Breeding Bambara Groundnut (*Vigna subterranea* (L.) Verdc) for Enhanced Yield and Nutritional Quality in South Africa

By

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Thesis Abstract

Bambara groundnut (*Vigna subterranea* (L.) Verdc.; 2n= 2x = 22) is a nutrient-dense grain legume cultivated in sub-Saharan Africa (SSA) and Asia. The current food systems in tropical and subtropical regions depend on the cultivation and use of a few commodity crop species. This causes most indigenous crop species, such as Bambara groundnut, to remain neglected by researchers and underutilised in the value chains. Underutilised crop species have received limited research and development attention from researchers and policymakers, and hence, their economic value, production methods, seed enterprises, product development and commercialisation are not yet fully explored. Due to a lack of systematic genetic improvement, the yield of most underutilised crops is low (<0.85 ton $ha^{-1)}$ and stagnant. Unlocking Bambara groundnut's economic and value-adding potential as an essential multipurpose food and cash crop will enhance food and nutritional security in developing countries. Research on Bambara groundnut in South Africa is relatively peripheral and there are no known improved varieties of this crop with high yield and nutritional quality. Therefore, specific objectives of this study were: (1) to document the progress made on Bambara groundnut production, utilisation and genetic improvement in SSA to discern the key production constraints, genetic resources and analysis, breeding methods and gains on yield and nutrition to guide breeding; (2) to assess the genotype-by-environment interaction (GEI) effect on grain yield and to select best adapted Bambara groundnut genotypes in South African target production areas for breeding; (3) to determine the compositions of phytochemicals and mineral elements present in Bambara groundnut genetic pool to identify superior and contrasting genotypes to guide product development and breeding; (4) to determine the magnitude of the genetic diversity and population structure of Bambara groundnut collections of South Africa using high throughput single nucleotide polymorphisms (SNP) markers to complement phenotypic and nutrition profile data for genotype selection and breeding; and (5) to determine the combining ability effects and gene action conditioning yield and related traits in Bambara groundnut genotypes to identify the best combiner donor parents and progenies for genetic advancement, cultivar development and release.

The first part of the study reviewed progress on Bambara groundnut production, utilisation and genetic improvement in SSA. The study presented key production constraints, genetic resources and analysis, breeding methods and genetic gains on yield and nutritional quality. Modern crop management, production technologies, and value chains are yet to be developed in Africa to achieve economic gains

from Bambara groundnut production and marketing. Improved crop management and post-harvest handling technologies, modern varieties with high yield and nutritional quality, value addition and market access are among the key considerations in current and future Bambara groundnut research and development programs. Information presented will guide sustainable production and effective crop breeding to pursue food and nutrition security and improved livelihoods through Bambara groundnut enterprises.

In the second chapter of the study, 75 Bambara groundnut genotypes were evaluated across seven selected environments using a 5 x 15 alpha lattice design with three replications. The study revealed significant ($p<0.05$) differences among genotypes (G), environments (E) and GEI effects on grain yield. A high proportion of the observed variation was due to GEI (36.62%), followed by environment (35.63%) and genotype (24.16%) effects. Grain yield across environments ranged from 1.4 ton ha^{-1} for ARC Bamb-68 to 0.10 ton ha^{-1} for ARC Bamb-74. Genotype ARC Bamb-68 (0.96 ton ha^{-1}), ARC Bamb-9 (0.88 ton ha^{-1}) and ARC Bamb-54 (0.84 ton ha^{-1}) attained the highest grain yield across locations, while ARC Bamb-74 exhibited the lowest grain yield of 0.16 ton ha^{-1}. The genotype and genotype-by-environment biplot identified ARC Bamb-17, ARC Bamb-14, ARC Bamb-20, ARC Bamb-18, ARC Bamb-14, and ARC Bamb-26 as the most stable genotypes across locations, while ARC Bamb-18 and ARC Bamb-54 were specifically adapted to Loskop and Brits. The Mafikeng site was ideal for Bambara groundnut evaluation, genotype differentiation, and large-scale seed production. The selected genotypes with high grain yields and stability are valuable genetic resources as breeding parents for Bambara groundnut improvement in South Africa.

In the third chapter of the study, 75 genetically diverse Bambara groundnut genotypes were field evaluated across four environments using a 15 x 5 alpha lattice design with three replications during the 2020-2021 cropping season. Genotypes were profiled for fat, phenolic and flavonoids contents at the Agricultural Research Council (ARC) analytical laboratory in South Africa. Further, the genotypes were assessed for the contents of the following minerals: calcium (Ca), iron (Fe), potassium (K), phosphorus (P), zinc (Zn) and nitrogen (N). The nutritional content of the test genotypes varied significantly ($P<0.05$), which were affected by the genotype and environment interactions. The Ca, Fe, K and Zn content varied from 150.70 to 216.53, 4.30 to 16.77, 771.99 to 1155.89 and 5.50 to 7.17 mg.100 g^{-1} dry seed sample, respectively. Genotypes, including ARC Bamb-2, ARC Bamb-19, ARC Bamb-73, ARC Bamb-56, ARC Bamb-37, ARC Bamb-3 and ARC Bamb-69 exhibited the highest fat content (>6.00 %). ARC Bamb-40 and ARC Bamb-59 recorded a higher mean Fe content of 16.00

mg.100 g^{-1}. ARC Bamb-2 was the top-performing genotype with high fat content (6%), Ca (211.93 mg.100 g^{-1}), and Zn (7.17 mg.100 g^{-1}). Ca, K, and N contents displayed strong correlations ($r>0.60$, $P<0.05$). Phosphorus and Zn contents exhibited moderate correlations with Ca. Overall, the study selected genotypes ARC Bamb-73, ARC Bamb-19, ARC Bamb-9 and ARC Bamb-2 with high compositions of essential nutrients for product development or breeding. The selected genetic resources are valuable for trait integration and developing new breeding populations with enhanced nutrient compositions and agronomic and market-preferred traits.

In the fourth part of the study, the magnitude of the genetic diversity and population structure of South Africa Bambara groundnut collections was determined using high throughput single nucleotide polymorphisms (SNP) markers. Ninety-three genotypes were genotyped with 2286 SNP markers and phenotyped with some unique complementary morpho-agronomic traits of the crop. The mean genetic diversity value was 0.32, revealing moderate genetic differences among the assessed genotypes. Cluster and structure analyses grouped the tested genotypes into two distinct categories. Further, the analysis of molecular variance partitioned the total genetic variation into among genotypes (90%), within genotypes (8%) and among populations (2%). The results revealed two heterotic groups for hybridisation and selection programs. The following unique genotypes were selected: ARC Bamb-37 (with spreading growth type), ARC Bamb-49 (bunch type), ARC Bamb-61 (semi-bunch) and ARC Bamb-83 (spreading) using the SNP markers and desirable agronomic traits. The study provided new insight on Bambara groundnut genetic profiles of South African collections, which will assist in conservation strategy and management of the crop for effective breeding.

The final part of the study assessed combining ability effects and gene action conditioning yield and related traits in Bambara groundnut genotypes to identify the best combiner donor parents and progenies for genetic advancement and breeding. Ten contrasting parents were selected and crossed using a 10 × 10 half-diallel mating design, and 45 progenies developed. The progenies and their parents were field evaluated using a 5 × 11 alpha lattice design with two replications in two contrasting locations in South Africa. Data was collected on agronomic traits and subjected to statistical analyses to compute genetic parameters. Genotype × location interaction effect was significant ($P < 0.05$) for the studied agronomic traits. General combining ability (GCA) and specific combining ability (SCA) effects were significant in most assessed agronomic traits, including yield per plant. The GCA × location and SCA × location interaction effects were significant for most traits. A Baker's ratio of < 1 were recorded for most assessed traits, indicating the preponderance of non-additive gene effects conditioning the traits. The parental lines such as ARC Bamb-25, ARC Bamb-8 and ARC Bamb-55 recorded positive and desirable GCA effects for yield per plant. The progenies ARC25×ARC8,

ARC44×ARC9 and ARC6×ARC9 had desirable SCA effects for yield per plant, ARC44×ARC8, ARC44×ARC68, ARC42×ARC8 for higher number of secondary branches per stem, ARC25 ×ARC8 for early maturity, ARC42×ARC55 for higher number of pods per plant and ARC42 ×ARC57 for increased seed width. The new families selected in the current study are useful breeding populations and will be subjected to selection and multilocation evaluation to release the best-performing varieties.

Overall, the present study appraised the present production constraints, genetic resources and analysis, breeding methods and genetic gains on yield and nutritional quality to guide future breeding. Moreover, new Bambara groundnut breeding populations were developed with enhanced yield and nutritional compositions for genetic advancement and multilocation selection for variety release and adoption in South Africa.

Declaration

I, Nomathemba Gloria Majola, declare that:

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other University.
3. This thesis does not contain other persons' data, pictures, graphs or other Information, unless specifically acknowledged as being sourced from other persons.
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Signed

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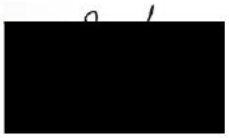

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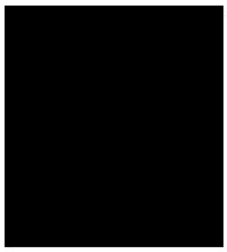

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I would like to thank God for his immense grace in my life and for granting me the opportunity to complete my studies.

Dedication

This thesis is dedicated to my late grandmother, Matshidiso Majola, I know you would have been proud as you began this journey with me.

Table of contents

ABBREVIATIONS

ARC	Agricultural Research Council
AMMI	Additive main effects and multiplicative interaction
ANOVA	Analysis of variance
CV	Coefficient of variation
DF	Degrees of freedom
DM	Days to 75% maturity
GCA	General combining ability
G×E	Genotype x environment interaction
GGE	Genotype main effects and genotype × environment interaction
H^2	Broad sense heritability
h^2	Narrow sense heritability
ha^{-1}	Per hactare
HSW	100 seed weight
IPCA	Interaction principal component analysis
LSD	Least significant difference
N	Nitrogen
NSB	Number of secondrary branches
NPP	Number of pods per plant
NSP	Number of seeds per pod
PCA	Principal component analysis
R^2	Coefficient of determination
SCA	Specific combining ability
SNP	Single nucleotide polymorphism
SSA	Sub-Saharan Africa
UKZN	University of KwaZulu-Natal

Introduction to thesis

Background

Bambara groundnut (*Vigna subterranea* [L.] Verdc.; 2n= 2x = 22) is a grain legume rich in dietary nutrients and cultivated in sub-Saharan Africa (SSA) and Asia. The crop is mainly under-researched and underutilised. It is regarded as a complete food with a high potential to alleviate food and nutrition insecurity (Soumare et al. 2022). The grains have balanced nutrient compositions including the following macro-nutrients : nitrogen (17000 - 26000), phosphorus (81 - 563), potassium (1545 - 2200), and micro-nutrients such as magnesium (32 - 335), calcium (30 - 128), iron (2 - 9) and zinc (11 - 40 per 100 g dry sample grain) (Maphosa et al. 2022; Chelangat et al. 2023). Also, the grains contain carbohydrates (~72%), protein (~25%), fibre (~ 6.3%), and oil of ~7% (Ibny et al. 2019). Thus, the crop can contribute significantly to nutrition security, sustainable food systems and cash income through novel product development and commercialisation strategies (De Valença et al. 2017).

The current crop production systems in tropical and subtropical regions depend on the cultivation and use of a few commodity crop species. This causes most indigenous crop species remain neglected by researchers and underutilised in the value chains of each crop. The underutilised crop species such as Bambara groundnut (*V. subterranean* L.), groundnut (*Arachis hypogaea* L.) and cowpea (*Vigna unguiculata* L.) have unique benefits in food, nutritional and health security to humankind (Alhassan et al. 2013). Furthermore, underutilised crop plant species have a long agricultural history and are vital components of smallholder farming systems. Underutilised crops are considered as future food security crops (Hillocks et al. 2020). These species have intrinsic quality attributes and resilience to biotic and abiotic stresses associated with climate change. They have adapted and coevolved under the prevailing farming and environmental conditions, pest and disease pressure and low-input farming systems in Africa. However, underutilised crop species have received limited research and development attention from researchers and policymakers, and hence, their economic value, production methods, seed enterprises, product development and commercialisation are not yet fully explored (Akpalu et al. 2015). Due to a lack of systematic genetic improvement, the grain yield of most underutilised crops is low (<0.85 t ha^{-1}) and stagnant compared to the limited number of food security crop species such as rice, maize, wheat, barley and sorghum.

Annual production of Bambara groundnut is estimated to be 0.2 million tonnes from an area of 0.25 million hectares worldwide (FAOSTAT, 2022). Sub-Saharan Africa is the largest producer of Bambara groundnut, while a small quantity is produced in Southeast Asia (e.g. Thailand and Indonesia), the United States of America (USA) and Australia. West Africa is the main Bambara groundnut production region in SSA, where Burkina Faso, Niger and Cameroon are the leading producers contributing to 74% of world's production (FAOSTAT, 2022).

Bambara groundnut is popularly cultivated and marketed in southern Africa including Swaziland, Botswana, Zimbabwe and South Africa. Hillock et al. (2020) estimated market supply of 1800 - 4200 tonnes in 2020 in South Africa, with a substantial amount imported from Zimbabwe. In southern Africa, Zimbabwe is the largest exporter of Bambara groundnut, contributing about 2000 - 3000 tonnes per year to the Southern African countries. Bambara groundnut is a relatively prominent legume crop in Botswana, cultivated over an estimated area of 1500 hectares with a total grain production of about 400 tonnes since 2006 (Khan et al. 2020). The main Bambara groundnut producing provinces in South Africa include Limpopo, Mpumalanga, North West, Gauteng and KwaZulu-Natal. Most production is undertaken by smallholder farmers for food and income generation (Gbaguidi et al. 2018).

Bambara groundnut farmers use farm-saved seeds of landraces, which is often poor-quality and low-yielding (Sedibe et al. 2020). The seed is acquired from the informal sector via farmer-to-farmer seed exchange or local markets. Unlike the traditional legume crops (e.g. common bea, groundnut and soybean), there is limited formal Bambara groundnut breeding and improved seed delivery systems in SSA, hindering production and productivity of the crop (Ibrahim et at. 2018). Value-added products are yet to be developed to enhance human nutritional benefits and market and investment opportunities from Bambara groundnut enterprises.

Genetically diverse Bambara groundnut collections are available in SSA for pre-breeding and breeding programs. For instance, wild relatives of the crop have been reported in northeastern Nigeria and northern Cameroon, which are regarded as the centres of diversity for the crop. The International Institute of Tropical Agriculture in Nigeria has maintained about 2000 collections of Bambara groundnut. Various national and international research programs in Africa and Southeast Asia have been actively involved in the breeding,

selection, and genetic advancement of novel Bambara groundnut lines. This has resulted in the release of few varieties, such as 'Mana' and 'Kazuma' in Zimbabwe in 2004 (Mayes et al. 2019a). Bambara groundnut populations derived from the variety Kazuma as founder parent enabled the development of a draft Bambara groundnut genomic sequence (Mayes et al. 2019a).

The primary goal of Bambara improvement programs focuses on grain yield and nutritional quality traits. The nutritional benefits of underutilised food crops such as Bambara groundnut is an economic and affordable way to decrease malnutrition in Africa (Harris et al. 2018). Integrating conventional breeding with molecular breeding techniques can fast-track cultivar development and deployment in Bambara groundnuts. Next-generation sequencing (NGS) and genotype-by-sequencing (GBS) technologies have reduced genotyping costs drastically, making these technologies the most feasible for the development of molecular markers and genotyping (Bhat et al. 2016; Zou et al. 2016). Sequencing-based diversity array technology (DArTseq) has assisted in identifying haplotype blocks or single nucleotide polymorphism (SNP) signatures that are significantly correlated with quantitative trait variations (Valdisser et al. 2017). These markers have been broadly sutilised in plant breeding research, including genetic differentiation, the development of dense genetic and physical maps and population structure analyses. Knowledge of genetic diversity among Bambara groundnut genotypes provides baseline genetic information to assemble a genetic pool for breeding and utilisation in South Africa.

Rationale of the study

In Southern Africa, micronutrient deficiency, usually referred to as the 'hidden hunger', is a major problem, while stunted growth remains the most common nutritional disorder affecting marginalised communities. There is rampant malnutrition due to over-reliance on starch-based cereal porridges, tubers and roots as staple food crops. Providing nutritious food for the poor and undernourished populations has been a major challenge for the developing world, and acute shortage, unreliable supply, and elevated costs of protein-rich foods of animal origin in developing and underdeveloped countries have resulted in the search for inexpensive and reliable alternative sources of minerals and proteins of plant origin. However, despite its importance as a source with good nutritional benefits, Bambara groundnut is still a neglected, underutilised, under-researched crop that is only produced in

some parts of South Africa. Unlocking this crop's economic and value-adding potential as an essential multipurpose food and cash crop will enhance food and nutritional security in developing countries.

Research on Bambara groundnut in South Africa is relatively peripheral and there are no known improved varieties of this crop with high yield and nutritional quality. The pre-breeding efforts involving Bambara groundnut in South Africa have been directed towards the selection of high-yielding genotypes and identifying anti-nutritional factors (Unigwe et al. 2016; Mohale et al. 2014; Amara et al. 2018). Previous studies used a minimal number of genotypes tested in limited environment (Ahmad et al. 2017; Oludare et al. 2017; Absattar et al. 2018). Therefore, there is an urgent need to bolster the pre-breeding and breeding of the crop to develop high-yielding and nutritionally enhanced new varieties with desired traits.

Overall research goal

The overall goal of this study was to develop improved Bambara groundnut genotypes with increased yield and nutritional traits for sustainable production in South Africa

Specific objectives

The specific objectives of this study were to:

I. To document the progress made on Bambara groundnut production, utilisation and genetic improvement in SSA to discern the key production constraints, genetic resources and analysis, breeding methods and genetic gains on yield and nutrition-related traits to guide breeding.
II. To assess the genotype-by-environment interaction (GEI) effect on grain yield and to select best adapted Bambara groundnut genotypes in South African target production areas for breeding and cultivation.
III. Determine the compositions of phytochemicals and mineral elements present in Bambara groundnut genetic pool to identify superior and contrasting genotypes to guide product development and breeding.
IV. Determine the magnitude of the genetic diversity and population structure of Bambara groundnut collections of South Africa using high throughput single

nucleotide polymorphisms (SNP) markers to complement phenotypic and nutrition profile data for genotype selection and breeding.

V. Determine the combining ability effects and gene action conditioning yield and related traits in Bambara groundnut genotypes to identify the best combiner donor parents and progenies for genetic advancement, cultivar development and release.

Research hypotheses

The hypotheses of this study included the following:

I. The current review will identify key constraints to Bambara groundnut production and effective breeding methods on yield and nutritional quality to guide crop improvement.

II. Changes in the environment affect the performance of the Bambara groundnut genotypes, and that can be exploited to identify genotypes with broad or specific adaptation.

III. There is an adequate genetic variation in phytochemicals and mineral elements among the Bambara groundnut genotypes for parental selection.

III. There is extensive genetic diversity among selected Bambara groundnut genotypes revealed by SNP markers to provide a broad genetic base for selection.

IV. The selected Bambara groundnut parents and their progenies show good combining ability for yield, early maturity and pod width to guide variety development and release.

Thesis outline

This thesis comprises five chapters, developed according to the objectives set above. Chapter 1 is written as a separate review paper, while Chapters 2 to 5 are written as discrete research papers, each following a stand-alone research paper format. The literature review and the four experimental chapters of the study are combined into thesis chapters that are discrete but interdependent papers according to the University of KwaZulu-Natal's dominant thesis format. As such, some unavoidable repetitions of references and introductory information among chapters exist. Chapter 1 was published in Agronomy Journal, 11(7), 268-289, DOI: https://www.doi.org//10.3390/agronomy11071345; Chapter

2 was published in the South African Journal of Botany, 150, 1061-1068, DOI: https://www.doi.org//10.1016/j.sajb.2022.09.008 .

The outline of the thesis is, therefore, as follows:

1. Thesis introduction
2. Chapter One: Review of the Literature
3. Chapter Two: Genotype by environment interaction effects and stability analysis of Bambara groundnut (*Vigna subterranea* [L.] Verdc) for grain yield in South Africa.
4. Chapter Three: Analyses of the compositions of phytochemicals and minerals in Bambara groundnut genotypes.
5. Chapter Four: Genetic diversity and population structure analyses of South African Bambara groundnut (*Vigna subterranea* [L.] Verdc)
6. Chapter Five: Combining ability and gene action in Bambara groundnut (*Vigna subterranea* (L.) Verdc.) genotypes for agronomic traits.

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CHAPTER 1. Bambara groundnut (*Vigna subterranea* [L.] Verdc) production, utilisation and genetic improvement in sub-Saharan Africa

Abstract

Bambara groundnut (*Vigna subterranea* [L.] Verdc) is nutritionally rich grain legume crop indigenous to Africa. It is tolerant to drought stress and adapted to grow under low input and marginal agricultural production systems in Africa and Asia. Bambara groundnut is an orphan crop, which is underutilised and under researched plant genetic resource. Modern crop management practices, production technologies, and value chains are yet to be developed in Africa to achieve economic gains from Bambara groundnut production and marketing. In sub-Saharan Africa (SSA) the production and productivity of Bambara groundnut is low and stagnant because of diverse abiotic and biotic stresses and socio-economic constraints. Improved crop management and post-harvest handling technologies, modern varieties with yield and nutritional quality, value addition and market access are among the key considerations in current and future Bambara groundnut research and development programs. This paper presents progress on Bambara groundnut production, utilisation and genetic improvement in SSA. It presents the key production constraints, genetic resources and analysis, breeding methods and genetic gains on yield and nutritional quality and outlook. Information presented will guide sustainable production and effective breeding of the crop to pursue food and nutrition security, and improved livelihoods through Bambara groundnut enterprises.

Keywords: Bambara groundnut, breeding methods, genetic resources, nutrition security, Southern Africa, underutilised crops

1.1 Introduction

Lack of modern agricultural production technologies and improved varieties are among the main factors affecting crop production and leading to food insecurity and impoverished livelihoods in sub-Saharan Africa (SSA). A larger proportion of essential human food is derived from only some 10 crop plant species (Oerke, 2012). Most nutritionally rich and unique hidden crop genetic resources are yet to be used, explored and promoted for human wellbeing and a niche market.

A restricted number of commercial crop species are cultivated and used in the tropical and subtropical areas of existing agricultural production systems. As a result, most native agricultural species continue to be underutilized in each crop's value chain and ignored by research and development initiatives. Underutilized crop species, including cowpea (*Vigna unguiculata* L.), groundnut (*Arachis hypogaea* L.), and Bambara groundnut (*Vigna subterranea* [L.] Verdc), offer unique advantages for the world's food, nutrition, and health security (Alhassan et al. 2013). Moreover, underutilized crop plant species are valuable parts of smallholder farming systems with a long agricultural history and are anticipated to be important crops for food security in the future. These species are resilient to biotic and abiotic challenges related to climate change and possess innate qualities that make them good food. They have adapted and coevolved under the prevailing farming and environmental conditions, pest and disease pressure and low-input farming systems in Africa. However, because underutilized agricultural species have not recived enough attention from researchers and policymakers for research and development, their economic importance, production techniques, product development, and commercialization have not yet been thoroughly studied (Akpalu et al., 2015). In contrast to the limited number of food security crop species such as rice, maize, wheat, barley, sorghum, etc., the grain yield of the majority of underutilized crops is low (<0.85 t ha^{-1}) and static as a result of a lack of systematic genetic improvements (Alhassan et al. 2013).

Bambara groundnut (2n = 2x = 22) is one of the grain legume crops indigenous to Africa. Bambara groundnut is known by different names in SSA. For instance, it is referred to as earth pea, jugo bean, nyimo beans or ditloo in Southern Africa. It is believed to have originated from Timbuktu areas in central Mali, West Africa (Nandini, 2019). However, the primary centre of genetic diversity of Bambara groundnut is the north-eastern region of

Nigeria and northern Cameroon. Also, a secondary centre of diversity exists outside Africa including Sri-Lanka, Malaysia, Philippines and India (Unigwe et al.2018). It is predominantly self-fertilizing crop with cleistogamous flowers. It is cultivated extensively by small-scale farmers in the drier regions of sub-Saharan Africa (SSA) under the

The grain of Bambara groundnut is regarded as a source of complete and balanced diet. It contains carbohydrates (51-71%), crude protein (18-24%), oil (4-12%), fibre (3-12%), and ash (3-12%) (Mayes et al. 2019b). Furthermore, it has essential and non-essential amino acids at 32.72% and 67.28%, respectively per 100-gram grain (Gonné et al.2013). Lysine is the major essential amino acid (10.3%) present in the grain. Due to higher protein and amino acid contents, Bambara groundnut is an ideal food to complement most cereal-based diets in SSA (Alake and Ayo-Vaughan, 2017). The total energy gains from Bambara groundnut grain consumption is the highest compared with other legumes such as cowpea (*V. unguiculate* L.), pigeon pea (*Cajanus cajan* L.) and lentil (*Lens culinaris* L.) (Haleegoah et al.2015). Bambara groundnut grain possess some anti-nutritional factors (ANFs) that may limit bioavailability. Hence, variety selection with low ANFs, grain soaking and cooking, and optimal consumption ensure nutritional benefits (Unigwe et al., 2018).

Bambara groundnut grain is consumed fresh or roasted and served as snack when young and immature. During maturity, the grain develops a hard seed coat hence it has to be boiled prior to processing and consumption. In Benin, the grains are processed into flour to make bread, cake and dumpling. In Eastern African countries, the crushed grains are used to prepare soup. Bambara groundnut flour is also processed to make bread in Zambia (Cook, 2017). In South Africa and Swaziland, the grains are utilised to add flavour in boiled cowpea grain (Mubaiwa et al.2017). It is also processed to produce milk similar to soybean. The derived milk is used as the weaning food in several African countries. Bambara groundnut milk has lighter colour compared with soybean milk (Yao et al. 2015). The biomass (stem, leaves and the haulm) has been extensively used for livestock feed. Also, the seed cake is used for livestock feed, while the grains are fed to pig and poultry. Young and succulent leaves contain essential mineral elements including nitrogen and phosphorus useful for animal health (Keller, 2004). In Nigeria, tilapia fish are fed with Bambara groundnut leaf protein (Mazahib et al.2013).

Bambara groundnut is cultivated in altitudes ranging from of 1100 m to 1600 m above sea level with mean temperatures of 20 to 28C^{o} (Azam-Ali et al.2014). The crop is a relatively

drought tolerant and requires limited agricultural inputs for production. It thrives with limited rainfall and under poor soil fertility conditions where many crop species would fail to produce (Dansi et al.2012). It requires 110 to 150 days for growth and physiological maturity depending on the genotype and environment. Bambara groundnut prefers well drained soils. Sandy or loamy soil with pH ranging from 5.0 to 6.5 for its establishment, high pod yields and ease of harvest (Figure 1.1A). Unshelled yields of 500 to 800 kg ha^{-1} were reported under poor soil conditions with reduced nutrients and without the use of inorganic fertilisers. This is attributed to its ability to fix atmospheric nitrogen through a symbiotic association with a *Rhizobium* bacteria (Singh et al. 2016). Bambara groundnut is grown as a cover crop to protect the topsoil from erosion and to supress weed infestations, making it significant for crop rotation with major cereal crops (Azam-Ali et al.2014).

Bambara groundnut comprises of a highly established tap root system and highly branching stem bearing numerous horizontal branches, where the leaves arise. The leaf consists of an extensive petiole with green or purple base. It has irregularly developing flowers that arise from an elongated peduncle beneath the soil. The harvestable yield of Bambara groundnut is a matured unshelled pod (Figure 1.1B) developed underground. The pod is harvested after the plants are dug out of the soil. In plants with a bunched growth type, the pod remains attached to the root crown and manually harvested.

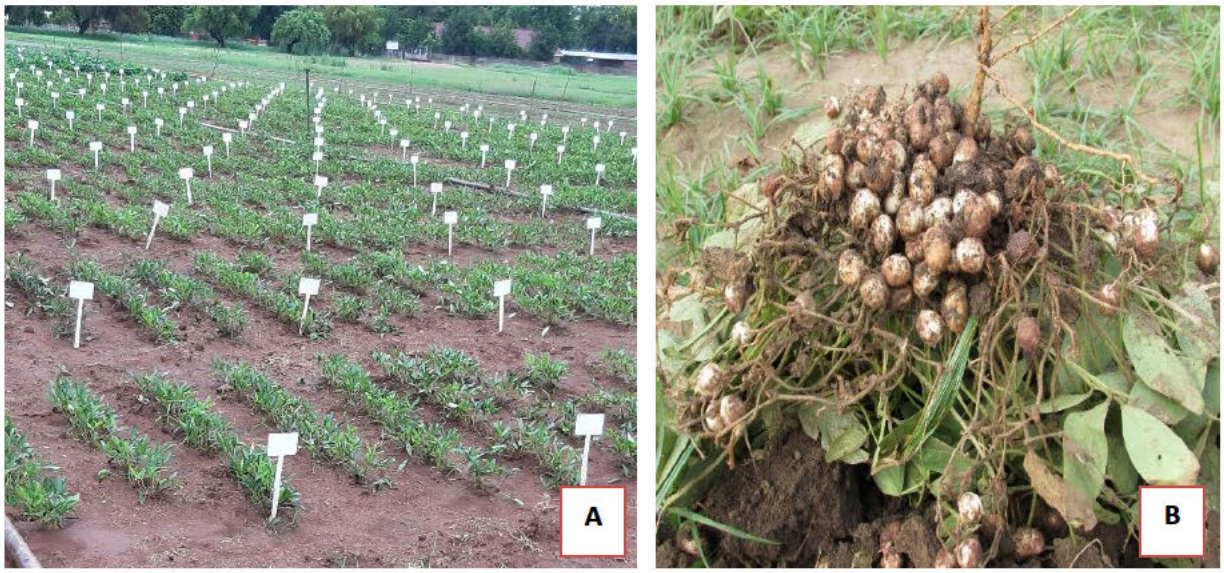

Figure 1.1 Bambara groundnut: (A) field established crop stand at the Loskop site and (B) uprooted plant with matured pods at the Roodeplaat research site, South Africa.

Bambara groundnut pods are formed underground spanning some 4 cm deep in the soil. Some Bambara groundnut genotypes bear single seed per pod, while others can produce two to three seeds. The pods are formed in a clump (Figure 1.1B) with yellow or dark red colour. The grain colour is diverse including cream, yellow, brown, red, purple and black (Mkandawire, 2017).

The International Institute of Tropical Agriculture (IITA) maintains the majority of Bambara groundnut germplasm accessions collected from various SSA countries (Smýkal et al.2015). In SSA, the crop is still cultivated using genetically unimproved landraces. There is a need for a concerted research effort on germplasm assembly, evaluation, product profiling, variety design and development. Bambara groundnut has not been fully explored, which is key to utilise the available genetic variation for breeding and cultivar development.

Despite the various nutritional and economic of the crop, Bambara groundnut is one of the underutilised and under researched plant genetic resources. Modern production technologies and value chains are yet to be developed in Africa to achieve economic gains from Bambara groundnut production, product development and commercialisation. There is limited research and development efforts globally on Bambara groundnut. The average) grain yield of the crop is 0.85 t ha^{-1} despite a potential yield of over 3 t ha^{-1} (Mayes et al.2019a). The main yield limiting factors include a lack of improved cultivars with high

yield and resistance to insect pests and diseases. In most SSA countries, the crop is cultivated using unimproved varieties or landraces that were developed through mass selection from wild relatives. There are no released cultivars emanated from modern breeding programs in SSA. Improving the production methods, yield levels, nutritional quality, processing and marketing of Bambara groundnut will enhance food and nutrition security, and improve livelihoods and contributes to economic development in SSA. In light of the above background, the aim of this paper was to document the progress made on Bambara groundnut production, utilisation and genetic improvement in SSA through highlighting the key production constraints, genetic resources and analysis, breeding methods and gains on yield and nutrition. Information presented in this paper will guide production and genetic improvement of the crop to pursue food and nutrition security, and improved livelihoods from Bambara groundnut enterprises.

1.2 Production status of Bambara groundnut

Total global Bambara groundnut production was estimated at 0.23 million tonnes in the 2022 production year (FAOSTAT, 2022). A larger quantity of Bambara groundnut is produced by West African countries with a total production of 0.18 million tonnes in 2022 from an estimated area of 0.19 million hectares (FAOSTAT, 2022). The top three world Bambara groundnut producing countries are Cameroon, Niger and Burkina Faso, and (Table 1.1).

Table 1.1: The top six Bambara groundnut-producing countries and corresponding total production in 2022 globally (FAOSTAT, 2022).

	Countries						
Variable	Cameroon	Niger	Burkina Faso	Mali	Democratic Republic of the Congo	Togo	**World**
Total production (million tonnes)	0.065	0.053	0.044	0.04	0.026	0.014	**0.230**
Area harvested (ha)	49049	45640	40640	37329	35043	27819	**65325**

Yield (million tons/ha)	0.098	0.088	0.086	0.074	0.070	0.064	**0.074**

In SSA Bambara groundnut is mainly produced by small-scale farmers across fragmented and remote farm lands. Most production areas are inaccessible to collect production and productivity data. This makes it challenging to accurately assess the total production and size of production areas in SSA. The yield level of Bambara groundnut in Africa varies from 0.6 - 1 t ha^{-1} depending on variety and production conditions (Smýkal et al.2015). However, unshelled mean yields of up to 3 t ha^{-1} was reported when cultivating some landraces in the transition agro-ecological zone in Nigeria (Jørgensen et al. 2010). Low mean yields of 0.85 t ha^{-1} was reported in Ghana under good management practices close to yield levels of other legumes such as cowpea (0.80 t ha^{-1}) and pigeonpea (0.78 t ha^{-1}) (Ayamdoo et al. 2013). There is emerging need for Bambara food products due to population growth, climate change, urbanisation and life style changes in SSA. Presently, there is limited market supply of Bambara groundnut unlike other legumes such as groundnut (Keller, 2014).

Figure 1.2 presents Bambara groundnut production status. The productivity of the crop in SSA has remained stagnant and low that ranged from 0.65 t ha^{-1} to 0.78 t ha^{-1} from 1999 to 2018. This was mainly due to low yield gains associated with the use of low yielding varieties and poor-quality seed. Higher productivity (0.9 t ha^{-1}) was achieved in 1999 (Figure 1.2) notably through use of better management practices (Karunaratne et al. 2015). The production trend exhibited a significant improvement with 10% increase in area harvested and 8% increase in yield from 2012 to 2018 (Figure 1.2). This was mostly attributed to increased area of production and the favourable dought and heat tolerant attributes of the crop. The area of production has increased owing to its wide acceptance by the farmers and the market place signalling a higher scope for the expansion of Bambara groundnut production in SSA (FAOSTAT, 2020).

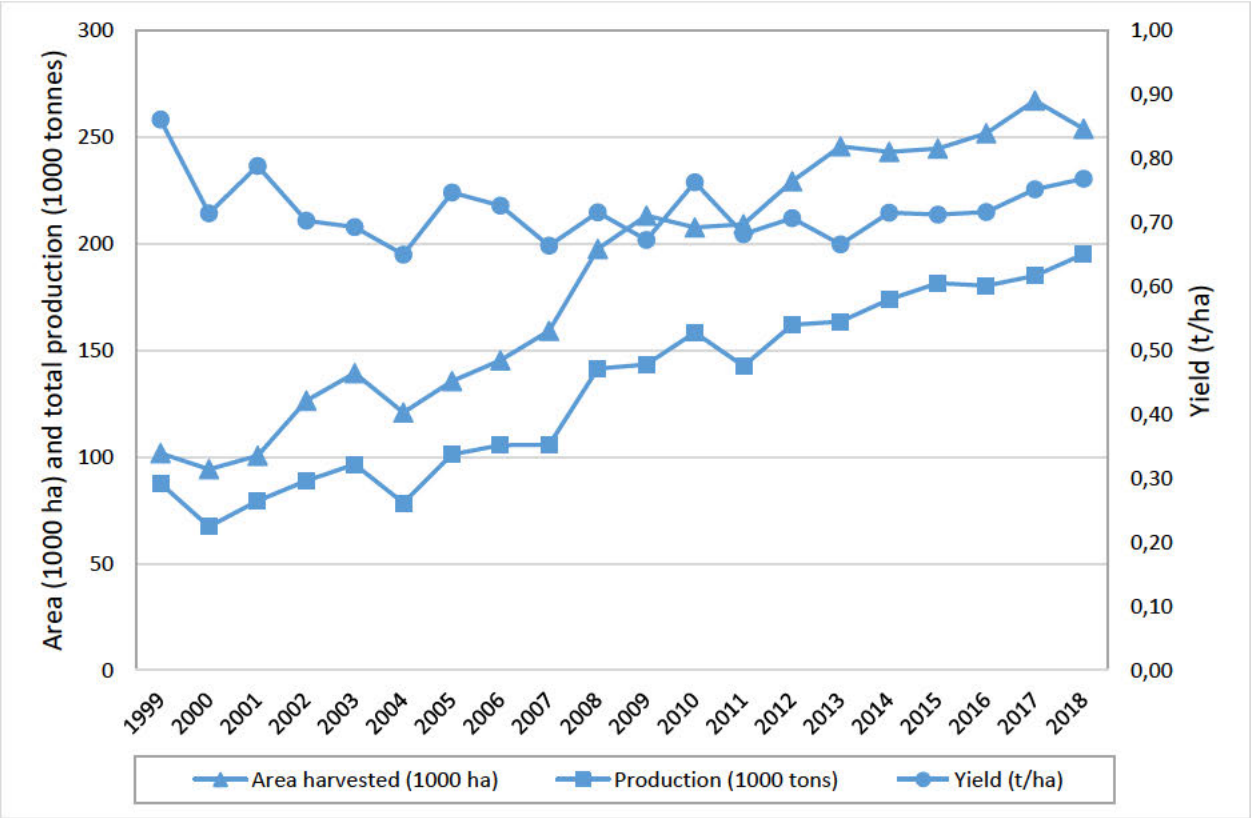

Figure 1.2 Total production (1000 tonnes), area of production (1000 ha) and yield (t/ha) of Bambara groundnut in sub-Saharan Africa during 1999 to 2018 (FAOSTAT, 2020).

Bambara groundnut is popularly cultivated and marketed in southern Africa including Swaziland, Botswana, Zimbabwe and South Africa. Hillock et al.(2012) estimated market supply of 1500 - 4000 tons in 2005 in South Africa with a substantial amount imported from Zimbabwe. In southern Africa, Zimbabwe is the largest exporter of Bambara groundnut contributing to about 200 - 3000 tons per year to the southern African countries. Bambara groundnut is relatively the prominent legume crop in Botswana cultivated over an estimated area of 1500 ha with a total grain production of about 400 tons since 2006 (Khan et al. 2020). In South Africa, the main Bambara groundnut producing provinces include Limpopo, Mpumalanga, North West, Gauteng and KwaZulu-Natal. Most production is undertaken by smallholder farmers for food and income generation (Gbaguidi et al.2018).

1.3 Constraints to Bambara groundnut production

Productivity of Bambara groundnut is affected by a number of production challenges (Alake and Ayo-Vaughan, 2017). The leading production constraints include biotic stresses (e.g.

diseases caused by fungi, bacteria, and viruses, insect pests and nematodes), abiotic stress (e.g. drought, extreme temperatures and poor soil fertility*)* and socio-economic factors (Sesay et al. 2018). The key production constraints of the crop are outlined below.

1.3.1 Biotic stresses

Field insect pests and diseases affect Bambara groundnut production. Bambara groundnut pods are developed in the soil (Figure 1.1), hence it is self-protected from aerial insect pests and diseases when compared with other common legumes. Pathogenic fungi, viruses, insect pests, nematodes and weeds inflict yield loss in Bambara groundnut (Zongo et al.2018).

1.3.1.1 Pathogenic fungi

Several fungal pathogens attack Bambara groundnut crops under dry and humid conditions. Fungal diseases of the crop in SSA comprises of the leaf spot caused by *Cercospora canescens (Ellis and Martin),* Fusarium wilt (*Fusarium oxysporum* Schlechtend f.sp. *voandzeia*), rust (*Puccinia graminis* f.sp. Tritici) and leaf blight (*Colletotrichum graminicola* [Ces.] G.W. Wils) (Mkandawire, 2017). Leaf blotch caused by the *Phomopsis sp*. and powdery mildew (*Erysiphe sp*) are key diseases reported in Zimbabwe attacking mainly immature leaves (Obagwu, 2013). In Malaysia Bambara groundnut is reportedly affected by the dieback disease (*Lasiodiplodia theobromae* [Pat.] Griff. and Maubl*).* Asiwe (2019) reported *Aspergillus* genera as the main fungus causing seed borne diseases on both Bambara groundnut and cowpea. *Sclerotium rolfsii* (Sacc.) in South Africa (Cilliers et al.2003) and powdery mildew (*Erysiphe polygony*) in Ghana are other key pathogens of the crop (Mkandawire and Sibuga, 2005). Fungal pathogens reduce seed germination, seed quality and lead to mycotoxin production harmful to both humans and livestock.

1.3.1.2 Viral diseases

Bambara groundnut is affected by viral diseases especially in regions where other grain legumes such as cowpea are grown. Thottappilly and Rossel (2007) reported some common viral diseases ravaging Bambara groundnut production in Nigeria including cowpea aphid-borne mosaic virus (CABMV), black-eye cowpea mosaic virus (BECMV), peanut mottle potyvirus (PMV), cowpea mottle comovirus (CMV), and cowpea yellow mosaic virus (CYMV). Rosette virus disease has also been reported in Tanzania, although no drastic yield damages occurred (Jonah et al.2013). There is no yield loss data recorded due to diseases on Bambara production (Feldman et al.2019). However, disease severity is dependent on genotype, the environment and genotype x environment interaction (Hasan et al.2018).

1.3.1.3 Insect pests and nematodes

Bambara groundnut is less affected by field insect pests compared with other legumes such as cowpea. However, storage pests such as the cowpea weevil (*Callosobruchus maculatus* F.) cause severe damage on stored grains (Tlankka et al.2020). The cowpea weevil is classified as the main postharvest insect pest affecting major legumes (Duodu and Apea-Bah, 2017). This pest reportedly caused 99% losses on pulse crops during storage. Bruchids (*Callosobruchus maculatus* Boh.) are the most devastating insect pests of Bambara groundnut stored grains (Mkandawire et al.2019). Akpalu et al.(2018) reported field crop losses due to leafhoppers [*Hilda patruelis* (Stal) and *Empoasca facialis* (Jacobi)]. Aphids led to a 65% yield damage under high rainfall conditions (Adu-Dapaah et al.2014). Other insect pests of the crop include groundnut jassid (*Empoasca kerri* Pruthi) and brown leaf beetles (*Ootheca mutabilis* Schönherr) (Ombima, 2016).

The root-knot nematode [*Meloidogynae javanica* Treub] is among the main field pests leading to low yield gains on Bambara groundnut (Lusser et al., 2012). Severe damages by the root knot nematode leads to leave chlorosis, slow growth, restricted root systems and reduced pod weight thus inflicting yield loss. Nematode infestations have been reported in several African countries including Botswana, Zimbabwe and South Africa (Yao et al.2015).

1.3.1.4 *Management of Bambara groundnut diseases and insect pests in*

Bambara groundnut producers in SSA do not use crop protection chemicals due to limited access and prohibitive costs. Instead, cultural practices such as crop rotation, burning of previous season debris and application of ash on storage seeds are practiced as preventative measures against several diseases and pests (Keller, 2014). Earthing up after the development of flowers had positive effect on yield and facilitate easy harvesting. Use and effectiveness of biological control agents have not been reported against Bambara groundnut pests and diseases. Resistance breeding is the most economic and sustainable approach to control common diseases and pests of legume crops including Bambara groundnut. There is need to exploit the existing genetic variation to develop disease and pest resistant cultivars (Nyau et al. 2015).

1.3.2 Abiotic stress

Bambara groundnut has become the favoured drought-tolerant crop. It has unique ability to grow in a wide range of agro-ecological zones that differ in soil conditions. It has the ability to produce a significant amount of yield under moderate or extreme drought stress. Nonetheless, research has shown that drought stress on the crop leads to serious reductions in pod dry matter and ultimately reduced grain yield especially if it occurs during flowering stage (Abu-Muriefah, 2013). The total minimum rainfall requirement of the crop is estimated to be 300 mm (Azam-Ali et al.2011). However, excessive rainfall conditions especially during harvest results in drastic yield losses. Production is most suitable in regions with temperatures of 19℃ to 30℃. Singh and Basu (2016) reported the highest seedling emergence at 30℃. Temperatures above 30℃ result in heat stress.

Bambara groundnut grows well on well-drained soils such as sandy and sandy loam soils. Some reports suggest that Phosphorus mineral elements is crucial for growth and yield performance of the crop (Mitran et al.2018). Use of superphosphate fertilizer was reported to increase pod yields under poor soil conditions in Nigeria (Toungos et al.2018). Bambara groundnut fixes 32 to 81 kg nitrogen ha^{-1} which is sufficient amount for good pod set and grain production. Several studies have shown that temperature, humidity and day length have variable effects on the vegetative, phenological and reproductive developmental stages of Bambara groundnut (Feldman et al.2019).

1.3.3 Socio-economic constraints

Bambara groundnut is yet a minor crop widely grown by women smallholder farmers in SSA. The crop has not received research support on breeding, seed systems and agronomic management methods. Bambara groundnut breeding projects are required to develop farmer- and market-preferred and superior cultivars for food security, enhanced livelihoods and for return on investment (Baoua et al.2015). Furthermore, farmers have limited access to finance to expand production through the use of new seed varieties, crop inputs such as irrigation systems, fertilizers and crop protection resources, effective harvesting systems and postharvest storage facilities. Also, smallholder farmers should have the access to regional markets for economic gains from Bambara groundnut production (Shimelis et al.2010; Cook et al.2017).

1.3.4 Lack of coordinated seed system and market

Bambara groundnut farmers use farm saved and poor-quality seed of low yielding landraces (Sedibe et al.2020) acquired from the informal sector via farmer-to-farmer seed exchange or from local markets. Unlike the traditional legume crops, there is limited formal Bambara groundnut breeding and improved seed delivery systems in SSA hindering production and productivity of the crop (Ibrahim *et at*. 2018; Agyeman et al.2021). Value-added products are yet to be developed from Bambara groundnut. This will enhance human nutritional benefits, and market and investment opportunities from Bambara groundnut enterprises.

1.4 Genetic resources and genetic analysis in Bambara groundnut

Demand-driven variety development and product profiles are required for Bambara groundnut breeding and product development. Bambara groundnut is an important source of food products and cash income along the value chains. Genetic variation, germplasm acquisition and evaluation, new progeny development, and maintenance are critical components to design and release farmer-preferred variety. Effective selection of target traits and enhanced response to selection is dependent on genetic variation (Smýkal et al.2015). Also, adequate genetic variation is required to select parental lines with complementary traits for breeding. Key traits required in Bambara groundnut breeding include grain yield and gain quality, disease resistance, heat and drought tolerance, early cooking time and low levels of antinutritional components such as phenolics, tannins and phytates to enhance digestion and bioavailability of minerals (Qaku et al.2020).

Genetically diverse Bambara groundnut collections are available in SSA for pre-breeding and breeding programs (Uba et al. 2021). For instance, wild relatives of the crop have been reported in north eastern Nigeria and northern Cameroon, which are regarded as the centres of diversity for the crop. The International Institute of Tropical Agriculture in Nigeria has maintained about 2000 Bambara groundnut collections. These were largely sourced from farmers' fields across SSA countries (Table 1.2). Furthermore, 972 accessions are maintained by various national gene banks of the Southern Africa Development Community (SADC) member countries (Saka et al.2004).

Various national and international research programs in Africa and Southeast Asia have been actively involved in breeding, selection and genetic advancements of novel Bambara groundnut lines. This has resulted in the release of few varieties such as 'Mana' and

‘Kazuma’ in Zimbabwe in 2004 (Mayes et al.2019b). Bambara groundnut populations derived from variety Kazuma as founder parent enabled development of a draft Bambara groundnut genomic sequence (Mayes et al.2019b). Several Kazuma derived varieties were under evaluation for future variety release and commercialisation (Ho et al. 2016).

Table 1.2: Number of Bambara groundnut accessions collected from various countries or institutions in Africa (Gbaguidi et al.(2018).

Country/Institution	Number of accessions
National Centre for Genetic Resources and Biotechnology (NCGRB)/Nigeria,	2035
National Plant Genetic Resource Centre (NPGRC)/Zambia,	124
Genetic Resources and Biotechnology Institute (GRBI)/Zimbabwe	129
International Plant Genetic Resource Institute (IPGRI)/Cameroon	207
Information Centre for Genetic/Togo	129
Plant Genetic Resource Research Institute (PGRRI)/Ghana	166
Central African Republic	103
Mozambique	12
Burkina Faso	143
Chad	65
Plant Genetic Resource Centre of Malawi (PGRC)	59
Congo	42
Madagascar	49
Senegal	34
Niger	33
National Plant Genetic Resource of Tanzania (NPGRT)	23
Mali	70
Benin	3
Swaziland	11
Gambia	7
Sudan	5
Botswana, National Genetic Resource Centre (NGRC)	4
Cote d' coire	4
Kenya Agricultural Research Institute	2
Agricultural Research Council (ARC)/South Africa	1
Ethiopian Biodiversity Institute (EBI)	1
Unidentified	101

Well-characterised germplasm collection is a pre-requisite for plant-breeding programmes. Bonny et al.(2019) reported two genetic clusters when investigating the genetic diversity among 101 Bambara groundnut accessions in Côte d'Ivoire using 13 morphological traits. The authors reported significant differences for days to 50% flowering, days to 50% maturity, 100-seed weight and grain yield. Phenotypic traits and molecular markers have become complementary tools in genotype selection programs (Abberton and Marshall, 2005). Several molecular markers have been used to examine genetic diversity in Bambara groundnut (Adeniji et al.2022; Odongo et al.2015; Ahmad et al.2016; Fatimah and Ardiarini, 2018; Redjeki et al.2020). The key markers system reported were Random Amplified Polymorphic DNA (RAPD) (Amadou et al.2001; Mukakalisa et al.2011; Rungnoi et al.2012; Ardiarini and Fatimah, 2018), Restriction Fragment Length

Polymorphism (RFLP) (Massawe et al.2013; Mukakalisa et al.2011; Benson et al.2015; Puozaa et al.2017), Simple Sequence Repeats (SSR) (Somta et al.2011; Molosiwa, 2012; Ahmad et al.2016), single nucleotide polymorphism (SNP) (Ahmad et al.2017; Oludare et al.2017; Absattar et al.2018; Akohoue et al.2020) and diversity array technology (DArT) (Haleegoah et al.(2020). These studies reported the presence of high genetic variability and distinct genetic groups for marker- assisted selection. There are limited reports on genetic analysis using SNP markers for Genome Wide Associations (GWAS) studies on Bambara groundnut in Africa. Genetic linkage maps have been constructed for Bambara groundnut based on wild and domesticated accessions (Bhanu et al.2016). The genomic sequence of the crop has been recently published (Hendre et al.2019). Bambara groundnut is one of the crops selected for research by the African Orphan Crop Consortium (AOCC) which ensures effective research and development of the crop. The AOCC comprises 101 traditional food crops with multiple economic traits and enhanced nutrition (Azam-Ali et al.2014). Various studies reported nutrient compositions of Bambara groundnut (Pahane et al.2017; Adebiyi et al.2019; Hussin et al.2020; Hardy and Jideani, 2020; Lin Tan et al.2020). Figure 1.3 summarises key macronutrients present in the root, leaf and seed of Bambara groundnut. These included nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg) and calcium (Ca). The seed is a rich source of N, K and P compared with leaf and root. Bambara groundnut also contains micronutrients such as zinc, iron, calcium and potassium. These are vital for a balanced diet and to combat malnutrition prevalent in SSA.

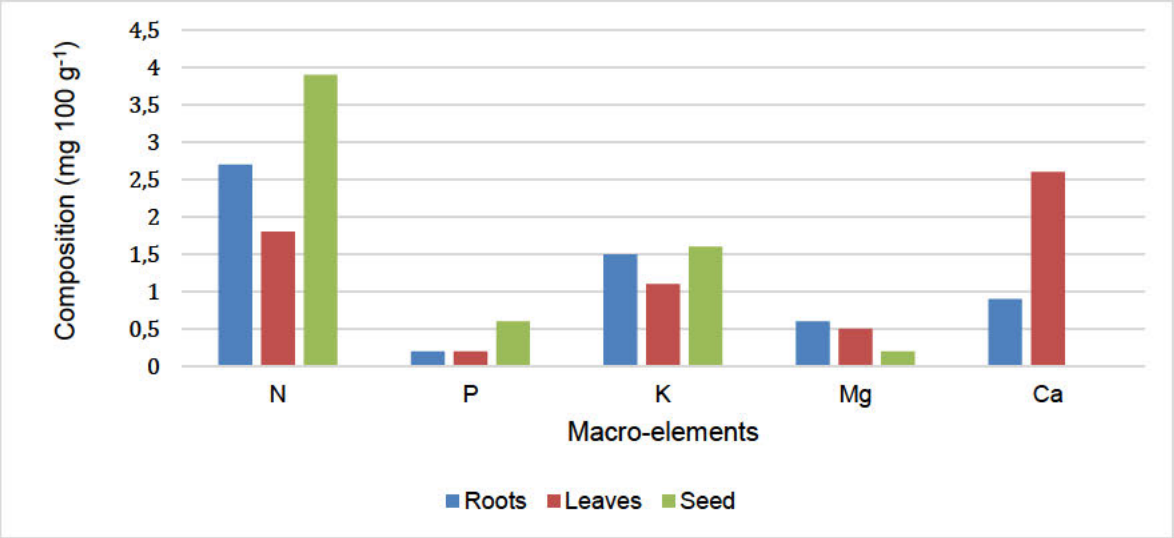

Figure 1.3: Macronutrient composition of Bambara groundnut (Lin Tan et al.2020)

1.5 Genetic diversity

Limited achievements are made on genetic advancement and gains on yield and quality relatedtraits in Bambara groundnut (Uma et al.2009). The hitherto limited progress includes breeding for yield potential (Dwivedi et al.2007), nutritional composition (Ijarotimi and Esho, 2009), drought tolerance (Berchie et al.2012), adaptations to marginal soils (Kendabie et al.2016) and N-fixation ability (Taffouo et al.2010). Breeding progress and genetic gain on the crop is relatively slow when compared to other traditional legumes. This is attributable to various challenges such as lack of adequate genetic variation, poorly coordinated or non-existent seed systems, complex flowering biology and pod formation, lack of value additions and poor market systems of the crop (Bitire et al. 2023).

The primary goal of Bambara improvement programs focuses on grain yield and nutritional quality traits. Oyeyinka et al.(2015) compared nutrition, functional and phytochemical properties of different selections. The results exhibited high protein content among the test genotypes. Similarly, high levels of essential fatty acid, thiamine, ribovin and vitamin K were recorded (Tsamo et al. 2018). Halimi et al.(2019) examined the chemical properties of starches in Bambara groundnut. The results revealed that seed source/origin and crop management practice affected chemical composition (Olaleye et al.2013). Food fortification, use of artificial supplements and food imports are among the strategies used to overcome the problem of malnutrition in Africa . Adoption of traditional plant breeding methods to enhance nutritional benefits of underutilised food crops such as Bambara groundnut is an economic and affordable means to decrease malnutrition in Africa (Harris et al.2018).

Integrating conventional breeding with molecular breeding techniques can fast track cultivar development and deployment. This could significantly reduce the breeding cycles and enable varieties to be released rapidly. Rajendrakumar (2016) reported that morphological markers have been successfully used to select best parents in Bambara for development of early maturing varieties. Morphological markers are not expensive, less automated and easy to analyse, therefore can be successfully used in Bambara groundnut breeding. However, phenotypic traits are subject to genotype x environment interaction limiting the reproducibility of selection gains (Nti, 2009).

The use of molecular markers and development of genetic tools improve the understanding of genetic control of agricultural traits, leading to quality control, marker-assisted selection,

and genomics-enabled breeding (Mayes et al. 2019). Genetic linkage maps based on the diversity arrays technology (DArT), simple sequence repeats (SSR) and single nucleotide polymorphisms (SNP) markers were made for Bambara groundnut. Four founder populations including wild type and domesticated Bambara groundnut genotypes were used to develop progenies segregating for growth habit and drought resistance (Basu et al. 2017; Ahmad 2012; Ahmad et al. 2015, 2016;Khan et al. 2022; Mayes et al. 2015a; Ho et al. 2017; Chai et al. 2017).

Molecular markers overcome the limitations of biochemical and morphological markers (Kumar et al.2009). Microsatellites markers were used to evaluate a population of 80 Bambara groundnut landraces from Ghana (Jones et al. 2019). About 32 Bambara genotypes were genotyped using SNP markers in Burkina Faso. A total of 186 alleles were detected with a mean of two alleles per locus and an average genetic similarity of 75.8% (Mafakheri et al.2017).

Diverse Bambara groundnut genotypes were finger printed to establish their genetic purity and for estimation of residual heterozygosity using 20 selected SSR markers (Ahmad et al.2011). Ho et al. (2016) reported quantitative trait loci (QTL) analysis and construction of linkage map of Bambara groundnut using DArT and SSR markers. This has been regarded as a significant milestone for integrated breeding of the crop (Mayes et al.2019a). Molecular markers complement phenotypic selection by reducing selection generation cycles. Marker-assisted breeding is well recognised on traditional legume crops such as soybean, groundnut and common beans. Recent research interest has shifted to orphan crops such as Bambara groundnut due to its potential to cope with climate change and provide nutritional security (Mohammed et al.2015). Adaptation to local growing conditions, crop phenology and market preferences are the drivers of Bambara groundnut breeding and adoption in SSA. The growing demand for new cultivars of Bambara groundnut can be met using the conventional breeding and genomic selection methods (Pandey et al.2016).

1.6 Outlook and recommendation

There is limited research progress on Bambara groundnut improvement and product development in Africa. Also, modern crop management, production technologies, and value chains are yet to be developed in SSA to achieve economic gains from Bambara groundnut

production and marketing. Improved crop management and post harvest handling technologies, modern varieties with high yield and nutritional quality, value addition and market access are among the key research and development drivers of Bambara groundnut.

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CHAPTER 2. Genotype by environment interaction effects and stability analysis of Bambara groundnut (Vigna *subterranea* [L.] Verdc) for grain yield in South Africa

Grain yield is a wining trait in Bambara groundnut breeding and production. However, the selection response is diminished by genotype-by-environment interaction effects on yield expression. Therefore, it is crucial to evaluate genotype-by-environment interaction (GEI) to select high yielding and stable genotypes for effective breeding and variety recommendation. The objectives of this study were to assess the GEI effect on grain yield and to select the best adapted genotypes in target production environments in target production regions of South Africa. Seventy-five Bambara groundnut genotypes were evaluated across seven selected environments using a 5 x 15 Alpha lattice design with three replications. The study revealed significant ($p<0.05$) differences among genotypes (G), environments (E) and GEI effects on grain yield. A high proportion of the observed variation was due to GEI (36.62%), followed by environment (35.63%) and genotype (24.16%) effects. Grain yield across environments ranged from 1.4 ton ha^{-1} for ARC Bamb-68 to 0.10 ton ha^{-1} for ARC Bamb-74. Genotype ARC Bamb 68 (0.96 ton ha^{-1}), ARC Bamb9 (0.88 ton ha^{-1}) and ARC Bamb-54 (0.84 ton ha^{-1}) attained the highest grain yield across locations, while ARC Bamb-74 exhibited the lowest grain yield of 0.16 ton ha^{-1}. The genotype and genotype-by-environment biplot identified ARC Bamb-17, ARC Bamb-14, ARC Bamb-20, ARC Bamb-18, ARC Bamb-14, and ARC Bamb-26 as the most stable genotypes across locations, while ARC Bamb-18 and ARC Bamb-54 were specifically adapted to Loskop and Brits. The Mafikeng site was ideal for Bambara groundnut evaluation, genotype differentiation, and large-scale seed production. The selected genotypes with high grain yields and stability are useful genetic resources as breeding parents for Bambara groundnut improvement in South Africa.

Keywords: Additive main effect and multiplicative interaction (AMMI), Bambara groundnut, GGE biplot, Genotype and genotype x environment interaction, Yield stability.

2.1 Introduction

Bambara groundnut (Vigna. subterranea [L.] Verdc; 2n = 2x = 22) is an important source of protein and other important nutrients for human consumption especially in Southern Africa. However, the existing Bambara groundnut varieties have low yield potentials. This is due to the extensive cultivation of unimproved cultivars under the traditionally low-input systems (Mayes et al. 2020). Furthermore, diseases and pests, draught and cold stresses contribute to the current yield gap. Breeding efforts towards improvement of Bambara groundnut in South Africa have been directed towards the selection of high-yielding genotypes usually evaluated at a single site (Gerrano et al. 2021; Amara et al. 2018). However, genotype performance should be evaluated over multiple locations due to fluctuations in environmental conditions, drought, low soil fertility, non-uniform management practices and occurrence of diseases and pests that affect the genotype performance. The differential performance of a genotypes over multiple locations is caused by genotype by environment interactions (GEI). The variable performance due to GEI provides opportunities and challenges during genotype evaluation and selection. The GEI reduces selection efficiency estimation of gene effects and combining ability for quantitative traits such as grain yield (Yan and Kang, 2012). The best and most stable genotypes for cultivar recommendation can be difficult to find due to the GEI's interference with the breeding selection process.

Multi-environment trials (MET|) are conducted and analysed to identify mega-environments, superior and stable genotypes, and as to ideal testing locations within mega-environments (Yan and Kang, 2012; Yan and Tinker, 2015; 2016). Evaluating genotype performance over multiple locations increases the confidence of identifying superior genotypes and suitable test locations. Multiple environment trials give the breeder an opportunity to quantify the different components contributing to the observed performance. While GEI can confound genotypes performance, it allows the separation of heritable from non-heritable components of variation in genotypes performance (Grada and Ciulca, 2013).

The AMMI and GGE-biplot analyses are complementary and have been used extensively due to their ability to identify genotypes with broad or specific adaptation (Kaya et al. 2006). The two models incorporate a multivariate approach for the multiplicative effect of GEI with univariate methods for the environment and genotype additive main effects. (Zobel et al. 1988). The AMMI model functions efficiently for evaluating genotype stability and

adaptation. (Carvalho et al. 2016). Principal component analysis and ordinary ANOVA are employed together to assess the main effects of genotype and environment, along with genotype × environment interactions. An overview of the relationship between surroundings and genotypes is provided by AMMI. (Zobel et al. 1988; Crossa 1990). Furthermore, in comparison to a generic ANOVA, it provides a more comprehensive analysis of the data, which increases its application in breeding programs (Zobel et al. 1988; Crossa, 1990).

Moreover, the AMMI model incorporates the AMMI stability value (ASV) for identification of genotypes that are reasonably stable across range of environments. The relative distance between a genotype and the origin of an interaction principal component axes (IPCA) biplot is the derives the ASV. The ASV is used to identify stable genotypes across environments. ASV levels that are lower suggest that genotype stability is higher. (Purchase 1997; Anley et al. 2013). The GGE biplot complements the AMMI approach through identifying environmental similarities and evaluating grain yield potential and stability utilizing average environment coordination (AEC). The genotype × environment interaction and genotype performance are identified by means of the GGE biplot. The method makes it easier to view the intricate GEI accurately on a graph (Yan et al. 2001). The GGE model separates the environment effects and merges the genotype main effects with the genotype × environment interaction and as a result, genotype similarities across selected environments are identified. The present study was set out to assess the genotype-by-environment interaction (GEI) effect on grain yield and to select best adapted Bambara groundnut genotypes in South African target production areas for breeding

2.2 Materials and methods

2.2.1 Plant material

Seventy-five Bambara groundnut genotypes (Table 2.1) sourced from the Agricultural Research Council, Vegetable, Industrial and Medicinal Plants (ARC_VIMP) gene-bank, Pretoria, South Africa, (Latitude 25.604˚S and Longitude 28.345˚E) were used in this study. These lines were principal collections widely cultivated and evaluated for nutritional quality and genetic diversity for crop improvement programs in South Africa (Majola et al. 2022).

Table 2.1. Description of genotypes used in the study in South Africa.

Genotype	Genotype designation/name	Source	Origin	Genotype	Genotype designation/name	Source	Origin
G1	ARC Bamb-1	ARC-VIMP	South Africa	G39	ARC Bamb-39	ARC-VIMP	South Africa
G2	ARC Bamb-2	ARC-VIMP	South Africa	G40	ARC Bamb-40	ARC-VIMP	South Africa
G3	ARC Bamb-3	ARC-VIMP	South Africa	G41	ARC Bamb-41	ARC-VIMP	South Africa
G4	ARC Bamb-4	ARC-VIMP	South Africa	G42	ARC Bamb-42	ARC-VIMP	South Africa
G5	ARC Bamb-5	ARC-VIMP	South Africa	G43	ARC Bamb-43	ARC-VIMP	South Africa
G6	ARC Bamb-6	ARC-VIMP	Botswana	G44	ARC Bamb-44	ARC-VIMP	Botswana
G7	ARC Bamb-7	ARC-VIMP	South Africa	G45	ARC Bamb-45	ARC-VIMP	South Africa
G8	ARC Bamb-8	ARC-VIMP	South Africa	G46	ARC Bamb-46	ARC-VIMP	South Africa
G9	ARC Bamb-9	ARC-VIMP	Botswana	G47	ARC Bamb-47	ARC-VIMP	Botswana
G10	ARC Bamb-10	ARC-VIMP	South Africa	G48	ARC Bamb-48	ARC-VIMP	Namibia
G11	ARC Bamb-11	ARC-VIMP	South Africa	G49	ARC Bamb-49	ARC-VIMP	South Africa
G12	ARC Bamb-12	ARC-VIMP	South Africa	G50	ARC Bamb-50	ARC-VIMP	South Africa
G13	ARC Bamb-13	ARC-VIMP	South Africa	G51	ARC Bamb-51	ARC-VIMP	South Africa
G14	ARC Bamb-14	ARC-VIMP	South Africa	G52	ARC Bamb-52	ARC-VIMP	South Africa
G15	ARC Bamb-15	ARC-VIMP	South Africa	G53	ARC Bamb-53	ARC-VIMP	Namibia
G16	ARC Bamb-16	ARC-VIMP	South Africa	G54	ARC Bamb-54	ARC-VIMP	Namibia
G17	ARC Bamb-17	ARC-VIMP	South Africa	G55	ARC Bamb-55	ARC-VIMP	South Africa
G18	ARC Bamb-18	ARC-VIMP	South Africa	G56	ARC Bamb-56	ARC-VIMP	South Africa
G19	ARC Bamb-19	ARC-VIMP	South Africa	G57	ARC Bamb-57	ARC-VIMP	Swaziland
G20	ARC Bamb-20	ARC-VIMP	South Africa	G58	ARC Bamb-58	ARC-VIMP	South Africa
G21	ARC Bamb-21	ARC-VIMP	South Africa	G59	ARC Bamb-59	ARC-VIMP	South Africa
G22	ARC Bamb-22	ARC-VIMP	South Africa	G60	ARC Bamb-60	ARC-VIMP	South Africa
G23	ARC Bamb-23	ARC-VIMP	South Africa	G61	ARC Bamb-61	ARC-VIMP	Swaziland
G24	ARC Bamb-24	ARC-VIMP	South Africa	G62	ARC Bamb-62	ARC-VIMP	South Africa
G25	ARC Bamb-25	ARC-VIMP	South Africa	G63	ARC Bamb-63	ARC-VIMP	South Africa
G26	ARC Bamb-26	ARC-VIMP	South Africa	G64	ARC Bamb-64	ARC-VIMP	Zimbabwe
G27	ARC Bamb-27	ARC-VIMP	South Africa	G65	ARC Bamb-65	ARC-VIMP	Zimbabwe
G28	ARC Bamb-28	ARC-VIMP	South Africa	G66	ARC Bamb-66	ARC-VIMP	South Africa
G29	ARC Bamb-29	ARC-VIMP	South Africa	G67	ARC Bamb-67	ARC-VIMP	South Africa
G30	ARC Bamb-30	ARC-VIMP	South Africa	G68	ARC Bamb-68	ARC-VIMP	South Africa
G31	ARC Bamb-31	ARC-VIMP	South Africa	G69	ARC Bamb-69	ARC-VIMP	South Africa
G32	ARC Bamb-32	ARC-VIMP	South Africa	G70	ARC Bamb-70	ARC-VIMP	South Africa
G33	ARC Bamb-33	ARC-VIMP	South Africa	G71	ARC Bamb-71	ARC-VIMP	South Africa
G34	ARC Bamb-34	ARC-VIMP	South Africa	G72	ARC Bamb-72	ARC-VIMP	South Africa
G35	ARC Bamb-35	ARC-VIMP	South Africa	G73	ARC Bamb-73	ARC-VIMP	South Africa
G36	ARC Bamb-36	ARC-VIMP	South Africa	G74	ARC Bamb-74	ARC-VIMP	South Africa

G37	ARC Bamb-37	ARC-VIMP	South Africa	G75	ARC Bamb 75	ARC-VIMP	South Africa
G38	ARC Bamb-87	ARC-VIMP	South Africa				

ARC-VIMP= Agricultural Research Council,Vegetable, Industrial and Medicinal Plants, Bamb= Bambara groundnut G= Genotype

2.2.2 Description of the study environment

The field experiments were set up in October 2019/2020 and November 2020/2021 summer cropping season at five selected environment representing different agro-ecological regions of South Africa. The experimental environment included Loskop, Brits, Polokwane, Rustenburg and Mafikeng. Loskop is located at Groblersdal in the Mpumalanga province, Brits, Rustenburg and Mafikeng are situated in the North West, while Polokwane is located in Limpopo province. These provinces are located in the north, north-eastern and north central zones of South Africa and are regarded as the major belts of Bambara groundnut production and marketing (Figure 2.1). Individual seasons and a combination of the different locations provided unique environmental conditions due to rainfall and temperature fluctuations. Thus, a total of seven environments were identified for genotypic evaluations as a result of site × season combinations. The tests environments were defined as E1 = Loskop 2019/2020, E2 = Brits 2019/2020, E3 = Polokwane 2019/2020, E4 = Rustenburg 2019/2020, E5 = Loskop 2020/2021 E6 = Brits 2020/2021 and E7 = Mafikeng 2020/2021. Geographic locations, altitude, weather and soil characteristics of the studied environments are presented in Table 2.2.

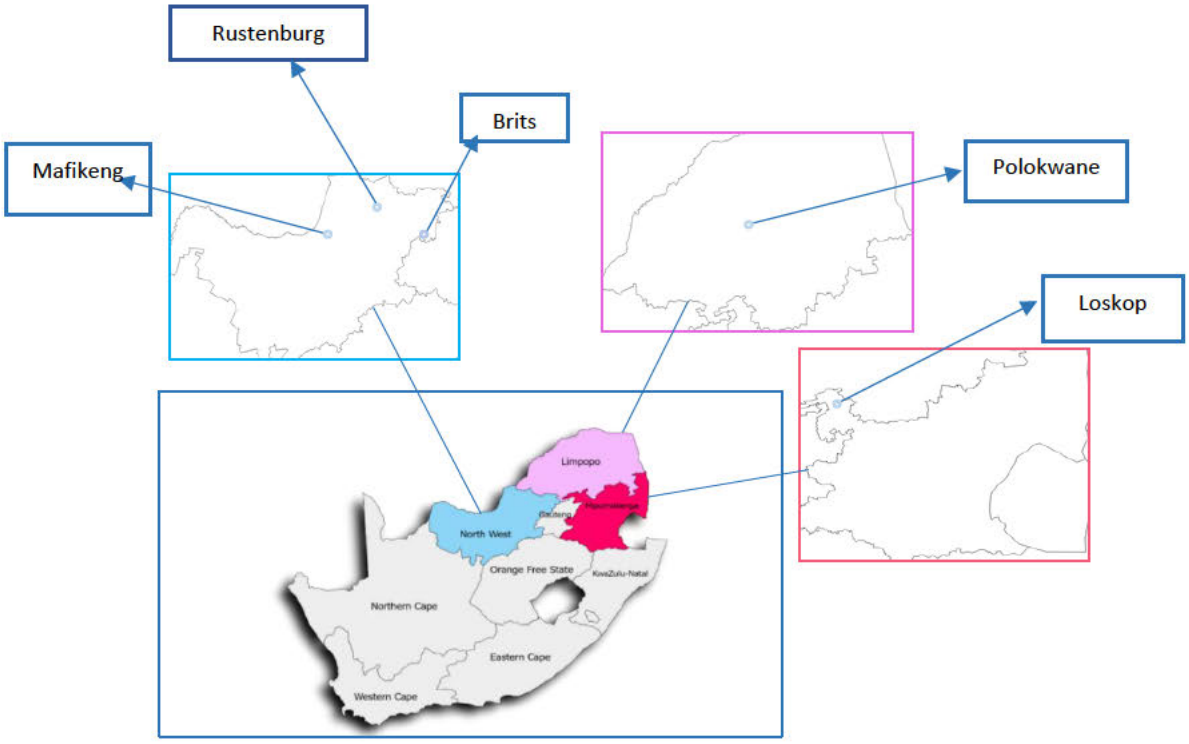

Figure 2.1: Map of study areas in South Africa

Table 2.2: Characteristics of the five locations used to evaluate 75 Bambara groundnut genotypes

Location	**Soil texture**	**Altitude (masl)**	**Rainfall (mm)**	**T_{Min} (°C)**	**T_{Max} (°C)**	**Latitude**	**Longitude**
Loskop	Loamy	920	497	18,0	28,0	25.17° S	29.39° E
Brits	Clay loam	1083	629	18.6	28,9	25.61° S	27.79° E
Rustenburg	Clay	1170	602	17,5	28.0	25.65° S	27.25° E
Polokwane	Clay loam	1310	495	17.6	27.6	23.89° S	29.44° E
Mafikeng	Sandy	1359	571	16.7	27.6	25.85° S	25.60° E

Masl = metres above sea level, mm = millimetres, min = minimum, max = maximum, T = temperature, °C = degrees Celsius.

2.2.3 Trial design and management

The experiment at each site was laid out in a 5 x 15 alpha lattice design with three replications. Each genotype was planted in a plot consisting of two 2m rows with intra- and inter-row spacings of 0.5 and 1 m, respectively. Sowing was carried out manually with one seed hand sown per hole of 2cm depth. The plants were cultivated under rainfed conditions,

and spray irrigation was used to provide extra moisture when the rainfall was insufficient for good growth and development. Weeding was done manually after crop establishment.

2.2.4 Data collection

Data was collected using the International Plant Genetic Resources Institute's descriptors. (IPGRI 2000). Yield per pod (YPP) was measured by weighing the dried pods from each plot and was recorded in grams per plot. Grain yield (per hectare) was calculated as:

$$\left(\frac{Plot\ weight\ (g)}{Plot\ area\ (m)} * \frac{100-14}{100-mc}\right) * 10\,000$$

Where; mc is moisture content measured at harvesting, 14 % is the standard constant for moisture content in legumes (Parker and Namuth-Covert, 2017), and 10 000 is the conversion factor for a hectare.

2.2.5 Statistical analysis

The data from all five locations were analyzed utilizing the linear mixed effects model (LMM) in GenStat version 20 following the results of the Bartlett's test for uniformity of variance (Payne, 2017). Data was subjected to the combined analysis of variance (ANOVA) according to the alpha lattice design using GenStat 20th version (Payne et al. 2017) for evaluation of the significance of G x E interaction prior to further analysis.

Sequential computations of the AMMI and GGE biplot models were made in order to analyze the G x E interaction and produce genotype stability. AMMI analysis was computed to deduce the effects due to G x E interaction and yield stability of the genotypes.

The AMMI analysis was carried out in GenStat version 20 (Payne, 2017). The following AMMI model was adopted following Zobel et al. (1988):

$$Yij = \mu + \alpha i + \beta j + \Sigma n\ \lambda n\ \delta in\ yjn + Pij + \varepsilon ij$$

Where Yi is the mean yield of the ith genotype effect in jth environment in all replications; and the additive components are μ (the grand mean), αi (the ith genotype effect) and βj (the jth environment effect). The multiplicative effect consists of terms $\Sigma n\ \lambda n\ \delta in\ yjn$ and Pij where, Σn is the singular value, λn is the interaction principal component, δin is eigen vector for the genotype principal component, yjn is the environment principal component, Pij is the AMMI residuals and εij is the random error.

AMMI stability values (ASVs) were calculated using GenStat statistical software (20th Edition) based on the following formula:

$$ASV = \sqrt{[\frac{IPCA1ss}{IPCA2ss}\ (IPCA1score)^2 + [\ IPCA2score]^2}$$

Where $\frac{SSIPCA1}{SSIPCA1}$ denotes the weighted value assigned to the first interaction principal component score due to its high contributions in the GE model. SSIPCA1 and SIPCA2 are the sum of squares for IPCA1 and IPCA2, respectively (Purchase et al. 2000).

Using GenStat statistical software (20th Edition), the GGE biplot analysis was carried out based on the singular value decomposition (SVD) of the first and second principal components (PC1 and PC2, respectively. (Yan et al. 2001).

Performance Stability Index (YSI): The stability index is normally applied to yield data and is referred to as the YSI. This stability index was calculated for each genotype by summing the overall average performance ranking for each trait and the ASV ranking for each trait. YSI was calculated as suggested (Bose et al. 2014).

YSI = RASV + RY where, RASV is the rank of the AMMI stability value and RY is the rank of the average grain yield of the genotypes in all environments. YSI incorporates both average yield and stability into one criterion. The low values of the parameter show desirable genotypes with higher yield.

2.3 Results

2.3.1 Combined analysis of variance

The Bartlett's test showed that the variance among the seven environments were homogenous and subsequently a combined ANOVA was conducted. The combined analysis of variance of 75 genotypes evaluated across seven environments is presented in Table 2.2. The results revealed highly significant differences ($p<0.001$) for grain yield among genotypes. Highly significant differences ($p<0.001$) were also present among environments and genotype x environment interaction (G x E). The results showed a great range of variation for grain yield across all locations (Table 2.3). Grain yield across environments ranged from 1.4 ton ha^{-1} for genotype 68 (ARC Bamb- 68) to 0.10 ton ha^{-1} for genotype 74 (ARC Bamb -74) which had the lowest grain yield among all the genotypes tested across

locations. Genotype 68 (ARC Bamb- 68), genotype 9 (ARC Bamb- 9) and genotype 54 (ARC Bamb 54) attained the highest grain yield across locations, while genotype 74 (ARC Bamb 74) and genotype 64 (ARC Bamb 64) exhibited the lowest grain yield.

Table 2.2 Analysis of variance for grain yield among 75 Bambara groundnut genotypes evaluated across seven environments in South Africa

Source of variation	DF	Mean squares
Environment (E)	6	16.7***
Replication	2	0.3ns
Block (Rep)	4	0.2
Genotype (G)	74	0.4***
G x E	444	0.3***
Residual	424	0.2
Trial statistics		
R^2	75%	
CV	30.76%	

DF= degrees of freedom, *** = significant differences at 0.001 probability level, CV = coefficient of variation and R^2 = Coefficient of determination

Table 2.3. Mean performance of 75 Bambara groundnut genotypes for grain yield (ton ha^{-1}) evaluated at Loskop, Brits, Polokwane and Rustenburg in South Africa during 2019 cropping season

Genotype	**E1**	**E2**	**E3**	**E4**	**E5**	**E6**	**E7**
Top ten genotypes							
			Grain yield (ton ha^{-1})				
G68	0,86	0,9	0,87	0,96	0,82	1,40	0,93
G9	0,85	0,81	0,76	0,94	0,79	1,08	0,92
G54	0,62	0,78	0,74	0,91	0,75	1,07	0,92
G17	0,56	0,73	0,65	0,81	0,63	1,06	0,91
G3	0,54	0,68	0,63	0,78	0,62	1,03	0,90
G5	0,52	0,68	0,63	0,77	0,52	1,02	0,90
G11	0,52	0,62	0,62	0,76	0,52	0,99	0,88
G69	0,5	0,62	0,62	0,75	0,42	0,95	0,87
G39	0,45	0,54	0,62	0,74	0,4	0,44	0,86
G19	0,43	0,54	0,53	0,66	0,36	0,34	0,83
Bottom five genotypes							
G30	0,12	0,19	0,36	0,13	0,14	0,25	0,38
G47	0,12	0,16	0,29	0,13	0,13	0,24	0,37
G60	0,12	0,15	0,26	0,12	0,12	0,24	0,36
G64	0,11	0,12	0,20	0,11	0,12	0,23	0,33
G74	0,1	0,08	0,18	0,11	0,10	0,23	0,32
Mean	0.58	0.69	0.66	0.80	0.58	1.06	
LSD (5 %)	0.45	0.58	0.62	0.75	0.45	0.85	1.30
CV (%)	25.6	22.7	35.5	36.8	20.8	37.8	38.2

CV = coefficient of variation; LSD= least significance difference at 0.05; E1 = Loskop 2019/2020; E2 = Brits 2019/2020; E3 = Polokwane 2019/2020; E4 = Rustenburg 2019/2020; E5 = Loskop 2020/2021; E6 = Brits 2020/2021; E7 = Mafikeng 2020/2021; ton ha^{-1} = tonnes per hectare.

2.3.2 Additive main effect and multiplicative interaction (AMMI)

The environment, genotype and genotype by environment interaction had highly significant ($p<0.01$) effects for grain yield (Table 2.4). The environment and genotype effects contributed 35.63 and 24.16 % to the total variation in grain yield, respectively. The interaction effects accounted for 36.62% of the total variance. The interaction sum of squares was further partitioned into two interaction principal components analysis (IPCA) scores by the AMMI model. All the IPCAs were highly significant ($p<0.01$). The first IPCA captured 66.00 % of genotype by environment interaction sum of squares. The second contributed 12.26 % to the observed variation. The model managed to explain all the

variations by two IPCAs. AMMI stability values (ASV) recorded variations in yield stability among the 75 genotypes (Table 2.5). According to Purchase, (1997), a stable variety exhibits low ASV. Genotype G60 (ARC Bamb-60) with ASV value of 0.95 was the most stable, followed by genotype G53 (ARC Bamb-53) and genotype G68 (ARC Bamb-68). The least stable genotypes were G18 (ARC Bamb-48), G48 (ARC Bamb-48) and G34 (ARC Bamb-34) with high ASV values.

Table 2.4. AMMI analysis of variance for Bambara groundnut grain yield (ton ha^{-1}) evaluated at Loskop, Brits, Polokwane and Rustenburg in South Africa during 2019 cropping season

Sources of variation	DF	SS	MS	Total variation explained (%)	G x E explained (%)
Total	1574	177.70	0.13		
Treatments	524	97.98	0.18***		
Genotype	74	4.07	0.55***	24.16	
Environments	6	67.49	11.24***	35.63	
Block	6	20.38	1.45	0.85	
Interactions	444	26.42	0.05***	36.62	
IPCA 1	79	17.43	0.22***		66.00
IPCA 2	77	3.24	0.04***		12.26
Residual	288	5.75	0.02		

***Significant at probability level 0.001, DF= degrees of freedom, SS= sum of squares, MS= mean square error, G x E= genotype by environment interaction, IPCA= interaction principal component axis, ton ha^{-1} = tonnes per hectare

Table 2.5. AMMI adjusted mean grain yield (ton ha^{-1}), IPCA scores and AMMI stability value (ASV) of Bambara groundnut genotypes evaluated across seven environments in South Africa

Genotype	Mean	IPCA1	IPCA2	ASV	YSI
10 most stable genotypes					
G60	1.10	0,01	0,82	0,95	0.39
G53	1.36	0,05	0,86	1,05	0.42
G68	1.36	0,07	0,88	2,54	0.36
G23	0.95	0,05	0,91	2,87	0.56
G62	0.86	0,03	1,1	2,98	0.57
G15	0.79	0,01	-0,56	3,01	0.68
G12	0.76	-0,8	-0,54	3,25	1.20
G60	0.72	-0,9	-1,41	3,35	1.80
G61	0.70	-0,89	-1,62	3,65	1.81
G14	0.70	-0,9	-2,12	3,87	2.80
5 least stable genotypes					
G18	0.45	12,33	2,35	65,2	3.52
G30	0.42	10,14	-1,53	61,5	3.90
G34	0.32	8,56	-1,45	56,2	4.54
G44	0.24	7,52	-1,15	42,3	4.80
G48	0.15	-7,35	8,12	39,5	5.20

AMMI = Additive main effect and multiplicative interaction; IPCA= interaction principal component analysis; IPCA1 = first interaction principal component axis; IPCA2 = second interaction principal component axis.

2.3.3 Stability and performance of genotypes

Figure 2.2 depicts genotype-focused biplot displaying genotypic comparisons based on average performance and stability across environments within a mega environment. The first two principal components explained 76.22% of the total variation. Genotype G37 (ARC Bamb-37), G49 (ARC Bamb-49), G59 (ARC Bamb-59) and G30 (ARC Bamb-30) were the most stable genotypes as they were located in close proximity to the average-environment coordination (AEC) indicating consistency across environments. On the contrary, genotype G23 (ARC Bamb-23) and G14 (ARC Bamb-14) exhibited below average mean performance and were least stable.

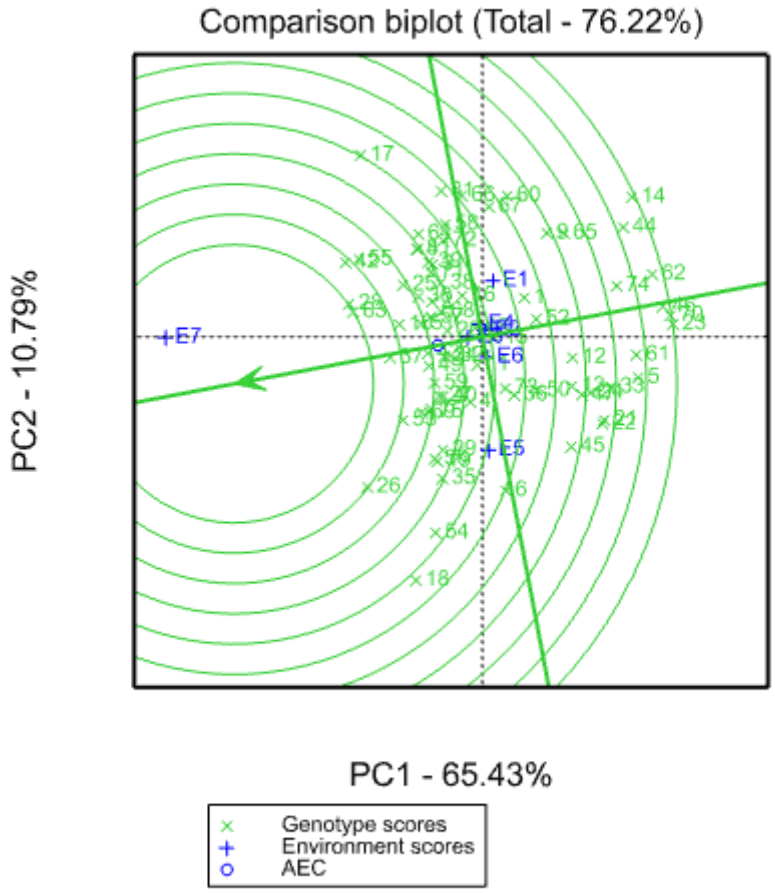

Figure 2.2: The biplot for average-environment coordination (AEC) vdisplays genotypes in relation to ideal genotypes. Blue numbers represent environments: E1 = Loskop 2019/2020; E2 = Brits 2019/2020; E3 = Polokwane 2019/2020; E4 = Rustenburg 2019/2020; E5 = Loskop 2020/2021; E6 = Brits 2020/2021; E7 = Mafikeng 2020/2021. Genotypes codes (1-75) are presented in Table 1.1.

2.3.4 Identification of mega- environments and high- performance genotypes

The GGE biplot polygon view, showing the "which-won-where" pattern is presented in (Figure 2.3). PC1 explained 65.43% and PC2 explained 10.79% and, thus cumulatively explained for 76.22% of the total variation for grain yield. The lines perpendicular to each side of the hexagon clustered the environments into ten sectors. Moreover, environment E3 (Polokwane 2019/2020) and E4 (E4 = Rustenburg 2019/2020) clustered into one sector forming

a mega-environment, and another mega- environment was made of environment E5 (E5 = Loskop 2020/2021) and E6 (E5 = Loskop 2020/2021). Rustenburg and Mafikeng solely discerned into one sector and formed one separate environment. The GGE biplot also identified genotypes that had specific adaptation and grain yield in the respective environments. Each sector had a vertex genotype, representing the winning and most responsive genotype in the environment that fell in the same sector. Genotype 17 (ARC Bamb-17), G14 (ARC Bamb-14), G 20 (ARC Bamb-20), G18 (ARC Bamb-18), G14 (ARC Bamb-14), G26 (ARC Bamb-26) and G25 (ARC Bamb-25) were the best performing and most adapted to the mega-environment comprised of environments Brits (E2), Loskop (E3) and Polokwane (E5). The genotype 18 (ARC Bamb-18) and G54 (ARC Bamb-54) and G6 (Bamb-6) were specifically adapted to Loskop 2020/2021 (E5) and Brits 2020/2021 (E6) mega- environment. Similarly, G25 (ARC Bamb-25) and G37 (ARC Bamb-37) were best suited for Mafikeng (E7).

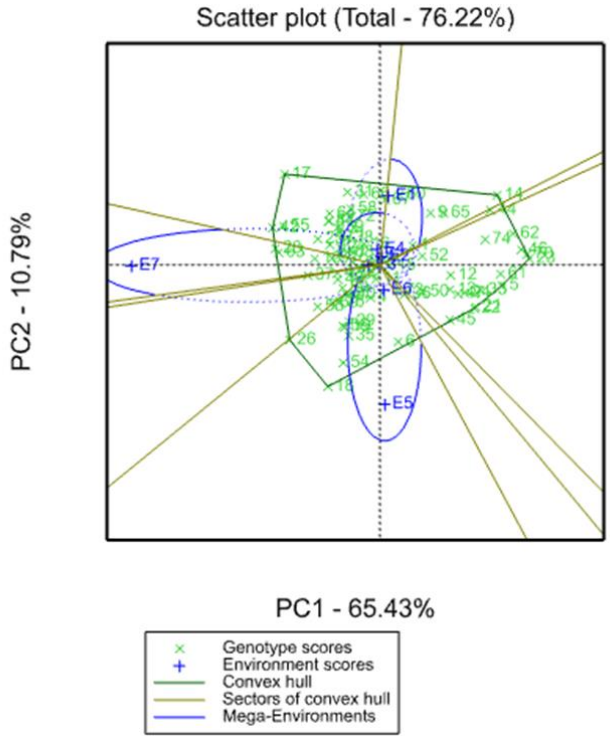

Figure 2.3: The "which-won-where" polygon view of GGE biplot based on grain yield of 75 Bambara groundnut genotypes. Blue numbers represent environments: E1 = Loskop 2019/2020; E2 = Brits 2019/2020; E3 = Polokwane 2019/2020; E4 = Rustenburg 2019/2020; E5 = Loskop 2020/2021; E6 = Brits 2020/2021; E7 = Mafikeng 2020/2021. Genotypes codes (1-75) are presented in Table 1.1.

2.3.5 Ideal environment for high grain yield

An ideal environment is defined as the one that can distinguish between genotypes and represent various locations within the mega-environments. An environment with the longest vector is identified as a discriminating environment, which provides more information regarding differences among other environments. In contrast, minimal genotypic information is provided by a non-discriminating environment.. The biplot accounted for 76.22 % of the total variation relative to genotype and genotype-by-environment interaction. Mafikeng (E7) was located closer to the concentric centre of the ideal environment indicating it provided the ideal conditions for testing and selecting superior genotypes as it (Figure 2.4). Loskop 2020/2021 (E5) was the next ideal environment. Environment E2 (Brits 2019/2020) and E6 (Brits 2020/2021) were classified as the least favourable testing environments for genotype evaluation.

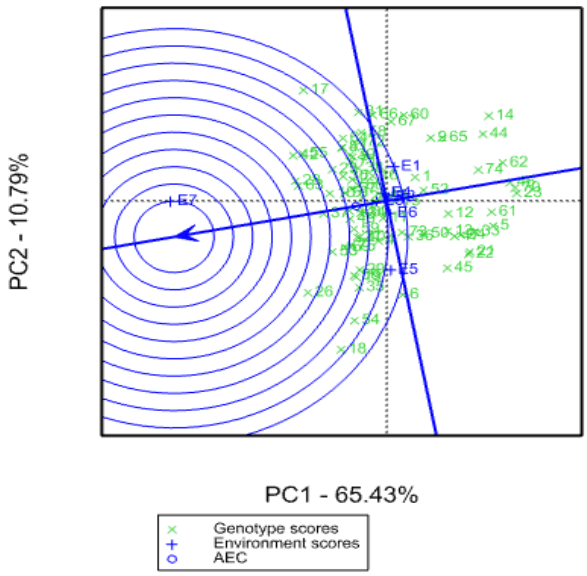

Figure 2.4: GGE biplot showing the discriminating ability and representativeness of test environments. Blue numbers represent environments: E1 = Loskop 2019/2020; E2 = Brits 2019/2020; E3 = Polokwane 2019/2020; E4 = Rustenburg 2019/2020; E5 = Loskop 2020/2021; E6 = Brits 2020/2021; E7 = Mafikeng 2020/2021. Genotypes codes (1-75) are presented in Table 2.1.

2.4 Discussion

Genetic variation

Highly significant genotypes x environment interaction was recorded among Bambara groundnut genotypes evaluated across seven environments in South Africa. This suggests the presence of differential responses of genotypes to environmental changes staggering genotypic selections study environments. Inconsistencies in genotypic performance across environments exacerbates selections of superior genotypes, thus delaying the breeding process. The test environments differed in altitude, precipitation, temperature, distribution of insect pests and diseases and chemical and physical properties of soils which expectedly influenced the different trait expressions among the genotypes. Many studies have reported significant genotype x environment interaction for agronomic performance and yield potential of Bambara groundnut (Jahanshiri et al. 2022; Hlanga et al. 2022; Nhamo et al. 2022).

AMMI analysis determines the percentage of variation attributed to environment, genotype, and their interacton. The mean squares and AMMI model showed highly significant differences among all the genotypes, environments and their interactions. The environment and genotype effects contributed 35.63 and 24.16 % to the total variation in grain yield, respectively. This suggests that majority of the variation exhibited among Bambara groundnut genotypes was due to differences in the environment and significantly affected grain yield. Yield is a quantitative trait governed by multiple genes that respond to environment factors causing a bottleneck in selection efficiency (Esan et al. 2023). Several factors, including humidity, temperature and rainfall conditions affect agronomic performance and yield potential of crops (Suhairi, Jahanshiri et al. 2018, Nhamo, Paterson et al. 2022). These findings are in agreement with results by Linua et al. (2013), Khan et al. (2021) and Olanrewaju et al. (2021), who reported large magnitude of environmental variation based on GEI analysis. For example, Olanrewaju et al. (2021) found that the environment contributed 62% of variation in grain yield among 92 Bambara groundnut accessions evaluated at Ibadan. GEI contributed 20% on grain yield which provides provide opportunities and challenges for selecting superior or desirable genotypes at distinct environments. Moreover, GEI leads to minimal correlations among genotype and phenotypic expression, resulting in extended breeding cycles or the inability to detect better

genotypes. The low contribution of genotypic variance indicates that the environmental factor restricted genotypic expression.

The distance between the coordinate point and the origin in a two dimensional of IPCA1 against IPCA2 scores in the AMMI model is known as ASV. Genotypes exhibiting minimal ASV values are the most stable, thus genotype G60 (ARC Bamb-60) with ASV value of 0.95 was the most stable, followed by genotype G53 (ARC Bamb-53) and genotype G68 (ARC Bamb-68). The least stable genotypes were G18 (ARC Bamb-48), G48 (ARC Bamb-48) and G34 (ARC Bamb-34) with high ASV values. The most stable genotypes are the best candidates for breeding of high yielding and most adapted varieties.

Identification of mega- environments and high- performance genotypes

Newly bred cultivars must exhibit inceased yield potential and stability in a variety of environments. According to Yan and Tinker (2016), an ideal genotype has outstanding average performace and great stability across environments. In the present study, Genotype G37 (ARC Bamb-37), G49 (ARC Bamb-49), G59 (ARC Bamb-59) and G30 (ARC Bamb-30) were the most stable genotypes as they were located in close proximity to the average-environment coordination (AEC) indicating consistency across environments. These genotypic rankings were based on the general stability of the genotypes across the environments within the four mega environments. Genotype 17 (ARC Bamb-17), G14 (ARC Bamb-14) and G 20 (ARC Bamb-20) were the best performing and most adapted to the mega-environment comprised of environments Brits (E2), Loskop (E3) and Polokwane (E5). This indicates that these genotypes can be selected for breeding in each respective environment due to high adabtability.

Environment 5 (Polokwane) is characterised by minimum rainfall averages and drought. These suggests that these genotypes are best suited for parental selections in drought tolerance breeding program. Sari et al. (2021) and Nwokeocha et al. (2015) successfully identified adapted and stable Bambara genotypes using GGE model for varietal selections. Highly stable genotypes in each location can be made available following preliminary yield trials for evaluations against the widely used standard checks.

Four mega-environments were established for the test environments. Mega-environments are a collection of environment that frequently share the most prolific genotypes yearly. Identification of homogeneous environments is essential for decreasing breeding costs

because assessments might be carried out at one of the locations found in a mega-environment.

Environment E3 (Polokwane 2019/2020) and E4 (E4 = Rustenburg 2019/2020) clustered into one sector forming a mega-environment, and the second mega- environment was made of environment E5 (E5 = Loskop2020/2021) and E6 (E5 = Loskop 2020/2021). These findings suggest the breeder can select for grain yield at either of the testing environment for single season evaluation. Thus, suggesting that resources will be saved in resource-poor breeding programs since Bambara groundnut genotypes can be evaluated in one environment for grain yield and still give accurate results and effective selection. Similarly, the seven environments were positively correlated and were clustered into mega-environments based on the predominant atmospheric and environmental conditions including variations in temperature and rainfall. An ideal environment is the one with high ability to discriminate and with great representative capacity (Yan et al. 2006). Therefore, Mafikeng environment permitted the genotypes to show their full genetic potential thus allowing precision for selection of parents in developing breeding populations. The genotype 18 (ARC Bamb-18) and G54 (ARC Bamb-54) and G6 (Bamb-6) were specifically adapted to Loskop 2020/2021 (E5) and Brits 2020/2021 (E6) mega- environment. Similar findings were reported by Linus et al. (2023) which assessed genotypic performance of Bambara groundnut genotypes by identifying three mega-environments.

In identifying optimal environments for genotype performance, the GGE-biplot technique can be used to prioritize available resources for testing new genotypes. Test locations that are both representative and discriminating are favourable sites for selecting generally adapted genotypes. According to Sousa et al. (2018), the ideal sites tend to discriminate the performance among different genotypes easily, hence it provides a basis for selecting superior genotypes. In this study, Mafikeng was regarded as the most discriminating environment (ideal environment) and representative of other environments. An ideal environment allows all genotypes to express their full potential, thus providing opportunities for the identification of the best-performing genotypes (Sousa et al. 2018).

2.5 Conclusions

The study assessed the genotype-by-environment interaction (GEI) effect on grain yield and to select best adapted Bambara groundnut genotypes in South African target production areas for breeding. Significant genetic variation was observed for grain yield among Bambara groundnut genotypes. Both AMMI and GGE analyses demonstrated that there were implications in the ranking of genotypes due to the genotype x environment interaction effect. The GGE biplots grouped the test environments into four mega-environments. Grain yield across environments ranged from 1.4 ton ha^{-1} for ARC Bamb-68 to 0.10 ton ha^{-1} for ARC Bamb74. Genotype ARC Bamb 68 (0.96 ton ha^{-1}), ARC Bamb- 9 (0.88 ton ha^{-1}) and ARC Bamb- 54 (0.84 ton ha^{-1}) attained the highest grain yield across locations. The Mafikeng site was ideal for Bambara groundnut evaluation, genotype differentiation, and large-scale seed production. The selected genotypes with high grain yields and stability will be useful genetic resources as breeding parents for Bambara groundnut cultivation and breeding in South Africa. However, this study must be accompanied by genetic analysis for clarifications of genetic control for the development of improved varieties.

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Chapter 3. Analyses of the compositions of phytochemicals and minerals in Bambara groundnut genotypes

Abstract

Bambara groundnut (*Vigna subterranea* (L.) Verdc) grains have variable phytochemical compositions and minerals vital for the food and feed industry. There is a need to exploit the genetic diversity of Bambara groundnut for breeding new varieties with enhanced grain yield, nutritional quantity and quality to develop various products. The objective of this study was to determine the compositions of phytochemicals and mineral elements present in Bambara groundnut genetic pool to identify superior and contrasting genotypes to guide product development and breeding. Seventy-five genetically diverse Bambara groundnut genotypes were field evaluated across four environments using a 15 x 5 alpha lattice design with three replications during the 2020-2021 cropping season. Genotypes were profiled for fat, phenolic and flavonoids contents at the Agricultural Research Council (ARC) analytical laboratory in South Africa. Further, the genotypes were assessed for the contents of the following minerals: calcium (Ca), iron (Fe), potassium (K), phosphorus (P), zinc (Zn) and nitrogen (N). The nutritional content of the test genotypes varied significantly ($P<0.05$), which were affected by the genotype and environment interactions. The Ca, Fe, K and Zn content varied from 150.70 to 216.53, 4.30 to 16.77, 771.99 to 1155.89 and 5.50 to 7.17 mg.100 g^{-1} dry seed sample, respectively. Genotypes, including ARC Bamb-2, ARC Bamb-19, ARC Bamb-73, ARC Bamb-56, ARC Bamb-37, ARC Bamb-3 and ARC Bamb-69 exhibited the highest fat content (>6.00 %). ARC Bamb-40 and ARC Bamb-59 recorded a higher mean Fe content of 16.00 mg.100 g^{-1}. ARC Bamb-2 was the top-performing genotype with high fat content (6%), Ca (211.93 mg.100 g^{-1}), and Zn (7.17 mg.100 g^{-1}). Ca,K, and N contents displayed strong correlations ($r>0.60$, $P<0.05$). Phosphorus and Zn contents exhibited moderate correlations with Ca. Overall, the study selected genotypes ARC Bamb-73, ARC Bamb-19, ARC Bamb-9 and ARC Bamb-2 with high compositions of essential nutrients for product development or breeding. The selected genetic resources are valuable for trait integration and developing new breeding populations with enhanced nutrient compositions and agronomic and market-preferred traits.

Keywords: Bambara groundnut, mineral composition, nutrient analysis, phytochemicals, product development

3.1 Introduction

Hidden hunger or a lack of micronutrients in human diets is the major challenge in sub-Saharan Africa (SSA) (Qaku et al. 2020). An estimated 1.1 million child fatalities are attributed to micronutrient deficiencies annually (Black et al. 2013). Cereal- and starch-based diets in SSA are the primary food sources providing daily dietary calories. Hidden hunger is exacerbated by a lack of balanced diets that contain essential nutrients such as minerals, proteins and microelements. Sustainable production of nutrient-dense and climate-resilient legumes, including Bambara groundnut, enables the diversification of human nutrition in the increasingly drier and hotter climate in Africa and internationally.

Bambara groundnut (*Vigna subterranea* (L.) Verdc) originated in West Africa, and it is widely grown in arid and semi-arid agroecologies in Africa and Asia. It is one of the best protein sources (9.6 to 40%) and other nutrients (e.g. magnesium, iron, zinc, and potassium). Bambara groundnut seed contains approximately 65% carbohydrates, 25% protein, and 7.4% oil (Brink et al. 2006). In addition, the seeds contain 32.72% essential amino acids (e.g. lysine, methionine) and 66.10% non-essential amino acids (e.g. glutamic and aspartic acids) (Onimawo et al. 1998; Minka and Brunetau, 2000; Amarteifio et al. 2006). High amounts of essential minerals (e.g. calcium [95.8-99 mg.100 g^{-1}], iron [4.9- 48], potassium [11.44-19.35] and sodium [2.9-12.0 mg.100 g^{-1}] are present in Bambara groundnut making it a complete food of choice.

Anti-nutritional factors (e.g. tannins, phenolics and flavonoids) are present in Bambara groundnut seed (Grant, 1989; Welch and Graham, 2004; Soetan, 2008). Anti-nutritional factors (ANFs) reduce nutrient uptake and absorption by humans and animals after consumption. Bambara groundnuts' nutritional and ANF compositions vary across genotypes and growing environments (Gerrano et al. 2019). A study by Akpalu (2010) assessed the nutritional value of Bambara groundnut landraces and reported a higher magnitude of protein (18 to 24 %), carbohydrate (65.86 to 68.63 %), fat (6.00 to 6.20 %) and fibre (2.90 to 3.00 %) vital for human dietary requirements. Likewise, Alake and Alake (2016) reported that essential minerals such as Zn, Mg, K, Fe, and Ca are present in Bambara groundnut collections. Bambara groundnut contained 37.00, 4.70, 430.00 and 125.00 mg 100 g^{-1} of Ca, Zn, P and K, respectively (Carnovale et al.1990). Amarteifio et al. (2010) assessed Bambara groundnut landraces of Botswana and reported higher nitrogen content (20.05 to 22.00 %) than previously reported in Namibia (19.28 to 20.53 %) and

Swaziland (17.10 to 19.91 %). Diverse Bambara groundnut genotypesfrom the International Institute of Tropical Agriculture (IITA)/Nigeria was profiled by Atoyebi et al. (2017) who grouped the lines into low to medium classes based on the contents of nitrogen, moisture, carbohydrate, crude fibre, total sugar, fat and minerals. The previous reports indicate variable nutrient compositions across the test genotypes and environments, necessitating targeted selection for variety design and product development. There is a need to exploit the genetic diversity of Bambara groundnuts for breeding new varieties with enhanced grain yield, nutritional quantity, and quality to develop various products.

Pre-breeding and breeding for enhanced nutrition quality, agronomic and farmer-preferred traits, and product development require adequate genetic variation (Nguni et al. 2012; Shegro et al. 2012; Gerrano et al. 2015). Previous efforts on Bambara groundnut breeding have focused on evaluating a limited number of collections on the agronomic performance with less emphasis on nutritional quality. There is marked genetic variation among landraces and introductions from diverse geographic origins of Bambara groundnut for targeted selection and to introgress multiple traits, including nutritional compositions. Detailed analyses of the compositions of phytochemicals such as fat, phenolics, flavonoids and mineral elements of Bambara groundnut enable ideotype selection and deduce trait correlations to guide breeding and product development. Also, there is a need to gain insight into multi-trait relationships for simultaneous selection. In light of the above background, the objective of this study was to determine the compositions of mineral elements and phytochemicals present in Bambara groundnut genetic pool to identify superior and contrasting genotypes to guide product development and breeding.

3.2 Materials and methods

3.2.1 Plant materials and site description

The study used 75 Bambara groundnut genotypes presented in Chapter 2 (Table 2.1). The test genotypes were obtained from the Agricultural Research Council (ARC) genebank collection. The genotypes were previously evaluated for grain yield, agronomic traits and genetic diversity for developing new breeding populations (Majola et al. 2022). Most of the evaluated genetic resources were initially collected from Bambara groundnut farmers from Kwa-Zulu Natal Province in South Africa, while some are introductions from other countries in southern Africa.

3.2.2 Trial establishment and experimental design

The genotypes were evaluated across five locations (Brits, Loskop, Mafikeng and Polokwane) in South Africa during the 2020-2021 main cropping season. The test environment represented unique environmental conditions for the production of Bambara groundnut in terms of agroecology, soil characteristics and climate (Table 3.1). Each genotype was planted on 2m row plots with intra- and inter-row spacings of 0.5 and 1 m, respectively. One seed was hand-sown per hole at a depth of 5cm. The trials were irrigated during the critical germination and seedling emergence stages to establish a uniform plant stand. Agronomic practices, including weeding, diseases and insect pests' management, followed standard procedures for Bambara groundnut production (BAMNET, 2000). No fertilizer was applied during plant growth. The trials were laid out in a 15 x 5 alpha lattice design with three replications at each location.

Table 3.1. Description of the environment for seed production and sampling to study phytochemical and nutrient compositions of Bambara groundnut genotypes

Site	Soil texture	Altitude (masl)	Rainfall (mm)	T_{Min} (°C)	T_{Max} (°C)	Latitude	Longitude
Loskop	Loamy	920	497	18,0	28,0	25.17° S	29.39° E
Brits	Clay loam	1083	629	18.6	28,9	25.61° S	27.79° E
Rustenburg	Clay	1170	602	17,5	28.0	25.65° S	27.25° E
Polokwane	Clay loam	1310	495	17.6	27.6	23.89° S	29.44° E
Mafikeng	Sandy	1359	571	16.7	27.6	25.85° S	25.60° E

m.a.sl = meter above sea level; mm = millimeter; min = minimum; max = maximum; °C = Celsius

3.2.3 Determination of nutritional contents

The test genotypes were field-grown to harvest maturity. Mature grains were harvested per the two-row plots to determine nutritional content. The mature seeds were dried at 12.5 °C moisture content. The dried seeds were ground into fine powder using an electric seed blender in preparation for analysis. The different nutrients were determined using various methods. Three replications per genotype were analysed. The antinutritional (phenolics and flavonoids) concentrations and fat and mineral contents such as calcium (Ca), iron (Fe), potassium (K), phosphorus (P), zinc (Zn) and nitrogen (N) were determined at the ARC analytical laboratory in South Africa. The fat content was determined using the Soxhlet method using diethyl ether (AOAC Method 920.39) (Norhayati et al. 2018). Total phenolic content was quantified as described by Makkar (1999). Gallic acid was used for the calibration curve, and the results were expressed in milli-gram gallic acid equivalent per gram dry weight (mg GAE/g DW). Flavonoid content was quantified using the aluminum chloride spectrophotometric method as described by Zhishen et al. (1999). Flavonoids content was described in milligram catechin equivalent per gram dry weight (mg CE/g DW) following the use of catechin in plotting the calibration curve. Major minerals (Ca, K, and P) were analysed using an atomic absorption spectrometer (AAS) (Elmer, 2023). The concentrations of Fe and Zn were determined as described in AOAC (2000). Approximately 0.5 g of finely ground dried samples were wet digested using a mixture of nitric acid (65%) and hydrochloric acid (37%) (1:3 v/v). Digestion was conducted on a 95 ◦C hot plate. Mineral elements in the digested plant materials were determined using the inductively coupled plasma optical emission spectrometry (ICP-OES) measured in mg 100 g^{-1}. The percentage of nitrogen content was analysed by combustion using an Elementar Rapid N III instrument (Elementar, Germany). At high temperatures and in the presence of pure oxygen,

nitrogen was liberated by combustion and nitrogen was isolated from other combustion products. A thermal conductivity detector measured the nitrogen content in each sample (Horneck and Miller. 1998).

3.3 Data analysis

The collected data were subjected to analysis of variance (ANOVA), and means were separated by the Fisher's Least Significant Difference (LSD) in R software using the “nlm” and “lm” packages (R Core Team, 2022). Mean values and coefficients of variation were computed for each mineral element and nutrient to compare variability within significantly different nutrients. Subsequently, the Pearson correlation and unweight pair group method using arithmetic averages (UPGMA) on the similarity indices and principal coordinate analysis (PCA) was performed to group and identify genotypes and to deduce trait associations. A combination of R packages, including FactoMineR, factoextra, devtools, ggpubr, tidyverse and cluster (Lê et al. 2008; Josse and Husson, 2007) analyses were used for data summary and to construct multivariate graphs. All the statistical tests were performed at 5% significance level using R (R CoreTeam, 2019).

3.4 Results

3.4.1 Analysis of variance

There were significant genotype x environment interaction effects on nutrient contents except for Zn and N (Table 3.2). Further, the genotype effect was significant for all nutrients except Zn and N, while the environment (Loskop) significantly influenced all the nutrients except for phenolic content.

3.4.2 Genotype performance for fat, phenolic, flavonoid and mineral contents

Genotype Bamb-2 was ranked the top for the contents of fat (6%), Ca (211.93 mg.100 g^{-1}) and Zn (7.14 mg.100 g^{-1}). Genotypes Bamb-2, Bamb-19, Bamb-73, Bamb-56, Bamb-37, Bamb-3 and Bamb-69, had high fat content above 6.00 % (Table 3.3). Nitrogen content was the highest in Bamb-62 (3.51 mg.100 g^{-1}) and Bamb-9 (3.50 mg.100 g^{-1}). Genotypes Bamb-40 and Bamb-59 had >16.00 mg.100 g^{-1} Fe, making them the highest-performing genotypes for Fe content. Conversely, genotype Bamb-29 was ranked among the lowest-performing genotypes for fat (3.80 %), Ca (166.17 mg.100 g^{-1}) and P (609.85 mg.100 g^{-1}) contents.

Table 3.2. Mean squares and significance tests for 75 Bambara groundnut genotypes evaluated across four locations in South Africa

Sources of variation	Df	Fat	Phenolics	Flavonoids	Ca	Fe	K	P	Zn	N
Locations (Loc)	3	680.2***	4.3	68.6***	4255657.0***	1023.8***	230469942.0***	99958379.0***	803.4***	8.2***
Replication (Rep) in locations	2	0.2	2.9	0.2	6642.0**	6.8	46073.0	315249.0*	16.2***	0.7**
Incomplete blocks	4	6.2**	9.1	3.5	1579.0	57.3**	133989.0***	306967.0*	3.7	0.2
Genotype (Gen)	74	6.7***	14.6***	2.3*	3221.0***	121.7***	86200.0***	429435.0***	1.9	0.2
Gen x Loc	222	8.6***	12.0***	2.4***	2525.0***	52.1***	79696.0***	394478.0***	1.5	0.1
Residuals	819	1.5	4.2	1.7	989.0	13.0	28642.0	101852.0	1.9	0.2

*** significant at 0.01 probability levels, respectively. Df= degrees of freedom; Ca =calcium; Fe =iron; K= potassium; P = phosphorous; Zn = zinc; N= Nitrogen; ENV=environment

Table 3.3. Mean values of fat, phenolic, flavonoid and mineral contents for 75 Bambara groundnut genotypes evaluated in four locations in South Africa

Genotype	Fat (%)	Phenolics	Flavonoids	Ca	Fe	K	P	Zn	N
		(mg GAE/g DW)	(mg CE/g DW)	mg.100 g^{-1}					
ARC Bamb-1	5.25	5.15	3.20	196.41	12.97	1003.1	727.21	7.22	3.37
ARC Bamb-10	5.51	4.25	3.12	192.52	8.30	938.15	989.72	6.31	3.23
ARC Bamb-11	5.07	5.45	3.01	177.21	10.7	1021.07	1066.39	6.44	3.29
ARC Bamb-12	5.78	5.27	2.84	150.70	5.55	1053.81	1064.03	6.38	3.17
ARC Bamb-13	5.36	2.86	2.67	183.77	7.74	996.60	828.00	6.98	3.33
ARC Bamb-14	4.80	5.73	2.61	204.86	9.19	902.28	1076.43	6.43	3.38
ARC Bamb-15	5.50	3.44	2.56	191.02	6.42	924.35	741.67	6.48	3.46
ARC Bamb-16	4.03	6.10	2.52	199.56	5.97	932.46	624.48	6.60	3.30
ARC Bamb-17	4.75	3.62	2.52	186.87	5.77	1161.21	952.56	6.47	3.35
ARC Bamb-18	4.47	4.15	2.50	194.58	12.04	1043.31	798.1	6.27	3.28
ARC Bamb-19	6.11	4.46	2.42	174.63	14.28	893.28	709.47	5.71	3.31
ARC Bamb-2	6.00	4.31	2.36	211.93	9.65	1042.27	729.29	7.17	3.34
ARC Bamb-20	3.77	4.06	2.34	230.24	13.7	829.02	949.54	7.14	3.22
ARC Bamb-21	5.18	6.53	2.28	191.94	9.14	1042.68	1104.03	6.62	3.26
ARC Bamb-22	5.26	4.90	2.27	194.90	4.78	810.85	731.94	5.73	3.30
ARC Bamb-23	5.66	5.56	2.23	168.89	5.34	890.62	729.42	5.79	3.19
ARC Bamb-24	4.87	3.87	2.23	183.00	12.15	1041.47	671.22	6.64	3.42
ARC Bamb-25	5.15	4.32	2.23	205.46	13.14	977.90	680.18	6.09	3.15
ARC Bamb-26	4.84	4.42	2.22	215.27	9.36	918.80	646.74	5.50	3.25
ARC Bamb-27	4.36	3.75	2.20	190.60	11.39	1014.40	693.28	6.13	3.27
ARC Bamb-28	4.27	4.53	2.19	175.68	8.93	981.48	761.21	6.19	3.26
ARC Bamb-29	3.80	6.27	2.19	166.17	10.91	1055.74	609.85	6.15	3.22
ARC Bamb-3	6.50	3.90	2.18	176.59	15.02	978.37	848.79	6.77	3.24
ARC Bamb-30	5.08	3.53	2.18	199.51	13.70	973.73	736.75	6.13	3.35
ARC Bamb-31	5.41	5.20	2.17	205.01	6.45	942.80	573.96	7.00	3.30
ARC Bamb-32	5.08	6.56	2.17	172.81	11.52	956.42	826.94	7.07	3.40
ARC Bamb-33	5.58	4.65	2.16	208.72	9.31	1042.33	975.94	6.39	3.21
ARC Bamb-34	4.78	5.03	2.15	178.70	14.32	958.55	690.84	6.99	3.32
ARC Bamb-35	5.40	4.82	2.13	216.53	7.62	1017.78	734.22	6.42	3.24
ARC Bamb-36	5.27	5.59	2.12	210.70	7.48	1034.6	668.67	6.10	3.44
ARC Bamb-37	6.26	4.99	2.09	175.18	11.43	906.04	629.08	6.94	3.46
ARC Bamb-38	4.2	4.21	2.09	186.01	11.61	951.94	888.46	6.19	3.24
ARC Bamb-39	5.26	6.68	2.08	184.01	11.84	1032.31	1080.06	6.43	3.29
ARC Bamb-4	4.88	3.92	2.07	184.09	10.57	921.00	649.27	6.58	3.33
ARC Bamb-40	5.10	7.36	2.06	171.58	16.34	1027.91	778.44	5.94	3.13

ARC Bamb-41	5.76	3.10	2.06	177.45	8.90	949.47	673.42	6.17	3.25
ARC Bamb-42	5.27	6.69	2.06	192.39	9.29	991.26	680.29	6.04	3.14
ARC Bamb-43	3.96	5.21	2.05	176.80	5.60	922.45	816.85	6.73	3.11
ARC Bamb-44	5.42	5.10	1.99	188.44	9.22	771.99	914.84	6.52	3.28
ARC Bamb-45	4.17	3.68	1.96	204.16	9.34	1008.41	532.53	6.80	3.36
ARC Bamb-46	5.87	3.87	1.95	168.37	12.46	1025.49	1477.82	6.81	3.20
ARC Bamb-47	4.60	5.15	1.94	183.37	11.14	1010.73	890.09	6.55	3.15
ARC Bamb-48	4.76	5.15	1.94	184.09	5.75	928.15	741.93	6.58	3.37
ARC Bamb-49	5.07	5.90	1.93	157.29	7.46	1001.94	971.44	6.26	3.32
ARC Bamb-5	5.30	4.45	1.93	180.29	10.33	1044.53	632.02	6.48	3.39
ARC Bamb-50	4.34	5.61	1.91	182.17	12.81	1031.95	888.38	6.62	3.20
ARC Bamb-51	5.26	4.53	1.91	195.50	12.61	979.76	656.04	6.39	3.16
ARC Bamb-52	5.27	3.29	1.88	190.64	10.58	1100.73	1058.77	6.62	3.36
ARC Bamb-53	4.62	3.73	1.86	172.91	13.93	947.36	701.21	7.06	3.43
ARC Bamb-54	5.19	5.58	1.86	187.71	11.8	910.09	874.88	6.33	3.38
ARC Bamb-55	3.85	5.02	1.84	197.61	8.13	904.37	1058.65	6.71	3.30
ARC Bamb-56	6.22	5.00	1.81	194.91	13.68	1148.91	785.52	6.27	3.30
ARC Bamb-57	5.95	4.63	1.79	195.32	10.81	911.78	681.03	6.82	3.36
ARC Bamb-58	4.55	4.50	1.79	196.34	12.14	919.66	904.20	6.61	3.31
ARC Bamb-59	4.98	5.99	1.79	153.77	16.77	1059.03	786.49	6.34	3.26
ARC Bamb-6	5.54	3.58	1.78	190.69	8.05	975.6	1077.27	6.94	3.36
ARC Bamb-60	5.00	5.65	1.77	196.79	10.87	1079.98	1091.94	6.27	3.31
ARC Bamb-61	5.09	4.39	1.77	173.82	4.42	996.33	882.98	6.38	3.35
ARC Bamb-62	5.34	4.73	1.75	203.06	11.63	977.04	674.75	6.61	3.51
ARC Bamb-63	4.58	5.47	1.73	176.57	9.82	884.45	563.85	6.32	3.44
ARC Bamb-64	5.44	3.56	1.64	174.87	10.72	928.51	667.75	6.36	3.43
ARC Bamb-65	4.43	6.31	1.63	191.70	9.76	1068.62	1053.07	6.49	3.31
ARC Bamb-66	4.20	4.39	1.63	174.45	7.10	979.49	731.46	6.29	3.32
ARC Bamb-67	3.94	4.40	1.62	173.06	10.32	1097.13	958.82	6.23	3.37
ARC Bamb-68	4.95	5.71	1.62	185.91	10.59	915.22	1117.82	6.82	3.48
ARC Bamb-69	8.90	6.66	1.59	188.71	10.38	1002.52	991.48	6.89	3.15
ARC Bamb-7	5.22	3.83	1.57	183.62	12.6	1136.66	926.72	6.92	3.29
ARC Bamb-70	5.44	4.69	1.52	170.41	10.95	1116.89	825.56	6.73	3.36
ARC Bamb-71	4.39	3.39	1.51	196.79	8.21	865.3	668.96	7.17	3.35
ARC Bamb-72	5.74	6.50	1.49	200.21	5.98	917.44	792.94	6.19	3.41
ARC Bamb-73	6.11	5.84	1.47	181.30	4.30	883.81	771.25	6.32	3.31
ARC Bamb-74	5.17	6.07	1.44	184.84	10.28	992.52	738.95	6.39	3.23
ARC Bamb-75	4.77	5.05	1.44	175.81	9.38	981.31	727.16	5.81	3.29
ARC Bamb-8	4.63	4.38	1.37	171.95	9.01	1033.40	969.19	6.29	3.24
Bamb-9	4.36	4.02	1.17	206.09	5.20	1155.89	887.72	6.34	3.50

Mean	5.06	4.84	2.04	187.09	9.94	983.86	823.28	6.47	3.31
CV (%)	24.40	42.52	64.21	16.78	36.17	17.18	38.71	21.06	10.38

ARC= Agricultural Research Council, Bamb= Bambara; CV= Coefficient of variation; Ca =calcium; Fe =iron; K= potassium; P = phosphorous; Zn = zinc; N= Nitrogen

3.4.3 Correlations of fat, phenolic, flavonoid and mineral contents

The Pearson correlation analysis showed that the nutrient compositions exhibited moderate to weak correlations (Table 3.4). Fat was positively and highly significantly correlated with Ca ($r=0.32$, $p<0.001$), K ($r=0.36$, $p<0.001$), P ($r=0.27$, $p<0.01$) and significantly associated with Zn ($r=0.24$, $p<0.05$). Phenolic contents were significantly correlated with K ($r=0.4$, $p<0.05$) and P ($r=-0.26$, $p<0.05$) and negatively correlated with N ($r=-0.36$, $p<0.001$). Flavonoids exhibited positive correlations with Ca ($r=0.31$, $p< 0.01$) and Zn ($r=0.26$, $p< 0.05$) only. Calcium had strong positive correlations with K ($r=0.63$, $p<0.001$) and N ($r=0.62$, $p<0.001$). The other strong positive significant correlations ($p< 0.01$) were for Ca with P. The strongest and negative correlation was recorded for K and N ($r=-0.68$, $p<0.001$), while P and Zn had positive correlations with K. Nitrogen mainly exhibited negative correlations with the other minerals, including Zn ($r=-0.47$, $p<0.001$). Iron content did not correlate significantly with any of the assessed nutrients in this study.

Table 3.4. Pearson correlation coefficients showing the associations of fat, phenolic, flavonoid and mineral contents recorded for 75 Bambara groundnut genotypes evaluated in four environment in South Africa

Parameters	Fat	Phenolics	Flavonoids	Ca	Fe	K	P	Zn	N
Fat	1.00								
Phenolics	0.30	1.00							
Flavonoids	0.19	0.21	1.00						
Ca	0.32***	0.18	0.31***	1.00					
Fe	-0.02	-0.05	-0.21	-0.12	1.00				
K	0.36***	0.24*	0.15	0.63***	0.03	1.00			
P	0.27**	0.26*	0.14	0.34***	-0.05	0.52***	1.00		
Zn	0.24*	0.01	0.26*	0.40***	0.07	0.33***	0.29**	1.00	
N	-0.34***	0.36***	-0.19	0.62***	0.04	-0.68***	-0.47***	-0.17	1.00

** and *** significant at 0.01 and 0.001 probability level, respectively. Ca =calcium; Fe =iron; K= potassium; P = phosphorous; Zn = zinc; N= Nitrogen,

3.4.4 Principal component analyses of fat, phenolic, flavonoid and mineral contents

The three principal components (PC) with Eigen values ≥1 that accounted for 62% of the total variation the 75 Bambara groundnut genotypes (Table 3.5). Principal component 1 (PC1) explained 37% of the variation, while PC2 and PC3 explained 13% and 12%, respectively. Potassium accounted for 20% of the variation, N (19%), Ca (18%) and P (13%) were the major contributors of PC1. Iron had a non-significant contribution to PC1. The variation for PC2 was dominated by Fe, which accounted for 55%, followed by flavonoids (30%). Phenolic and Zn contents explained most of the variations for PC3, contributing to 35 and 43% , respectively.

Table 3.5. Principal components (PC), the proportion of variance and contributions of fat, phenolic,flavonoid and mineral contents measured in 75 Bambara groundnut genotypes

	Principal components		
Parameters	**PC1**	**PC2**	**PC3**
Eigenvalues	3.34	1.19	1.08
Varaince (%)	37.14	13.19	12.02
Cumulative variance (%)	37.14	50.32	62.34
Fat	9.68	0.04	1.73
Phenolics	6.31	4.18	34.77
Flavonoids	5.18	30.42	9.41
Ca	18.46	0.05	4.74
Fe	0.28	55.09	0.02
K	20.25	5.34	0.07
P	13.11	1.84	0.90
Zn	7.29	2.31	42.66
N	19.44	0.73	5.70

PC= Principal component; var= variation, Ca =calcium; Fe =iron; K= potassium; P = phosphorous; Zn = zinc; N= Nitrogen

3.4.5 Biplot analyses of fat, phenolic, flavonoid and mineral contents

Figure 3.2 shows the summary of biplot analyses for fat, phenolic, flavonoid and mineral contents of 75 Bambara groundnut genotypes. Genotypes that are plotted near a vector of a particular parameter are highly correlated with it. The length of the vector is relative to the mean of the parameter, while the direction indicates the relation with other parameters and genotypes. Fat content had a relatively small absolute mean compared to the other nutrients.

Genotypes positively correlated with fat content were Bamb-69, Bamb-75, and Bamb-65, among others, in the respective quadrant. Similarly, genotypes positively correlated to fat were also likely to correlate with phenolic content as they were plotted in the same quadrant. Genotypes such as Bamb-22, Bamb-26 and Bamb-72 were closely associated with the vector for flavonoids. Calcium, N and Zn were in the same quadrant and shared similar genotype-trait correlations with genotypes such as Bamb-9, Bamb-59 and Bamb-1. For Fe, K and P, the genotypes associated with these nutrients are Bamb-7, Bamb-70 and Bamb-56, among others.

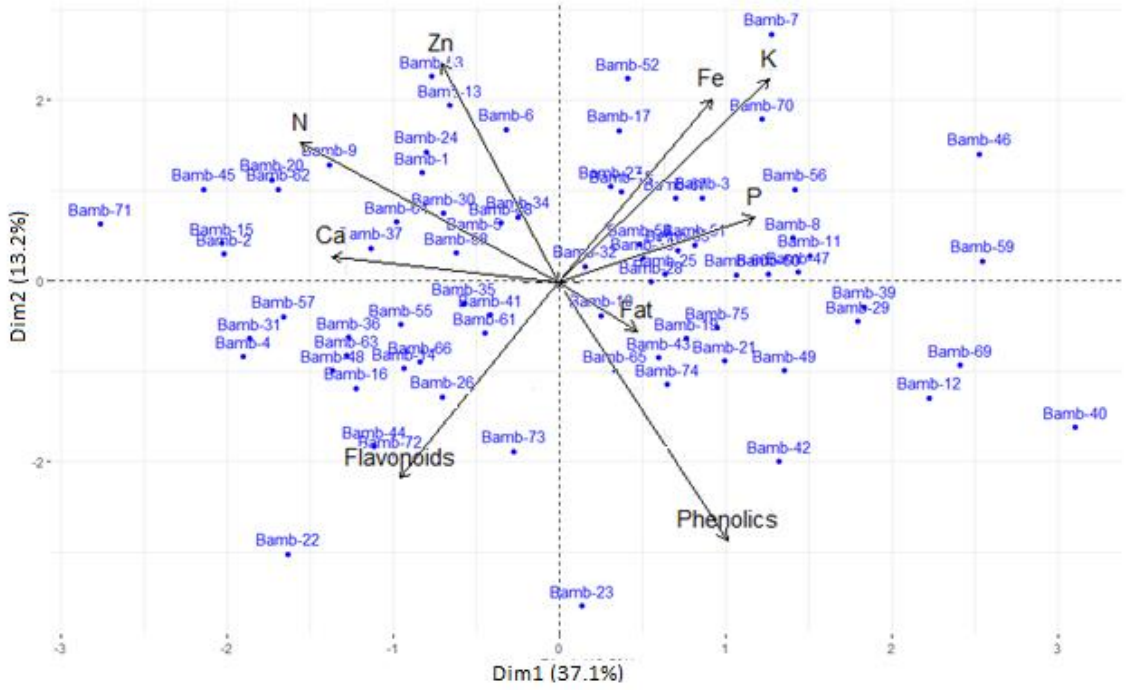

Figure 3.2. Multivariate relationships for fat, phenolic, flavonoid and mineral contents among 75 Bambara groundnut genotypes. Note: Dim1 and Dim2 denote dimensions 1 and 2, respectively.

3.4.6 Cluster analysis

Based on UPGMA analysis , three major clusters were identified with variable constituent genotypes (Figure 3.3). The biggest cluster (Cluster I) had 30 genotypes, including Bamb-72, Bamb-73, Bamb-49, Bamb-5, Bamb-54, Bamb-10, Bamb-14, Bamb-16, and Bamb-19. This

cluster was characterised by high flavonoids, Ca and N contents. The second biggest cluster (Cluster II) had 24 genotypes that included Bamb-11, Bamb-12, Bamb-21, Bamb-23, Bamb-43, Bamb-47, Bamb-50, Bamb-51, Bamb-33 and Bamb-35. The genotypes in this cluster generally had high phenolic and K contents. The smallest cluster (Cluster III) had 21 genotypes such as Bamb-17, Bamb-18 and Bamb-24, Bamb-68, Bamb-7, Bamb-70, Bamb-8 and Bamb-9 that typically had high K, N and Zn contents.

A further cluster analysis using the scatterplot shows that there were overlaps in genotypes performance for some genotypes that belonged to different clusters (Figure 3.4). Genotypes such as Bamb-59, Bamb-40, Bamb-22, Bamb-23 and Bamb-72 (Cluster I), Bamb-46, Bamb-7 and Bamb-52 (Cluster III), and Bamb-53, Bamb-45 and Bamb-71 (Cluster II), did not overlap with genotypes from other clusters (early maturing genotype) while the rest of the genotypes overlapped in performance in one trait or another (Figure 3.4). An overlap of genotypes coincides with high or mediocre performance in multiple parameters, or the cluster being characterised by multiple traits.

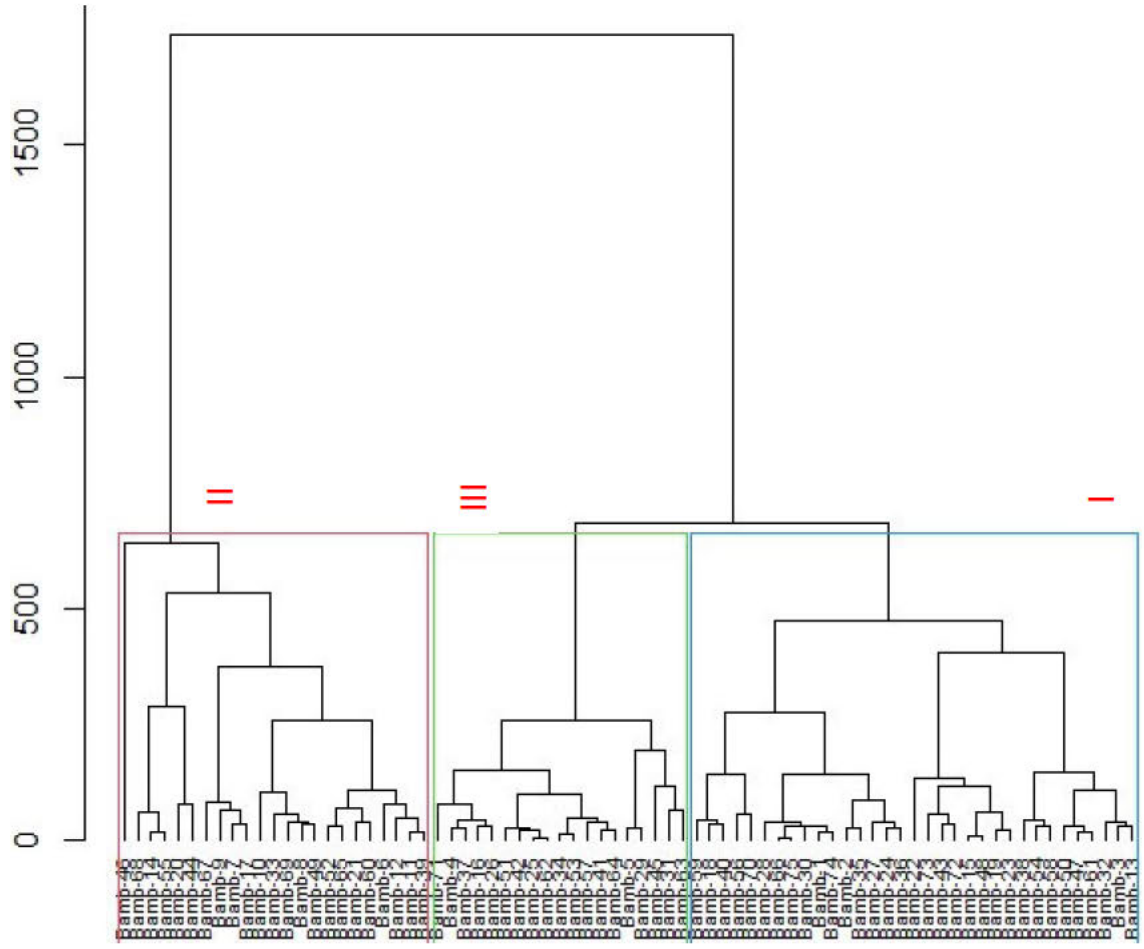

Figure 3.3. Dendrogram depicting the interrelatedness of 75 Bambara groundnut genotypes based on fat, phenolic, flavonoid and mineral contents

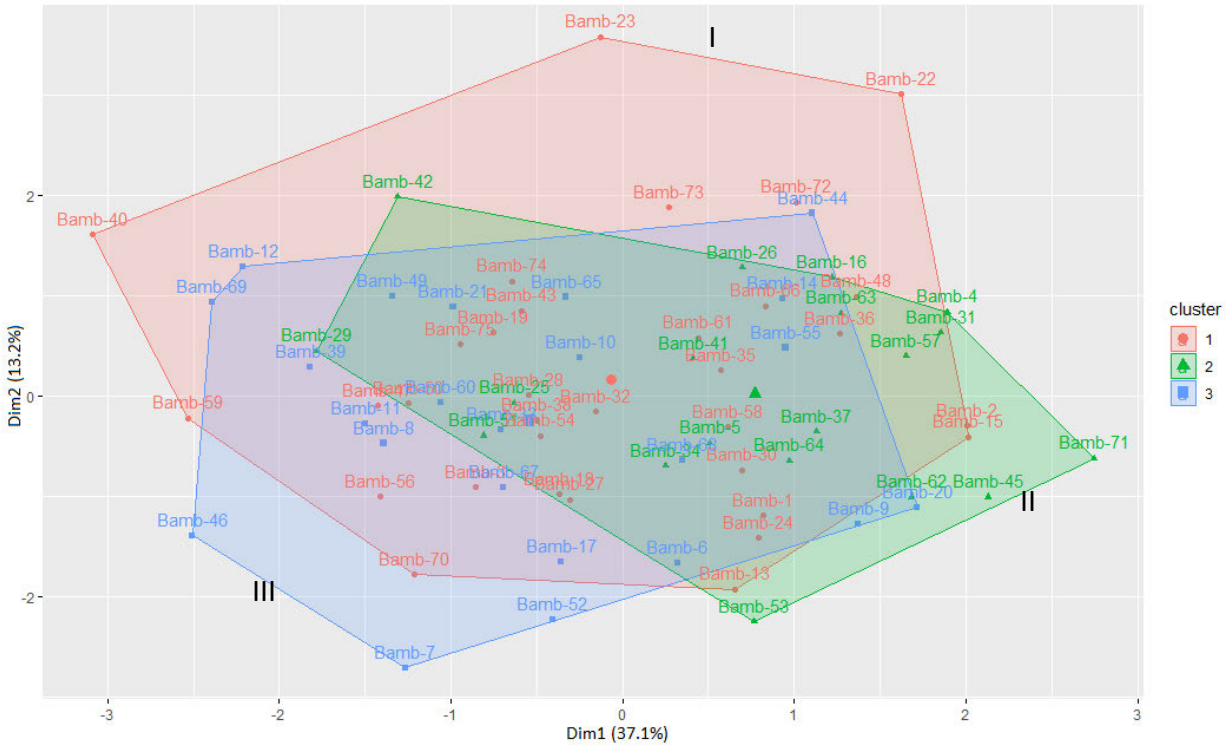

Figure 3.4. A scatter plot depicting the clusters of 75 genotypes of Bambara groundnut based on fat, phenolic, flavonoid and mineral contents. Note: Dim1 and Dim2 denote dimensions 1 and 2, respectively.

3.5 Discussion

3.5.1 Variation for nutrients and mineral content

There were significant differences among the Bambara groundnut accessions for phytochemical and nutrient contents due to genotype x location interaction. This suggests the need to investigate Bambara groundnut genotypes for adaptability and nutrient composition under diverse environments to guide variety recommendation and breeding. Mineral composition and biomass allocation in plant tissues is a function of physiological processes that are controlled by the plant's intrinsic genetics and extrinsic biotic and abiotic factors such as ambient temperature, soil nutrients and pests and diseases (Pittelkow et al. 2015; Sanchez et al. 2014; Mubaiwa et al. 2018; Ramatsetse et al. 2023). Further, the genotypic differences and environmental influence on most nutrients showing that superior genotypes can be identified in specific environments. Genotype differences in mineral accumulation are caused by differences in physiology and resource utilization as consequences of genetic makeup (Chimna et al. 2021). The genetic constitution dictates biochemical synthesis in plant tissues, which

directly impacts nutrient composition. The test genotypes' genetic constitutions were different, partly contributing to the variation in their mineral element content. Conversly, environmental factors such as soil fertility and radiation affect plant physiology and contribute to the differential accumulation of micronutrients in plants (Okebalama et al. 2022). The differences in soil fertility at the different locations affected mineral uptake by the different Bambara groundnut genotypes. For instance, air temperature and radiation affect plant growth and synthesis of biological compounds, which can be reflected in mineral element content (Husson, 2013). Temperature and moisture deficit stresses elevate the production of plant hormones such as abscisic acid and cytokinins (Gujjar and Supaibulwatana, 2019). These hormones affect plant tissues' accumulation and transportation of primary elements such as Fe and Mg (Mune et al. 2018). The differences in environmental conditions across the four locations and variations in the genetic constitution of the genotypes contributed to the observed variation in mineral content.

The average N, Ca, Zn, phenolics and flavonoid contents found in this population of Bambara groundnut were comparable to previous reports (Hlanga et al. 2021; Ndidi et al. 2014). The nutritional content of genotypes ARC Bamb-2 (for fat, Ca and Zn), and ARC Bamb-40 and ARC Bamb-59 (for Fe) suggest that these genotypes for cultivar development. The high content of essential elements such as Fe, Ca and Zn makes this population of Bambara groundnut important for addressing hidden hunger in South African communities, especially the poor and rural communities with limited access to a balanced diet. For instance, Zn deficiency is recognized as a global public health challenge, and promoting Bambara utilization can alleviate this challenge (Hambridge, 2000; Stein, 2010).

3.5.2 Bivariate correlations

The Pearson correlation analysis showed that the nutrients exhibited moderate to weak correlations among each other. Additionally, previous reports have indicated variable correlations among the mineral elements (Ndidi et al. 2014; Hlanga et al. 2021; Olanrewaju et al. 2022). The differences between correlations results in previous studies and those reported in this study could emanate from differences in germplasm and attendant environmental conditions. The positive correlations exhibited between fat and Ca, K, P, and Zn is important for simultaneous selection since selecting any of these traits could lead to an indirect improvement in the other traits. Simultaneous improvement of correlated traits has been

identified as a strategy for efficient and effective breeding strategy (Makanda et al. 2019). However, challenges will be encountered during selection where the correlations are unfavourable. Such unfavourable correlations often result in an indirect reduction in performance in one or more traits after the selection of another trait. In this instance, selection for high N could result in reduced accumulation of N due to their negative correlation. Similarly, the negative correlations between K and N and phenolic content and N would present challenges for Bambara groundnut improvement if conventional selection methods are used. Differential correlations are caused by genetic linkages, and environmental factors. Some nutrients are inherently linked possibly because they are controlled by the same or related loci (Arogundade et al. 2023). In some instances, the nutrients may share similar pathways for accumulation or be affected by related enzymes or catalysts for accumulation (Hamadou et al. 2022). Molecular or mutation breeding methods could help break unfavourable linkages or induce mutations with increased concentrations of particular traits (Alake, 2018).

Phenolic and flavonoids have been reported to be antinutrients that reduce Fe availability in monogastric livestock and humans (Thavarajah, 2013). Therefore, the non-significant correlations between flavonoids and other traits are conducive to its reduction in Bambara groundnut without negatively affecting the accumulation of essential nutrients. Similarly, phenolics were negatively correlated with Fe, indicating that genotypes with high Fe content could be developed without elevating the concentration of phenolics or flavonoids. The correlations exhibited in this study will be useful for developing appropriate strategies for biofortification of Bambara groundnut genotypes through genetic improvement.

3.5.3 Principal components and trait contributions

The first two PCs accounted for 62% of the variation among the genotypes for nutrient content, showing that the majority of the differences could be explained by genotype, environment and genotype x environment interaction effects. An ability to account for a large proportion of the variation being accounted in a few PCs allows the breeder an opportunity to embark on further analysis to identify major contributors to the identified variation. Potassium (K) (20% of the variation), nitrogen (19%), calcium (18%) and phosphorous (13%) were the major contributors of the variation explained by the PC1, indicating that were important for assessing the genetic variation among the genotypes. The high contribution of these elements to the first PC shows that they must be used as target traits for evaluating genotypes for conservation purposes and

identifying superior genotypes. However, their importance in discriminating the genotypes does not imply that they are more important than the other mineral elements in terms of dietary requirements. The elements that contributed most to PC2 included Fe and flavonoids, suggesting that they could be used as the next important characterization criterion for Bambara groundnut. Mbuma et al. (2022) and Chelangat et al. (2023) found that different sets of mineral elements with different contributions to PCs. The relative importance of the traits on different PCs varies between studies due to differences in germplasm evaluation and environmental conditions.

3.5.4 Multivariate associations among genotype and nutrient variables

Genotypes closely associated with vectors of specific nutrients can be selected as parental genotypes for improving such nutrients. For fat content, ARC Bamb-69, ARC Bamb-75, and ARC Bamb-65 could be identified. ARC Bamb-9, ARC Bamb-59 and ARC Bamb-1 could be selected for their high accumulation of Calcium, N and Zn while ARC Bamb-7, ARC Bamb-70 and ARC Bamb-56 were suitable for Fe, K and P improvement. The use of the multivariate biplot has been used in selecting suitable candidates for crop improvement by several researchers (Jonah et al., 2010: Maunde et al. 2015). Conversely, genotypes not close to a vector of any particular nutrient were not exceptional in accumulating any of the nutrients.

3.5.5 Genotype relationships

The cluster analyses found that the genotypes could be grouped in different clusters, indicating similarities and differences among them. The differential grouping can be exploited since crop improvement hinges on genetic diversity among genotypes. Genotypes in a cluster share similar performances for specific nutrients while those belonging to different clusters are expected to perform differently. The genotypes in the biggest cluster could be good candidates for improving Ca and N content, while the smallest clusters were ideal for K and Zn improvements, respectively. However, the overlapping of genotypes in some clusters shows that some genotypes have good or bad performance in multiple nutrients, making them belong to different clusters at the same time. Clustering based on biochemical traits has been used successfully to identify divergence among genotypes (Song et al. 2013). The selection of genotypes for crossing should focus on divergent genotypes while recommending genotypes

for production can risk of biotic and abiotic stresses, especially in stress-prone environments of sub-Saharan Africa.

3.6 Conclusion

The study used a significantly higher number of Bambara groundnut and determined the phytochemical and mineral elements compositions. The test genoty pesrepresent a genetic pool enabling the identification of superior and contrasting genotypes for product development and breeding. The following genotypes: ARC Bamb-2, ARC Bamb-19, ARC Bamb-73, ARC Bamb-56, ARC Bamb-37, ARC Bamb-3 and ARC Bamb-69 exhibited the highest fat content (>6.00 %). ARC Bamb-40 and ARC Bamb-59 recorded a higher mean Fe content (16.00 mg.100 g^{-1}). ARC Bamb-2 was the top-performing genotype with higher fat content (6%), Ca (211.93 mg.100 g^{-1}), and Zn (7.17 mg.100 g^{-1}). Calcium, K, and N contents strongly correlated ($r>0.60$, $P<0.05$). Phosphorus and Zn contents exhibited moderate correlations with Ca. Overall, the study selected genotypes ARC Bamb-73, ARC Bamb-19, ARC Bamb-9 and ARC Bamb-2 with high compositions of essential nutrients for product development or breeding. The selected genetic resources are valuable for trait integration and developing new breeding populations with enhanced nutrient compositions and agronomic and market-preferred traits.

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CHAPTER 4. Genetic diversity and population structure analyses of South African Bambara groundnut (*Vigna subterranea* [L.] Verdc)

Abstract

Bambara groundnut (*Vigna subterranea* [L.] Verdc**)** is a highly nutritious grain legume with a significant potential to develop various commercial food and feed products. It is a resilient crop able to grow under harsh climates and poor soil conditions. Bambara groundnut productivity in South Africa is low (0.62 t/ha) compared to the attainable yield of the crop reaching up to 3 t/ha. The low productivity is attributable to a lack of improved and high yielding cultivars that are locally bred and adapted in the country. Genetically diverse and locally adapted Bambara groundnut landrace varieties and exotic collections are present in South Africa for strategic breeding to improve yield and yield-related traits and nutritional quality. The objective of this study was to determine the magnitude of the genetic diversity and population structure of Bambara groundnut collections of South Africa using high throughput single nucleotide polymorphisms (SNP) markers. Ninety-three genotypes were assessed using genotyping with 2286 SNP markers and some unique complementary morpho-agronomic traits of the crop. The mean genetic diversity value was 0.32, revealing moderate genetic differences among the assessed genotypes. Cluster and structure analyses grouped the test genotypes into two distinct categories. Further, the analysis of molecular variance partitioned the total genetic variation into among genotypes (90%), within genotypes (8%) and among populations (2%). The results revealed two heterotic groups for hybridisation and selection programs. The following unique genotypes were selected: ARC Bamb 37 (with spreading growth type), ARC Bamb 49 (bunch type), ARC Bamb 61 (semi-bunch) and ARC Bamb 83 (spreading) using the SNP markers and desirable agronomic traits. The study provided new insight on Bambara groundnut genetic profiles of South African collections which will assist in conservation strategy and management of the crop for effective breeding.

Keywords: Bambara groundnut, Genetic diversity, Population structure, SNP.

4.1 Introduction

The genus *Vigna* belonging to the family Leguminosae is one of the most economically significant taxon of legumes. There are approximately 90 species within the genus, of which seven are cultivated as economic crops globally. Bambara groundnut (*Vigna subterranea* [L.] Verdc; 2n = 2x = 22) is Africa's third most important legume crop after peanut (*Arachis hypogaea* L.) and cowpea (*Vigna unguiculata* L.). Bambara groundnut is widely grown in the drier areas of sub-Saharan Africa (SSA), including South Africa (Tan et al.2020)

Bambara groundnut is highly nutritious, consisting of carbohydrates (51-71%), crude protein (18-24%), oil (4-12%), and fibre (3-12%) (Agajie, 2021). These balanced nutrients make Bambara groundnut products a complete food. It fixes atmospheric nitrogen through a symbiosis with soil bacteria, *Rhizobia* populations. Bambara groundnut is a climate-resilient crop adapted to grow under drought and heat stress and poor soil fertility conditions compared with other common legumes. The crop has the potential to produce a reasonable yield under low moisture environments, making it a valuable component in the cropping systems in dry regions (Minnaar-Ontong et al.2021).

Approximately 0.37 million hectares of Bambara groundnut is cultivated globally, with an annual production of approximately 0.22 million tons of shelled grain (FAOSTAT 2022). West Africa is the center of the genetic diversity of Bambara groundnut. The region accounts for about 65% of global production. However, most African countries do not produce enough quantities of Bambara groundnut without meeting their domestic demand (Mayes et al.2019). Hence, most Bambara groundnut produce is sold locally or regionally with limited supply to the global market.

Bambara groundnut is one of the legume crops cultivated in South Africa. The productivity in South Africa is low (0.62 t/ha) compared with the attainable yield of the crop reaching up to 3 t/ha. The low productivity in the country, including other SSA countries, is attributable to a lack of improved and high yielding cultivars, poor agronomic practices and biotic and abiotic production constraints (Abady et al.2019). Genetically diverse locally adapted Bambara groundnut landrace varieties and some introductions are grown in South Africa. The genotypes are yet to be systematically evaluated to develop improved varieties and commercial products. Characterising the phenotypically diverse germplasm available in South Africa would contribute to the pre-breeding program to facilitate parental selections for cultivar development (Shegro et al.2013).

Southern African countries, including Zimbabwe, Botswana, Namibia, Zambia, Malawi, and South Africa, are the possible centres of genetic diversity of Bambara groundnut. In these countries, some wild relatives of Bambara groundnut are reported (Catarino et al.2021). Bambara groundnut collections of diverse genetic backgrounds are preserved in the genebank of the Agricultural Research Council (ARC), South Africa, Southern African Development Community (SADC) in Zambia and the various National Plant Genetic Resource Centres in Southern Africa. The germplasm collections are predominantly landraces which are unimproved and locally adapted varieties with intrinsic farmer-preferred quality traits. The landraces differ in agro-morphological traits such as growth type, plant height, number of pods per plant, flowering rate, maturity days, tolerance to drought and heat stress, and insect pests and diseases.

Knowledge of the extent of genetic variability among genotypes and populations is critical for selecting superior genotypes for production and breeding. Genetic gain through breeding is dependent on the selection of genetically divergent and complementary parental genotypes (Feldman et al.2019). Hence, assessing the genetic diversity present in diverse populations is a prerequisite for any crop breeding programme. The level of genetic variability existing within the population defines the success and the efficiency of the genetic improvements (Alhassan and Egbe, 2014). Various methods such as phenotypic, biochemical and molecular approaches can be used to characterize the genetic diversity among genotypes and populations.

Previous studies reported the presence of genetic diversity of Bambara groundnut mainly using phenotypic traits (Berchie et al.2012; Unigwe et al.2016). Significant phenotypic variations have been reported in economic traits such as grain yield and yield components in Bambara groundnut (Gao et al.2020). There are marked phenotypic variations in global Bambara groundnut collections for selecting distinct phenotypes for large-scale production or breeding (Gao et al.2020). Quantitative morphological traits are affected by the genotype, environment, and genotype x environment interactions, limiting the accuracy of selection and the degree of trait heritability. Genotype screening using morphological and molecular makers would enhance the response to selection (Somta et al.2011). Assessing the genetic variation and population structure of Bambara groundnut genetic resources using high throughput molecular markers is critical for accelerated breeding and genetic gains.

Recent advances in molecular marker technology have brought significant developments and innovations in plant breeding through generating unique DNA profiles of assessed genotypes

for marker-assisted breeding and genomic selection (Bhat et al.2016). Several genetic diversity studies were reported in Bambara groundnut, including the use of microsatellite markers (Beena et al.2012; Molosiwa et al.2015; Minnaar-Ontong et al.2021), restriction fragment length polymorphism (RFLP) (Basu et al.2007) and amplified fragment length polymorphism (AFLP) (Odongo et al.2015). The choice of the marker system is influenced by factors such as ease of application, genome coverage, costs, and automation compatibility. The next-generation sequencing platform involving single nucleotide polymorphism (SNP) has improved the accuracy and efficiency of genotyping of crop species, including Bambara groundnut. SNPs are regarded as the most abundant molecular markers due to their wide distribution across the genome (Anđelković et al.2020).

Next-generation sequencing (NGS) and genotype-by-sequencing (GBS) technologies have reduced genotyping costs drastically, making these technologies the most feasible for the development of molecular markers and genotyping (Bhat et al.2016; Zou et al.2016). Diversity array sequencing technology (DArTseq) is one of the most widely used SNP based genotyping platforms for genome sequencing employing both NGS and GBS. This method involves three crucial steps: array development, genotyping, and data analysis. The first step is a complexity reduction method using methylation-sensitive restrictive enzymes to reduce the number of fragments present in the genomic representation (Bonin et al.2004). The second step involves labelling and hybridisation of the target genome of individuals with a reference DNA fragment. The last step extracts the microarray data and identifies polymorphism (Nawaz et al.2017). DArTseq has assisted in identifying haplotype blocks or single nucleotide polymorphism (SNP) signatures that are significantly correlated with quantitative trait variations (Valdisser et al.2017). These markers have been broadly utilized in plant breeding research, including genetic differentiation, development of dense genetic and physical maps and population structure analyses (Nadeem et al.2018). Hence, the objectives of this study were to determine the extent of genetic diversity and population structure among a collection of Bambara groundnut genotypes of South Africa using single nucleotide polymorphisms markers to select genetically unique genotypes for breeding.

4.2 Materials and Methods

4.2.1 Plant material

This study evaluated the genetic diversity and population structure of 93 Bambara groundnut genotypes (Table 4.1) using DArT SNP markers. The genotypes were acquired from the Agricultural Research Council-Vegetables, Industrial and Medicinal Plants (ARC-VIMP) genebank, Pretoria, South Africa. The test genotypes are core accessions that are adapted to South African agro-ecologies based on preliminary evaluations (Shegro et al.2013). The genotypes were selected from different growth types, in which 48% had bunching growth habits and were 26% each for semi-bunching and spreading types.

Table 4.1: List of Bambara groundnut genotypes used in the study and corresponding growth type

Entry No.	Acc. Code	Entry name	Growth type	Entry No.	Acc. Code	Entry name	Growth type
1	9	ARC Bamb 1	Bunch	48	144	ARC Bamb 48	Bunch
2	19	ARC Bamb 2	Bunch	49	145	ARC Bamb 49	Bunch
3	21	ARC Bamb 3	Bunch	50	146	ARC Bamb 50	Spreading
4	25	ARC Bamb 4	Semi-bunch	51	148	ARC Bamb 51	Bunch
5	49	ARC Bamb 5	Semi-bunch	52	149	ARC Bamb 52	Semi-bunch
6	52	ARC Bamb 6	Bunch	53	150	ARC Bamb 53	Bunch
7	53	ARC Bamb 7	Semi-bunch	54	151	ARC Bamb 54	Semi-bunch
8	55	ARC Bamb 8	Bunch	55	156	ARC Bamb 55	Bunch
9	61	ARC Bamb 9	Semi-bunch	56	160	ARC Bamb 56	Bunch
10	62	ARC Bamb 10	Semi-bunch	57	161	ARC Bamb 57	Bunch
11	66	ARC Bamb 11	Spreading	58	165	ARC Bamb 58	Spreading
12	74	ARC Bamb 12	Semi-bunch	59	166	ARC Bamb 59	Semi-bunch
13	77	ARC Bamb 13	Bunch	60	168	ARC Bamb 60	Semi-bunch
14	78	ARC Bamb 14	Bunch	61	169	ARC Bamb 61	Semi-bunch
15	79	ARC Bamb 15	Bunch	62	173	ARC Bamb 62	Bunch
16	80	ARC Bamb 16	Semi-bunch	63	174	ARC Bamb 63	Bunch
17	81	ARC Bamb 17	Spreading	64	175	ARC Bamb 64	Semi-bunch
18	82	ARC Bamb 18	Semi-bunch	65	176	ARC Bamb 65	Bunch
19	84	ARC Bamb 19	Spreading	66	177	ARC Bamb 66	Bunch
20	86	ARC Bamb 20	Semi-bunch	67	179	ARC Bamb 67	Semi-bunch
21	87	ARC Bamb 21	Spreading	68	180	ARC Bamb 68	Bunch
22	89	ARC Bamb 22	Bunch	69	181	ARC Bamb 69	Bunch
23	90	ARC Bamb 23	Semi-bunch	70	183	ARC Bamb 70	Bunch
24	92	ARC Bamb 24	Bunch	71	184	ARC Bamb 71	Bunch
25	94	ARC Bamb 25	Spreading	72	185	ARC Bamb 72	Semi-bunch
26	95	ARC Bamb 26	Bunch	73	188	ARC Bamb 73	Bunch
27	96	ARC Bamb 27	Semi-bunch	74	189	ARC Bamb 74	Bunch
28	97	ARC Bamb 28	Semi-bunch	75	190	ARC Bamb 75	Bunch
29	99	ARC Bamb 29	Bunch	76	191	ARC Bamb 76	Bunch
30	100	ARC Bamb 30	Bunch	77	192	ARC Bamb 77	Bunch
31	101	ARC Bamb 31	Bunch	78	193	ARC Bamb 78	Bunch
32	102	ARC Bamb 32	Bunch	79	194	ARC Bamb 79	Spreading
33	105	ARC Bamb 33	Bunch	80	195	ARC Bamb 80	Spreading
34	107	ARC Bamb 34	Semi-bunch	81	196	ARC Bamb 81	Spreading
35	114	ARC Bamb 35	Semi-bunch	82	197	ARC Bamb 82	Spreading
36	116	ARC Bamb 36	Bunch	83	198	ARC Bamb 83	Spreading

37	117	ARC Bamb 37	Spreading	84	199	ARC Bamb 84	Spreading
38	118	ARC Bamb 38	Spreading	85	200	ARC Bamb 85	Bunch
39	121	ARC Bamb 39	Bunch	86	201	ARC Bamb 86	Spreading
40	123	ARC Bamb 40	Bunch	87	202	ARC Bamb 87	Bunch
41	127	ARC Bamb 41	Semi-bunch	88	203	ARC Bamb 88	Spreading
42	129	ARC Bamb 42	Spreading	89	204	ARC Bamb 89	Spreading
43	131	ARC Bamb 43	Spreading	90	205	ARC Bamb 90	Spreading
44	136	ARC Bamb 44	Bunch	91	206	ARC Bamb 91	Spreading
45	140	ARC Bamb 45	Bunch	92	207	ARC Bamb 92	Bunch
46	141	ARC Bamb 46	Semi-bunch	93	208	ARC Bamb 93	Spreading
47	142	ARC Bamb 47	Spreading				

ARC = Agricultural Research Council, Bamb= Bambara

4.2.2 DNA isolation and genotyping

The seeds of the 93 Bambara groundnut genotypes were planted in plastic pots in a greenhouse at the ARC-VIMP. Leave samples were shipped to Biosciences eastern and central Africa-International Livestock Research Institute (BecA-ILRI) hub in Nairobi, Kenya for genotyping. Plant samples were collected from the shoot base from randomly selected four plants in each genotype four weeks after germination. DNA extraction was done using the DNeasy Plant Mini Kit (QIAGEN, 69104). The sampled leaves of test genotypes were processed and placed into a 96-well plate. Each well represented an individual genotype. Genotyping was done using 6563 DArT SNP markers following DArtseqTM technology (https://www.diversityarrays.com).

4.2.3 Data analysis

Monomorphic alleles with <30% missing data and rare SNPs with minor allele frequency of less than 2% were filtered out. Only 2286 (34.8%) polymorphic SNP markers were maintained for further analysis. Genotypic data were subjected to analysis of molecular variance (AMOVA) and various measures of genetic diversity within and among inferred subpopulations using GenAlex software version 6.5 (Peakall and Smouse, 2012). Genetic diversity parameters such as Shannon's Information Index (I), observed heterozygosity (H_o) and expected heterozygosity (H_e) and polymorphic information content (PIC) were determined using the protocol of Nei and Li (1979). The genotypic data was used to obtain a dissimilarity matrix using the Jaccard index as described by Debener et al.(1990). The matrix was then used to run a cluster analysis based on a neighbour-joining algorithm using the unweighted pair group method with arithmetic average (UPGMA) in DARwin 6.0 software (Perrier and Jacquemoud-Collet, 2006). Bootstrap analysis was performed for node construction using 10,000 bootstrap values. The number of genotypes represented by each predetermined

subpopulation based on growth type was unbalanced. Hence, allelic richness was corrected for sample size differences and estimated by using the rarefaction method implemented in HP-Rare (Kalinowski, 2005).

The Bayesian genotypic clustering approach of STRUCTURE version 2.3.4 (Pritchard et al.2000) was used to infer the population structure of the germplasm. An admixture model with independent allele frequencies without prior population information was used to simulate the population. The STRUCTURE program was set as follows: a burn-in period length of 100,000 and after burn-in 100,000 Markov chain Monte Carlo repetitions. This model assumes that each individual's genome is a mixture of genes originating from K unknown ancestral populations. For joint inference of the population substructure, K ranging from 1 to 10 was set up, with ten independent runs for each K. The most probable value of K for each test was detected by ΔK (Evanno et al. 2005), using the STRUCTURE HARVESTER (Earl and Vonholdt, 2012). Each genotype was grouped into a given cluster using 'membership coefficient' for each cluster interpreted as a probability of membership. The genotype membership was determined and visualized by the computer program CLUMPAK (Kopelman et al.2015).

4.3 Results

4.3.1 Marker characterisation

The genetic diversity analysis was conducted using 2286 polymorphic SNPs after filtering out monomorphic and SNPs with a minor allele frequency of <2%. The distribution of expected genetic diversity, inbreeding coefficient, polymorphic information content and genetic distance for 2286 SNP markers are summarised in Figure 4.1. The distribution of values for H_o, H_e and PIC were skewed towards the lower values, while the distribution for F_{IS} skewed towards the higher values (Figure 4.1). The observed heterozygosity of the 2286 SNP loci ranged from 0.0 to 0.685, with a mean of 0.042. The expected heterozygosity of the SNP loci ranged from 1.1% to 50%, with a mean of 21.2%. The PIC values varied from 0.01 to 0.50, with a mean value of 0.21, and approximately 31% of the markers used in this study had PIC values exceeding 0.30. The fixation index (F_{IS}) values ranged from 0.44 to 1.0, with a mean value of 0.73. The low value of observed heterozygosity and high fixation index level demonstrated that most of the SNP loci were fixed. The distribution of genetic distance values were skewed to the right in which 11% of the values were below 0.30 (Figure 4.1). The genetic distance among the Bambara groundnut genotypes ranged from 0.095 to 0.545, with a mean of 0.437 (data not

shown). Genotypes such as ARC Bamb 37 (spreading), ARC Bamb 49 (Bunching), ARC Bamb 61 (semi-bunching) and ARC Bamb 83 (spreading) revealed the highest average distance compared with the rest of the genotypes. This signifies that these genotypes were distantly related with the rest of the genotypes and can be used as parental genotypes as they are agronomically suitable (high yield and average plant height). The unique agronomic attributes for these genotypes include late maturity, a high number of branches, longer pod side, average plant height and grain yield.

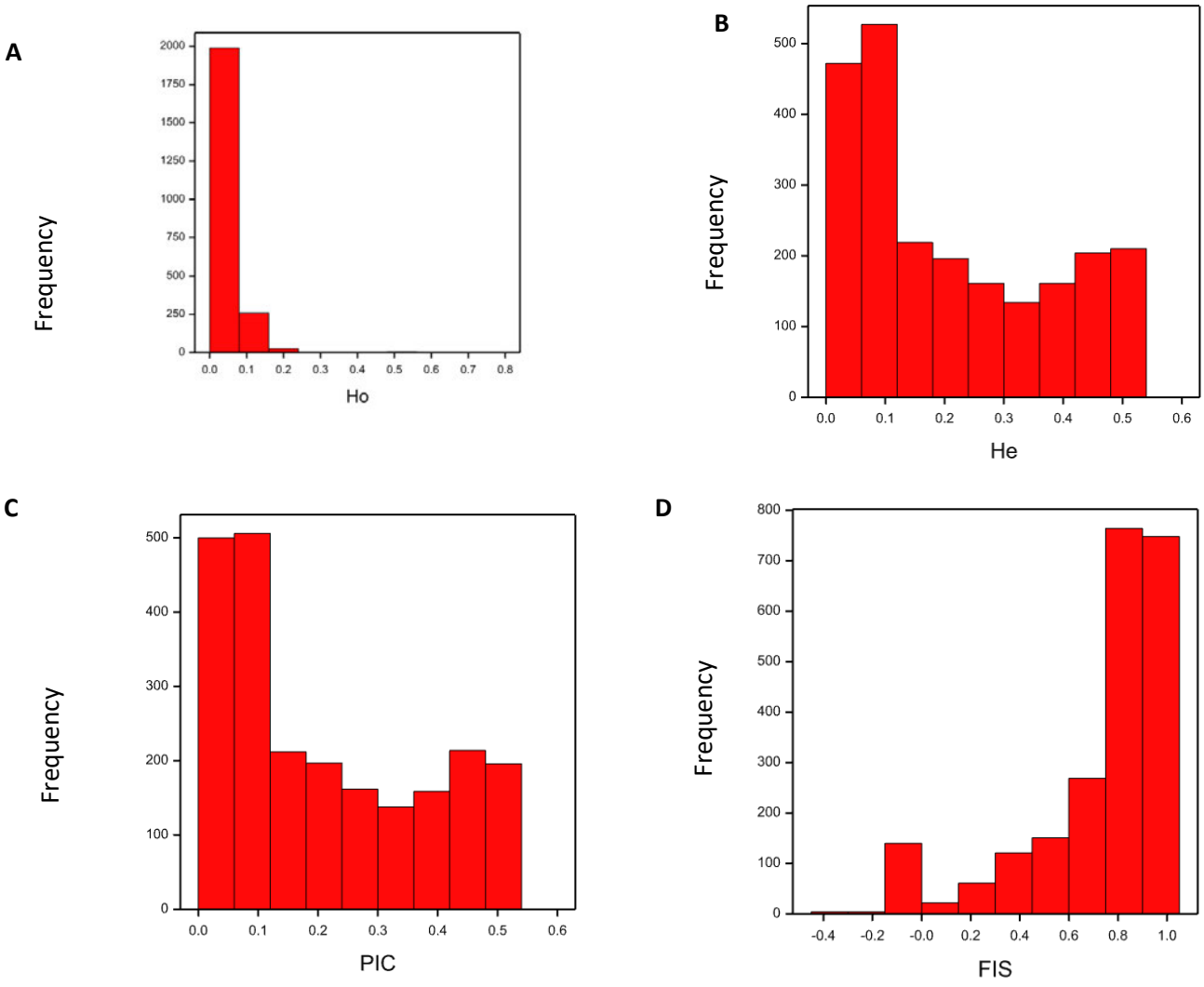

Figure 4.1: Frequency distribution for (A) observed heterozygosity (H_o), (B) expected genetic diversity (H_e), (C) polymorphic information content (PIC), and (D) inbreeding coefficient (F_{IS}), values for 2286 SNP markers used in assessing 93 Bambara groundnut collections of South Africa.

4.3.2 Population structure and genetic diversity analyses

Population structure revealed that the maximum value of delta K was at K = 2, suggesting two possible subpopulations among the 93 Bambara groundnut genotypes studied (Figure 4.2A). In this analysis, 16 genotypes were assigned in subpopulation 1 (Figure 4.2B). In the second subpopulation, 77 genotypes were grouped (Figure 4.2B). Structure analysis further categorized genotypes as either 'pure' or 'admixture'. Individuals with a probability score of above 80% for a given cluster were considered 'pure', whereas those with less than 80% were labelled 'admixture'. Out of the 93 genotypes, 65.6% of the genotypes were tagged as 'pure' and 34.4% as 'admixture'. In subpopulation 1 (Figure 4.2B, orange), the majority of the genotypes (81.3%) were admixtures, while only three genotypes were found to be pure, whereas in subpopulation 2, only 25% of the genotypes were found to be admixtures (Table 4.2). This signifies that there was gene flow between two or more genetically distinct populations of Bambara groundnut genotypes.

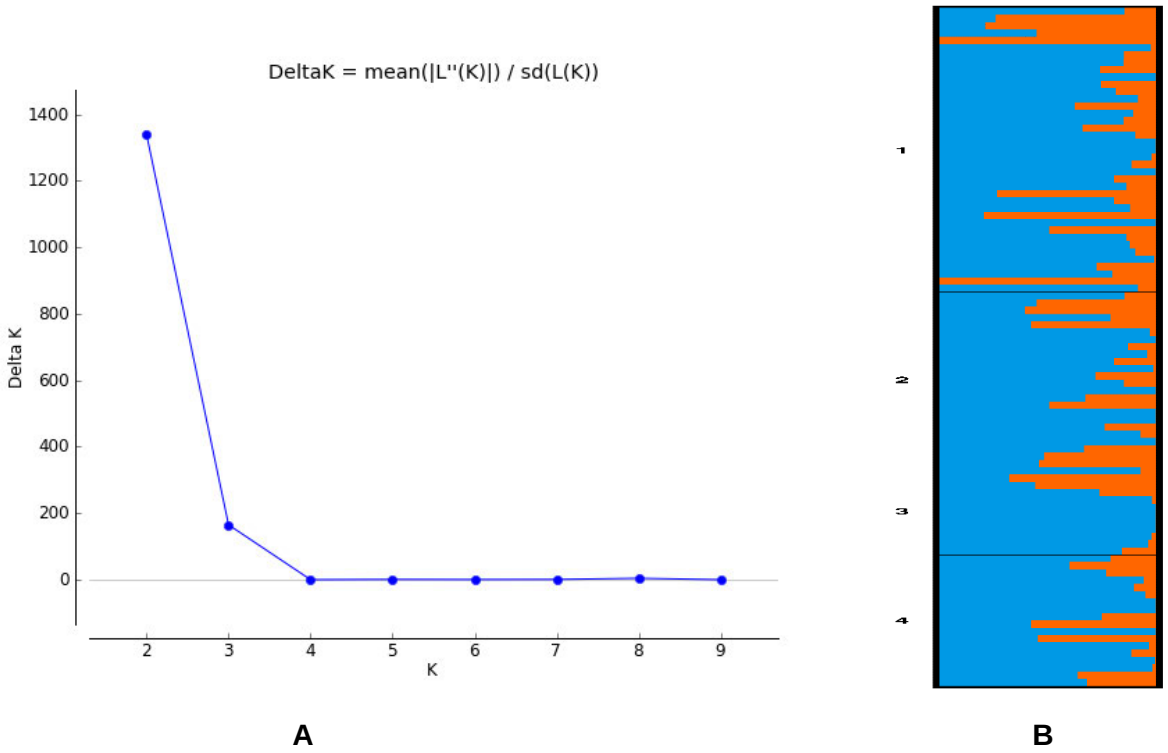

Figure 4.2. (**A**) Population structure analysis using a Bayesian-based approach. Estimation of hypothetical subpopulations using K-values showing the highest Delta K value was observed at K = 2. (**B**) Population structure analysis of 93 Bambara groundnut genotypes at K = 2 based on inferred ancestry (Q matrix).

Table 4.2 List of 93 Bambara groundnut genotypes classified based on the Bayesian genotypic clustering and Neighbour-joining based on Unweighted Pair Group Method with Arithmetic mean. Boldfaced genotypes are admixtures.

Population	Number of genotypes	Structure classification based on Bayesian approach	Population	Number of genotypes	Neighbour-joining based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA)
Pop 1	16	**ARC Bamb-2**, ARC Bamb-**3, ARC Bamb-4,** ARC Bamb-5, **ARC Bamb-26**, ARC Bamb-29, ARC Bamb-38, **ARC Bamb-41, ARC Bamb-42, ARC Bamb-44, ARC Bamb-62, ARC Bamb-63, ARC Bamb-65, ARC Bamb-66, ARC Bamb-85, ARC Bamb-87**	Cluster 1	23	ARC Bamb-2, ARC Bamb-3, ARC Bamb-5, ARC Bamb-6, ARC Bamb-7, ARC Bamb-8, ARC Bamb-10, ARC Bamb-11, ARC Bamb-19, ARC Bamb-36, ARC Bamb-41, ARC Bamb-46, ARC Bamb-55, ARC Bamb-62, ARC Bamb-64, ARC Bamb-65, ARC Bamb-67, ARC Bamb-72, ARC Bamb-74, ARC Bamb-77, ARC Bamb-85, ARC Bamb-87, ARC Bamb-92,
Pop 2	77	ARC Bamb-1, ARC Bamb-6 , ARC Bamb-7, ARC Bamb-8, **ARC Bamb-9**, ARC Bamb-10, **ARC Bamb-11**, ARC Bamb-12, ARC Bamb-13, **ARC Bamb-14**, ARC Bamb-15, ARC Bamb-16, **ARC Bamb-17**, ARC Bamb-18, ARC Bamb-19, ARC Bamb-20, ARC Bamb-21, ARC Bamb-22, ARC Bamb-23, ARC Bamb-24, ARC Bamb-25, ARC Bamb-27, ARC Bamb-28, ARC Bamb-30, **ARC Bamb-31**, ARC Bamb-32, ARC Bamb-33, ARC Bamb-34, ARC Bamb-35, **ARC Bamb-36**, ARC Bamb-37, ARC Bamb-39, ARC Bamb-40, **ARC Bamb-43**, ARC Bamb-45, ARC Bamb-46, ARC Bamb-47, ARC Bamb-48, ARC Bamb-49, ARC Bamb-5, **ARC Bamb-51**, ARC Bamb-52, ARC Bamb-53, **ARC Bamb-54**, **ARC Bamb-55**, ARC Bamb-56, ARC Bamb-57, **ARC Bamb-58**, ARC Bamb-59, ARC Bamb-60, **ARC Bamb-61**, ARC Bamb-64, **ARC Bamb-67**, ARC Bamb-68, ARC Bamb-69, ARC Bamb-70, ARC Bamb-71, ARC Bamb-72, ARC Bamb-73, ARC Bamb-74, ARC Bamb-75, **ARC Bamb-76**, **ARC Bamb-77, ARC Bamb-78**, ARC Bamb-79, ARC Bamb-80, ARC Bamb-81, ARC Bamb-82, ARC Bamb-83, **ARC Bamb-84**, ARC Bamb-86, ARC Bamb-88, ARC Bamb-89, ARC Bamb-90, ARC Bamb-91, **ARC Bamb-92**, **ARC Bamb-93**	Cluster 2	70	ARC Bamb-1, ARC Bamb-4, ARC Bamb-9, ARC Bamb-12, ARC Bamb-13, ARC Bamb-14, ARC Bamb-15, ARC Bamb-16, ARC Bamb-17, ARC Bamb-18, ARC Bamb-20, ARC Bamb-21, ARC Bamb-22, ARC Bamb-23, ARC Bamb-24, ARC Bamb-25, ARC Bamb-26, ARC Bamb-27, ARC Bamb-28, ARC Bamb-29, ARC Bamb-30, ARC Bamb-31, ARC Bamb-32, ARC Bamb-33, ARC Bamb-34, ARC Bamb-35, ARC Bamb-37, ARC Bamb-38, ARC Bamb-39, ARC Bamb-40, ARC Bamb-42, ARC Bamb-43, ARC Bamb-44, ARC Bamb-45, ARC Bamb-47, ARC Bamb-48, ARC Bamb-49, ARC Bamb-50, ARC Bamb-51, ARC Bamb-52, ARC Bamb-53, ARC Bamb-54, ARC Bamb-56, ARC Bamb-57, ARC Bamb-58, ARC Bamb-59, ARC Bamb-60, ARC Bamb-61, ARC Bamb-63, ARC Bamb-66, ARC Bamb-68, ARC Bamb-69, ARC Bamb-70, ARC Bamb-71, ARC Bamb-73, ARC Bamb-75, ARC Bamb-76, ARC Bamb-78, ARC Bamb-79, ARC Bamb-80, ARC Bamb-81, ARC Bamb-82, ARC Bamb-83, ARC Bamb-84, ARC Bamb-86, ARC Bamb-88, ARC Bamb-89, ARC Bamb-90, ARC Bamb-91, ARC Bamb-93

ARC_Bamb = Agricultural Research Council Bambara Groundnut line

Likewise, cluster analysis using neighbour joining based on UPGMA grouped the genotypes into two distinct clusters (Figure 4.3). The distinctiveness of the clusters was confirmed by the high cophenetic correlation coefficient (r = 0.94). Cluster I consisted of 23 genotypes, while Cluster II consisted of 70 genotypes. There were no distinctive clustering patterns observed based on the growth habit. Both the clusters had a mixture of genotypes from all the growth types. The majority of the genotypes (57%) grouped in Cluster I were from bunching growth types. In Cluster 2, 46%, 31% and 23% of the genotypes had bunching, spreading and semi-bunching growth habits, respectively.

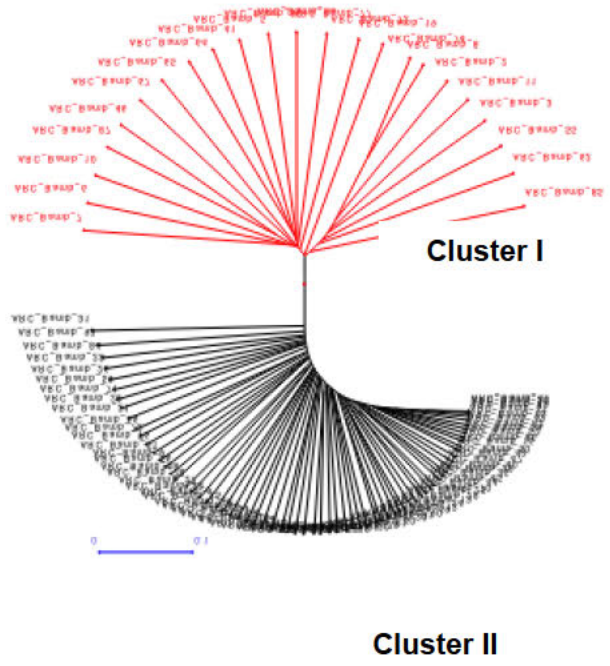

Figure 4.3. Neighbour joining analysis showing the genetic relationships among 93 Bambara groundnut genotypes tested using 2286 SNP markers.

The genetic parameter estimates of the 93 Bambara groundnut genotypes based on growth type are presented in Table 4.3. In terms of dispersion of Bambara groundnut genotypes based on growth type, the bunching type was more common than the spreading and semi-bunching types. The within-population diversity analysis conducted on the predetermined subpopulations based on growth types revealed significant difference for all the genetic parameters studied. The genotypes with bunching growth habits revealed higher values for all of the genetic diversity measures except

for H_o and were followed by the spreading types. The highest mean number of private alleles (174) (92.99%) was observed among bunching subpopulation demonstrating wide genetic diversity. The spreading types revealed high-observed heterozygosity (H_o) and low fixation index (F_{IS}). The proportion of polymorphic loci (%P) indicates the level of allele diversity and frequency. The percentage of polymorphic loci varied from 74.47% for semi-bunching growth type to 92.99% for bunching type, with a mean of 84.44%.

Table 4.3 Genetic diversity within and among 93 Bambara groundnut subpopulations classified based on growth type using 2286 SNPs markers.

Pop	N	I	H_o	H_e	F_{IS}	P_A	%P
Based on growth type							
Bunching	45	0.340	0.037	0.212	0.74	174.00	92.99%
Semi-bunching	24	0.314	0.039	0.197	0.69	56.00	74.47%
Spreading	24	0.319	0.052	0.201	0.63	36.00	80.87%
Overall	93	0.324	0.043	0.207	0.69	-	84.44%
SE	-	0.003	0.001	0.002	0.005	-	4.30%
Based on structure classification							
Population 1	16	0.33	0.037	0.220	0.76	34	73.60%
Population 2	77	0.34	0.043	0.209	0.72	603	98.51%
Overall	93	0.33	0.040	0.215	0.73	-	86.06%
SE		0.003	0.001	0.003	0.005	-	12.46%

N = number of genotypes tested per subpopulation; I = Shannon information index; H_o = observed heterozygosity per subpopulation; H_e = unbiased expected heterozygosity per subpopulation; F_{IS} = inbreeding coefficient, PA = number of private alleles; %P = percentage of polymorphic loci.

4.3.3 Analysis of molecular variance

Table 4.4 summarizes the results of the analysis of molecular variance (AMOVA) among 93 Bambara groundnut genotypes based on growth type and the Bayesian approach using structure. The AMOVA showed highly significant ($P< 0.001$) genetic variation among Bambara groundnut subpopulations, among genotypes, and within genotypes. In both classification approaches, the

between population variation was 2% and about 90% of the genetic variation was attributed to variation among genotypes, while 8% was explained by variation within genotypes. According to Wright (1984) standard guidelines for the interpretation of genetic differentiation, all pairs of subpopulations showed low level of population differentiation. However, the bunching type showed a relatively higher degree of differentiation (0.021) from the rest of the subpopulations. Gene flow among the subpopulation was very high (>11) (Table 4.5). Gene flow varied from 11.48 (between bunching and semi-bunching) to 12.92 (between bunching and spreading). The observed low genetic differentiation among the subpopulations could be explained by the high gene flow among subpopulations.

Table 4.4. Analysis of molecular variance among 93 Bambara groundnut genotypes classified based on growth types using 2286 SNPs

Source	DF	SS	MS	Est. Var.	Per. Var.	F- statistics
Based on growth type						
Among populations	2	2675.45	1337.72	6.89	2%	P < 0.001
Among genotypes	90	83926.83	932.52	446.14	90%	P < 0.001
Within genotypes	93	3742.50	40.24	40.24	8%	P < 0.001
Total	185	90344.77	-	491.18	100%	-
Based on structure classification						
Among populations	1	1390.92	1390.92	8.58	2%	P = 0.011
Among genotypes	91	85211.36	936.39	448.07	90%	P < 0.001
Within genotypes	93	3742.50	40.24	40.24	8%	P < 0.001
Total	185	90344.77		496.89	100%	-

DF = degree of freedom, SS= sum of squares, MS = mean sum of squares, Est. var. = estimated variance, Per. Var. = Percentage variation.

Table 4.5. Pair-wise estimates of genetic differentiation (F_{ST}) (above diagonal off brackets), gene flow (N_m) (above diagonal within brackets); genetic distance GD (lower diagonal off brackets) and genetic identity (GI) (lower diagonal within brackets).

Population	Bunch	Semi-bunching	Spreading
Bunching		0.021 (11.48)	0.019 (12.92)
Semi-bunching	0.008 (0.992)		0.020 (12.25)
Spreading	0.006 (0.994)	0.05 .995)	

4.4 Discussion

Molecular characterisation is the most effective and efficient approach to detect and quantify genetic variation within and among germplasm collections. The present study efficiently evaluated genetic diversity and population structure among 93 Bambara groundnut genotypes using more than 2K SNPs markers. In this study, the Shannon diversity index values ranged from 0.31 to 0.34, with a mean value of 0.32. Further, the two subpopulations generated based on structure classification showed an overall Shannon diversity index of 0.33. The mean Shannon diversity index value recorded in this study was higher than the 0.28 value reported by Odongo et al. (2015). However, the value was less than 0.60 reported by Somta et al.(2011). In population genetics analysis, the Shannon diversity index has been used to distinguish the level of variation between populations with the same number of loci. In some populations, variation is dominated by only a few common alleles, while in others, variation is contributed evenly by all alleles. The Shannon diversity index is more sensitive to rare variants (Sherwin et al.2021). The relatively low Shannon diversity index observed in this study might be attributed to the high level of private allele detected in each subpopulation.

The low observed heterozygosity and the relatively high fixation values exhibited by this population was due to the inherent self-pollination of Bambara groundnut. Konate et al.(2019), on the other hand, reported a high level of (H_o = 0.27) in 92 Bambara groundnut landraces due to frequent exchange of seeds between farmers and selection of varieties for a particular agronomic trait in Burkina Faso. The studied germplasm exhibited low levels of gene diversity (mean H_e = 0.207), signifying a low level of genetic diversity. Redjeki et al.(2020) also reported the existence

of a narrow genetic diversity among Bambara groundnut accessions attributable to few crossings made and a non-significant number of introductions. There has also been a lack of germplasm exchange among Bambara groundnut breeding programmes. The mean polymorphic loci for the overall population was 84.44%, and one-third of the SNPs used in this study showed high PIC values indicating the ability of the markers to discriminate among Bambara groundnut genotypes. This indicates that the SNPs used in the study were highly informative especially for an indigenous crop like Bambara groundnut with no reference genomes.

Clustering based on neighbor-joining and structure analyses based on the Bayesian approach generated two distinct populations. However, there was no relationship between SNP clusters and growth habits. Thus, clustering patterns did not follow the predetermined population classification based on growth type suggesting growth habits should not be used as sole criteria in classifying genotypes (Khan et al.2021a). These findings are consistent with reports by Ntundu et al.(2004) and Massawe et al.(2003). Such diverse genotypes cold be useful for breeding through direct selections and as parents for crosses with genotypes from different cluster. In this study, inter-population assessment of genetic diversity showed that the bunching type had the widest genetic variation for most of the genetic diversity measures. This was also supported by the extremely high private alleles recorded in the bunching type compared to the other types. Understanding population structure and clustering patterns of genotypes allow an effective choice of parental lines to enhance genetic gain from selection. The two subpopulations revealed significant ($P<0.001$) but slight variation (2%). These sub-populations are regarded as distinct genetic or heterotic groups. Selection of distantly related parental genotypes from the two heterotic groups would improve genetic diversity and breeding gain.

The observed low genetic differentiation among the subpopulations could be attributed to the high level of gene flow (Hartl et al.1997). The results are concurrent with (Minnaar-Ontong et al.2021) in their evaluation of 78 South African Bambara groundnut genotypes. The proportion of variance explained among genotypes is usually higher than among populations variance in self-pollinated crops due to the high level of homozygosity within individual genotypes (Massawe et al.2002; Aliyu et al.2016). The observed low genetic diversity among the South African Bambara groundnut genotypes requires further genetic enhancement.

Population genetic structure is affected by population size, population bottlenecks and gene flow. Gene flow among the growth types varied from 11.48 (between bunching and semi-bunching types) to 12.92 (between bunching and spreading). These results agree with the report of Waziri et al.(2013). Ketema et al.(2020) suggested that gene flow > 1 is adequate to neutralise the genetic differentiation between two populations. For self-pollinated crops like Bambara groundnut, gene flow is likely to be human-mediated (Govindaraju, 2002). In this study, the observed high level of gene flow among the different subpopulations could be associated with various factors that are related to the movement of seed through humans over space and time.

4.5 Conclusion

Knowledge of genetic diversity among Bambara groundnut genotypes is important for conservation and utilization of the available genetic resources. In the present investigation, 2286 polymorphic SNP markers were used for genotyping 93 Bambara groundnut genotypes to assess genetic diversity and determine population structure. Population structure and cluster analyses grouped the assessed genotypes into two distinct genetic clusters. The study depicted a high genetic variation among genotypes and a relatively low variation among sub-populations which was attributable to the self-pollinating characteristics of the crop and small population size as South Africa has the most limited number of Bambara landraces with very few production areas. Low genetic diversity among the South African Bambara groundnut genotypes requires further improvements through targeted crosses. The following unique genotypes were selected: ARC Bamb 37 (with spreading growth type), ARC Bamb 49 (bunching), ARC Bamb 61 (semi-bunching) and ARC Bamb 83 (spreading) based SNP markers and desirable agronomic traits. This study provides baseline genetic information for ARC Bambara groundnut germplasm collections for breeding and conservation.

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CHAPTER 5. Combining ability and gene action in Bambara groundnut (*Vigna subterranea* (L.) Verdc.) genotypes for agronomic traits

Abstract

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is a highly nutritious legume crop supporting sustainable food systems in arid and semi-arid agroecologies. However, modern varieties with high yield and desirable product profiles are yet to be bred in sub-Saharan Africa (SSA). Therefore, the objectives of this study were to determine the combining ability effects and gene action conditioning yield and related traits in Bambara groundnut genotypes to identify the best combiner donor parents and progenies for genetic advancement and breeding. Ten contrasting parents were selected and crossed using a 10 × 10 half-diallel mating design, and 45 progenies developed. The progenies and their parents were field evaluated using a 5 × 11 alpha lattice design with two replications in two contrasting locations in South Africa. Data was collected on agronomic traits and subjected to statistical analyses to compute genetic parameters. Genotype × location interaction effect was significant ($P < 0.05$) for the studied agronomic traits. General combining ability (GCA) and specific combining ability (SCA) effects were significant in most assessed agronomic traits, including yield per plant. The GCA × location and SCA × location interaction effects were significant for most traits. A Baker's ratio of < 1 were recorded for most assessed traits indicating the preponderance of non-additive gene effects conditioning the traits. The parental lines such as ARC Bamb25, ARC Bamb8 and ARC Bamb55 recorded positive and desirable GCA effects for yield per plant. The progenies ARC25×ARC8, ARC44×ARC9 and ARC6×ARC9 had desirable SCA effects for yield per plant, ARC44×ARC8, ARC44×ARC68, ARC42×ARC8 for higher number of secondary branches per stem, ARC25 ×ARC8 for early maturity, ARC42×ARC55 for higher number of pods per plant and ARC42 ×ARC57 for increased seed width. The new families selected in the current study are useful breeding populations and will be subjected to selection and multilocation evaluation to release the best-performing varieties.

Keywords: Bambara groundnut, genetic analysis, general combining ability effect, gene action, specific combining ability effect.

5.1 Introduction

Bambara groundnut (*Vigna subterranea* (L.) Verdc.; 2n= 2x = 22) is a nutrient-dense grain legume cultivated in sub-Saharan Africa (SSA) and Asia but largely under-researched and under-utilised. It is regarded as a complete food with a high potential to alleviate food and nutrition insecurity (Soumare et al. 2022). It can fix atmospheric nitrogen and improve soil fertility. In terms of total production, it is among the top three most vital legumes after groundnut (*Arachis hypogea* L.) and cowpea (*Vigna unguiculata* L.) (Mayes et al. 2019). Global production of Bambara groundnut is estimated at 240 000 tonnes per year from 406 911 hectares worldwide (FAOSTAT, 2023). Africa is the largest producer (239 607 tonnes per annum), while small quantities are produced in South-east Asia (e.g. Thailand and Indonesia), the United States of America, and Australia. West Africa is the centre of genetic diversity and the main Bambara groundnut production region in SSA, where Burkina Faso, Niger, and Cameroon are the leading producers, contributing to 74% of global production (FAOSTAT, 2023).

Bambara groundnut grains are nutrient-dense and ideal candidates to develop complete and value-added food products. The grains have balanced nutrient compositions including the following macro-nutrients (in mg per 100 g dry sample grain): nitrogen (17000 - 26000), phosphorus (81 - 563), potassium (1545 - 2200), and micro-nutrients such as magnesium (32 - 335), calcium (30 - 128), iron (2 - 9) and zinc (11 - 40) (Maphosa et al. 2022; Chelangat et al. 2023). Also, the grains contain carbohydrates (~72%), protein (~25%), fibre (~ 6.3%), and oil of ~7% (Ibny et al. 2019). Thus, the crop can contribute significantly to nutrition security, sustainable food systems and cash income through novel product development (De Valença et al. 2017).

Bambara groundnut enhances the productivity of agricultural soils by fixing atmospheric nitrogen through the symbiotic association with the microorganism *Rhizobia* (Taffouo et al. 2010; Obayelu et al. 2016). Therefore, it is a suitable crop in regenerative agriculture and reduces the cost of inorganic fertilisers under smallholder farming systems. Crop rotation involving Bambara groundnut aids the restoration and maintenance of nitrogen supply in agricultural soils. Bambara groundnut is a relatively drought-tolerant crop capable of withstanding long drought spells, making it a crop of choice in water-constrained environments (Mubaiwa et al. 2017).

The Bambara groundnut yield levels in SSA vary between 0.3 and 3.0 tonnes per hectare, with a mean of 0.85 tonnes per hectare (Mayes et al. 2019). In South Africa, the average crop yield is 0.62 tonnes per hectare, which is lower than the global average (0.2 tonnes per hectare). Other countries, such as Zimbabwe and Zambia, reported mean yields of 2.40 and 2.20 tonnes per hectare, respectively (Majola et al. 2022). The low yields are caused by biotic and abiotic stresses, poor farming practices and a lack of improved varieties (Hochholdinger and Baldauf 2018). Abiotic constraints include limited water availability, high temperatures, soil moisture stress and infertile soils leading to limited yield potential (Ziervogel et al. 2014). Smallholder farmers, notably women, are the primary producers of the crop who lack modern production inputs. Smallholder farmers cultivate using traditional ,low-yielding landrace varieties (Mbosso et al. 2020; Maphosa et al. 2022). Modern varieties with high yields and desirable product profiles are yet to be bred in sub-Saharan Africa. The lack of improved varieties has been reported widely as the main yield-limiting factor for sustainable and profitable crop production (Adzawla, 2016; Donkoh et al. 2016; Kendabie et al. 2020; Rafii et al. 2021). Consequently, low yields of Bambara groundnut render the crop less attractive for commercial production and product development.

There is a marked genetic variation for important agronomic traits (i.e. number of pods per plant, seeds per pod, days to maturity and yield potential) in Bambara groundnut genetic resources for pre-breeding and breeding (Beket et al. 2019; Uba et al. 2023). Pure line development is the common approach in strictly self-pollinating species, including Bambara groundnut until effective hybridization methods are available for hybrid breeding to exploit heterosis (Odesola et al. 2023). Bambara groundnut genetic diversity facilitates the identification of heterotic groups and the best parents for breeding. There is a need to identify the best combiner donor parents and progenies for genetic advancement and breeding of new generation varieties.

The concept of combining ability was proposed by Sprague and Tatum (1942). Combining ability analysis allows the selection of potential parents through progeny performance evaluation (Sawarkar et al. 2015). Good parental lines should be able to be used in a wide range of combinations with other lines to produce progenies that have high average performance. Combining abilities are distinguished as general and specific combining ability effects. The general combining ability (GCA) effect refers to the average performance of a candidate line based on all its progenies generated from different cross combinations (Begna 2021). A line with a

desirable GCA value for a trait of interest has a high potential to transmit the trait to its progenies and can be used in further crossing programs for varietal development. Conversely, the specific combining ability (SCA) effect is a cross or family's average performance (better or worse) than the mean of parental lines. The progenies that exhibit good SCA effects are primed for genetic advancement to develop stable breeding pure-line cultivars in self-pollinating crops. The ratios of GCA and SCA effects derived through combining ability analysis are used to deduce the gene action involved in the expression of quantitative characters (Mutari et al. 2022; Nassourou et al. 2022). The GCA effects are associated with additive gene action, while the SCA effects relate to non-additive gene action. Both genetic effects are essential in the development of breeding populations or in devising a suitable selection method (Viana and Matta. 2003). Different mating designs, including diallel are used to analyse combining ability effects and to deduce gene action controlling the inheritance of quantitative traits.

The diallel mating design is the most commonly used genetic design to determine the GCA of effects of parents and the SCA effects of progenies (Griffing 1956). It is a widely used design for self-pollinated species as the success rate for generating complete crosses is often lower (Signh 2007). It has been used in genetic analysis of traits of various legume crop species such as cowpea (Tchiagam et al. 2011, Rodrigues et al.2018, Owusu et al. 2020), soybean (El-Garhy et al. 2015, Kurasch et al. 2017, Adewale et al. 2023) and chickpea (Verma et al. 2020, Gaur et al. 2020, Lakhote et al. 2020). There is a lack of information on combining ability and genetic analyses of Bambara groundnut for agronomic traits. These could have partly contributed to limited breeding efforts to develop high-performing varieties with farmer-and-consumer-preferred traits for commercialization in SSA. The combining ability effects of the selected parents and their progeny should be assessed to develop new breeding populations adapted to South Africa. The objectives of this study were to determine the combining ability effects and gene action conditioning yield and related traits in Bambara groundnut genotypes to identify best combiner donor parents and progenies for genetic advancement and breeding.

5.2 Materials and methods

5.2.1 Plant materials

The study used 10 Bambara groundnut parental lines selected from preliminary evaluation trials based on agronomic, nutritional and genetic analysis using SNP markers (Majola et al. 2022). The names and attributes of the selected parental lines are presented in Table 5.1. The selected lines displayed high yield potential, better nutritional profiles and contrasting yield-related agronomic attributes, including maturity, pod length, stem thickness and seed colour.

Table 5. 1: Description of Bambara groundnut genotypes used in the study

Genotype code	Genotype designation/ Name	Source	Country of origin	Attributes
G6	ARC Bamb 6	ARC-VIMP	Botswana	Medium maturing, red seed colour, high Zn
G8	ARC Bamb 8	ARC-VIMP	South Africa	Early maturing, high yielding, high Fe and Ca
G9	ARC Bamb 9	ARC-VIMP	Botswana	High yield, thick stems, low grain tannins, high K
G25	ARC Bamb 25	ARC-VIMP	South Africa	Early maturing, high yielding, long pods, high Fe
G5	ARC Bamb 42	ARC-VIMP	South Africa	Large grain size, wide pods, high Zn and Ca
G7	ARC Bamb 44	ARC-VIMP	Botswana	Early maturing, mottled dry seeds, high Fe
G10	ARC Bamb 55	ARC-VIMP	South Africa	High yield, medium maturing, high P
G11	ARC Bamb 57	ARC-VIMP	Swaziland	Medium yield, quality fresh pods, high Mg
G12	ARC Bamb 66	ARC-VIMP	South Africa	Early maturing, cream seed colour, high Zn
G13	ARC Bamb 68	ARC-VIMP	South Africa	Wide pods, black seed colour, high Ca

ARC-VIMP = Agricultural Research Council – Vegetable, Industrial and Medicinal Plants Institute, Bamb = Bambara groundnut, Zn = Zinc, Fe = Iron, K = Potassium, P = Phosphorus Ca = Calcium

5.2.2 Development of crosses

The 10 parental lines were established in 10 L capacity polyethylene pots, with two seeds planted per pot under a glasshouse environment at the Agricultural Research Council – Vegetable, Industrial and Medicinal Plants Institute, Pretoria, South Africa. A half-diallel mating design was used to generate 45 F_1 progenies. The parental lines were stagger planted at weekly intervals to facilitate emasculation and cross-pollination. The crossing procedure was done following the protocol developed by Morales and Singh (1991). Crosses were carried out manually and flowers were hand-emasculated to remove the anther sacs from the staminal column using fine forceps

before the flowers opened up. Five flower buds per branch were emasculated, and pollen from a male donor was gently rubbed onto the stigma to facilitate cross-pollination. Pollination was conducted 24 hours after emasculation to allow pollen receptiveness of the stigma. o. Both emasculation and pollination were carried out in the morning before 10:00 am. Each pollinated flower was tagged and labelled, indicating the parents involved in the cross and the crossing date. Since the success rate of crosses in Bambara groundnut is generally low (<2%), multiple crosses were done to obtain sufficient seed for the field trials. At maturity, the seeds were harvested successively. After harvest, the seeds were stored under optimal conditions for later use.

5.2.3 Experimental environment and design

The 10 parental lines and 45 F_1 progenies were evaluated using a 5 × 11 alpha lattice design with two replications at two locations during the 2021/22 summer season (January 2021 to May 2022). The two locations were Brits (25.61° S 27.79° E) and Loskop (25.17° S 29.39°E). Brits is situated in the North-West province, while Loskop in the Mpumalanga province of South Africa. Brits' minimum and maximum temperatures were 18.6 and 28.9 °C, whereas Loskop experienced a minimum temperature of 18.0°C and a high temperature of 28.0°C. The total rainfall at Brits was 629 mm, and it was 497 mm at Loskop. The land was ploughed, disked and rotated in both environment to establish a fine seedbed. No basal or topdressing fertilisers were applied at ploughing and during plant growth. After land preparation, two seeds were hand-planted per hole. The planting holes were 30 cm apart in a row of 1 m length. The rows were spaced 1 m apart. The crop was established under rainfed conditions with no supplementary irrigation. Standard Bambara groundnut production practices in South Africa were followed as outlined by Bambara groundnut descriptors of the International Plant Genetic Resources Institute (IPGRI, 2000). The fields were manually weeded where required.

5.2.4 Data collection

Data was collected on the following agronomic traits: number of days to 75% maturity (DM) determined as the number of days from sowing until when 75% of the pods in a plot turned brown, number of nodes per stem (NNS) were counted, number of secondary branches per stem(NSB) were counted at maturity, number of stems per plant (NSP) were counted at harvest, after shelling the average number of seeds were counted from 10 randomly selected and tagged plants, pod length (PodL) and pod width (PodW) were measured in centimetres at physiological maturity and

recorded as the averages of 15 pods per genotype, stem thickness (ST) was measured using a Vernier calliper and recoded as an average of a random sample of five plants per plot, number of pods per plant (Npods) were counted at maturity and recorded as an average of 5 plants per plot, number of seeds per pod (NSPod) recorded as an average from a random sample of 10 pods from the plot, hundred seed weight (HSW) was determined as the weight of 100 randomly sampled seeds per plot after shelling, seed length (SL) and seed width (SW) recorded as averages of 10 random seeds per plot. SL was measured longitudinally aiming the centre of the seed. SW was measured transversally from the hilum to the dorsal region in the central area of the seed. All the plants in a plot were harvested by digging the entire root system and pods from the ground. Grain yield per plant (YPP) was recorded as the average of grain weight per plot divided by the number of harvested plants in a plot. Yield per plant was converted to kg ha^{-1} using the following formula:

$$\left(\frac{Plot\ weight\ (g)}{Plot\ area\ (m)} * \frac{100-14}{100-mc}\right) * 10\ 000$$

where mc is moisture content measured at harvesting, which was 14% (Parker A and Namuth-Covert D 2017), and 10 000 is the conversion factor for a hectare.

5.2.5 Data Analyses

5.2.5.1 Analysis of variance

Tests for normality and homogeneity of variance were conducted on the data from the individual locations prior to analysis of variance (ANOVA). The tests were conducted in GenStat® Version 18 (VSN International, Hempstead, UK). ANOVA was conducted using Genstat to estimate statistical significance among genotypes and environments. The mean values were separated using Fisher's Least Significant Difference (LSD) test procedure at a 5% significance level. The following general linear model was used for the combined analysis of variance following Smith et al. (2005):

$$Y_{ijkl} = \mu + G_i + E_j + GE_{ij} + E(R)_{jk} + E[R(B)]_{jkl} + \varepsilon_{ijkl}$$

where Y_{ijkl} is the observed performance of the i^{th} genotype grown in the j^{th} location of k^{th} replication within the lth incomplete block , μ is the grand mean, G_i is the effect of the ith

genotype, E_j the effect of the *j*th location, GE_{ij} is the effect of the interactions of the *i*th genotype with the *j*th location; $E(R)_{jk}$ is the effect of the *k*th replication in the *j*th location, $E[R(B)]_{jkl}$ is the effect of the *l*th block within the *k*th replication in the *j*th location, and ε_{ijkl} is the residual effect associated with each Y_{ijkl}.

5.2.5.2 Combining ability analysis

Combining ability analysis was computed using the lmDiallel (Onofri et al. 2021) and Diallel AnalysisR (Yaseen and Eskridge, 2020) packages in R software version 4.2.1 (R Core Team 2022) following the random effects model II and method II (i.e. parents and their progenies without reciprocals) according to Griffing (1956).

Combining ability analysis was carried out according to Kempthorne and Curnow (1961):

$$Y_{ij} = \mu + r_i + g_i + g_j + s_{ij} + \varepsilon_{ijk}$$

where Y_{ij} is the mean of the cross of between i^{th} and $_j{}^{th}$ parents, μ is the overall mean of the trial, r_i is the effects due to replication, g_i and g_j are the GCA effects of the i^{th} and j^{th} individuals, respectively, s_{ij} is the SCA effect for the cross between the i^{th} and $_j{}^{th}$ parents and Ɛijk is the residual error. The GCA effects were calculated as follows:

$$GCA = X_{ij} - \mu$$

Where, X_{ij} is the mean of the i^{th} parent across j crosses and μ is the overall mean.

The SCA effects were calculated as follows:

$$SCA = X_{ij} - (GCA_i + GCA_j + \mu)$$

Where, X ij is the mean of the cross between i^{th} and j^{th} parents, GCA_i and GCA_j are the GCA effects of the i^{th} and j^{th} parents and μ is the overall mean. The significance of GCA effects were tested based on the t-test in R software using the Diallel Analysis R package (Yaseen and Eskridge, 2020) as follows: $t = \frac{GCA}{se}$ and for SCA effects as $t = \frac{SCA}{se}$

Where se, is the standard error of the GCA or SCA estimates.

5.2.5.3 Estimates of genetic effects

The GCA and SCA variances were deduced from the analysis of variance, according to Agaba et al. (2017). The ratio of GCA to SCA variance (σ^2_{gca} / (σ^2_{gca}+ σ^2_{sca}) was used to test the relative importance of additive versus non-additive gene action according to Baker (1978). Broad-sense heritability values were estimated as $H^2 = \frac{(\delta^2_{GCa}+\delta^2_{SCA})}{(\delta^2_{GCA}+\delta^2_{SCA}+\delta^2_{\hat{e}})}$

Where: [$\boldsymbol{\delta}^2$ GCA + $\boldsymbol{\delta}^2$ SCA] = Total genetic effects, and [$\boldsymbol{\delta}^2$ GCA + $\boldsymbol{\delta}^2$ SCA+ $\boldsymbol{\delta}^2$e] = Phenotypic effects following Agba et al. (2017).

5.3 Results

5.3.1 Analysis of variance and genotypic mean performance for agronomic traits

The ANOVA mean squares showed that location, genotype and genotype × location effects were significant (P < 0.05) for DM, NNS, NSB, NSP, PodL, PodW, Npods, SL, SW and YPP (Table 5.2). Significant genotypic variation was observed for (P < 0.01) DM, NNS, NSB, PodL, PodW, Npods, SL, SW, HSW and YPP. Location was significant for all the studied traits except (P < 0.01) HSW.

The mean performance for the assessed agronomic traits for the parental lines and their crosses across the two test locations are presented in Table 5.3. DM varied between 168 and 176 days for ARC Bamb 9 recording fewer days to flowering (168 days) and ARC44 being late maturing (176 days). Crosses ARC66 × ARC44, ARC8 × ARC25, ARC55 ×ARC44 were early maturing compared to late-flowering crosses such as ARC8 × ARC55 and ARC8 × ARC66. The parents ARC Bamb 57 and ARC Bamb 68 recorded the highest NSB (≥20). Among the crosses, ARC8 × ARC25, ARC8 × ARC42, ARC8 × ARC44 recorded the maximum NSB of 26.

Pod length varied widely among the parental lines. The parent ARC Bamb 68 exhibited the longest pods (21.72 cm), followed by ARC Bamb 9 (20.44 cm) and ARC Bamb 57 (19.89 cm). Parental lines ARC Bamb 57 and ARC Bamb 68 also recorded the highest pod width of 15.34 cm and 14.92, respectively. The crosses ARC57 × ARC25, ARC68 × ARC25, ARC8 × ARC42 had long pods exceeding 22 cm. ARC57 × ARC25 had the highest pod width of 21.39 cm. Npods varied significantly from 83 to 170, which was generally lower for the crosses than the parental lines.

Npods were recorded for ARC Bamb 44 (171), ARC Bamb 25 (124) and ARC Bamb 8 (121) parental lines. The maximum number of pods was 169 recorded for cross ARC6 × ARC57, while the minimum of 78 pods per plant was recorded for cross ARC66 × ARC44.

For HSW, parental lines ARC Bamb 68 and ARC Bamb 25 recorded heavier seeds exceeding 50 g. Among the crosses, ARC66 × ARC25, ARC68 × ARC6 and ARC9 × ARC6 recorded the mean HSW of 84.14 g, 70.48 g and 66.41 g, respectively. The yield per plant varied from 78.72 to 122.33 kg ha^{-1} across parents and crosses. Parental lines ARC Bamb 55, ARC Bamb 25 and ARC Bamb 68 were the top-yielding lines with mean values of 122.33, 109.55 and 108.25 kg ha^{-1}, respectively. The lowest-yielding parent was ARC Bamb 44, recording grain yield of 78.72 kg ha^{-1}. In general, the mean yield of the progenies was higher than the parental genotypes, with only ARC68 × ARC44 (80.46 kg ha^{-1}) and ARC9 × ARC42 (78.94 kg ha^{-1}) having similar or lower yields than the lowest-yielding parental genotypes. Cross ARC8 × ARC25 had the highest YPP (170.78 kg ha^{-1}), followed by ARC9 × ARC44 (133.25 kg ha^{-1}) and ARC6 × ARC25 (132.28 kg ha^{-1}).

Table 5.2: Mean squares and significant tests for grain yield and yield components among Bambara groundnut genotypes evaluated across two locations

Sources of variation	Df	DM	NNS	NSB	NSP	PodL	PodW	ST	Npods	NSPod	HSW	SL	SW	YPP
Location (Loc)	1	1364.4***	1400.4***	80.2***	1436.0***	72.3***	90.3***	46.5***	2418.0***	2826.8***	69ns.0	500.8***	14.9***	2521.0***
Replication (Rep)	2	1663.1***	1745.9***	1105.4***	117.6***	34.7*	453.1***	2.5*	14960.0***	332.2ns	9007.0***	505.7***	194.6***	11393.0***
Block (Rep)	4	24.5ns	130.7*	24.9ns	0.9ns	19.1ns	11.0ns	0.7ns	2310.0***	265.9ns	226.0ns	142.3**	7.4*	576.0ns
Genotype (Gen)	54	67.7***	201.7***	74.9***	11.3***	16.4**	16.3***	0.7ns	2360.0***	113.6ns	298.0ns	130.3***	9.1***	1627.0***
Gen x Loc	54	76.7***	157.8***	91***	17.0***	18.5***	12.1***	0.6ns	3289.0***	114.6ns	273.0ns	132.7***	8.4***	1284.0***
Residuals	214	34.5	43.9	13.90	5.8	9.8	5.2	0.6	420.0	145.8	334.0	33.8	3.0	554.0

*, **and *** denote significant at 0.05, 0.01 and 0.001 probability levels, respectively. ns = Non-significant, DF = degrees of freedom, DM = days to 75% maturity, NNS = Number of nodes per stem, NSB = number of secondary branches per stem, NSP = number of stems per plant, PodL = Pod length (cm), PodW = Pod width (cm), ST = Stem thickness (1/100 mm), Npods = Number of pods per plant, NSPod = Number of seeds per pod, HSW = Hundred seed weight (g/100 seed), SL = Seed length (cm), SW = Seed width (cm) and YPP = yield per plant (kg ha^{-1}).

Table 5.3: Mean values for agronomic traits among 45 F1 families and 10 parents of Bambara groundnut genotypes evaluated at Brits and Loskop in South Africa.

	Traits												
Genotype	**DM**	**NNS**	**NSB**	**NSP**	**PodL**	**PodW**	**ST**	**Npods**	**NSPod**	**HSW**	**SL**	**SW**	**YPP**
Parents													
ARC Bamb 6	174.00	21.17	18.83	9.50	19.43	12.80	0.77	110.17	16.15	51.03	10.49	8.56	97.41
ARC Bamb 8	171.17	25.78	19.78	8.17	17.51	13.83	0.65	121.17	16.17	51.65	12.49	8.66	99.03
ARC Bamb 9	168.67	20.17	14.00	7.17	20.44	12.13	0.63	112.50	21.25	49.75	10.95	8.81	95.52
ARC Bamb 25	171.33	21.05	15.00	8.33	19.08	15.34	0.46	123.67	22.00	57.26	15.03	9.40	109.55
ARC Bamb 42	174.00	46.67	19.17	8.05	18.03	13.98	0.52	83.00	13.17	51.07	10.64	8.64	95.22
ARC Bamb 44	176.33	26.83	16.17	8.83	17.65	14.07	0.49	170.67	17.17	47.09	12.03	8.50	78.72
ARC Bamb 55	169.83	27.17	15.33	8.00	17.14	14.27	0.71	100.83	18.33	45.84	12.56	10.43	122.33
ARC Bamb 57	172.83	29.00	20.17	8.33	19.89	14.92	0.68	102.67	24.67	48.76	10.34	8.95	104.92
ARC Bamb 68	172.00	25.70	21.12	9.00	21.72	14.67	0.75	116.00	26.33	54.11	15.32	9.44	108.25
ARC Bamb 66	169.00	26.00	16.17	8.00	18.93	13.12	0.55	93.83	15.17	58.00	11.00	8.84	89.40
Mean	168.23	24.63	15.48	8.42	18.32	12.74	0.42	102.62	15.32	42.32	10.25	8.62	98.32
Crosses													
ARC8×ARC25	164.00	35.33	26.17	11.83	20.41	17.17	2.12	79.50	19.33	54.57	10.78	10.37	170.78
ARC9×ARC44	172.60	33.08	16.83	8.67	19.60	14.50	0.65	90.67	21.33	56.14	13.35	10.57	133.25
ARC6×ARC25	170.83	38.50	22.50	12.50	18.45	17.80	0.65	125.75	21.68	54.44	14.46	13.00	132.28
ARC9×ARC6	170.00	20.50	14.50	7.50	18.68	13.31	0.72	111.17	26.00	66.41	11.88	9.52	129.34
ARC9×ARC25	170.55	19.05	19.32	10.83	19.82	15.99	0.93	100.25	12.43	49.44	10.71	11.08	124.82
ARC44×ARC25	167.50	29.08	20.67	11.17	20.70	12.51	0.85	102.00	24.50	50.41	11.39	10.97	122.80
ARC9×ARC55	169.50	20.83	16.42	8.00	20.07	13.48	0.64	121.17	18.73	50.24	11.56	8.18	121.02
ARC8×ARC6	177.33	26.33	16.05	9.08	20.48	14.28	0.59	132.00	19.75	55.64	14.70	12.38	116.45
ARC9×ARC8	176.17	21.67	15.67	10.83	19.00	13.63	0.68	135.50	23.50	48.24	13.46	11.49	113.27
ARC68×ARC55	169.17	29.33	19.83	8.83	18.64	15.13	0.76	116.50	23.50	66.09	11.71	8.87	113.23
ARC55×ARC44	165.50	26.50	13.75	7.33	18.73	14.51	0.75	104.50	22.83	50.43	10.80	10.20	112.67
ARC8×ARC55	177.33	26.83	20.00	8.83	21.82	15.55	0.41	90.17	13.50	50.47	12.41	10.80	112.18
ARC42×ARC25	171.30	24.27	15.03	7.83	20.81	18.34	0.78	97.17	17.33	50.44	10.89	10.14	110.80
ARC6×ARC55	171.00	27.83	18.17	7.17	19.26	13.61	0.52	89.92	14.42	44.61	11.36	9.83	108.28

ARC8×ARC66	178.00	22.17	19.83	8.67	22.22	15.16	0.44	132.50	19.50	55.49	9.44	9.59	107.95
ARC57×ARC55	172.83	17.17	18.33	8.33	20.45	15.10	0.42	113.00	22.33	59.75	13.75	9.36	105.22
ARC66×ARC57	169.33	23.00	14.83	9.67	18.10	13.45	0.59	101.50	12.75	55.59	11.11	9.32	104.95
ARC66×ARC55	174.83	30.83	22.17	8.17	19.34	15.15	0.58	121.17	17.00	57.74	11.94	9.40	103.07
ARC6×ARC42	167.33	29.47	15.92	8.05	17.54	13.50	0.51	94.38	26.33	51.48	11.07	9.66	102.88
ARC6×ARC44	168.67	20.67	17.37	8.50	19.35	15.92	0.37	105.25	15.00	48.98	12.87	8.98	102.78
ARC8×ARC44	174.00	19.33	25.93	7.75	19.58	16.86	0.82	123.95	19.17	57.70	14.24	9.84	102.28
ARC8×ARC42	175.50	24.00	26.00	11.33	22.42	15.24	0.58	96.50	16.00	57.09	14.05	9.81	101.68
ARC66×ARC25	169.33	25.70	22.77	8.33	19.64	14.23	0.68	102.50	30.10	84.14	12.49	10.97	101.51
ARC8×ARC68	171.83	27.70	20.27	7.83	20.60	17.09	0.73	116.67	19.17	61.72	12.38	10.08	100.62
ARC55×ARC25	168.08	28.23	22.30	8.13	19.85	13.15	0.93	115.17	17.05	51.02	13.54	10.58	99.90
ARC9×ARC57	170.67	21.83	17.33	8.33	17.90	15.27	0.77	112.37	25.50	49.64	11.31	8.46	98.33
ARC68×ARC6	170.00	21.67	14.75	8.67	22.17	13.60	0.42	122.67	21.00	70.48	11.03	9.49	97.70
ARC66×ARC42	167.17	25.67	16.50	7.00	18.97	14.67	0.57	115.00	24.67	61.66	11.27	9.07	97.53
ARC57×ARC44	167.17	27.50	15.17	8.50	19.23	14.76	0.57	127.67	18.17	55.78	11.89	9.35	96.80
ARC68×ARC57	170.17	22.50	18.67	7.83	17.39	15.43	0.64	110.67	20.83	48.54	10.17	8.56	96.47
ARC8×ARC57	173.50	34.00	17.50	7.83	18.06	13.08	0.73	162.33	13.17	45.12	10.01	5.63	96.20
ARC66×ARC6	170.17	23.83	13.50	7.50	17.56	12.66	0.56	125.33	20.83	59.29	12.14	9.45	96.10
ARC44×ARC42	171.17	25.83	18.83	8.33	19.65	14.19	0.35	111.50	23.00	50.81	10.67	8.69	95.33
ARC6×ARC57	172.67	18.83	13.33	7.83	21.91	14.64	0.60	169.00	16.25	56.83	11.48	9.33	94.93
ARC57×ARC42	167.50	41.50	13.50	7.33	16.37	13.61	0.37	98.25	15.67	44.50	11.28	12.85	93.38
ARC55×ARC42	171.50	20.17	17.78	9.17	19.01	13.88	0.59	146.83	10.83	45.41	12.31	8.66	91.63
ARC9×ARC66	175.83	24.17	16.67	8.50	18.05	16.58	0.59	156.33	15.67	53.01	11.17	10.21	90.44
ARC68×ARC66	168.67	21.07	21.23	8.33	17.32	15.18	0.56	107.33	15.67	52.22	10.20	8.72	89.92
ARC68×ARC25	168.00	23.50	25.00	10.17	23.35	17.27	0.76	90.92	15.75	53.89	12.44	10.99	88.70
ARC68×ARC42	165.83	24.67	16.67	7.17	21.28	16.14	0.49	106.20	20.05	54.79	12.31	10.90	87.40
ARC66×ARC44	163.83	14.83	13.67	8.33	21.37	14.06	0.66	78.50	16.00	46.37	12.26	9.20	84.53
ARC57×ARC25	170.50	26.92	25.17	13.50	23.91	21.39	0.88	122.67	12.50	58.67	11.45	9.49	83.10
ARC68×ARC44	175.00	20.67	23.83	7.83	18.76	15.76	0.57	123.33	14.50	44.29	12.13	10.31	80.46
ARC9×ARC42	175.83	23.00	21.50	9.00	17.56	12.93	2.43	108.50	21.33	47.16	14.34	11.45	78.94
ARC9×ARC68	169.83	23.33	17.00	9.00	20.23	16.06	0.58	107.00	17.00	53.91	13.31	8.82	69.46
Mean	171.03	25.50	20.33	8.70	19.55	14.82	0.68	113.74	18.95	53.73	12.55	9.72	102.94

CV (%)	3.47	25.71	20.33	23.87	13.37	15.33	13.17	17.79	34.11	25.76	36.60	17.87	22.86
LSD (5%)	85.30	10.60	29.50	95.60	1.40	1.30	0.90	3.60	1.40	2.70	0.50	1.30	7.20

ARC-VIMP = Agricultural Research Council, Bamb = Bambara, CV = coefficient of variation, LSD = least significant difference, DM = days to 75% maturity, NNS = Number of nodes per stem, NSB = number of secondary branches per stem, NSP = number of stems per plant, PodL = Pod length (cm), PodW = Pod width (cm), ST = Stem thickness (1/100 mm), Npods = Number of pods per plant, NSPod = Number of seeds per pod, HSW = Hundred seed weight (g/100 seed), SL = Seed length (cm), SW = Seed width (cm) and YPP = yield per plant (kg ha^{-1})

5.3.2 Analysis of variance for combining ability effects for agronomic traits

Analysis of variance showing GCA and SCA mean squares for the studied agronomic traits amongst Bambara groundnut parental lines and progenies are summarized in Table 5.4. Location × GCA interaction effects were significant ($P < 0.001$) for all the traits except NSP, DM, PodL, ST, NSPod, HSW and SW. Similarly, location × SCA effects were significant for most traits except for ST, NSPod, HSW and SL. There were significant ($P < 0.001$) GCA effects for NNS, NSB, NPods and SL. Significant ($P \leq 0.001$) SCA effects were observed for most traits except for ST, NSPod, HSW and SL. The high SCA variance resulted in lower GCA/SCA ratios for all traits, which were less than 1. Broad-sense heritability varied from 2.91% to 60.20% across the studied traits. SL recorded the highest heritability estimate (60.20%), followed by NNS (52.09 %) and YPP (40.82%), whereas NSPod showed the lowest heritability (2.91%).

5.3.3 General combining ability effects of parental lines for agronomic traits

The GCA effects of the parents for the studied agronomic traits across the test locations are presented in Table 5.5. The parental lines ARC Bamb 25 and ARC Bamb 8 recorded negative and desirable GCA effect for DM. Significant and positive GCA effects for NSP were recorded for ARC Bamb 25, ARC Bamb 57, ARC Bamb 68 and ARC Bamb 8. Significant and desirable GCA effects for NNS, NSB, NSP PodL ST and YPP were computed for ARC Bamb 25. Parental line ARC Bamb 57 and ARC Bamb 8 exhibited significant and positive GCA effects for NSP, PodL, Npods and YPP. Significant and desirable GCA effects were recorded for HSW, YPP, SL and SW by ARC Bamb 66 and ARC Bamb 68. Parental lines ARC Bamb 25, ARC Bamb 55, ARC Bamb 57, ARC Bamb 66, ARC Bamb 68 and ARC Bamb 8 were the best combiners for YPP, recording high and positive GCA effects.

Table 5.4: Combining ability analysis of variance for agronomic traits of 45 Bambara groundnut families evaluated at Brits and Loskop in South Africa

Source of variation	DF	DM	NNS	NSB	NSP	PodL	PodW	ST	Npods	NSPod	HSW	SL	SW	YPP
Location (Loc)	1	1364.4***	1400.4***	80.2***	1436.0***	72.3**	90.3***	46.4***	2418.0***	2826.8***	69.0ns	500.8***	14.9***	2521.0***
Replication (Rep)	2	1663.1***	1745.9***	1105.4***	117.6***	34.7	453.1***	2.5***	14960.0***	332.2ns	9007.0***	505.7***	194.6***	11393.0***
Block	4	24.5ns	130.7*	24.9ns	0.9ns	19.1ns	11.0ns	0.6ns	2310.0***	265.9ns	226.0ns	142.3**	7.3*	576.0ns
SCA	45	74.3***	177.0***	82.9***	13.1***	17.3***	18.5***	0.8ns	2201.0***	112.2ns	341.0ns	24.2ns	10.5***	1770.0***
GCA	9	34.5ns	325.6***	35.3***	2.5ns	11.7ns	5.3ns	0.1ns	3155.0***	120.8ns	83.0ns	660.6***	1.8ns	909.0ns
Loc x SCA	45	88.0***	132.3***	97.8***	20.2***	18.4**	11.4***	0.7ns	2988.0***	115.7ns	300.0ns	22.6ns	9.3***	1305.0***
Loc x GCA	9	19.9ns	285.4***	57.1***	1.1ns	18.4ns	16.0**	0.1ns	4793.0***	109.0ns	139.0ns	682.9***	3.5ns	1183.0*
Residuals	214	34.50	43.90	13.90	5.80	9.80	5.20	0.61	420.0	145.80	334.00	33.80	3.00	554.00
σ^2sca		5.81	68.64	16.93	0.96	1.79	3.02	0.03	270.90	3.12	43.15	12.65	2.10	410.73
σ^2gca		0.76	18.30	5.67	0.49	0.67	1.63	0.01	300.20	1.01	29.97	65.02	0.86	127.30
Residual		54.24	79.96	37.25	8.62	11.87	9.76	0.62	1100.50	138.00	369.50	51.36	5.42	779.99
Baker's ratio		0.12	0.21	0.25	0.34	0.27	0.35	0.26	0.53	0.24	0.41	0.84	0.29	0.24
Heritability (%)		10.81	52.09	37.76	14.42	17.15	32.27	5.76	34.16	2.91	16.52	60.20	35.34	40.82

*, **and *** denote significant at 0.05, 0.01 and 0.001 probability levels, respectively. ns = Non-significant, DM = days to 75% maturity, NNS = Number of nodes per stem, NSB = number of secondary branches per stem, NSP = number of stems per plant, PodL = Pod length (cm), PodW = Pod width (cm), ST = Stem thickness (1/100 mm), Npods = Number of pods per plant, NSPod = Number of seeds per pod, HSW = Hundred seed weight (g/100 seed), SL = Seed length (cm), SW = Seed width (cm) and YPP = yield per plant (kg ha^{-1}), SCA = specific combining ability, GCA = general combining ability, Loc = location, σ^2sca = specific combining ability variance, σ^2gca = general combining ability variance.

Table 5.5: General combining ability effects for agronomic traits in Bambara groundnut parental lines evaluated at Brits and Loskop in South Africa

Parents	DM	NNS	NSB	NSP	PodL	PodW	ST	Npods	NSPod	HSW	SL	SW	YPP
ARC Bamb 25	-1.79*	1.81*	2.86***	1.52***	0.99**	1.41***	0.21*	-7.81**	0.33ns	2.47ns	0.01ns	0.90***	11.19***
ARC Bamb 42	-0.22ns	3.17***	-0.43ns	-0.41ns	-0.44ns	-0.26ns	0.02ns	-8.03**	-0.09ns	-2.51ns	-0.41ns	0.19ns	-7.75**
ARC Bamb 44	-0.76ns	-0.92ns	-0.30ns	-0.21ns	-0.14ns	-0.19ns	-0.08ns	0.03ns	0.23ns	-3.15ns	-0.13ns	-0.13ns	-2.26ns
ARC Bamb 55	0.01ns	0.13ns	-0.11ns	-0.54*	-0.17ns	-0.52ns	-0.05ns	-1.84ns	-1.08ns	-1.79ns	-0.10ns	-0.16ns	5.72*
ARC Bamb 57	-0.22ns	0.87ns	-1.12*	0.23*	0.28*	0.25ns	-0.06ns	8.24**	-0.75ns	-1.64ns	-1.01ns	-0.66**	5.80*
ARC Bamb 6	0.25ns	-0.47ns	-2.03***	-0.11ns	-0.12ns	-0.69*	-0.11ns	4.79ns	0.80ns	1.96ns	-0.15ns	0.22ns	4.58ns
ARC Bamb 66	-0.32ns	-1.62*	-0.78ns	-0.49ns	-0.45ns	-0.48ns	-0.11ns	-0.36ns	-0.20ns	4.39*	0.19*	0.31*	6.69*
ARC Bamb 68	-0.89ns	-1.34ns	1.31**	0.27*	0.53ns	0.72*	-0.06ns	-2.04ns	0.44ns	2.04*	2.80***	0.17*	10.01**
ARC Bamb 8	2.94***	0.96ns	2.19***	0.47*	0.60*	0.28ns	0.08ns	5.25*	-1.01ns	-0.18ns	0.09ns	0.07ns	8.81**
ARC Bamb 9	1.02ns	-2.59**	-1.59**	0.04ns	-0.47ns	-0.51ns	0.17ns	1.77ns	1.33ns	-1.56ns	-0.09ns	0.06ns	2.20ns

ARC-VIMP = Agricultural Research Council, Bamb = Bambara, *, **and ***Significant at 0.05, 0.01 and 0.001 probability level, respectively. ns = Non-significant, DM = days to 75% maturity, NNS = Number of nodes per stem, NSB = number of secondary branches per stem, NSP = number of stems per plant, PodL = Pod length (cm), PodW = Pod width (cm), ST = Stem thickness (1/100 mm), Npods = Number of pods per plant, NSPod = Number of seeds per pod, HSW = Hundred seed weight (g/100 seed), SL = Seed length (cm), SW = Seed width (cm) and YPP = yield per plant (kg ha^{-1}).

5.3.4 Specific combining ability effects of crosses for agronomic traits

Table 5.6 presented the SCA effects for agronomic traits of 45 Bambara groundnut crosses evaluated across two locations. Crosses ARC25 × ARC8, ARC44 × ARC55 and ARC44 × ARC66 had significant and negative SCA effects for DM, which were in the desirable direction. Three crosses, namely ARC42 × ARC57, ARC25 × ARC8 and ARC44 × ARC9 exhibited the highest and positive SCA effects for NNS. For branching capacity, there were seven crosses that exhibited positive and significant effects for NSB. Crosses including ARC25 × ARC57, ARC57 × ARC6, and ARC42 × ARC8 exhibited positive GCA effects for PodL. Only one cross ARC25 × ARC66 recorded positive and significant SCA effects for NSPods. Significant and positive SCA effects for YPP were recorded for three crosses: ARC25 × ARC8, ARC44 × ARC9 and ARC6 × ARC9. Crosses ARC25 × ARC55, ARC25 × ARC57, ARC25 × ARC9, ARC42 × ARC9, and ARC68 × ARC9 had undesirably high and negative SCA effects for YPP.

Table 5.6: Specific combining ability effects for grain yield and yield-related traits in 45 newly developed hybrids of Bambara groundnut evaluated at Brits and Loskop in South Africa

Crosses	DM	NNS	NSB	NSP	PodL	PodW	ST	Npods	NSPod	HSW	SL	SW	YPP
ARC25×ARC42	2.38ns	-6.06*	-5.92***	-2.01*	0.65ns	2.28*	-0.15ns	-0.75ns	-1.83ns	-3.46ns	-1.01ns	-0.75ns	4.12ns
ARC25×ARC44	-0.87ns	2.84 ns	-0.42 ns	1.11ns	0.24ns	-3.61***	0.02ns	-3.99ns	5.00ns	-2.85ns	-0.79ns	0.40ns	10.64 ns
ARC25×ARC55	-1.07ns	0.93 ns	1.02 ns	-1.58*	-0.56ns	-2.64**	0.08ns	11.05ns	-1.13ns	-3.61ns	1.32ns	0.03ns	-20.24*
ARC25×ARC57	1.58ns	-1.11ns	4.89**	3.22***	3.60***	4.81***	0.03ns	8.46ns	-6.01ns	3.88ns	0.15ns	-0.54ns	-25.52**
ARC25×ARC6	1.43ns	11.81***	3.13*	2.34**	-2.02*	2.17*	-0.13ns	14.99ns	1.60ns	-3.95ns	2.29ns	2.07**	13.27ns
ARC25×ARC66	0.51ns	0.16ns	2.16ns	-1.43ns	-0.50	-1.60ns	-0.11ns	-3.08ns	11.03*	23.31***	1.16ns	0.58ns	-6.22ns
ARC25×ARC68	-0.25ns	-2.32ns	2.29ns	0.17ns	2.21*	0.22ns	-0.07ns	-13.00ns	-3.96ns	-4.58ns	-2.67ns	0.47ns	-15.71ns
ARC25×ARC8	-8.08***	7.20**	2.57ns	1.09ns	-0.78ns	0.56ns	1.13***	-31.71***	1.07ns	-1.66ns	-1.63ns	-0.40ns	47.54***
ARC25×ARC9	0.38ns	-5.52*	-0.47ns	0.52ns	-0.31ns	0.19ns	-0.14ns	-7.48ns	-8.17ns	-5.42ns	-1.51ns	0.31ns	8.18ns
ARC42×ARC44	1.21ns	-1.77ns	1.04ns	0.22ns	0.62ns	-0.26ns	-0.28ns	5.73ns	3.93ns	2.52ns	-1.07ns	-1.16ns	2.12ns
ARC42×ARC55	0.77ns	-8.49**	-0.19ns	1.38ns	0.02ns	-0.24ns	-0.07ns	42.94***	-6.92ns	-4.23ns	0.52ns	-1.16ns	-9.56ns
ARC42×ARC57	-2.98ns	12.1***	-3.46*	-1.00ns	-2.50*	-1.29ns	-0.28ns	-15.72*	-2.41ns	-5.29ns	0.41ns	3.52***	3.70ns
ARC42×ARC6	-3.63ns	1.41ns	-0.14ns	-0.16ns	-1.49ns	-0.45ns	-0.09ns	-16.14*	6.68ns	-1.91ns	-0.66ns	-0.54ns	2.81ns
ARC42×ARC66	-3.22ns	-1.23ns	-0.80ns	-0.83ns	0.26ns	0.50ns	-0.03ns	9.63ns	6.02ns	5.82ns	0.38ns	-0.59ns	8.74ns
ARC42×ARC68	-3.98ns	-2.51ns	-2.73ns	-0.88ns	1.58ns	0.77ns	-0.16ns	2.50ns	0.76ns	1.30ns	-2.37ns	1.09ns	1.93ns
ARC42×ARC8	1.84ns	-5.48*	5.71***	2.53**	2.65**	0.30ns	-0.22ns	-14.49ns	-1.82ns	5.83ns	2.07ns	-0.24ns	-2.61ns
ARC42×ARC9	4.09ns	-2.93ns	5.00**	0.63ns	-1.12ns	-1.19ns	1.54***	0.99ns	1.15ns	-2.71ns	2.55ns	1.40*	-18.75*
ARC44×ARC55	-4.69*	1.93ns	-4.35**	-0.64ns	-0.55ns	0.32ns	0.19ns	-7.45ns	4.75ns	1.42ns	-1.26ns	0.70ns	5.98ns
ARC44×ARC57	-2.78ns	2.19ns	-1.93ns	-0.03ns	0.06ns	-0.20ns	0.02ns	5.62ns	-0.24ns	6.61ns	0.74ns	0.35ns	1.63ns
ARC44×ARC6	-1.76ns	-3.29ns	1.17ns	0.08ns	0.01ns	1.9*ns	-0.11ns	-13.34ns	-4.97ns	-3.78ns	0.85ns	-0.90ns	-2.76ns
ARC44×ARC66	-6.01*	-7.97**	-3.76*	0.29ns	2.37*	-0.17ns	0.16ns	-34.93***	-2.96ns	-8.82ns	1.09ns	-0.14ns	-9.74ns
ARC44×ARC68	5.71*	-2.42ns	4.29**	-0.41ns	-1.23ns	0.31ns	0.02ns	11.57ns	-5.11ns	-8.55ns	-2.83ns	0.82ns	-10.48ns
ARC44×ARC8	0.88ns	-6.06*	5.51***	-1.25ns	-0.48ns	1.86*	0.12ns	4.88	1.01ns	7.09ns	1.98ns	0.11ns	-7.49ns
ARC44×ARC9	1.40ns	11.24***	0.21ns	0.09ns	0.61ns	0.30ns	-0.13ns	-24.91**	0.82ns	6.90ns	1.28ns	0.84ns	30.08**
ARC55×ARC57	2.10ns	-9.19***	1.04ns	0.12ns	1.30ns	0.46ns	-0.14ns	-7.16ns	5.23ns	9.22ns	2.57ns	0.39ns	2.06ns
ARC55×ARC6	-0.21ns	2.81ns	1.78ns	-0.91ns	-0.04ns	-0.07ns	0.01ns	-26.80**	-4.24ns	-9.51ns	-0.67ns	-0.02ns	-5.26ns
ARC55×ARC66	4.2ns	6.97**	4.54**	0.46ns	0.36ns	1.24ns	0.06ns	9.61ns	-0.65ns	1.19ns	0.74ns	0.08ns	0.80ns
ARC55×ARC68	-0.89ns	5.18*	0.10ns	0.91ns	-1.32ns	0.02ns	0.19ns	6.6ns1	5.20ns	11.88*	-3.28ns	-0.58ns	14.29ns

ARC55×ARC8	3.43ns	0.38ns	-0.60ns	0.16ns	1.79ns	0.88ns	-0.30ns	-27.01**	-3.34ns	-1.49ns	0.11ns	1.10ns	-5.58ns
ARC55×ARC9	-2.48ns	-2.06ns	-0.39ns	-0.23ns	1.11ns	-0.38ns	-0.16ns	7.46ns	-0.46ns	-0.35ns	-0.53ns	-1.51*	9.85ns
ARC57×ARC6	1.69ns	-6.91**	2.03*	-0.80ns	2.71**	0.16ns	0.09ns	42.19***	-2.73ns	2.55ns	0.35ns	-0.02ns	-7.08ns
ARC57×ARC66	-1.05ns	-1.59ns	-1.77ns	1.40ns	-0.76ns	-1.23ns	0.07ns	-20.14*	-5.23ns	-1.12ns	0.82ns	0.50ns	14.21ns
ARC57×ARC68	0.34ns	-2.38ns	-0.04ns	-0.64ns	-2.46*	-0.46ns	0.07ns	-9.30ns	2.20ns	-5.82ns	-3.91ns	-0.39ns	9.04ns
ARC57×ARC8	-0.15ns	6.81**	-2.09ns	-1.39ns	-1.86ns	-2.36**	0.01ns	35.06***	-4.00ns	-7.00ns	-1.36ns	-3.56***	-10.04ns
ARC57×ARC9	-1.07ns	-1.80ns	1.53ns	-0.45ns	-0.94ns	0.62ns	-0.02ns	-11.42ns	5.97ns	-1.11ns	0.12ns	-0.73ns	-1.31ns
ARC6×ARC66	-0.70ns	0.58ns	-2.20ns	-0.63ns	-1.46ns	-1.07ns	0.10ns	7.13ns	1.29ns	-1.02ns	0.99ns	-0.24ns	-5.02ns
ARC6×ARC68	-0.30ns	-1.87ns	-3.05*	0.31ns	2.14*	-1.33ns	-0.09ns	6.14ns	0.81ns	12.52*	-3.91ns	-0.35ns	-0.10ns
ARC6×ARC8	3.19ns	0.49ns	-2.63ns	-0.02ns	0.39ns	-0.21ns	-0.06ns	8.17ns	1.01ns	-0.08ns	2.45ns	2.29**	-0.17ns
ARC6×ARC9	-2.22ns	-1.79ns	-0.39ns	-1.17ns	-0.32ns	-0.37ns	-0.02ns	-9.17ns	4.92ns	12.05*	-0.17ns	-0.56ns	19.31*
ARC66×ARC68	-1.05ns	-1.32ns	2.18ns	0.35ns	-2.36*	0.02ns	0.04ns	-4.02ns	-3.51ns	-8.17ns	-3.90ns	-0.58ns	3.38ns
ARC66×ARC8	4.44ns	-2.52ns	-0.09ns	-0.05ns	2.47*	0.45ns	-0.22ns	13.84ns	1.77ns	-2.67ns	-1.95ns	0.04ns	2.59ns
ARC66×ARC9	4.19ns	3.03ns	0.53ns	0.20ns	-0.62ns	2.67**	-0.15ns	41.15***	-4.40ns	-3.77ns	-0.04ns	0.66ns	-8.30ns
ARC68×ARC8	-1.15ns	2.72ns	-1.76ns	-1.10ns	-0.14ns	1.17ns	0.02ns	-0.32ns	0.79ns	5.90ns	-2.81ns	0.38ns	-1.41ns
ARC68×ARC9	-1.23ns	1.91ns	-1.23ns	0.49ns	0.55ns	0.94ns	-0.22ns	-6.50ns	-3.71ns	-0.53ns	-1.69ns	-0.86ns	-25.96**
ARC8×ARC9	1.26ns	-2.05ns	-3.45*	1.57*	-0.73ns	-1.03ns	-0.26ns	14.69ns	4.23ns	-3.96ns	1.15ns	1.55*	-0.98ns

ARC-VIMP = Agricultural Research Council, *, **and *** denote significant at 0.05, 0.01 and 0.001 probability levels, respectively. ns=non-significant; DM = days to 75% maturity, NNS = Number of nodes per stem, NSB = number of secondary branches per plant, NSP = number of stems per plant, PodL = Pod length, PodW = Pod width, ST = Stem thickness (1/100 mm), Npods = Number of pods per plant, NSPod = Number of seeds per pod, HSW = Hundred seed weight (g), SL = Seed length, SW = Seed width and YPP = yield per plant.

5.4 Discussion

Bambara groundnut has excellent adaptation to drought with nutrient profile,making it an ideal candidate for developing various food products. In continental Africa, the crop is cultivated using genetically diverse landrace varieties, which are low-yielding (< 2.3 ton/ha), thus limiting the crop's large-scale production and commercial value. There is a need for dedicated breeding to develop high-performing Bambara groundnut varieties with farmer-and-consumer preferred traits for adoption by growers and industry.

The present study determined the combining ability effects controlling yield and related traits in Bambara groundnut to identify superior genotypes as donor parents and to develop breeding populations for selection and genetic advancement. The studied germplasm varied for agronomic traits, including days to maturity, pod length, pod width and grain yield (Table 5.2). These indicated the selected parents and the novel crosses could be useful for breeding, targeting high-yield potential and other desirable agronomic traits. For example, the early maturing parental lines and crosses can be used in developing drought-escape varieties for cultivation in short-season production environments characterised by drought stress. Early maturing crosses ARC8 × ARC25, ARC68 × ARC42, ARC66 × ARC44 and ARC55 × ARC44 (Table 5.3) are selected as best recombinants to enhance early flowering and maturity. Farmers in sub-Saharan Africa are challenged by increasingly short rainy seasons due to climate change (Onyango and Nzengya, 2023). Developing genotypes that can escape terminal drought stress would increase the productivity of Bambara groundnut.

Developing varieties with high grain yield potential is a desirable trait for increasing the productivity of Bambara groundnut. Thus, selecting newly developed crosses with high yield potential will be a matter of choice for genetic advancement. Also, these crosses must incorporate other important traits such as secondary branching, earliness and seed quality to satisfy different market segments. Crosses ARC8 × ARC25 and ARC6 × ARC25 exhibited higher grain yield potential than their parental lines (Table 5.3). These indicated a favourable recombination of alleles donated by the individual parents.

Also, the yield of the newly-developed crosses is higher than the yield of the popular variety MPB 51 (Brianbeck), which is widely cultivated in South Africa (Mabhaudhi et al. 2014). Also, the yield attained for most crosses is comparable to MPB39 landrace, which is popularly grown

in KwaZulu-Natal (Musango et al. 2022). However, the new crosses have desirable shorter days to 75% maturity than this landrace.

Quantitative agronomic traits are governed by multiple genes, allowing differential genotype response to environment factors (Esan et al. 2023). The test locations influenced the parents' and crosses' different trait expressions (Table 5.4). The test locations is reportedly the largest contributor to the expression of the number of secondary branches, pod length, number of pods per plant and grain yield traits in Bambara groundnut (Jonah et al. 2010). Therefore, careful selection of production areas and appropriate agronomic practices are recommended to farmers to achieve high yields. Genetic potential for high yield must be complemented with appropriate technologies such as pest and weed control, fertilization and water management and appropriate planting time to reach the potential yields.

Genotype-by-environment interaction is the leading cause of the varied performance of genotypes when grown under different production environments. Many studies have reported significant genotype × environment interaction for Bambara groundnut agronomic performance and yield potential (Sari et al. 2021, Modi et al. 2022, Paulos et al. 2022). In the present study, genotype × location (environment) interaction effects were notable for studied agronomic traits (Table 5.2). These provide opportunities and challenges for selecting superior/desirable genotypes. Genotypes that perform consistently across all the test locations are considered widely adaptable. However, some genotypes perform extremely well in one location. These have specific adaptations to an environment. Parental lines, including ARC Bamb 55, ARC Bamb 109 and ARC Bamb 104 had an overall high mean performance, but ARC Bamb 55 was specifically adapted to Loskop while ARC Bamb 104 was superior at Brits. Among the crosses, ARC9 × ARC44, ARC6 × ARC25 and ARC9 × ARC6 were the overall best genotypes for yield potential but ARC9 × ARC44 was superior at Loskop and ARC6 × ARC25 was the best at Brits. The crosses that exhibit specific adaptation should be used for developing cultivars for the target production environment. However, broad adaptation should be sought in breeding populations to allow the germplasm to wider production. Germplasm with limited areas of adaptation will limit germplasm exchange.

The variances for GCA effects of the parental lines on several traits were significant, suggesting that the traits were conditioned by additive gene effects (Onwubiko et al. 2019). However, the variances for location × GCA interaction effects were also significant, indicating that the

expression of the additive genetic effects was affected by environmental factors. Traits with significant and positive GCA variances can be exploited by selecting parental lines with desirable characteristics to develop higher-performing progenies through hybridization. Grain yield, branching and maturity are important traits in developing suitable Bambara groundnut varieties. Two parents, ARC Bamb 25 and ARC Bamb 66 were good general combiners for grain yield per plant and hundred seed weight (Table 5.4), which indicates that these parents can be selected for developing breeding populations. Parental lines with desirable GCA effects can be selected for varietal development in self-pollinating species.

Traits with significant SCA variances indicate that non-additive gene effects partly control their inheritance. Non-additive gene effects arise from the interaction of alleles from of diverse parents (Hochholdinger and Baldauf 2018). If the allelic interaction is favourable, the resultant progeny performs superior to the parents involved due to dominance, overdominance or epistasis. SCA effects represent the non-additive proportion of variance that is difficult to exploit in trait improvement in self-pollinating crops due to low heritability and unpredictability of reshuffling of genes. Specific combining ability effect relates to the performance of some crosses relatively better or worse than would be expected based on the average performance of the parents involved. Osei et al. (2014) reported that selecting crosses with superior performance compared to their parents enhanced genetic gain in breeding through favourable gene recombination.

For developing early maturing breeding populations, crosses with negative SCA effects for number of days to 75% maturity are desirable for selection. These crosses include ARC25×ARC55, ARC25×ARC55, ARC25×ARC68, ARC25×ARC8, ARC42×ARC57, ARC42×ARC6, ARC42×ARC66, ARC42×ARC68, ARC44×ARC55, ARC44×ARC57, ARC44×ARC6 and ARC44×ARC66 (Table 5.5). Early maturing varieties are ideal for marginal environments such as those mostly found in sub-Saharan Africa, characterised by inadequate rainfall and high temperatures. However, earliness to maturity can lead to yield penalties in environments where soil moisture and other growing conditions are favourable.

High SCA effects for yield and related traits are desirable as they transmit additive genes during selection (Dholariya et al. 2014). The best hybrids selected in this study were ARC25×ARC8 for grain yield per plant and number of nodes per stem, ARC42×ARC8 for NSB, ARC25×ARC8 for early maturity, ARC42×ARC55 for number of pods per plant and ARC42×ARC57 for seed width. This indicates that the parents are good combiners; hence their

crosses are selected for improving yield and related trait. These crosses can generate novel breeding populations from which best-performing individuals with favourable agronomic characteristics can be selected for variety development. However, since the dominance, over-dominance and epistatic components are random and cannot be predicted, it will be difficult to breed for traits that are predominantly controlled by SCA effects (Wade 2001; Kristensen and Sørensen 2005). Furthermore, the SCA variance for most traits exhibited significant interactions with environmental effects. This means that the expression of SCA effects varied over the test locations, which makes breeding efforts even more complicated when attempting to exploit this component of trait inheritance.

Understanding the genetic control of agronomic traits is vital to guide selection in crop breeding programs. The ratio of GCA to SCA variances provides a valuable measure of trait control (Shaibu et al. 2018). GCA/SCA ratio higher than a 0.50 indicates additive gene effects, while the reverse is true for traits controlled largely by non-additive gene effects. In the present study, the majority of traits exhibited GCA/SCA ratios below 0.50, indicating that non-additive gene effects were preponderant. In terms of selection, these suggested that trait improvement will only be effective after selection in the advanced generations and that the selection of these traits in subsequent early generations could lead to unpredictable genetic gains. The non-additive gene action found in this study were in concurrence with Shiferaw (2022), who reported that grain yield was controlled by non- additive gene action. Heritability is a useful measure of the proportion of variance observed among genotypes due to genetic differences and is expressed in a broad and narrow sense genetic control of traits (Oppong-Sekyere et al. 2019). Robinson et al. (1949) classified heritability values less than 30% as low, between 30 and 60% as moderate and, above 60% as high. In the present study, heritability estimates for the number of nodes per stem, seed length, number of pods per plant and yield per pod were moderate, suggesting a high environmental influence on their expression. Similarly, Khaliqi et al. (2021) reported high heritability estimates for plant height, 100 seed weight, and harvest count in Bambara groundnut populations. High heritability estimates for grain yield were reported by (Rejeki et al. 2020). Conversely, the number of seeds per pod showed the lowest heritability (2.91%). Thissignifies that total phenotypic variance was primarily influenced by the environment, needing to delay the selection in advanced generations. The most suitable strategy for improving traits that are controlled by non-additive gene effects is through early

generation selection of transgressive segregants.Advanced generation family selection is required for polygenes with additive genetic effect (Wang et al. 2018).

5.5 Conclusion

The present study estimated combining ability effects and gene action controlling yield and related traits in Bambara groundnut to identify superior genotypes as donor parents and to develop breeding populations for selection and genetic advancement. The parental lines ARC Bamb25, ARC Bamb8 and ARC Bamb55 recorded positive and desirable GCA effects for YPP. The higher mean performance of progenies compared to their parents signalled possible genetic gain in yield and related traits through hybridization. The progenies ARC25×ARC8, ARC44×ARC9 and ARC6×ARC9 had desirable SCA effects for YPP, ARC44×ARC8, ARC44×ARC68, ARC42×ARC8 for number of branches per stem (NSB), ARC25 ×ARC8 for early maturity, ARC42×ARC55 for number of pods per plant and ARC42 ×ARC57 for seed width. Progenies selected in the current study are useful breeding populations and will be subjected to further selection and release. The SCA effects were preponderant, indicating that non-additive genes governed trait inheritance. The new families selected in the current study are valuable breeding populations and will be subjected to selection and multilocation evaluation to release the best-performing varieties.

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Overview of the research findings

Introduction and objectives of the study

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is indigenous to Africa and supports millions of smallholders, notably women farmers in arid and semi-arid regions. . The crop is drought-resistant and grown by subsistence farmers under traditional low-input and marginal agricultural systems. Bambara groundnut seeds are highly nutritious, containing ~72% of carbohydrates, ~25% of proteins and ~7% of fats. The seed comprised 32.72% essential amino acids and 66.10% non-essential amino acids. Lysine is the major essential amino acid representing 10.3% of the total essential amino acids. Bambara groundnut has intrinsic eating quality attributes and resilience to biotic and abiotic stresses associated with climate change. The crop has adapted and evolved under the prevailing farming and environmental conditions, pest and disease pressure, and low-input farming systems in Africa. However, the crop received limited research and development attention by researchers and policymakers, and hence, its economic value, production methods, product development, and commercialisation have not yet been fully explored. Due to a lack of systematic genetic improvement, the grain yield of Bambara groundnut is low (<0.85 ton ha^{-1}) and stagnant. Therefore, there is an urgent need to bolster the pre-breeding and breeding of the crop to develop high-yielding and nutritionally enhanced new varieties with desired traits. This study was executed with the following objectives:

- To document the progress made on Bambara groundnut production, utilisation and genetic improvement in SSA to discern the key production constraints, genetic resources and analysis, breeding methods and gains on yield and nutrition to guide breeding.
- To assess the genotype-by-environment interaction (GEI) effect on grain yield and to select best adapted Bambara groundnut genotypes in South African target production areas for breeding.
- To determine the compositions of phytochemicals and mineral elements present in Bambara groundnut genetic pool to identify superior and contrasting genotypes to guide product development and breeding Determine the magnitude of the genetic diversity and population structure of Bambara groundnut collections of South Africa using high throughput single nucleotide polymorphisms (SNP) markers to complement phenotypic and nutrition profile data for genotype selection and breeding.

- Determine the combining ability effects and gene action conditioning yield and related traits in Bambara groundnut genotypes to identify the best combiner donor parents and progenies for genetic advancement, cultivar development and release.

Research findings in brief

Bambara groundnut (*Vigna subterranea* [L.] Verdc) production, utilisation and genetic improvement in sub-Saharan Africa

A literature review study was conducted to identify key constraints to Bambara groundnut production and effective breeding methods on yield and nutritional quality to guide crop improvement. The main findings of the study were as follows:

- Grain yield of Bambara groundnut, is reportedly low (<0.85 ton ha^{-1}) and stagnant compared to the major food security crop species due a lack of dedicated genetic improvement of the crop.
- Key traits required for Bambara groundnut breeding are grain yield and quality gains, disease resistance, heat and drought tolerance, early cooking time and low levels of antinutritional components.
- Current research focus has moved to underutilised crops such as Bambara groundnut due to its potential to cope with climate change and provide nutritional security.
- Improved crop management and post-handling technologies, modern varieties with high yield and nutritional quality, value addition, and market access are among the key research and development drivers of Bambara groundnuts.
- Information presented will guide sustainable production, effective crop breeding to pursue food and nutrition security and improved livelihoods through Bambara groundnut enterprises in South Africa.

Genotype by environment interaction effects and stability analysis of Bambara groundnut (*Vigna subterranea* [L.] Verdc) for grain yield in South Africa.

Seventy-five Bambara groundnut genotypes were used in this study. The genotypes were evaluated across seven environments in South Africa using a 5 × 15 alpha lattice design with three replications. The main finding were:

- Significant ($p<0.05$) differences were recorded among genotypes (G), environments (E) and GEI effects on grain yield.
- A relatively higher proportion of the observed variation was due to GEI (36.62%), followed by environment (35.63%) and genotype (24.16%) effects.
- Genotype ARC Bamb 68 (0.96 ton ha^{-1}), ARC Bamb- 9 (0.88 ton ha^{-1}) and ARC Bamb-54 (0.84 ton ha^{-1}) attained the highest grain yield across locations, while ARC Bamb-74 exhibited the lowest grain yield of 0.16 ton ha^{-1}.
- The genotype and genotype-by-environment biplot identified ARC Bamb-17, ARC Bamb-14, ARC Bamb -20, ARC Bamb-18, ARC Bamb-14, and ARC Bamb-26 as the most stable genotypes across locations, while ARC Bamb-18 and ARC Bamb-54 were specifically adapted to Loskop and Brits.
- The Mafikeng site was ideal for Bambara groundnut evaluation, genotype differentiation, and large-scale seed production.

Analyses of the compositions of phytochemicals and minerals in Bambara groundnut genotypes

Seventy-five genetically diverse Bambara groundnut genotypes were field evaluated across four environments using a 15 x 5 alpha lattice design with three replications. Genotypes were profiled for fat, phenolic and flavonoids contents at the Agricultural Research Council (ARC) analytical laboratory in South Africa. Further, the genotypes were assessed for the contents of the following minerals: calcium (Ca), iron (Fe), potassium (K), phosphorus (P), zinc (Zn) and nitrogen (N). The major findings were as follows:

- The nutritional content of the test genotypes varied significantly ($P<0.05$), which were affected by the genotype and environment interactions.
- The Ca, Fe, K and Zn content varied from 150.70 to 216.53, 4.30 to 16.77, 771.99 to 1155.89 and 5.50 to 7.17 mg.100 g^{-1} dry seed sample, respectively.

- Genotypes, including ARC Bamb-2, ARC Bamb-19, ARC Bamb-73, ARC Bamb-56, ARC Bamb-37, ARC Bamb-3 and ARC Bamb-69 exhibited the highest fat content (>6.00 %).
- ARC Bamb-40 and ARC Bamb-59 recorded a higher mean Fe content of 16.00 mg.100 g^{-1}. ARC Bamb-2 was the top-performing genotype with high fat content (6%), Ca (211.93 mg.100 g^{-1}), and Zn (7.17 mg.100 g^{-1}).
- Ca,K, and N contents displayed strong correlations ($r>0.60$, $P<0.05$). Phosphorus and Zn contents exhibited moderate correlations with Ca.
- Overall, the study selected genotypes ARC Bamb-73, ARC Bamb-19, ARC Bamb-9 and ARC Bamb-2 with high compositions of essential nutrients for product development or breeding.
- The selected genetic resources are valuable for trait integration and developing new breeding populations with enhanced nutrient compositions and agronomic and market-preferred traits

Genetic diversity and population structure analyses of South African Bambara groundnut (*Vigna subterranea* [L.] Verdc).

This study determined the magnitude of the genetic diversity and population structure of Bambara groundnut collections in South Africa using high throughput single nucleotide polymorphisms (SNP) markers. Ninety-three genotypes (75 tests genotypes and 18 ARC collections) were assessed using 2286 SNP markers and some unique complementary morpho-agronomic traits of the crop. The research findings are below:

- The mean genetic diversity value was 0.32, revealing moderate genetic differences among the assessed genotypes.
- Cluster and structure analyses grouped the test genotypes into two distinct categories.
- The analysis of molecular variance partitioned the total genetic variation in to among genotypes (90%), within genotypes (8%) and among populations (2%).
- The results revealed two heterotic groups for hybridisation and selection programs.
- The following unique genotypes were selected: ARC Bamb-37 (with spreading growth type), ARC Bamb-49 (bunch type), ARC Bamb-61 (semi-bunch) and ARC Bamb-83 (spreading) using the SNP markers and desirable agronomic traits.

- The study provided insights into Bambara groundnut genetic profiles of South African collections and complemented phenotypic and nutrition profile data for genotype selection and breeding.

Combining ability and gene action in Bambara groundnut (*Vigna subterranea* (L.) Verdc.) genotypes for agronomic traits.

This part of the study determined the combining ability effects and gene action conditioning yield and related traits in Bambara groundnut genotypes to identify the best combiner donor parents and progenies for genetic advancement and breeding. Ten contrasting parents were selected and crossed using a 10 × 10 half-diallel mating design, and 45 progenies were developed. The progenies and their parents were field evaluated using a 5 × 11 lattice design with two replications in two contrasting locations in South Africa. Data was collected on agronomic traits and subjected to statistical analyses to compute genetic parameters. The key findings were:

- Genotype × location interaction effect was significant ($P < 0.05$) for the studied agronomic traits.
- General combining ability (GCA) and specific combining ability (SCA) effects were significant in most assessed agronomic traits, including yield per plant.
- The GCA × location and SCA × location interaction effects were significant for most traits.
- A Baker's ratio of < 1 were recorded for most assessed traits, indicating the preponderance of non-additive gene effects conditioning the traits.
- The parental lines such as ARC Bamb25, ARC Bamb8 and ARC Bamb55 recorded positive and desirable GCA effects for yield per plant.
- The progenies ARC25×ARC8, ARC44×ARC9 and ARC6×ARC9 had desirable SCA effects for yield per plant, ARC44×ARC8, ARC44×ARC68, ARC42×ARC8 for higher number of secondary branches per stem, ARC25 ×ARC8 for early maturity, ARC42 ×ARC55 for higher number of pods per plant and ARC42 ×ARC57 for increased seed width.
- The new families selected in the current study are valuable breeding populations and will be subjected to selection and multilocation evaluation for genetic advancement, cultivar development and release.

Implications of the study to breeding Bambara groundnut for high yield and nutritional quality.

- The review showed limited research progress on Bambara groundnut improvement and product development in Africa. Also, SSA has yet to develop modern crop management, production technologies, and value chains to achieve economic gains from Bambara groundnut production and marketing. Improved crop management and post-handling technologies, modern varieties with high yield and nutritional quality, value addition, and market access are among the vital research and development drivers of Bambara groundnuts.
- The study identified genotypes with high grain yield and stability to be adopted as genetic resources for cultivar development in South Africa.
- Superior genotypes with high mineral elements and nutrient content were identified as potential parents for the development of highly nutritious Bambara groundnut cultivars.
- Low genetic diversity among the South African Bambara groundnut genotypes requires further improvements through targeted crosses.
- Overall, the study identified new families with high combining ability effects for yield per plant and early maturity, which are recommended for genetic advancement and multilocation evaluation, cultivar development and release.