to 50% flowering, and plant spread. Multivariate analysis of the agro-morphological traits showed that the first three principal components (PC) with eigenvalues > 1 account for a total variability of 72% (PC1 = 39%; PC2 = 19% and PC3 = 14%). The PC biplot revealed a positive correlation between the yield and the yield-related traits (grain yield, unshelled grain yield, seed length, seed width, and shelling percentage) that were positively correlated with PC1. The two-way hierarchical cluster dendrogram categorized the 54 BGN genotypes into six morphotypes (clusters). Cluster I comprised 18.5% of the population, cluster II 3.7%, cluster III 12.9%, cluster IV 20.3%, cluster V 7.4%, and cluster VI 37%. This study proves that the BGN is highly amenable to conventional breeding, and breeders can initiate trait selection and recombination.

Keywords: Bambara groundnut; agro-morhological diversity; RILs

3.1 Introduction

Bambara groundnut (*Vigna subterranea* (L.) Verdc.), is a native model crop for future climate resistance, with the potential to sustain food systems in Africa (Karunaratne et al., 2015). Most subsistence farmers in sub-Saharan Africa (SSA) depend on Bambara groundnut (BGN) since it is a significant socioeconomic crop that plays a role in the semi-arid regions of the continent (Khaliqi et al., 2021). BGN is an essential legume in rural economies, and it provides a cheap source of all the essential nutrients in well-balanced proportions (Goudoum et al., 2016). The crop has a high protein content (16 - 21%), carbohydrate (50 - 60%) and is low in fat (4.5 - 6.5%) (Alhamdi et al., 2020). The legume is also revered for its inherent ability to grow in marginal environments that characterize low-input farming systems (Mabhaudhi et al., 2018). In addition to being fairly drought tolerant (Chibarabada et al., 2018), the groundnut can significantly improve soil fertility through the biological fixation of atmospheric nitrogen (Hasan et al., 2018).

Despite the nutritional and economic importance of BGN, production is still low (Majola et al., 2021). The crop is produced on an average area of 0.4 ha by relatively elderly farmers (Alhassan et al., 2013). One of the main reasons for the decrease of Bambara production is the absence of improved agronomic practices and high-yielding cultivars. BGN has insignificantly benefitted from any breeding programmes targeting the development of improved cultivars for sustainable production (Massawe et al., 2005).

The complex anatomy of the Bambara reproductive system has been a significant impediment to improving Bambara (Aliyu et al., 2016). The crop has minute florets (Abrol & Shankar, 2012), which are recalcitrant to emasculation and hand pollination. However, scientists at the University of Nottingham recently proved the amenability of Bambara to artificial hybridization (Chai et al., 2016). This novel discovery, coupled with the elucidation of the annotated Bambara genome, means that careful trait selection can now commence, leading to the development of high-yielding Bambara cultivars. Bambara groundnut's sustainable promotion and improvement will require developing and deploying improved cultivars adapted to the ever- changing climate conditions (Mayes et al., 2019; Mohammed et al., 2023).

Moreover, developing new cultivars requires a clear understanding of the existing diversity to inform breeding programmes and management strategies. In that regard, two Bambara pure-lines representing the available world-wide germplasm were recombined to develop bi-parental populations, advanced through single seed descent. Hence, this study is the first to evaluate the performance of true breeding BGN lines in South Africa and the region.

3.2 Materials and methods

3.2.1 Plant material

The genetic material used in this study comprised 44 BGN RILs and ten checks that included the parental lines. The RILS developed at the University of Nottingham, United Kingdom (UK), from a cross between single genotype Tiga Nicuru (maternal) and DipC (paternal) lines. The progenies were then advanced from F2 to F6 using the single seed descent method at the Ukulinga Research Farm. Most landrace checks were sourced from the Agriculture Research Council (ARC). The full description of the planting material is presented in Table 3.1.

Table 3.1: Fifty-four BGN planting materials used in the study

No	Code	Pedigree	SCC	No	Code	Pedigree	SCC
1	G1	Tiga nicuru/DipC -F7/E445	B	**28**	G28	Tiga nicuru/DipC -F7/E470	B
2	G2	Tiga nicuru/DipC -F7/E457	DB	**29**	G29	Tiga nicuru/DipC -F7/E498	DB
3	G3	Tiga nicuru/DipC -F7/E446	DB	**30**	G30	Tiga nicuru/DipC -F7/E453	P
4	G4	Tiga nicuru/DipC -F7/E461	BLK	**31**	G31	Tiga nicuru/DipC -F7/E453	B
5	G5	Tiga nicuru/DipC -F7/E460	BLK	**32**	G32	Tiga nicuru/DipC -F7/E465	B
6	G6	Tiga nicuru/DipC -F7/E473	B	**33**	G33	Tiga nicuru/DipC -F7/E481	DB
7	G7	Tiga nicuru/DipC -F7/E468	B	**34**	G34	Tiga nicuru/DipC -F7/E477	P
8	G8	Tiga nicuru/DipC -F7/E478	BLK	**35**	G35	Tiga nicuru/DipC -F7/E479	DB
9	G9	Tiga nicuru/DipC -F7/E495	B	**36**	G36	Tiga nicuru/DipC -F7/E487	P
10	G10	Tiga nicuru/DipC -F7/E490	BLK	**37**	G37	Tiga nicuru/DipC -F7/E486	BLK
11	G11	Tiga nicuru/DipC -F7/E467	DB	**38**	G38	Tiga nicuru/DipC -F7/E486	B
12	G12	Tiga nicuru/DipC -F7/E494	B	**39**	G39	Tiga nicuru/DipC -F7/E485	B
13	G13	Tiga nicuru/DipC -F7/E502	BLK	**40**	G40	Tiga nicuru/DipC -F7/E491	BLK
14	G14	Tiga nicuru/DipC -F7/E469	B	**41**	G41	Tiga nicuru/DipC -F7/E480	B
15	G15	Tiga nicuru/DipC -F7/E483	B	**42**	G42	Tiga nicuru/DipC -F7/E482	P
16	G16	Tiga nicuru/DipC -F7/E471	RED	**43**	G43	Tiga nicuru/DipC -F7/E472	B
17	G17	Tiga nicuru/DipC -F7/E493	DB	**44**	G44	Tiga nicuru/DipC -F7/E492	B
18	G18	Tiga nicuru/DipC -F7/E503	B	**45**	P1	IITA 686	BLK
19	G19	Tiga nicuru/DipC -F7/E489	P	**46**	P2	TIGD	CRM
20	G20	Tiga nicuru/DipC -F7/E458	B	**47**	P3	DIP-C	CRM
21	G21	Tiga nicuru/DipC -F7/E448	B	**48**	P4	LUNT	BLK
22	G22	Tiga nicuru/DipC -F7/E447	P	**49**	P5	ANKPA4	B
23	G23	Tiga nicuru/DipC -F7/E463	BLK	**50**	P6	S19	DB
24	G24	Tiga nicuru/DipC -F7/E474	BLK	**51**	LL1	Songkhla	RED
25	G25	Tiga nicuru/DipC -F7/E449	B	**52**	LL2	100SB16ANAMC	B
26	G26	Tiga nicuru/DipC -F7/E484	P	**53**	LL3	EXSOCOTO	CRM
27	G27	Tiga nicuru/DipC -F7/E500	BLK	**54**	LL4	UKZN-L1	CRM

SCC: Seed coat colour; **B**: Brown; **BLK**; Black; **CRM**: cream; **DB**: Dark brown; **P**: Purple; **RED**: RED

3.2.2 Study sites

The study was conducted during the summer cropping season of 2021 and 2022 in South Africa, KwaZulu-Natal province in Pietermaritzburg, at two research stations, using three environments treated as seasons. The full description of the study sites is presented in Table 3.2. Two successive experiments were conducted at Ukulinga Research Farm (29°37'S; 30°16'E; 750 m a.s.l) from 2021 and 2022. The experiment at Cedara College of Agriculture (29.53° S, 30.27° E; 1 104 m a.s.l) was only conducted in one season. Figure 3.1 shows the two sites used in KZN.

Table 3.2: Environmental characteristics of the three environments used to evaluate the 54 BGN genotypes during 2021/2022 cropping seasons

Code	**Latitude**	**Longitude**	**Altitude**	**Av Temp**	**RH**	**Rainfall**	**Planting date**
			m.a.s.l	**(°C)**	**(%)**	**(mm)**	
UKRF (ENV1)	29.6377051	30.3948151	705	25.9	53	37.4	02/12/2021
CRDA (EVN2)	29.5378197	30.2766917	1104	25.6	55	36.1	13/12/2021
UKRF (ENV3)	29.6377051	30.3948151	705	24.9	49.42	21.81	20/01/2022

UKRF: Ukulinga Research Farm; **ENV**: Environment; **CRDA**: Cedara College of Agriculture; **m.a.s.l**: Meter above sea level; **(°C)**: Degree Celsius; **(%)**: Percentage; **(mm)**: millimetre; **Av Temp**: average temperature; **RH**: Relative humidity.

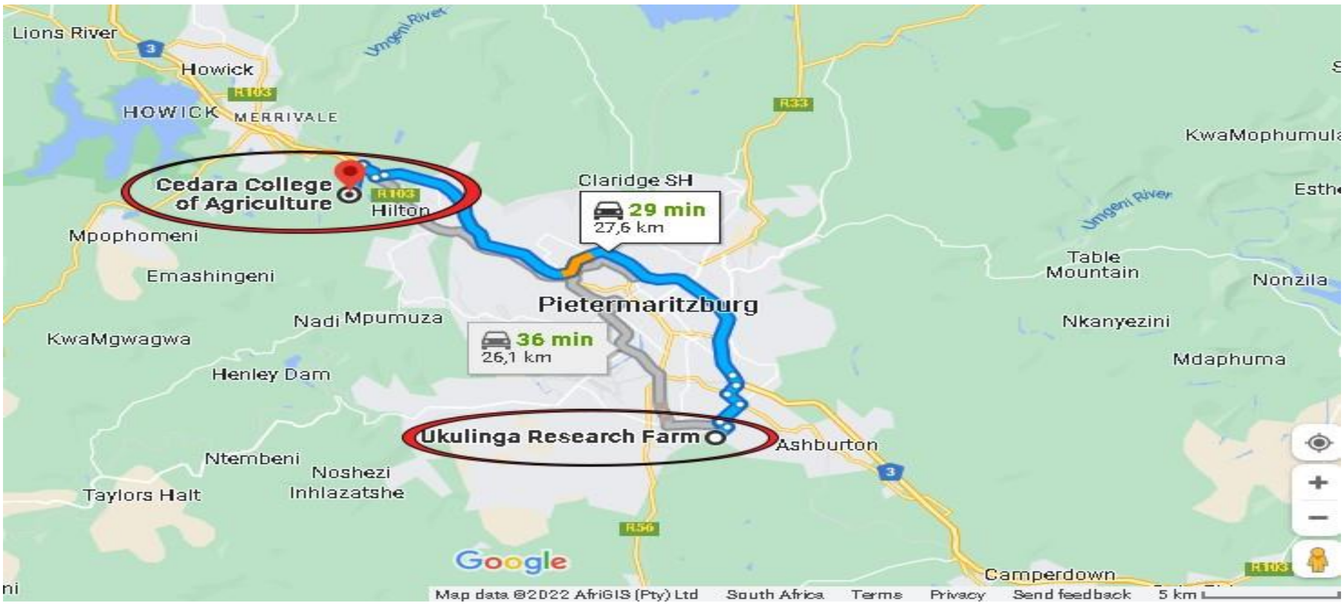

Figure 3.1:Two experimental sites used (URF and CRDA) to evaluate the 54 BGN in KwaZulu-Natal

3.2.3 Experimental design and field management

An alpha lattice block design with two replicates was used to evaluate the 54 BGN genotypes comprising 44 F_7 recombinant inbred lines and ten landrace checks. The plot area consisted of a single 1.5 m row, with intra-row spacing of 0.3 m and 0.9 m from plot to plot. The field was mechanically ploughed by a tractor. The hand hoes were used to prepare the seedbed and making of the ridges. All the seeds were sown on the top of the ridges. Mounding or earthing up was carried out before flowering, following the research findings by Ouedraogo et al. (2013). Mounding was recommended before the flowering period since mounding during flowering results in low grain yield; hence, mounding was carried out before flowering. Using manufacturer recommendations, a pre-emergence paraquat SL and springbok soluble concentrate herbicide was used to control the weeds. A gramoxone post-emergence herbicide was applied to control aggressive weeds and was supplemented with hand hoe weeding as an integrated pest management (IPM) strategy. Insects were controlled by karate Zeon insecticide, using 100g/l as a recommended measurement, and 45g per 16 litre coproxy with active ingredients of koperoskiscloried and copper oxychloride was used to control the diseases.

3.2.4 Data collection

The quantitative and qualitative data were collected using BGN classification and descriptors from the International Plant Genetic Resources Institute (IPGRI), International Institute of Tropical Agriculture (IITA), and BAMNET (IPGRI). Eleven quantitative and five qualitative traits were recorded following the BAMNET guidelines. The full description of all the traits is presented in Table 3.3.

Table 3.3: Qualitative and quantitative traits collected from the 54 BGN genotypes

Traits collected	Scale	Collection time and how traits were collected/measured
Growth habit (GH)	Bunch; Semi-Bunch; Spreading	Followed BAMNET guide refer (IPGRI)
Terminal leaflet shape (TLS)	Round Oval; Lanceolate Elliptic	Followed BAMNET guide refer (IPGRI)
Terminal leaflet colour (TLC)	Green; Red; Purple	Followed BAMNET guide refer (IPGRI)
Stem pigment colour (SPC)	Green; Purple; Reddish	
Seed coat colour (SCC)	Cream; Grey; Light red Dark red, Light Brown, etc.	Followed BAMNET guide refer (IPGRI)
Germination percentage (GP)	%	50% of the seedlings that emerged after sowing per plot were counted and recorded daily.
Days to 50% emergence (DTE)	Days	This trait was recorded as the number of days from the date of sowing to when 50% of plant emergence.
Days to 50% flowering (DTF)	Days	This trait was recorded as the number of days from 50% seedling emergence to 50% flowering plants.
Plant Height (PH)	Cm	Recorded ten weeks after planting, measured from the ground level to the tip of the highest point using a ruler.
Plant spread (PS)	Cm	Recorded ten weeks after planting. Using a measuring ruler, the average of 3 widest lengths between opposite points of plants.
Shelling percentage (SHLP)	%	The SHLP was calculated using the formula: SHLP = UGY/GY x 100
Unshelled grain yield (UGY)	kg/ha	Measurements were done using a weighing balance and then calculated from grams per plot to kg/ha, converted from unshelled grain yield per plot to kg/ha.
Grain yield (GY)	kg/ha	A weighing balanced was used to measure grain yield in grams per plot, then converted to kg per hectare.
Grain yield per plant (GYPP)	G	A weighing balanced was used to measure grain yield in grams per plant.
Seed width (SW)	mm	The seed width of 10 randomly sampled shelled seeds per plant was measured using a digital vernier calliper.
Seed length (SL)	mm	The seed length of 10 randomly sampled shelled seeds per plant was measured using a digital vernier calliper.

3.2.5 Statistical analysis

All data was subjected to analysis of variance using an unbalanced regression model in GenStat software (version 20.1) (Payne et al., 2011). Principal component analyses (PCA) and cluster analysis were performed to determine the interrelationship among all traits using the JMP pro 14th version (Sall et al., 2017). Pearson's correlation coefficients were also computed to determine the association among eleven qualitative traits and to observe the relationship among 11 quantitative traits with respect to grain yield.

3.3 Results

3.3.1 Analysis of variance

The analysis of variance (ANOVA) results for eleven agro-morphological quantitative traits revealed highly significant differences ($P < 0.01$) amongst the RILs genotypes for all quantitative traits except for days to 50% emergence (DTE), days to 50% flowering (DTF), and plant spread (PS) (Table 3.4). The effects of the environments showed highly significant differences ($P < 0.001$) for all studied traits. The interaction among the genotype × environment was highly significant ($P < 0.001$) for seed length (SL), seed width (SW), unshelled grain yield (UGY), grain yield (GY), grain yield per plant (GYPP), and shelling percentage (SHLP).

Table 3.4: Combined ANOVA for eleven quantitative BGN traits

SOV	ENV	REP (ENV)	BLOCK(REP)	GEN	GEN x ENV	ERROR
D.F	2	3	6	53	106	
DTE (Days)	2035.56***	9.04	35.17***	11.14	9.79	7.89
GP (%)	30238.30***	169.10	4381.50***	899.10**	493.30	574.20
DTF (Days)	5709.40***	49.53	99.39***	25.41	30.94	23.48
PH (cm)	1579.68***	15.18	27.48**	14.12**	8.79	7.71
PS (cm)	28526.08***	89.58	284.67***	80.86	66.26	66.24
GYPP (g)	5432.07***	330.27***	448.10***	173.92***	97.65***	32.81
UGY (Kg/ha)	9480368***	1425226***	838264***	242450***	102587**	68666
SL (mm)	51.43***	2.52	8.98***	3.64***	2.21***	1.14
SW (mm)	27.69***	0.49	2.93***	1.977***	1.47***	0.67
SHLP (%)	419.66**	60.29	79.29	225.31***	125.15***	71.72
GY (Kg/ha)	3229552***	485882***	323474***	87651***	37603**	22255

SOV: source of variation, **D.F:** degree of freedom, **ENV:** environment, **REP:** replication, **GEN:** genotype, **GP:** germination percentage, **DTE:** days to 50% emergence, **DTF:** days to 50% flowering, **PH:** plant height, **PS:** plant spread, **SL:** seed length, **SW:** seed width, **UGY:** unshelled grain yield, **GY:** grain yield, **GYPP:** grain yield per plant, **SHLP:** shelling percentage, ***** :P <0.05; ****** : P < 0.01; ******* : P < 0.001

3.3.2 Combined mean performance of eleven quantitative traits among 54 BGN genotypes

The combined mean performance of phenological, vegetative and yield traits of the 54 BGN genotypes is presented in Table 3.5. The days to 50% emergence (DTE) ranged from 10.95 (G20) to 16.75 (G17), with a mean of 14 days. Germination percentage (GP) ranged from 13.33% to 76.67%, with a mean of 46.45%. The genotypes that recorded the highest GP was G18 and G30, with a value of 76.67%, and the lowest GP was G3, which recorded 13.33%—the days to 50% flowering (DTF) had a mean of 60.59 days. The genotype G18 was the earliest flowering genotype, with a value of 55.88 days, while the G41 recorded the latest flowering genotype, with a value of 66.35. Plant height (PH) ranged from 16.12 cm for the genotype LL3 to 23.15 cm for the genotype G3, with an overall mean performance of 20.47 cm. Plant spread (PS) ranged from 28.76 cm for the genotype LL3 to 44.33 cm for the genotype G30, with a mean performance of 37.08 cm. Grain yield per plant (GYPP) ranged from 3.99 g (G4) to 30.87 g (G23), with a mean performance of 10.94 g. unshelled grain yield (UGY) ranged from 68.7 to 1329.5 kg/ha, with a mean of 372.37 kg/ha. The trait seed length (SL) recorded a range of 8.26 to 12.96 mm, with a mean performance of 11.22 mm. Seed width (SW) ranged from 6.44 mm (P6) to 9.31 mm (LL4), with a mean performance of 7.91 mm. Shelling percentage (SHLP) ranged from 41.60% (G4) to 69.04% (G25), with a mean performance of 56.58%. Grain yield (GY) ranged from 29.3 to 1276.6 kg/ha, with a mean performance of 219.7 kg/ha.

Table 3.5: Combined mean performance for the top 10 and bottom 5 BGN representing the 54 BGN ranked according to grain yield performance

GEN	DTE	GP	DTF	PH	PS	GYPP	UGY	SL	SW	SHLP	GY	Rank
	(Days)	(%)	(Days)	(cm)	(cm)	(g)	(Kg/ha)	(mm)	(mm)	(%)	(Kg/ha)	
G23	12.28	46.67	60.43	20.81	40.21	30.87	866.8	11.95	8.68	65.30	772.03	1
P3	14.31	39.92	60.10	19.05	32.37	22.72	1329.5	10.98	7.66	57.82	544.08	2
G40	14.49	43.33	63.02	22.29	39.89	21.65	532.7	12.96	9.31	58.59	437.00	3
LL2	13.89	56.67	60.01	19.51	40.06	20.73	625.6	11.76	8.68	62.52	386.00	4
P4	12.79	50.00	58.83	20.53	39.92	20.56	538.2	11.65	8.13	57.59	348.04	5
G8	15.10	40.00	61.88	19.81	41.26	16.91	568.6	12.14	8.74	61.80	346.01	6
LL4	15.13	46.67	62.19	19.39	43.95	14.98	602.1	12.04	8.71	58.22	330.06	7
G44	11.97	43.33	59.87	22.74	33.61	21.65	347.1	11.64	8.35	62.94	329.07	8
G43	16.32	36.67	60.01	19.83	33.61	13.96	346.6	11.55	7.78	60.23	327.01	9
G35	11.81	66.67	58.95	20.75	40.67	13.56	485.1	11.26	7.68	56.58	323.00	10
G15	14.80	33.33	60.44	18.94	34.83	5.00	98.4	10.91	8.05	52.26	54.00	50
G3	16.48	13.33	66.25	19.65	34.51	6.00	103.7	10.06	6.44	42.86	44.04	51
G2	14.37	33.33	62.89	19.00	31.05	5.66	76.2	10.79	7.20	55.03	41.08	52
P5	12.10	26.67	60.35	17.45	31.95	5.34	89.1	9.09	6.61	47.22	39.06	53
G4	15.53	43.33	58.86	21.13	34.52	3.99	68.7	9.70	6.55	41.60	29.03	54
Mean	**14.07**	**46.45**	**60.59**	**20.47**	**37.01**	**10.94**	**372.37**	**11.22**	**7.91**	**56.58**	**214.91**	-
%CV	**20.27**	**52.70**	**8.19**	**13.99**	**23.09**	**56.08**	**78.11**	**9.49**	**10.24**	**14.72**	**78.00**	-
SE	**2.85**	**24.56**	**4.96**	**2.87**	**8.58**	**6.28**	**299.08**	**1.07**	**0.814**	**8.35**	**173.00**	-
Min	**10.95**	**13.33**	**55.88**	**16.12**	**28.48**	**-8.54**	**68.7**	**8.26**	**6.44**	**40.53**	**29.03**	-
Max	**16.57**	**76.67**	**66.35**	**23.15**	**44.33**	**30.87**	**1329.5**	**12.96**	**9.31**	**69.04**	**772.03**	-

GEN: genotype, **CV**: coefficient of variance, **SE**: standard error, **GP**: germination percentage, **DTE**: days to 50% emergence, **DTF**: days to 50% flowering, **PH**: plant height, **PS**: plant spread, **SL**: seed length, **SW**: seed width, **UGY**: unshelled grain yield, **GY**: grain yield, **GYPP**: grain yield per plant, **SHLP**: shelling percentage, **Min**: minimum, **max**: maximum.

3.3.3 *Characterization of qualitative traits*

The results of the five qualitative traits collected from 54 Bambara groundnut genotypes (BGN) are displayed in Figure 3.1. Among the 54 BGN genotypes, three growth habits were identified: bunch type (51.4%), spreading type (45.5%) and semi-bunch type (3.0%), respectively. In terms of terminal leaflet colour (TLC), green (87.0%) was the most identified, followed by purple (11.9%) and red (0.07%). The terminal leaflet shape (TLS) varied from oval (39.9%), lanceolate (37.3%), and elliptic (21.3%) to round (1.4%). The stem pigment colour was dominated by purple (45%), followed by green (39.6%) and reddish (14.58%). The seed coat colour among the genotypes showed significant variability, with six distinct colours identified: brown (35%), black (28%), dark brown (14.8%), purple (13%), cream (5.5%), and red (3.7%).

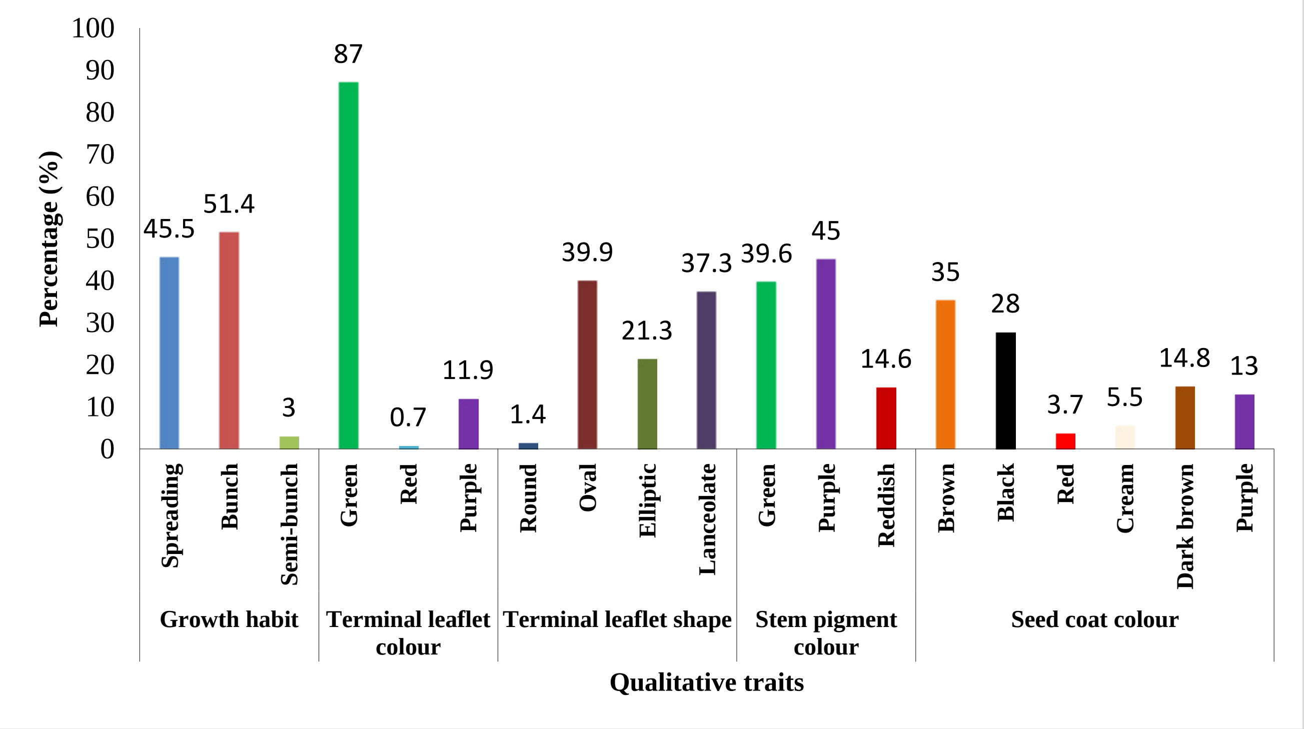

Figure 3.1: Percentage distribution of the phenotypic scale on 54 BGN qualitative traits

3.3.4 *Principal component analysis (PCA)*

The first three PCs accounted for a total variability of 72% (Table 3.6). The first principal component (PC) accounted for approximately 39% of the total variability. The first PC was contributed mainly by grain yield (GY), unshelled grain yield (UGY), grain yield per plant (GYPP), seed width (SW), and seed length (SL), while the Second PC accounted for 19.3% of the total variability. Traits with the highest loading in PC 2 were days to 50% emergence (DTE) and days to 50% flowering (DTF). PC3 accounted for 14% of the total variation.

Table 3.6: Eigenvectors and eigenvalues associated with each character with respect to 11 agro-morphological traits

Variables	**PC1**	**PC2**	**PC3**
DTE	-0.19	**0.43**	-0.22
GP	0.21	-0.53	0.13
DTF	-0.15	**0.45**	-0.31
PH	0.10	-0.34	-0.47
PS	0.25	-0.25	-0.41
GYPP	**0.39**	0.18	0.13
UGY	**0.40**	0.17	0.30
SL	**0.31**	0.08	-0.48
SW	**0.39**	0.17	-0.12
SHLP	0.29	0.16	-0.16
GY	**0.42**	0.19	0.27
Proportion of Variance (%)	39	19	14
Cumulative Proportion (%)	39	58	72
Eigenvalues	4.3	2.1	1.5

GP: germination percentage, **DTE:** days to 50% emergence, **DTF:** days to 50% flowering, **PH:** plant height, **PS:** plant spread, **SL:** seed length, **SW:** seed width, **UGY:** unshelled grain yield, **GY:** grain yield, **GYPP:** grain yield per plant, **SHLP:** shelling percentage.

3.3.5 Interrelationship amongst agro-morphological traits

The PCA biplot in Figure 3.3 shows the vector relationship among the studied traits. The first cluster in quadrant one is comprised of the traits: grain yield (GY), grain yield per plant (GYPP), unshelled grain yield (UGY), shelling percentage (SHLP), seed length (SL) and seed width (SW). The second cluster in quadrant two comprised the traits: days to 50% emergence (DTE) and days to 50% flowering (DTF). Lastly, the third cluster in quadrant four comprised the traits plant spread (PS), plant height (PH) and germination percentage (GP).

In quadrant 1, the traits GY, UGY, GYPP, SL, SW, and SHLP showed a highly positive correlation, as shown by the acute angles between their vectors. In quadrant 2, two traits, DTE and DTF, show a positive correlation, indicated by an acute angle between two vectors. In quadrant 4, PH, GP and PS are positively correlated to each other, proven by an acute angle between the vectors. Traits in the same quadrant are positively correlated. However, traits in different quadrants that display an acute angle are positively correlated; in this case, seed length is correlated to plant spread (PS).

Negative correlations were observed between the traits grain yield and (i) DTE and (ii) DTF, as depicted by an obtuse angle between vectors. There was a low association between yield traits (GY and UGY) with germination percentage (GP) and plant height (PH), as shown by the angle between vectors (Figure 3.3).

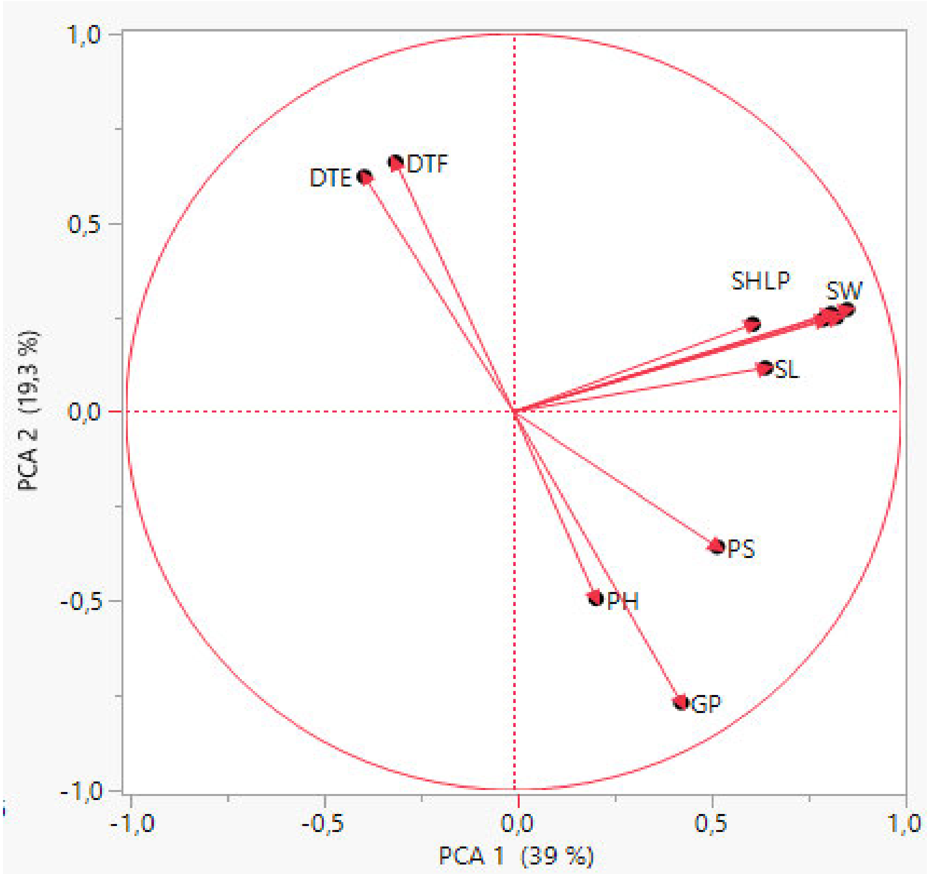

Figure 3.3: PCA biplot displaying 11 quantitative traits for 54 BGN and their loading vectors in four quadrants

3.3.6 Genotype agro-morphological trait association

The PCA biplot in Figure 3.4 shows the relationship between the quantitative traits and the performance of the 54 BGN genotypes. Genotypes in the same quadrant are related and perform more like the same; the genotypes P3, G23, G1, G8, LL4, G40, LL2 and G13 were associated with yield traits in quadrant 1; The genotypes G30, G18, G20, G22, G35 and G34 were associated with vegetative traits in quadrant 4. Clustered genotypes show similar performance across environments and are morphologically related (Figure 3.4).

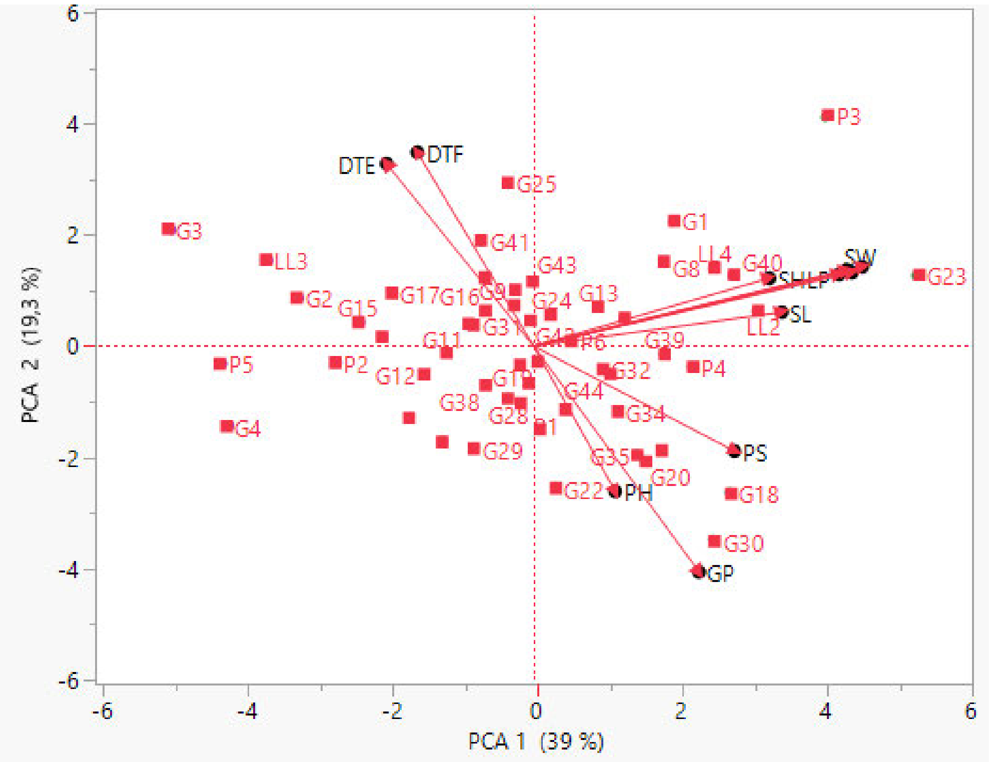

Figure 3.4: Biplot analysis shows the association between the 54 BGN genotypes and 11 quantitative traits

3.3.7 Correlational matrix

A person's correlation based on the means of the 54 BGN genotypes was generated to determine the relationship among eleven quantitative traits. The correlation coefficients for the traits studied are presented in Table 3.7. Among the studied traits there were a significant positive correlation between grain yield and grain yield per plant ($r = + 0.79$; $P < 0.01$), grain yield and unshelled grain yield ($r = + 0.99$; $P < 0.01$), grain yield and seed length ($r = + 0.37$; $P < 0.01$), grain yield and seed width ($r = + 0.64$; $P < 0.01$) and lastly grain yield and shelling percentage ($r = + 0.44$; $P < 0.01$). The results showed no negative significant correlation between grain yield and the other ten traits; however, a low negative correlation between grain yield and days to 50% emergence ($r = - 0.19$); grain yield and days to 50% flowering ($r = - 0.2$) and grain yield with plant height ($r = - 0.04$) was observed.

Table 3.7: Correlation analysis of 11 quantitative traits for the 54 BGN genotypes.

	DTE	**GP**	**DTF**	**PH**	**PS**	**GYPP**	**UGY**	**SL**	**SW**	**SHLP**	**GY**
DTE	1	- 0.63**	0.41**	- 0.184	- 0.22	- 0.21	- 0.19	- 0.04	- 0.09	- 0.184	- 0.19
GP	-	1	- 0.62**	0.31*	0.38**	0.16	0.19	0.11	0.18	0.117	0.18
DTF	-	-	1	- 0.146	- 0.21	- 0.08	- 0.22	0.02	- 0.08	0.071	- 0.2
PH	-	-	-	1	0.43**	0.04	- 0.06	0.32*	0.07	0.037	- 0.04
PS	-	-	-	-	1	0.26	0.2	**0.51****	0.35**	0.231	0.22
GYPP	-	-	-	-	-	1	**0.76****	0.40**	0.59**	1.51**	**0.79****
UGY	-	-	-	-	-	-	1	0.341*	**0.63****	0.35**	**0.99****
SL	-	-	-	-	-	-	-	1	**0.64****	0.49**	0.37**
SW	-	-	-	-	-	-	-	-	1	**0.50****	**0.64****
SHLP	-	-	-	-	-	-	-	-	-	1	0.44**
GY	-	-	-	-	-	-	-	-	-	-	1

** Correlation is significant at the 0.01 level, * Correlation is significant at the 0.05 level, **GP:** germination percentage, **DTE:** days to 50% emergence, **DTF:** days to 50% flowering, **PH:** plant height, **PS:** plant spread, **SL:** seed length, **SW:** seed width, **UGY:** unshelled grain yield, **GY:** grain yield, **GYPP:** grain yield per plant, **SHLP:** shelling percentage.

3.3.8 Cluster analysis among the 54 Bambara groundnut genotypes

Cluster analysis was performed to observe the similarities among the genotypes (Figure 3.5). The genotypes were categorized into six clusters. Cluster I comprised ten genotypes (18.5%), Cluster II comprised two genotypes (3.7%), Cluster III comprised seven genotypes (12.9%), Cluster IV comprised 11 genotypes (20.3%), Cluster V comprised four genotypes (7.4%), and the last cluster VI comprised 20 genotypes (37%). The quantitative traits were clustered into three clusters: A (DTE and DTF), B (GY, UGY, GYPP, SL, SW, SHLP), and C (GP, PS, PH).

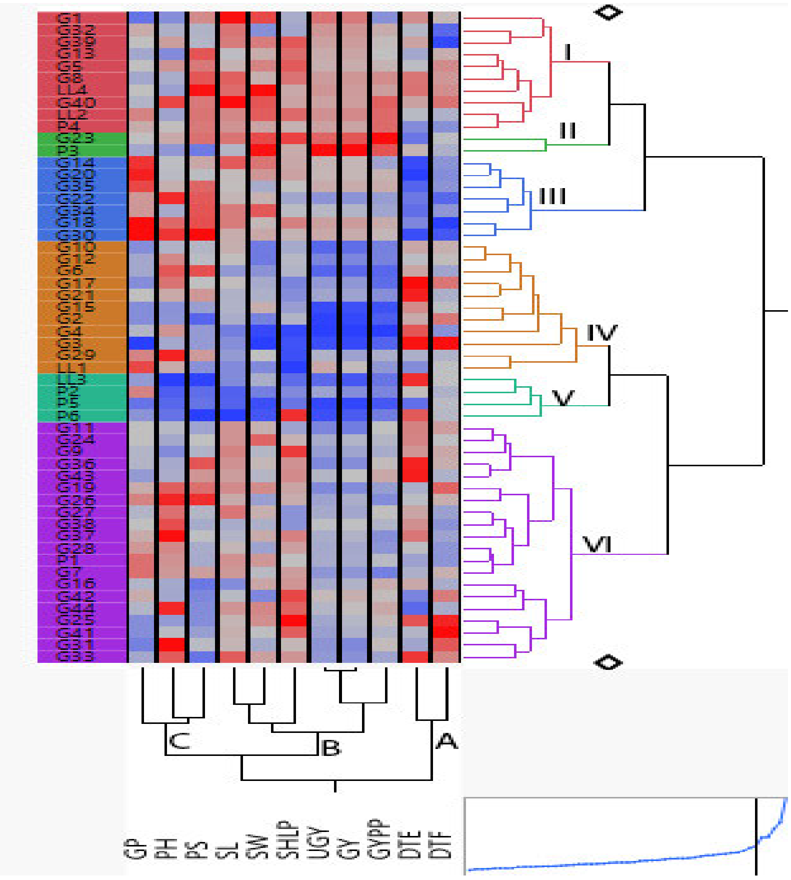

Figure 3.5: Cluster analysis revealed as dendrogram for 54 BGN genotypes generated using the Ward method on 11 quantitative traits.

BGN genotypes were clustered based on the performance of their traits and the association among the traits across different environments. There was a pattern which revealed some similarities among the genotypes. Figure 3.6 illustrates that the genotypes in cluster II outperformed genotypes in other clusters for the traits seed width (SW), unshelled grain yield (UGY), grain yield per plant (GYPP), shelling percentage (SHLP), and grain yield (GY). The genotypes in clusters I and II were high-yielding BGN candidates with outstanding performance in terms of yield and yield-related traits. However, the genotypes in cluster III were the top-performing genotypes for the traits germination percentage (GP), plant height (PH) and plant spread (PS). In cluster III, the early emerging genotypes reached flowering earlier than other BGN genotypes in other clusters. Genotypes in clusters IV and V were the least performing genotypes in terms of yield and yield-related traits and displayed low GP. Genotypes with inferior performance for the traits PH, PS and seed length (SL) were observed in cluster V (Figure 3.6).

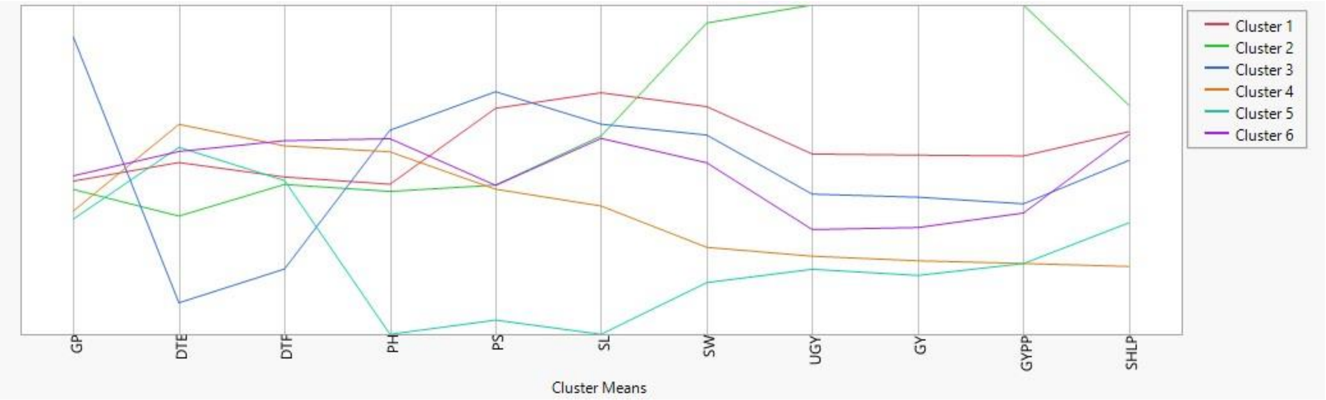

Figure 3.6: Quantitative traits mean performance generated from the six-cluster standard deviation of the 54 BGN genotypes (**GP**: germination percentage; **DTE**: days to 50% emergence; **DTF**: days to 50% flowering, **PH**: plant height; **PS**: plant spread; **SL**: seed length)

3.4 Discussion

In the present study, the evaluated agro-morphological traits revealed highly significant differences among the genotypes (Table 3.4). There was a highly significant difference (P < 0.01) among the IRLs genotypes except for the trait DTE, DTF and Plant spread, and this might be indicating breeding stability for these traits as they show no significant differences across evaluated environments. Yield and yield-related traits varied significantly (p < 0.01), and similar findings were reported by Ntundu et al. (2006), Unigwe et al. (2016), and Khan et al. (2021c). The environments were significantly different, as ANOVA indicated for all traits; this implies a significant variation among the environments, which is justified by the variation among the yield and yield-related traits. A study that evaluated the effects of G × E interaction on yield stability for 30 BGN genotypes in four Malaysian environments reported highly significant variations for the genotypes, locations (environment) and G x E interaction (Khan et al., 2021b). This suggests the need for selection for specific adaptation and classification of BGN growing environments.

Germination percentage (GP) ranged from 13.33% to 76.67%, with a mean of 46.61%, which was slightly lower than the findings reported by Hlanga et al. (2022), who reported a germination percentage that ranged from 16.3% to 100%, with a mean of 85.4%. The variation in GP might be that our study sites were established under rainfed conditions; this might influence GP since germination in our study depended on rainfall for moisture. However, the study by Hlanga et al. (200) was being irrigated, and it did not depend on rainfall for moisture. Plant spread, plant height, and seed length mean performance reported in this study were slightly higher than what is reported by Ntundu et al. (2006); these might infer that these traits were slightly influenced by the environment, meaning breeding outcome for these traits were success since they are not even significant different when looking at the G x E interaction. Comparable results on days to 50% flowering were reported by Unigwe et al. (2016), and Ntundu et al. (2006). The results on grain yield and associated component traits were comparable to reports by Valombola et al. (2019) and Bonny et al. (2019), showing similar output and interaction among these traits.

Three growth habits (GH) were identified in this study among the 54 BGN genotypes (Figure 3.2). Bunchy and spreading were the top frequent GH types, while the semi-bunchy type was the lowest recorded GH type among the genotypes evaluated. In a study by Ntundu et al. (2006) and Khan et al. (2013), semi-bunch and spreading types were the top dominating GH types; it was suggested that farmers may have selected against spreading GH as a result of not wanting to lose yield on the ground since spreading BGN spread wide making it difficult to harvest everything (Ntundu et al., 2006). In our study, this is not in line with our findings because the previous studies were conducted using landraces, and they are highly heterogenous, while our study used inbreed recombinant lines descending from bi-parental lines, and selection and breeding by breeders was not based on the type of GH. Breeders might have selected parental lines that will give them a wide range of variation to have a huge pool to select desirable BGN for future improvement.

The terminal leaflet colour identified in this study was in line with the findings reported by Ntundu et al. (2006). However, a low percentage of reddish terminal leaflet colour was observed among the IRLs in the present study. Khan et al. (2021c) only identified green and purple leaflet colours. Likewise, a similar high percentage of green and purple leaflet colours was observed in the present study (Figure 3.1). The leaflet shape identified in a study conducted by Alhamdi et al. (2020), comprised three leaflet shapes: oval, lanceolate, and elliptic. These leaflet shapes were associated with the seed coat and leaflet colour; these results showed similarities with our findings. Our findings identified four leaflet shapes: round, lanceolate, oval, and elliptic (Figure 3.1). However, the round shape was the least identified leaflet shape, while the dominating leaflet shape was lanceolate and oval. These findings are similar to the results reported by Ntundu et al. (2006) and Khan et al. (2021c). Mohammed et al. (2018), reported that among the accessions, there was a high variability in seed coat colour. This supports the findings in the present study, where six distinct seed coat colours were identified. Brown and black were the two dominating seed coat colours for our germplasm seed coat colour.

In the present study, the results for PCA revealed a total variability of 72% from evaluated traits (Table 3.6). Ntundu et al. (2006) reported the first four PCs with eigenvalues > 1, accounting for 63% and 65% of the total variability. In contrast, Hlanga et al. (2022) evaluated nineteen BGN lines in two research stations for agro-morphological traits and reported 52.31% variability from the first two PCAs. Our findings showed 72% for the first three PCAs with an eigenvalue > 1. These might suggest that good-yielding IRLs might be selected based on the traits with the highest PC loadings since they show high variability, allowing a vast pool of different IRL genotypes.

The principal components biplot (Figure 3.4) have shown the association among eleven traits and the performance of the 54 BGN genotypes. Some genotypes that have shown a considerable association with yield traits were G40, LL2, G23, G8 and G1, while the genotypes associated with plant spread and germination percentage were G18 and G30. Plant heights were seen associating with the genotypes G20, G35 and G22. This implies a vast pool of genetic variability among the genotypes.

The correlation results from the present study presented in Table 3.7 have revealed that grain yield is highly significant and has a strong positive relationship with the traits unshelled grain yield (r = 0.99), grain yield per plant (r = 0.79), seed width (r = 0.64), and seed length. Plant spread also had a positive association with grain yield, while plant height had a negative association with grain yield. Jonah et al. (2010), suggested that seed and pod yield per plant can be targeted when selecting for yield improvement in BGN because the two traits correlate positively with grain yield. Genetically correlating with this suggests that these traits can be targeted and utilized for direct and indirect selection to improve BGN grain yield. In a study by Johan et al. (2014), it is said that a positive significant correlation of seed yield with the traits pod yield per plant, seed yield per plant, pod number per plant, pod width and length, seed width and length can probably improve BGN. Targeting the traits that our study got a positive correlation with grain yield will improve the yield of BGN for future breeding programs.

3.5 Conclusion

This study evaluated the agro-morphological diversity of F7 BGN RILs across three environments in KwaZulu Natal. The study revealed a significant agro-morphological diversity among the 44 BGN RILs evaluated. Multivariate analysis of agro-morphological traits revealed that the first three principal components (PC) with eigenvalues > 1 accounted for a total variability of 72%, where PC1 accounted for 39%, PC2 = 19%, and PC3 = 14%, respectively. There was a positive correlation between yield-related components and the trait GY. The following BGN genotypes, P3, G23, G1, G8, LL4, G40, LL2, and G13 in quadrant 1, positively associated with GY. The hierarchical cluster dendrogram categorized the 54 BGN genotypes into six clusters. Among the six clusters, clusters 1 and 2 comprise high-yielding genotypes that can be explored for further breeding programs.

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Chapter 4: Selecting the best performing improved seventh-generation Bambara groundnut (*Vigna subterranea* L.) genotypes.

Abstract

The field experiments were conducted in two experimental sites, where 54 Bambara groundnut (BGN) RILs were evaluated in three environments during the 2021/2022 cropping season. The study aimed to determine the genetic variance components among BGN RILs to guide future selection. The experiment was laid as 6 by 18 alpha lattice block design with two replications. Eleven quantitative traits were collected following BGN classification and descriptors guidelines by The International Institute of Tropical Agriculture (IITA) and BAMNET. A combined analysis of variance (ANOVA) and restricted maximum likelihood (REML) procedure was executed to estimate the variance components. The combined ANOVA results showed a highly significant difference ($P < 0.01$) among 54 BGN genotypes for most quantitative traits except for days to 50% emergence, days to 50% flowering, and plant spread. The broad sense heritability (H^2) ranged from low (0%) to high (65%). The trait germination percentage (GP) (65%) recorded the highest H^2, followed by plant height (PH) (61.44%), with genetic advance (GA) recording 59.5% on grain yield (GY) and 57.6% for unshelled grain yield (UGY), respectively. For eleven quantitative traits, GY recorded the highest phenotypic coefficient of variance (PCV), 69.4%, and genotypic coefficient of variance (GCV), 44.8%. The lowest PCV and GCV values were recorded on the traits' GP (7.03%) and days to 50% flowering (0%), respectively. Based on the person’s correlation matrix, GY (kg/ha) revealed a highly significant positive association with the traits (i) unshelled grain yield ($r = 0.99$); (ii) seed width ($r = 0.61$); (iii) grain yield per plant ($r = 0.58$), and shelling percentage ($r = 0.40$). The genotypes P3, G23, G1, G8, LL2, and G18 were the top six performing across three different environments, ranked based on BLUP estimated values correlated with GY trait. Before being submitted for commercial registration, these genotypes should be recommended for further comprehensive evaluation.

Keywords: BGN; BLUP; genetic advance; heritability; variance components

4.1 Introduction

Increasing global demand for agricultural products puts massive pressure on water resources (Bonetti et al., 2022). The world's population of over seven billion is expected to reach nine billion by 2050. It is highly suggested that global crop production needs to increase two times the current production rate to meet the global food demand (Cheng et al., 2017). Furthermore, global warming significantly threatens food security since it results cause drought, desertification, salinization, floods, and a rise in temperature (Aliyu et al., 2015). Existing literature suggests combining staple and underutilized crops to support the agricultural system (Mabhaudhi et al., 2022). Most underutilised crops have the potential and are recognized as harsh tolerant crops that withstand drought stress. Minor and underutilised crops have been highly praised as potential future (food security) crops (Mabhaudhi & Modi, 2013; Aliyu et al., 2015).

Bambara groundnut (BGN) (*Vigna subterranea*) is a hardy crop that is exceptionally drought-resistant and can be grown wherever climate conditions suit drought-tolerant crops such as sorghum (Barimalaa et al., 2005). Multiple drought-tolerance mechanisms (drought escape and avoidance) have been shown to contribute to high water use efficiency (WUE) in BGN (Chibarabada et al., 2015). The protein-rich BGN crop is adapted to harsh environments and contributes significantly to the local diet, culture, and economy. BGN is well-known for its high nutritional content, functional properties, antioxidant capacity, and drought tolerance (Adetunji et al., 2015). Through a nodulation process, *Vigna subterranean* can fix atmospheric nitrogen in symbiosis with Brady rhizobium strains (Adu-Dapaah & Sangwan, 2004).

The production of BGN decreases with a decrease in water supply; as stated by Collinson et al. (1996), the pod yield of BGN decreases as the amount of irrigation water is reduced, as does the harvest index and total dry matter production. Additionally, clay soils limit the performance of BGN, and the lack of improved varieties negatively influences grain yield (Chibarabada et al., 2018). The legume BGN is influenced by sodium chloride, as salinity significantly decreases germination and plant growth increase (Ambede et al., 2012).

To evaluate new cultivars, plant breeders use multi-environment trial (MET) to evaluate the effect of genotype × environment interaction (GEI) and selection of stable cultivars. In the final phase of the genetic improvement program, it is relevant to evaluate promising genotypes under different environmental conditions of the producing areas (Araméndiz-Tatis et al., 2021). Genotype × environment interaction is the responses of genotypes across various environments (Tonk et al., 2011). Genotype × environment interaction is the differences in cultivar productivity in various environments; as a result, it becomes challenging to select the best-performing genotypes in several environments

(Alam et al., 2021). However, evaluating cultivar candidates in several environments is helpful as it enables the selection of genotypes with wide adaptation and stability across environments (Tonk et al., 2011; Araméndiz-Tatis et al., 2021).

In plant breeding, breeders employ a mixed model methodology that has been proven more accurate in the selection process (Tolhurst et al., 2019). This method can estimate variance components using the restricted maximum likelihood model (REML) and predict genotypic values using the best linear unbiased prediction (BLUP). In the case of unbalanced data, the REML method is an exceptional method for estimating variance components (da Costa et al., 2002; Santos et al., 2015). The Best Linear Unbiased Prediction (BLUP) method, commonly used in forestry and animal genetic evaluation, can also be employed for annual crop breeding (Viana et al., 2010). The study aimed to determine the genetic variance components among BGN RILs to guide future selection. The study further identifies superior BGN genotypes guided by the BLUP values.

4.2 Materials and methods

4.2.1 Plant material and field preparation

The plant material used in this study consisted of 54 BGN (Table 4.1), comprising 44 seventh generation (F7) inbreed recombinant lines (IRLs) derived from a cross between single BGN genotype Tiga Nicuru (maternal) and DipC (paternal). These materials were developed and sourced from Malaysia at the University of Nottingham (UK), and ten landrace checks were sourced from the Agriculture Research Council (ARC). In all experimental sites, the fields were disc-ploughed using a tractor. After ploughing, the rotavator was used to break down the clods to maintain a flat seedbed and incorporate the plant's residues with the soil. Hand hoe, rake, spades, and measuring tape were used to prepare the field and make the ridges.

Table 4.1: Fifty-four BGN germplasm used in the current study

No	Code	Pedigree	Entry	No	Code	Pedigree	Entry
1	G1	Tiga nicuru **x** DipC	E445	**28**	G28	Tiga nicuru **x** DipC	E470
2	G2	Tiga nicuru **x** DipC	E457	**29**	G29	Tiga nicuru **x** DipC	E498
3	G3	Tiga nicuru **x** DipC	E446	**30**	G30	Tiga nicuru **x** DipC	E453
4	G4	Tiga nicuru **x** DipC	E461	**31**	G31	Tiga nicuru **x** DipC	E464
5	G5	Tiga nicuru **x** DipC	E460	**32**	G32	Tiga nicuru **x** DipC	E465
6	G6	Tiga nicuru **x** DipC	E473	**33**	G33	Tiga nicuru **x** DipC	E481
7	G7	Tiga nicuru **x** DipC	E468	**34**	G34	Tiga nicuru **x** DipC	E477
8	G8	Tiga nicuru **x** DipC	E478	**35**	G35	Tiga nicuru **x** DipC	E479
9	G9	Tiga nicuru **x** DipC	E495	**36**	G36	Tiga nicuru **x** DipC	E487
10	G10	Tiga nicuru **x** DipC	E490	**37**	G37	Tiga nicuru **x** DipC	E486
11	G11	Tiga nicuru **x** DipC	E467	**38**	G38	Tiga nicuru **x** DipC	E496
12	G12	Tiga nicuru **x** DipC	E494	**39**	G39	Tiga nicuru **x** DipC	E485
13	G13	Tiga nicuru **x** DipC	E502	**40**	G40	Tiga nicuru **x** DipC	E491
14	G14	Tiga nicuru **x** DipC	E469	**41**	G41	Tiga nicuru **x** DipC	E480
15	G15	Tiga nicuru **x** DipC	E483	**42**	G42	Tiga nicuru **x** DipC	E482
16	G16	Tiga nicuru **x** DipC	E471	**43**	G43	Tiga nicuru **x** DipC	E472
17	G17	Tiga nicuru **x** DipC	E493	**44**	G44	Tiga nicuru **x** DipC	E492
18	G18	Tiga nicuru **x** DipC	E503	**45**	P1	IITA 686	IITA 686
19	G19	Tiga nicuru **x** DipC	E489	**46**	P2	TIGD	TIGD
20	G20	Tiga nicuru **x** DipC	E458	**47**	P3	DIP-C	DIP-C
21	G21	Tiga nicuru **x** DipC	E448	**48**	P4	LUNT	LUNT
22	G22	Tiga nicuru **x** DipC	E447	**49**	P5	ANKPA4	ANKPA4
23	G23	Tiga nicuru **x** DipC	E463	**50**	P6	S19	S19
24	G24	Tiga nicuru **x** DipC	E474	**51**	LL1	Songkhla	Songkhla
25	G25	Tiga nicuru **x** DipC	E449	**52**	LL2	100SB16ANAMC	100SB16ANAMC
26	G26	Tiga nicuru **x** DipC	E484	**53**	LL3	EXSOCOTO	EXSOCOTO
27	G27	Tiga nicuru **x** DipC	E500	**54**	LL4	UKZN-L1	UKZN-L1

4.2.2 *Experimental site*

The study was conducted during the 2021/2022 summer cropping season at KwaZulu-Natal (Pietermaritzburg) in South Africa. The study consisted of three environmental trials established in two research stations (the University of KwaZulu Natal experimental farm Ukulunga and Cedara College of Agriculture). The environments were considered as seasons. The first and the third environmental trials were established at Ukulinga Research Farm (29°37’S; 30°16’E; 750 m a.s.l) Pietermaritzburg, KwaZulu-Natal, South Africa. These two environmental trials were separated by planting dates (Table 4.2). The soil at this site was categorized as Cleveland, with an adequate rooting depth of 0.40 m (Chibarabada et al., 2019). The second research station is Cedara College of Agriculture (29.53° S, 30.27° E; 1 104 m a.s.l). In this research station, the second environmental trial was established on the 13th of December 2021. The climatic description of all three environments is summarized in Table 4.2. The table displays environmental coordinates (latitude and longitude), average temperature, relative humidity, rainfall, and planting dates for all three environments.

Table 4.2: Characterisations of three environments used to evaluate IRLs during 2021/2022 cropping season in two research stations

Code	Latitude	Longitude	Altitude	Av Temp	RH	Rainfall	Planting date
			m.a.s.l	**(°C)**	**(%)**	**(mm)**	
UKRF (ENV1)	29.6377051	30.3948151	705	25.9	53	37.4	02/12/2021
CRDA (EVN2)	29.5378197	30.2766917	1104	25.6	55	36.1	13/12/2021
UKRF (ENV3)	29.6377051	30.3948151	705	24.9	49.42	21.81	20/01/2022

UKRF: Ukulinga Research Farm; **ENV**: Environment; **CRDA**: Cedara College of Agriculture; **m.a.s.l**: Meter above sea level; **(°C)**: Degree Celsius; **(%)**: Percentage; **(mm)**: millimetre; **Av Temp**: average temperature; **RH**: Relative humidity. (**Sourced from (https://power.larc.nasa.gov/data-access-viewer/)**

4.3 Experimental design

A 6 by 18 alpha lattice block design was used to evaluate the 54 BGN genotypes comprised of 44 F_7 recombinant inbred lines (RILs) and ten landrace checks (Table 4.1). The layout was replicated twice in each environment. An experimental layout had two replications separated by a 2-meter space. Five seeds of BGN per genotype were sown in a single 1.5-meter row plot spaced at 0.9 m between rows, and the seeds were planted at a depth of about 5 cm deep using plant spacing of 0.3 m between plants within a 1.5 m plot.

4.4 Measuring parameter standard

The quantitative traits were collected following the BGN classification and descriptors guideline by IPGRI, The International Institute of Tropical Agriculture (IITA), and BAMNET (2000). The Eleven quantitative traits that were collected following the BAMNET guidelines include germination percentage (GP), days to 50% emergence (DTE), days to 50% flowering (DTF), plant spread (PS), plant height (PH), seed length (SL), seed width (SW), grain yield per plant (GYPP), grain yield (GY), unshelled grain yield (UGY) and shelling percentage (SHLP) presented in Table 4.3. The formulas used to calculate are presented below.

Grain yield was converted from total grain yield per plot (g) to total grain yield per hectare using the formula below, used by (Olanrewaju et al., 2021):

$$\text{Grain yield (kg/ha)} = \frac{\text{Plot GY x 10 000}}{\text{plot area}} \qquad \textbf{Equation 1}$$

Plot GY= Plot grain yield/plot (g)

The shelling percentage was calculated using the formula below:

$$\text{Shelling percentage (\%)} = \frac{\text{GY}}{\text{UGY}} \text{X } 100 \qquad \textbf{Equation 2}$$

GY = grain yield (kg/ha)

UGY = unshelled grain yield (kg/ha)

Table 4.3: Eleven agro-morphological quantitative traits of 54 BGN genotypes collected during the 2021/2022 season

Traits collected	**Scale**	**Collection time and how the trait was collected or measured**
Germination percentage (GP)	%	Emerged BGN seedlings after sowing per plot were counted and recorded daily
Days to 50% emergence (DTE)	Days	This trait was recorded as the number of days from the date of sowing to when 50% of plant emergence.
Days to 50% flowering (DTF)	Days	This trait was recorded as the number of days from 50% seedling emergence to 50% flowering plants.
Plant Height (PH)	cm	Recorded ten weeks after planting, measured from the ground level to the tip of the highest point using a ruler.
Plant spread (PS)	cm	Recorded ten weeks after planting. Using a measuring ruler, the average of 3 widest lengths between opposite points of plants.
Shelling percentage (SHLP)	%	The shelling percentage was calculated using formula (2) above.
Unshelled grain yield (UGY)	kg/ha	Measurements were done using a weighing balance and then calculated from grams per plot to kg/ha, converted from unshelled grain yield per plot to kg/ha.
Grain yield (GY)	kg/ha	A weighing balance was used to measure grain yield in grams per plot, then converted to kg per hectare using the formula (1) above.
Grain yield per plant (GYPP)	g	A weighing balance was used to measure grain yield in grams per plant.
Seed width (SW)	mm	The seed width of 10 randomly sampled shelled seeds per plant was measured using a digital vernier calliper.
Seed length (SL)	mm	The seed length of 10 randomly sampled shelled seeds per plant was measured using a digital vernier calliper.

4.4.1 Cultural practices

4.4.1.1 Planting and field layout preparation

The three experimental environments were established during the 2021 and 2022 summer seasons. The sowing dates were as follows: the first environment (02 December 2021) at Ukulinga Research Farm (UKRF), the second environment (13 December 2021) at Cedara College of Agriculture (CRDA), and the third environment was established on the 20th of January 2022 at UKRF. The field was mechanically ploughed by a tractor. The hand hoes were used to prepare the seedbed and making of the ridges. All the seeds were sown on the top of the ridges.

4.4.1.2 Mounding, fertilizer application, and irrigation

Mounding or earthing up was carried out before and after flowering, following the research findings by Ouedraogo et al. (2013). The study conducted by Ouedraogo et al. (2013) recommended that mounding in the BGN field should not be done during the flowering period of the crop as it results in low grain yield; hence, mounding was carried out before flowering. The side dressing method of fertilizer application was used, and fertilizers were applied at 70 kg/ha N, 40 kg/ha P_2O_5, and 30 kg/ha K_2O on the fifth week or just before flowering. All three environments were rain-fed experiments, and climatic data from 2021 to 2022 was accessed at power access climate data websites using the site's coordinates (https://power.larc.nasa.gov/data-access-viewer/).

4.4.1.3 Weed, pest, and disease control

A pre-emergence paraquat SL and springbok soluble concentrate herbicide was used to control the weeds using recommended factory measurements. A gramoxone post-emergence herbicide was applied to control aggressive weeds and was supplemented by hand hoe weeding as an integrated pest management (IPM) strategy. Insects were controlled by karate Zeon insecticide, using 100g/l as a recommended measurement, and 45 g/16L coproxy sup with active ingredients of koperoskiscloried and copper oxychloride was used to control the diseases on the crop.

4.4.1.4 Harvesting, storing and shelling

At maturity, the plants were harvested using a hand hoe when 90% were matured and ready for harvest. All Mature plants were identified by the leaves turning brown and drying out, and they were manually harvested by hand hoe and packaged separately in sacks. The harvested plants were then sun-dried in a tunnel for three weeks. The pods were trashed, and a counter-scale model (AQUA) weighing balance was used to measure the grain yield (g) per plant. Seed length and width were measured after shelling using the digital Vernia calliper (mm).

4.4.2 Statistical analysis

4.4.2.1 Analysis of variance and best linear unbiased predictors

A combined analysis of variance (ANOVA) was performed on the software GenStat 20th Edition using an unbalanced ANOVA design (Payne et al., 2011) following an alpha lattice design. The sources of variation tested were genotype (G), environment (E), and genotype by environment (G × E interaction), replication by environment (R × E interaction), and block by replication by environment (B × R × E interaction). Variance component estimates were generated using Deltagen (v 0.5-2e785a4), a free web-built software developed by Jahufer and Luo (Jahufer & Luo, 2018) accessed on the website (www.deltagen.agresearch.co.nz). Deltagen runs a linear mixed model fitted by residual maximum likelihood (REML) using the package “lmerMod.” Estimates of variance components and best linear unbiased predictors (BLUPs) were calculated using the model shown below (3), with genotypes (G), environments (E), replications (R), and blocks (B) nested in replication regarded as random variables.

$Y_{jklm} = \mu + E_j + G_k + R_l + B_{m(l)} + GE_{jk} + e_{jklm}$ **Equation 3**

Where: Y_{jklm} is the observed phenotypic value for genotype k in replicate l and block m at environment j, µ is the general mean, E_j is the effect of the kth environment, G_k is the effect of the jth genotype, R_l is the effect of the lth replicate nested in the $_j$th environment, $B_{m(l)}$ is the effect of the mth block nested in the lth replicate and the kth environment, GE_{jk} is the interaction effect between the kth genotype and the jth environment, and e_{jklm} is residual error.

4.4.2.2 Estimation of variance components and genetic parameters analysis

The Genotypic (σ^2G), Environmental (σ^2E) and error (σ^2e) Variance was estimated on Deltagen.

The phenotypic variance was calculated using the formula below.

$$\boldsymbol{\sigma^2 P = \sigma^2 G} + \frac{\boldsymbol{\sigma^2}\ \mathbf{G\ x\ E}}{\mathbf{E}} + \frac{\boldsymbol{\sigma^2}\ \mathbf{G\ x\ REP}}{\mathbf{REP}} + \frac{\boldsymbol{\sigma^2}\ \mathbf{BLOCK(REP*ENV)}}{\mathbf{REP\ x\ ENV}} + \frac{\boldsymbol{\sigma^2}\ \mathbf{E}}{\mathbf{E}} + \frac{\boldsymbol{\sigma^2}\ \mathbf{e}}{\mathbf{E\ x\ REP\ x\ BLOCK}} \qquad \textbf{Equation 4}$$

Where: $\boldsymbol{\sigma^2}\mathbf{G}$ = Genotypic variance

$\boldsymbol{\sigma^2}$ **G x E** = Genotype by environmental variance

$\boldsymbol{\sigma^2}$ **E** = The environmental variance

$\boldsymbol{\sigma^2}$ BLOCK(REP x ENV) = Block by replication variance

$\boldsymbol{\sigma^2}$ **e** = Error variance

Broad sense heritability (H^2) was calculated using the formula below, according to the calculation followed by Khan et al. (Khan et al., 2020)

$\mathbf{H^2}(\%) = \frac{\sigma^2 G}{\sigma^2 P}$ x 100 **Equation 5**

$\boldsymbol{\sigma^2}\mathbf{G}$ is genotypic variance.

$\boldsymbol{\sigma^2}\mathbf{p}$ is phenotypic variance.

The phenotypic coefficient of variance (PCV) was calculated using the formula below (Khan et al., 2010)

PCV (%) = $\frac{\sqrt{\sigma^2 P}}{\bar{x}}$ x 100 **Equation 6**

Where $\sqrt{\boldsymbol{\sigma}^2 P}$ Is the phenotypic standard deviation, and ($\bar{x}$) is the grand mean of the trait.

The genotypic coefficient of variance (GCV) was calculated using the formula below.

GCV (%) = $\frac{\sqrt{\sigma^2 G}}{\bar{x}}$ x 100 **Equation 7**

Where $\sqrt{\boldsymbol{\sigma}^2 G}$ Is the genotypic standard deviation, and ($\bar{x}$) is the grand mean.

CV$_e$ (%) = $\frac{\sqrt{\sigma^2 e}}{\bar{x}}$ x 100 **Equation 8**

Relative difference (%) and genetic advance (%) were calculated following the calculations done by Khan et al. (Khan et al., 2021c)

RD (%) = $\frac{\sigma^2 P - \sigma^2 G}{\sigma^2 p}$ x 100 **Equation 9**

The genetic advance was calculated using the formula below, according to Alake et al. (Alake and Ayo-Vaughan, 2017)

GA (%) = H^2 x K x $\frac{\sqrt{\sigma^2 P}}{\bar{x}}$ x 100 **Equation 10**

where K is the selection differential and is constant (2.06) at 5% selection intensity; H^2 is the broad sense heritability, and $\sqrt{\sigma^2 P}$ is the phenotypic standard deviation, and ($\bar{x}$) is the grand mean of a trait.

GCV/ CVe was calculated as the ratio of (**Equation 7** and **Equation 8**).

4.5 Results

4.5.1. Analysis of variance

The combined analysis of variance results for all agro-morphological traits is presented in Table 4.4. The performance of the genotypes (G) across three environments revealed a highly significant difference ($P < 0.01$) for most traits, except for days to 50% emergence (DTE), days to 50% flowering (DTF), and plant spread (PS). The environment (E) was highly significantly different ($P < 0.001$) for the performance of all agro-morphological traits. The genotype by environmental interaction (G x E) was highly significant ($P < 0.001$) for the following yield traits: seed length (SL), seed width (SW), grain yield (GY), unshelled grain yield (UGY), grain yield per plant (GYPP) and shelling percentage (SHLP). Across environments, replications were significantly different ($P < 0.001$) on the performance of yield traits (GY, UGY, and GYPP), and there was a highly significant difference ($P < 0.01$) within the blocks that were nested in replications for all the traits.

Table 4.4: Combined ANOVA showing mean square values and the significance level for 11 quantitative traits for 54 BGN genotypes

SOV	**D.F**	**DTE**	**GP**	**DTF**	**PH**	**PS**	**UGY**	**GYPP**	**SL**	**SW**	**SHLP**	**GY**
		(Days)	(%)	(Days)	(cm)	(cm)	(kg/ha)	(g)	(mm)	(mm)	(%)	(kg/ha)
ENV	2	2035.56***	30238.30***	5709.40***	1579.68***	28526.08***	9480368***	5432.07***	51.43***	27.69***	419.66**	3229552***
REP (ENV)	3	9.04 ^{ns}	169.10 ^{ns}	49.53 ^{ns}	15.18 ^{ns}	89.58 ^{ns}	1425226***	330.27***	2.52 ^{ns}	0.49 ^{ns}	60.29	485882***
BLOCK(REP)	6	35.17***	4381.50***	99.39***	27.48**	284.67***	838264***	448.10***	8.98***	2.93***	79.29	323474***
GEN	53	11.14 ^{ns}	899.10**	25.41 ^{ns}	14.12**	80.86 ^{ns}	242450***	173.92***	3.64***	1.98***	225.31***	87651***
GEN x ENV	106	9.79 ^{ns}	493.30 ^{ns}	30.94 ^{ns}	8.79 ^{ns}	66.26 ^{ns}	102587**	97.65***	2.21***	1.47***	125.15***	37603**
ERROR		7.89	574.20	23.48	7.71	66.24	68666	32.81	1.14	0.67	71.72	22255

SOV: Source of variation; D.F: Degree of freedom; **GP**: Germination percentage; **DTE**: Days to 50% emergence; **DTF**: Days to 50% flowering; **PH**: Plant height; **PS**: Plant spread; **SL**: Seed length; **SW**: Seed width; **UGY**: Unshelled grain yield; **GY**: Grain yield; **GYPP**: Grain yield per plant; **SHLP**: Shelling percentage; **ns**: $P >= 0.05$; ***** :$P <0.05$; ****** : $P < 0.01$; ******* : $P < 0.001$.

4.5.2. Performance of 54 BGN based on Best linear unbiased prediction (BLUP)

The best linear unbiased prediction (BLUP) for eleven agro-morphological traits for the 54 BGN genotypes evaluated in three environments is presented in Table 4.5. The best linear unbiased prediction (BLUP) method is widely used for estimating the random effects of a mixed model. When several traits are selected, the performance of BLUP is critically dependent on the availability of reasonable variance estimates (Piepho et al., 2008). The number of days to 50% emergence (DTE) ranged from 13.97 (G20) to 14.25 (G36) days, with a mean value of 14.13 days. The trait germination percentage (GP) ranged from 34.01% (G3) to 57.79% (G18), with a mean value of 46.60%. The average days to 50% flowering (DTF) recorded 60.436 days, and it ranged from 55.88 (G18) to 66.26 (G3) days, respectively. The trait plant height (PH) ranged from 18.97 cm (LL3) to 21.57 cm (G31), with a mean performance of 20.54 cm. The trait plant spread (PS) varied from 35.18 cm (P3) to 38.88 cm (LL4), with a mean of 37.31 cm.

The grain yield (GY) kg/ha mean value for the performance of BGN across three environments varied from 93.56 kg/ha for the genotype G15 to 542.96 kg/ha for the genotype P3, with an overall mean of 216.80 kg/ha. Unshelled grain yield had an overall mean of 375.65 kg/ha and varied from 166.38 (G15) to 944.37 kg/ha (P3). The trait grain yield per plant (GYPP) had an average of 10.97 g, with a mean range of 3.76 (G1) to 17.66 g (G23). The seed length ranged from 10.47 mm (P2) to 11.80 mm (G1), with a mean of 11.24 mm. The seed width (SW) varied from 7.53 (G4) to 8.36 mm (P3), with an average of 7.94 mm. The mean performance of the trait shelling percentage was 56.72 % and varied from 50.54 % (LL1) to 61.42 % (G25) (Table 4.6). The coefficient of variance (%) for all eleven quantitative traits varied from 7.95% (DTF) to 68.20% (UGY), showing a wide variation among the genotypes. The calculated standard deviation performance for all traits ranged from 0.07 (DTF) to 126.68 (UGY).

Table 4.5: The Best Linear Unbiased Prediction (BLUP) breeding estimate mean values showing the top 10 best-performing genotypes and bottom 5 BGN genotypes

GEN	DTE	GP	DTF	PH	PS	GYPP	UGY	SL	SW	SHLP	GY	Rank
P3	14.16	40.35	59.95	19.28	35.18	14.77	944.37	11.149	8.36	57.19	543.06	1
G23	14.03	47.97	60.43	20.64	38.02	17.66	658.47	11.443	8.17	60.00	403.23	2
G1	14.18	38.72	60.91	19.87	37.73	3.77	608.51	11.804	8.17	55.08	336.40	3
G8	14.18	45.85	61.88	20.34	38.40	13.52	539.38	11.628	8.00	58.66	323.35	4
LL2	14.12	51.59	60.01	20.18	37.89	14.06	475.16	11.436	8.15	58.95	298.65	5
G18	14.07	57.79	55.88	21.09	38.34	12.19	497.35	11.51	7.96	58.34	295.91	6
LL4	14.19	45.05	62.21	20.15	38.88	12.61	520.25	11.521	8.34	57.29	291.01	7
G32	14.10	49.30	57.37	20.30	37.11	11.45	495.46	11.446	7.74	55.47	284.00	8
G13	14.09	43.93	61.20	19.84	38.25	11.33	461.53	11.25	8.00	58.80	283.44	9
G40	14.16	44.60	63.02	21.14	37.83	14.43	450.91	11.802	8.14	57.43	267.11	10
G3	14.21	34.01	66.26	20.34	36.99	9.50	244.10	10.999	7.76	55.08	136.32	50
P5	14.09	41.12	60.35	19.53	36.42	9.37	240.50	10.472	7.55	53.10	134.01	51
G4	14.25	45.71	58.86	20.85	36.88	8.91	224.89	10.691	7.53	50.98	126.18	52
G2	14.18	41.21	62.90	19.97	35.98	9.30	202.52	11.103	7.74	56.06	118.42	53
G15	14.16	41.67	60.44	19.90	36.642	8.50	166.38	11.127	7.98	55.05	93.56	54
MEAN	**14.13**	**46.61**	**60.43**	**20.53**	**37.31**	**10.97**	**375.65**	**11.239**	**7.94**	**56.72**	**216.81**	
CV %	**19.69**	**45.14**	**7.95**	**13.34**	**21.71**	**48.12**	**68.20**	**9.070**	**10.30**	**14.19**	**67.48**	
SE	0.29	0.00	0.00	0.15	0.17	0.16	0.11	0.18	0.17	0.17	0.11	

4.5.3. Phenotypic (σ2 P), genotypic (σ 2 G), and genotype by environment variance (σ2 G x E)

All traits' overall phenotypic variance (σ2P) was higher than the overall genotypic variance (σ2G). The highest phenotypic and genotypic variance was recorded on the trait unshelled grain yield (UGY), with the values 61212 and 26510, respectively. The lowest phenotypic and genotypic variance were recorded on the traits' seed length (0.56) and days to 50% flowering (0), respectively. Seed length (SL) revealed the lowest (0.59) variance for genotype by environmental interaction (σ2 G x E), whilst the highest σ2 G x E was recorded on the trait unshelled grain yield (12072.00) (Table 4.6).

Table 4.6: Estimates for genotypic variance, phenotypic variance, heritability, genetic advance, covariance, and relative deference of eleven agro- morphological quantitative traits for 54 BGN genotypes

SOV	**DTE**	**GP**	**DTF**	**PH**	**PS**	**GYPP**	**UGY**	**SL**	**SW**	**SHLP**	**GY**
Unit	**(Days)**	**(%)**	**(Days)**	**(cm)**	**(cm)**	**(g/plant)**	**(Kg/ha)**	**(mm)**	**(mm)**	**(%)**	**(Kg/ha)**
Mean	14.13	46.61	60.43	20.54	37.31	10.97	375.65	11.24	7.94	56.72	216.80
$\sigma^2_{\text{G x E}}$	1.13	3.06	3.39	7.62	8.73	3.59	12072.00	0.59	3.96	30.86	5902.60
$\sigma^2_{\text{G X REP}}$	0.16	1.29	0	2.85	0.00	4.87	2991.00	0.09	0.00	5.51	925.30
σ^2_{G}	0.09	6.97	0	9.10	3.14	1.06	26510.00	0.24	1.02	14.70	9429.00
$\sigma^2_{\text{BLOCK(REP)}}$	0.06	8.49	2.12	5.41	5.88	1.13	27691.00	0.12	4.46	0.00	11100.70
$\sigma^2_{\text{E x REP}}$	0.00	0.00	0.00	1.58	1.90	2.26	10251.00	0.00	1.30	0.00	2634.60
σ^2_{E}	19.50	2.54	53.51	1.48	2.68	4.53	71292.00	0.43	2.26	2.74	24183.70
σ^2_{e}	7.74	4.43	23.06	7.50	6.56	2.79	65628.00	1.04	6.68	64.75	21401.40
SOV	**DTE**	**GP**	**DTF**	**PH**	**PS**	**GYPP**	**UGY**	**SL**	**SW**	**SHLP**	**GY**
σ^2_{G}	0.09	6.97	0.00	9.10	3.14	1.06	26510.00	0.24	1.02	14.70	9429.00
σ^2_{P}	7.89	8.55	21.68	10.84	5.12	3.03	61212.00	0.56	2.89	26.40	21057.13
H^2 %	1.23	65	0.00	61.44	38.00	16.00	41.61	35.00	25.00	47.00	41.65
GA %	0.48	9.41	0.00	**23.72**	6.03	7.61	57.60	5.32	12.99	14.19	59.55
PCV %	19.32	7.03	7.44	18.74	7.71	23.83	67.19	7.35	25.64	9.87	69.40
GCV %	2.12	5.66	0.00	14.69	4.75	9.39	43.34	4.36	12.72	6.76	44.79
RD %	89.01	19.39	100	21.61	38.38	60.62	35.49	40.74	50.40	31.53	35.46
CV_e %	19.69	4.52	7.95	13.33	6.87	15.23	68.20	9.07	32.55	14.19	67.48
GCV/CV_e	0.12	1.25	0.00	1.10	0.69	0.61	0.63	0.48	0.39	0.48	0.66

GP: germination percentage; **DTE:** days to 50% emergence; **DTF:** days to 50% flowering; **PH:** plant height; **PS:** plant spread; **SL:** seed length; **SW:** seed width; **UGY:** unshelled grain yield; **GY:** grain yield; **GYPP:** grain yield per plant; **SHLP:** shelling percentage.

4.5.4. Phenotypic coefficient of variance and genotypic coefficient of variance

The overall phenotypic coefficient of variance (PCV) values were higher than the alternative genotypic coefficient of variance (GCV) values. The trait grain yield recorded the highest PCV (69.4%) and GCV (44.08%) (Table 4.6). The lowest PCV and GCV values were recorded on the trait's germination percentage (7.03%) and days to 50% flowering (0%), respectively. A PCV estimate from the highest to the lowest values for all eleven traits was ranked as follows: grain yield 69.4%, unshelled grain yield 67.19%, seed width 25.64%, grain yield per plant 23.83%, days to 50% emergence 19.32%, plant height 18.74%, shelling percentage 9.87%, plant spread 7.71%, days to 50% flowering 7.44%, seed length 7.35%, and germination percentage 7.03% (GP), respectively. While GCV were ranked as follows: Grain yield (44.79%), unshelled grain yield (43.34%), plant height (14.69%), seed width (12.72%), grain yield per plant (9.39%), shelling percentage (6.76%), germination percentage (5.66%), plant spread (4.75%), seed length (4.36%), days to 50% emergence (2.12%), and days to 50% flowering (0), respectively.

4.5.5. Error coefficient of variances (CVe), GCV/CVe ratio, and relative difference (RD)

Coefficient of error variance (CVe) showed that UGY (68.2.0%) was the highest recorded trait (Table 4.6) , with non-explainable error in the experiment due to environmental factors, soil type, moisture, temperature and human error management, followed by the traits, GY, SW, DTE, GYPP, SHLP, PH, SL, DTF, PS, and GP (Table 4.6). The ratio of the genotypic coefficient of variance to the error coefficient of variance (GCV/ CVe) measures the relative importance of the genetic and environmental factors in determining the variation of a trait within a population. The GCV/CVe ratio findings showed that genetic factors influenced GP (1.25), as it had the highest GCV/CVe ratio of all ten traits, followed by PH (1.10), PS (0.69), GY (0.66), UGY (0.63), and GYPP. (0.06). The characteristic DTF (0) had the lowest GCV/ CVe ratio, indicating it was influenced mainly by environmental factors.

Relative difference (RD) estimates the genotypic coefficient of variance concerning a respective phenotypic coefficient of variance (Khan et al., 2020). The more significant PCV value with the corresponding trait's lower GCV value resulted in a high relative difference (RD%) value. This study revealed the highest RD% for the trait days to 50% flowering (100%), and the most negligible RD% value was revealed on the trait germination percentage with a value of 19.39%; all other traits had RD% value of greater than 20% (Table 4.6).

4.5.6. Broad sense heritability and genetic advanced % (GA)

Broad sense heritability (H2) and advanced genetic results for this study are presented in Table 4.6. The findings in the present study H^2 revealed heritability among traits ranging from high (65%) to low (0%). Germination percentage (GP) was the trait with the highest H^2, followed by plant height (61.44%), and the trait with low heritability was days to 50% flowering (DTF). The H^2 for eleven traits was classified into three levels (high > 60%, moderate = 30-60%, and low < 30%) as described by (Khan et al., 2020). The traits germination percentage (65%) and plant height (61.44%) were detected for high heritability. In contrast, five traits showed moderate broad sense heritability comprising shelling percentage (47%), grain yield (41.65%), unshelled grain yield (41.61%), plant spread (38%), and seed length (35%). Traits that were categorized for low, broad sense heritability estimates were seed width (25%), grain yield per plant (16%), days to 50% emergence (1.23%), and days to 50% flowering (0%).

Genetic advanced (GA) is categorised into three classes: low (0% to 10%), intermediate (10% to 20%), and high (> 20%) (Juangsamoot et al., 2012). For this study, GA results findings (Table 4.6) revealed that grain yield (GY) 59.5%, unshelled grain yield (UGY) 57.6%, and plant height (PH) 23.7% were categorised as high GA > 20%, while the trait seed width (SW) revealed intermediate (13.01%) GA (Table 4.6). The traits that showed low GA were shelling percentage (9.54%), germination percentage (9.04%), grain yield per plant (7.61%), plant spread (6.03%), days to 50% emergence (0.48%), and days to 50% flowering (0%) (Table 4.6).

4.5.7. Correlation matrix analysis for eleven agro-morphological traits of 54 BGN genotypes

Understanding the association between agro-morphological parameters is essential for crop improvement programs (Shegro et al., 2013). The correlation relationship among 11 agro-morphological traits for the 54 BGN genotypes is presented in Table 4.7. The person's correlation matrix revealed a highly significant positive association between the trait grain yield (GY) with (i) unshelled grain yield (r = 0.99; p < 0.001); (ii) seed width (r = 0.61; p < 0.001); (iii) grain yield per plant (r = 0.58; p < 0.001), and (iv) shelling percentage (r = 0.40; p < 0.001). The trait seed length (SL) had a positive significant correlation with the traits' seed width (r = 0.66; p < 0.001), shelling percentage (r = 0.44; p < 0.001), and plant spread (r = 0.045; p < 0.01). The traits' seed length (r = 0.29; p < 0.05) and shelling percentage (r = 0.31; p < 0.05) had a very low positive significant correlation with the trait grain yield. A very low negative significant correlation was observed between GY with the trait days to 50% emergence (r = - 0.28; p < 0.05). A non-significant positive low correlation was detected between the traits grain yield and germination percentage (r = 0.22; p > 0.05), as well as grain yield and plant spread (r = 0.17; p > 0.05). Germination percentage revealed a highly significant (p < 0.001) negative correlation with the trait days to 50% emergence (r = -0.66) and days to 50% flowering (r = - 0.55).

Table 4.7: A person's correlation analysis revealed the association of phenotypic and yield traits among the 54 BGN genotypes.

	DTE	GP	DTF	PH	PS	GYPP	UGY	SL	SW	SHLP
DTE	1	-	-	-	-	-		-	-	-
GP	**-0.66*****	**1**	-	-	-	-	-	-	-	-
DTF	0.36**	**-0.55*****	**1**	-	-	-	-	-	-	-
PH	-0.18 ^{ns}	0.26 ^{ns}	-0.13 ^{ns}	**1**	-	-	-	-	-	-
PS	-0.18 ^{ns}	**0.28***	-0.08 ^{ns}	**0.41****	**1**	-	-	-	-	-
GYPP	**-0.27***	**0.28***	-0.06 ^{ns}	0.05 ^{ns}	0.15 ^{ns}	**1**	-	-	-	-
UGY	**-0.28***	0.23 ^{ns}	-0.19 ^{ns}	-0.14 ^{ns}	0.15 ^{ns}	**0.53*****	**1**	-	-	-
SL	-0.05 ^{ns}	0.04 ^{ns}	0.01 ^{ns}	0.24 ^{ns}	**0.45*****	0.18 ^{ns}	**0.29***	**1**	-	-
SW	-0.08 ^{ns}	0.10 ^{ns}	-0.03 ^{ns}	-0.02 ^{ns}	0.26 ^{ns}	**0.40****	**0.60*****	**0.60*****	**1**	-
SHLP	-0.17 ^{ns}	0.06 ^{ns}	0.11 ^{ns}	-0.01 ^{ns}	0.17 ^{ns}	**0.44*****	**0.31***	**0.48*****	**0.45*****	1
GY	-0.28 ^{ns}	0.22 ^{ns}	-0.17 ^{ns}	-0.13 ^{ns}	0.17 ^{ns}	**0.58*****	**0.99*****	**0.32***	**0.61*** **	**0.40****

ns: P >= 0.05; ******* Correlation is significant at the 0.001 level**;** ****** Correlation is significant at the 0.01 level; ***** Correlation is significant at the 0.05 level; **GP:** germination percentage; **DTE:** days to 50% emergence; **DTF:** days to 50% flowering; **PH:** plant height; **PS:** plant spread; **SL:** seed length; **SW:** seed width; **UGY:** unshelled grain yield; **GY:** grain yield; **GYPP:** grain yield per plant; **SHLP:** shelling percentage.

4.6 Discussion

This chapter discusses the study's findings, and the study aimed to determine the genetic variance components among BGN RILs to guide future selection. The combined analysis of variance (ANOVA) for the 54 BGN genotypes (44 IRLs and ten landrace checks) is presented in Table 4.3. There was a highly significant variability on the genotypes (G), environment (E), genotype by environment interaction (G × E), replication, and blocks nested in replications for BGN yield traits. The environment was observed to be highly significant for all traits. In a multi-environmental trial (MET), a study by Khan et al. (2022a) explained that seasons and location are the most significant causes of yield heterogeneity among genotypes. The significant difference observed in grain yield is supported by the study conducted by Chibarabada et al. (2018) and Olanrewaju et al. (2021), where they also observed a highly significant difference in yield traits of BGN. The significance of GEI indicated that grain yield and yield-related traits of the genotypes were not consistent in different experimental environments, and the impact of the environment on the yield potential of the genotypes was also different. The genotype by environment (G × E) interaction for the traits GP, DTE, DTF, PH, and PS was insignificant; this implies that the performance of these traits was consistent across the environment, revealing that environmental factors had less influence on these traits.

Based on BLUP means, five BGN genotypes (G18, G20, G14, G30, and LL1) were the top performing for the trait germination percentage (GP). These genotypes could be suggested for selecting genotypes with good germination percentages. Germination percentage ranged from 34% to 57.79%, with a mean of 46.60%. In a study conducted by Hlanga et al. (2022), a higher emergence percentage mean (85.4% and 72.8%) was reported, significantly differing from our findings. These differences may be due to the different germplasm used in each study. Our study was rainfed, while the other study depended on irrigation for moisture, resulting in germination range variation.

For days to 50% emergence, BLUP means there was no significant difference between genotypes; this trait ranged from 13.97 days to 14.25 days with a mean of 14.13. The CV% (19%) showed low variability for days to 50% emergence. The observed Bambara genotypes with fewer days to 50% emergence were G14 and G20; these genotypes can be selected for genotypes that take less period to emerge and can be recommended for farmers who rely on rainfed farming practices. This study’s mean findings align with the study conducted by Bonny et al. (2019). Days to 50% flowering performance in this study ranged from 55.88 to 66.2 days with a mean of 60 days; similar findings were reported in a study conducted in South Africa (Unigwe et al., 2016), which reported a mean of 59 days to 50% flowering. A study by Shegro et al. (2013) & Valombola et al. (2019) found slightly lower days to 50% flowering, which differed from our findings, with a mean of 35 and 42 days, respectively. However, the genotypes G18, G39, G32, and G30 reached days to 50% flowering earlier; hence, they can be selected for early flowering as they might reach maturity fast.

Most studies have shown a positive correlation between plant height traits and grain yield (Shegro et al., 2013; Bonny et al., 2019; Valombola et al., 2019). This might indicate that the taller the plant, the higher the grain yield; selection of tall plants may significantly increase grain yield on BGN production. Most genotypes with the highest value of 21 cm were recorded on the genotypes G31, G6, G18, G19, G22, G30, and G38; these were the tallest genotypes in the collection. Plant height recorded on our findings ranged from 18 cm to 21 cm, with a mean of 20.5 cm. These findings align with the results reported by Unigwe et al. (2016) & Bonny et al. (2019).

The trait plant spread in this study showed a positive correlation with grain yield, ranging from 35.18 cm to 38.87 cm, with a mean of 37 cm. The mean range is within the range of mean findings reported by Ntundu et al. (2006). The reported mean in this study for the trait grain yield per plot is 10.97 g, slightly lower than the results reported by Unigwe et al. (2016). As KZN was affected by floods, most yield traits did not perform well compared to results reported by other studies. Regardless of the effect of floods, the grain yield reported in this study performed well compared to a study conducted by Valombola et al. (2019), which reported grain yield ranging from 39.76 to 470.9 kg/ha, with a mean of 176.07. Present study results revealed that grain yield varied from 93 kg/ha to 542 kg/ha, with a grand mean of 216.80 kg/ha.

All phenotypic variance estimates were higher than the corresponding genotypic variance estimates for all eleven traits evaluated. These findings were reported by Khan et al. (2020), Khan et al. (2022d), and Onwubiko et al. (2019), emphasizing that the trait's expression is influenced mainly by the environment. Among eleven agro-morphological traits evaluated in this study, phenotypic variance (σ^2_P) and genotypic variance (σ^2_G) estimated high variability for the trait grain yield (GY) and unshelled grain yield (UGY) (Table 4.6), which may reveal that there is a vast pool of genetic makeup that can be selected on these traits. In a study by Alake and Ayo-Vaughan (Alake & Ayo-Vaughan, 2017), the trait grain yield was the second highest estimated phenotypic and genotypic variance after the trait biomass for the studied crop Bambara groundnut. Similar findings were reported by Khan et al. (2022b), where yield was the highest estimated trait for both phenotypic and genotypic variance. The trait that recorded the lowest phenotypic variance in a study conducted by Khan et al. (2022b) is inter-node length, which is different from our findings, which recorded the traits' seed length (SL) and days to 50% flowering (DTF).

Phenotypic and coefficient of variability are categorised as low (less than 10%), 10–20% as moderate, and greater than 20% as high (Khaliqi et al., 2021; Donkor et al., 2022). From the studied numeric traits, the present study has identified high PCV on grain yield, unshelled grain yield, grain yield per plant, and seed width. Moderate PCV values were identified on the trait's days to 50% emergence and plant height, leaving the remaining traits categorized as low PCV. The traits grain yield and unshelled grain yield recorded high PCV and GCV, and plant height and seed width were moderate for both PCV and GCV. Similar findings were presented in a study conducted by Onwubiko et al. (2019), where seed yield recorded the highest GCV and PCV, respectively. It is reported by Donkor et al. (2022), that the production of high-yielding BGN varieties may, therefore, benefit from the application of phenotypic selection to such traits that fall into the category of high GCV and moderate PCV. Contrarily, characters with low GCV and PCV values (Table 4.6) demonstrated a low coefficient of variation < 20% (CV%) among the 11 evaluated characters. Comparable results were reported in a study conducted by Onwubiko et al. (2019).

All quantitative traits evaluated displayed a relative difference (RD%) greater than 20%, except for the trait germination percentage. This indicates that environmental factors influenced the traits, as it was reported in a study conducted by Onwubiko et al. (2019) and Khan et al. (2020), that the traits with high RD% are a result of environmental effects and direct selection becomes hard to perform on such traits. It is further reported that traits with low RD% are associated with genetic effects (Onwubiko et al., 2019). Hence, direct selection can be performed in such traits. Based on our results for RD%, only the germination percentage can undergo direct selection.

Among the eleven numerical traits evaluated, unshelled grain yield and grain yield showed a high error coefficient of variance (CVe) (Table 4.6). This portrays the high variability that is present in yield trait of BGN. The trait grain yield is influenced by several factors, such as genetic factors, environmental factors, and crop management (Alake, 2018). An error coefficient of variance (CVe) was estimated to measure the model's accuracy, and the results are presented in Table 4.6. Unshelled grain yield and grain yield recorded the highest CVe, indicating high variability with less accuracy for these traits. These traits are highly influenced by several factors, including environmental factors and crop management; hence, it is recommended that molecular makeup be assessed to target the traits of interest.

In definition, broad sense heritability is the proportion of the phenotypic variance ($\sigma^2{}_P$) that is attributable to an overall variance for the genotype ($\sigma^2{}_G$) (Schmidt et al., 2019). Broad-sense heritability is the percentage of the ratio of genetic variance to the phenotypic variance in a population. A more significant genotypic coefficient of variance (GCV), high heritability, and genetic advance offer more robust indicators than individual characters (Khaliqi et al., 2021). The estimated broad sense heritability for 11 quantitative traits exhibited in the present study ranged from high (65%) to low (0). Grain yield estimated a broad sense heritability of 41.65%, classified as moderate. Khan et al. (2022b) results reported a heritability estimate of 78.07 on yield for the same crop, which differs from our findings. Meanwhile, Khaliqi et al. (2021) reported broad sense heritability that ranged from 0 to 28.31 for yield and yield-related characters, which was slightly lower than our findings. Differences influence heritability estimated for yield and yield estimates in test environments (Shayanowako et al., 2018). When looking at genetic advance (GA) (Table 4.6), the traits grain yield (59.545), unshelled grain yield (57.06), and plant height (23.71) were the top three to record the highest GA. From these three traits that recorded high GA, grain yield is one trait that also recorded a high genotypic coefficient of variance; it is reported by Khan et al. (2020), that higher GCV, coupled with high heritability as well as high genetic advance, gives a superior sign of selection than the examination of individual genetics.

The five traits that showed strong to intermediate positive significant correlation between grain yield and (i) unshelled grain yield, (ii) seed width, (iii) grain yield per plant, (iv) shelling percentage, and (v) seed length (Table 4.7) suggests that these are the traits that can be targeted when selecting for yield improvement in BGN RILs for future breeding. Nzuve et al. (2014) also suggested that a positive relationship between traits can guide further breeding to aid seed availability. The trait grain yield was identified as having a strong correlation with yield components in a study conducted by Esan et al. (2023), and these findings are in line with our findings. Plant height had a negative, non-significant correlation with the trait grain yield. A negative significant correlation between grain yield and days to 50% emergence and days to 50% flowering was portrayed in the results. Khan et al. (2022b, c) reported some of these findings, revealing a positive correlation between grain yield with the traits seed width and length and a negative correlation with plant height.

4.7 Conclusion

The study aimed to determine the genetic variance components among BGN RILs to guide future selection. The performance of BGN RIL genotypes in three environments demonstrated a significant difference across the environments, as revealed by the ANOVA. The results showed that the traits' germination percentage (GP), plant height (PH), and grain yield (GY) were highly heritable as they demonstrated high and intermediate broad-sense heritability. However, the trait GP was associated with low genetic advance, a low phenotypic coefficient of variation (PCV), and a genotypic coefficient of variance (GCV). Predicting that the environment less influenced the trait. Plant height and GY were associated with strong genetic advance (GA), but only GY revealed high GCV and PCV. The traits GY displayed were intermediate heritability, coupled with high GA and GVC%. The traits GP, PH, PS, and GY were less influenced by the environment among the eleven evaluated traits, as guided by the ranks for the ratio of GCV/CVe and RD% results. The genotypes P3 (Dip-C), G23 (Tiga nicuru **x** Dip-C E463), G1 (Tiga nicuru **x** Dip-C E445), G8 (Tiga nicuru **x** Dip-C E478), LL2 (100SB16ANAMC), and G18 (Tiga nicuru **x** Dip-C E503) were the top six BGN with the highest grain yield guided by estimated BLUP values as compared to other BGN genotypes. Before being submitted for commercial registration, these genotypes should be recommended for further comprehensive evaluation. Further, the employment of marker-assisted selection could help to enhance choice efficiency and validate for identifying the optimum BGN features for improvement of this crop in the future.

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Chapter 5: General overview, summary, and conclusions

5.1 Introduction and objectives of the study

The world's agricultural techniques and food production are focused on a small number of crops, posing a severe risk to the food supply, more especially when you look at the impact of climate change, which is predicted to increase the frequency of extreme occurrences (Mayes et al., 2019). These challenges faced by farmers might be resolved by including a neglected and underutilized legume like the Bambara groundnut (BGN) in the food systems and commercial market. Incorporating the ignored and underutilised legume in the commercial market would considerably increase food availability and better-quality seeds for farmers.

Scouting the literature proved that BGN's value chain and production is impacted by a lack of processing facilities and non-improved commercialized BGN seeds (Mbosso et al., 2020). To date, farmers still depend on the landraces to produce BGN. Therefore, there is a need to improve and commercialize the neglected and underutilised BGN legume so it will be easily accessible and available to the farmers, with good seed quality. The 44 BGN recombinant inbred lines developed in Malaysia have not yet been evaluated in multi-environmental trials (MET) to assess the performance of the genotypes across different environments. This is why the RILs were evaluated in South Africa (KwaZulu Natal Province), to evaluate the performance of BGN RILs for selection of the best-performing BGN pure-lines.

The specific objectives of the study were to:

i. To evaluate the agro-morphological diversity of BGN recombinant inbred lines using quantitative and qualitative traits.
ii. To determine genetic variance components among BGN RILs to guide future selection.

5.2 Research findings in brief

Agro-morphological diversity of Bambara groundnut recombinant inbred lines evaluated across three environments in the KwaZulu-Natal province of South Africa

Fifty-four BGN genotypes comprising 44 RILs and ten checks were evaluated for their agro-morphological diversity using quantitative and qualitative traits. The study's first objective was to evaluate the agro-morphological diversity of BGN recombinant inbred lines using quantitative and qualitative traits. The following data were collected using BAMNET guideline (i) quantitative traits: days to 50% emergence (DTE), germination percentage (GP), days to 50% flowering (DTF), plant spread (PS), plant height (PH), seed length (SL), seed width (SW), shelling percentage (SHLP), grain yield per plant (GYPP), unshelled grain yield (UGY) and grain yield (GY); (ii) qualitative traits: growth habit (GH), terminal leaflet shape (TLS), terminal leaflet colour (TLC), stem pigment colour (SPC), and seed coat colour (SCC). The main findings of the study were as follows:

- Analysis of variance for eleven agro-morphological quantitative traits was highly significant ($P < 0.01$) different among BGN RILs for most traits except for the traits: days to 50% emergence, days to 50% flowering, and plant spread.
- The five evaluated qualitative traits were highly variable.
- Three growth habits, namely bunch (51.4%), spreading (45.5%) and semi-bunch type (3%) were identified.
- Multivariate analysis for eleven agro-morphological traits revealed the first three principal components (PC) with eigenvalues greater than one, accounting for 72% of the total variability (PC1 = 39%, PC2 = 19%, and PC3 = 14%).
- The principal component biplot showed a positive correlation between yield and yield-related traits, positively associated with PC1 (grain yield, unshelled grain yield, seed length, seed width, and shelling percentage).
- The two-way hierarchical cluster dendrogram categorized the 54 BGN genotypes into six morphological clusters.
- Cluster I comprised 18.5% of the populace, Cluster II 3.7%, Cluster III 12.9%, Cluster IV 20.3%, Cluster V 7.4%, and Cluster VI 37%, respectively.
- Eight BGN genotypes (P3, G23, G1, G8, LL4, G40, LL2, G13) were associated with yield and yield component traits in quadrant one of the PCA.

Selecting the best-performing improved seventh-generation Bambara groundnut (*Vigna subterranea* L.) genotypes

The study's second objective was to determine the genetic variance components among BGN RILs to guide future selection. The variance components estimate was computed using Deltagen, which runs a linear mixed model fitted by residual maximum likelihood (REML) using the package “lmerMod.” The main findings of this study were as follows:

- It was revealed that the overall total phenotypic coefficient of variance (PCV) values were higher than genotypic coefficients of variance (GCV) for the alternative traits.
- The trait grain yield had the highest PCV (69.4%) and GCV (44.8%).

- High broad sense heritability (H^2) values for the trait's germination percentage (65%) and plant height (61.44%) were observed.
- Moderate broad sense heritability values were recorded for the traits shelling percentage (H^2 = 47%), grain yield (H^2 = 41.65%), unshelled grain yield (H^2 = 41.60%), plant spread (H^2 = 38%) and seed length (H^2 = 35%).
- The GCV/CVe ratio findings indicated that genetic factors significantly influenced the germination percentage (1.25); it recorded the highest GCV/CVe ratio compared to the other ten agro morphological traits.
- According to Person's correlation matrix, there was a highly significant positive correlation between grain yield (kg/ha) and the following traits: (i) unshelled grain yield (r = 0.99); (ii) seed width (r = 0.61); (iii) grain yield per plant (r = 0.58); and shelling percentage (r = 0.40).
- As estimated by BLUP means, the top six BGN genotypes ranked based on yield performance are P3, G23, G1, G8, LL2, and G18.

5.3 Implications of the research findings

- The highly significant agro-morphological diversity observed amongst the BGN IRL will be the foundation of breeding features with a vast pool for different breeding goals.
- The traits with high broad sense heritability (germination percentage and plant height) estimate values can be targeted for direct selection, while the other traits can undergo indirect selection and be recommended for further molecular evaluation.
- BGN IRLs, with impressive yield, will significantly alleviate BGN's shortage of non-commercial seeds in the market.

5.4 Study limitations

The study had several limitations, the first of which was associated with other limitations, including the budget (funds). Due to project funds constraints, we could not perform soil sampling and testing. However, fertilizer application was applied based on soil history analysis and the fertilizer manufacturer's recommendations. A second limitation of the study, which had an association with research funds, was the use of two experimental sites (Ukulinga research farm and Cedara College of Agriculture) within KZN, with a total of three environmental trials; this limited the study to evaluate the 54 BGN genotypes in three environmental trials, limiting the genotypes to be evaluated in vastly different environments across provinces in South Africa. Sufficient funds would have allowed us to expand outside KZN province and evaluate the lines even in regions where BGN is consumed, e.g. Mpumalanga and Limpopo. However, with our limited number of seeds, we would only go for a maximum of 6 environmental trials. Hence, the trials were only replicated two times due to seed availability. During the harvesting approach, KZN experienced heavy storms and rainfall, which resulted in floods, making collecting other traits like days to maturity challenging since the crops immediately started to senesce and dry out.

5.5 Recommendations

- It is recommended that the genotypes be further assessed in multi-environmental trials (MET) outside KwaZulu Natal province to consider adaptation when the germplasm experiences a highly changeable environment with various rainfall, temperature, and other environmental factors.
- Furthermore, examining the nutritional content of the best-performing BGN genotype with high grain yield production is recommended.
- The BGN RILs should be analysed for molecular genetic diversity to comprehend the genetic variety of the lines and validate the morphological diversity of studied features.
- Farmers should evaluate the lines to constitute a part of the participatory varietal selection (PVS) to add their knowledge in selecting the suitable candidate they would prefer.

5.6 Conclusion

Using quantitative and qualitative parameters, this study successfully examined the agro-morphological variety of BGN recombinant inbred lines (RILs) and determined the genetic variance components across BGN RILs. The results demonstrated a considerable degree of genetic variety among BGN RILs, indicating a great potential for selection and enhancement. The study found critical features such as germination %, plant height, and yield traits like grain yield per plant, seed width, and seed length that might be targeted for future breeding projects. The findings of this work provide insights into the genetic makeup of BGN and display the potential of RILs as a valuable resource for crop variation. The results can guide future selection and breeding initiatives to strengthen the productivity and resilience of Bambara groundnut, subsequently contributing to sustainable food production and security in South Africa and regions where the crop is an essential source of nutrition.

5.7 References

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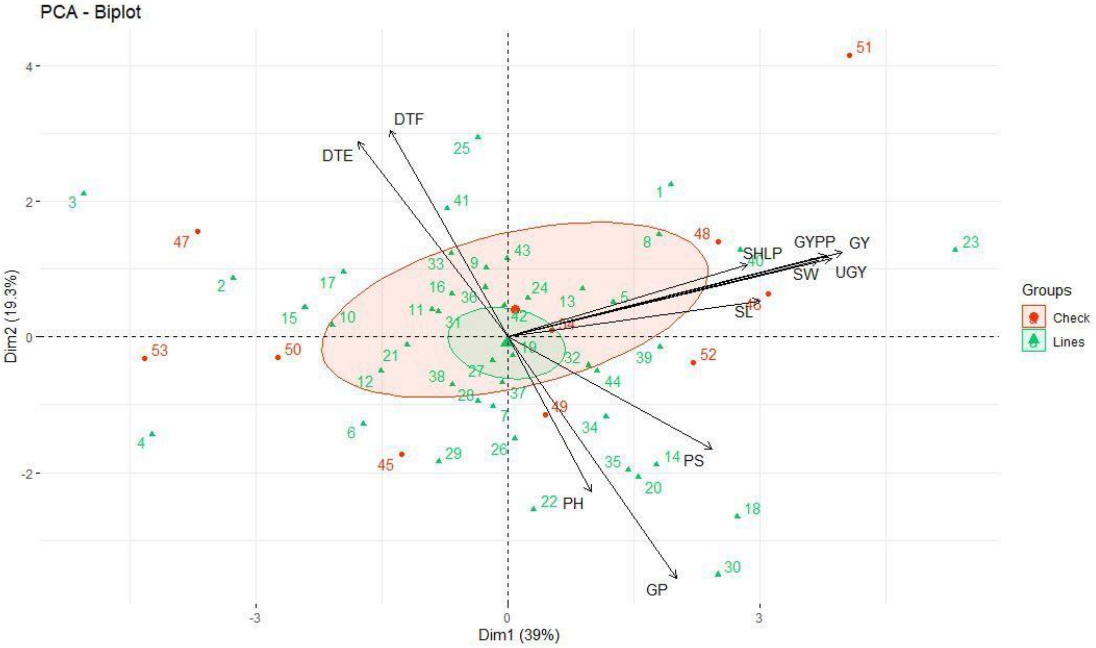
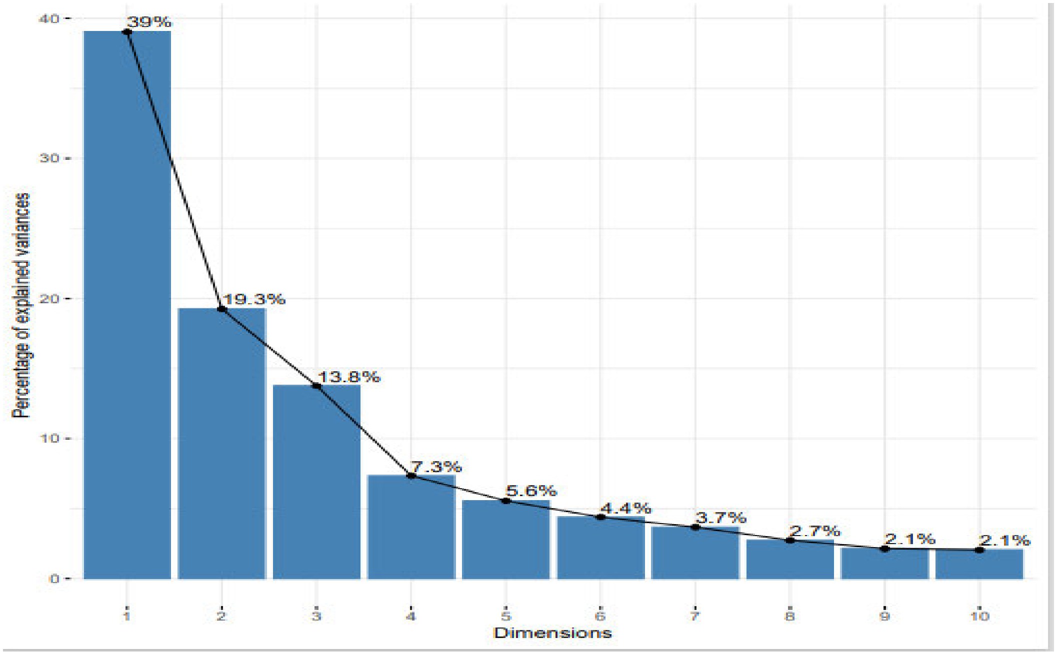
Appendix

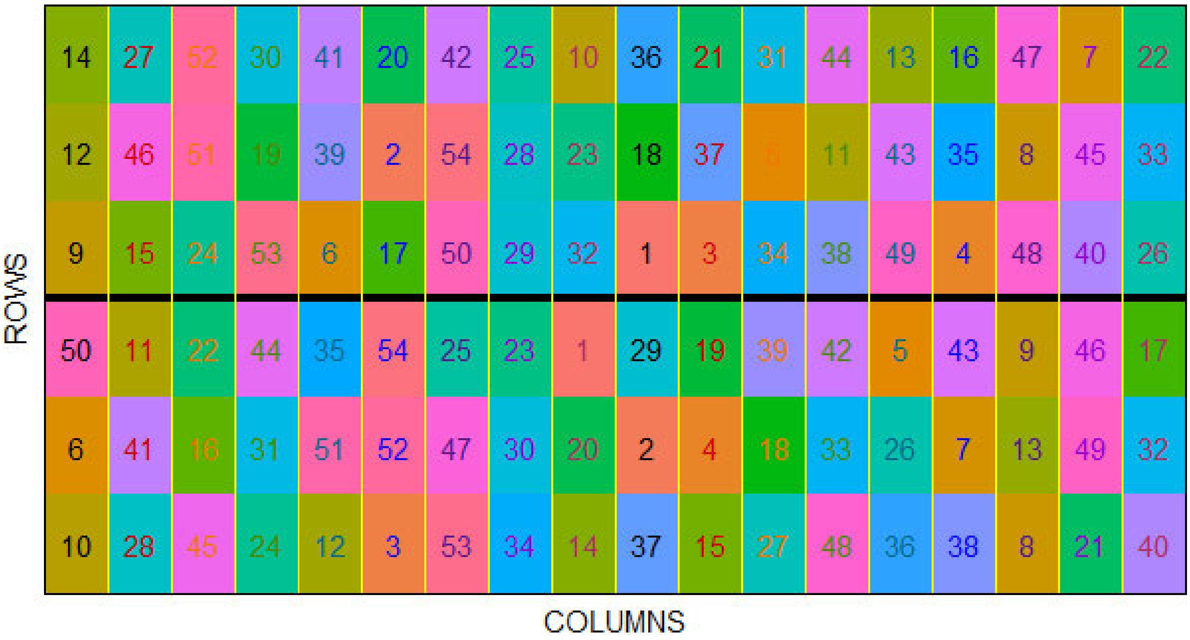


Appendix 1: PCA Biplot showing 44 Bambara groundnut RILs and ten landrace checks associated with eleven quantitative traits



Appendix 2: Scree plot explaining the percentage of variability among 11 quantitative BGN traits

Schematic drawing of Experimental Design



14	27	52	30	41	20	42	25	10	36	21	31	44	13	16	47	7	22
12	46	51	19	39	2	54	28	23	18	37	5	11	43	35	8	45	33
9	15	24	53	6	17	50	29	32	1	3	34	38	49	4	48	40	26
50	11	22	44	35	54	25	23	1	29	19	39	42	5	43	9	46	17
6	41	16	31	51	52	47	30	20	2	4	18	33	26	7	13	49	32
10	28	45	24	12	3	53	34	14	37	15	27	48	36	38	8	21	40

Appendix 3: Alpha lattice block design (6 x18)

Appendix 4: Descriptive statistics of eleven agro-morphological traits for 54 BGN genotypes

Traits	Minimum	Maximum	Mean	Std. Deviation	CV %
DTE	13.98 (G20)	14.25 (G36)	14.13	0.07	19.69
GP	34.01 (G3)	57.79 (G18)	46.60	4.85	45.14
DTF	55.88 (G18)	66.26 (G3)	60.44	2.02	7.95
PH	18.97 (LL3)	21.57 (G31)	20.54	0.58	13.34
PS	35.18 (P3)	38.88 (LL4)	37.31	0.83	21.71
GYPP	3.76 (G1)	17.66 (G23)	10.97	1.92	48.12
UGY	166.38 (G15)	944.37 (P3)	375.65	126.68	68.20
SL	10.47 (P2)	11.80 (G1)	11.24	0.30	9.07
SW	7.53 (G4)	8.36 (P3)	7.936	0.17	10.30
SHLP	50.54 (LL1)	61.42 (G25)	56.72	2.35	14.19
GY	93.56 (G15)	542.96 (P3)	216.80	75.21	67.48

GP: Germination percentage; **DTE**: Days to 50% emergence; **DTF**: Days to 50% flowering; **PH**: Plant height; **PS**: Plant spread; **SL**: Seed length; **SW**: Seed width; **UGY**: Unshelled grain yield; **GY**: Grain yield; **GYPP**: Grain yield per plant; **SHLP**: Shelling percentage.